Univerzita Karlova v Praze

2. lékařská fakulta

Studijní program: Experimentální chirurgie

MUDr. Petr Škapa

Patologické prognostické faktory v experimentální chirurgické léčbě zhoubných nádorů dolního pohlavního ústrojí žen (hrdlo děložní, vulva)

Pathologic prognostic factors in the experimental surgical therapy of malignant tumors of the lower female genital tract (uterine cervix, vulva)

Dizertační práce

Školitel: prof. MUDr. Josef Zámečník, Ph.D.

Praha, 2014

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem řádně uvedl a citoval všechny použité prameny a literaturu. Současně prohlašuji, že práce nebyla využita k získání jiného nebo stejného titulu.

Souhlasím s trvalým uložením elektronické verze mé práce v databázi systému meziuniverzitního projektu Theses.cz za účelem soustavné kontroly podobnosti kvalifikačních prací.

V Praze, 25.11.2014

MUDr. Petr Škapa

Podpis

Identifikační záznam:

ŠKAPA, Petr. *Patologické prognostické faktory v experimentální chirurgické léčbě zhoubných nádorů dolního pohlavního ústrojí žen (hrdlo děložní, vulva). [Pathologic prognostic factors in the experimental surgical therapy of malignant tumors of the lower female genital tract (uterine cervix, vulva)].* Praha, 2014. 69 s., 14 příl. Dizertační práce (Ph.D.). Univerzita Karlova v Praze, 2. lékařská fakulta, Ústav patologie a molekulární medicíny 2. LF UK. Školitel Zámečník, Josef.

Klíčová slova:

vulva, děložní hrdlo, HPV, karcinom, dlaždicobuněčný karcinom, CIN, VIN, imunohistochemie, p16, sentinelová lymfatická uzlina, fertilitu zachovávající operace

Klíčová slova anglicky:

vulva, uterine cervix, HPV, carcinoma, squamous cell carcinoma, CIN, VIN, immunohistochemistry, p16, sentinel lymph node, fertility sparing surgery

Poděkování

Na tomto místě bych rád poděkoval v první řadě svému školiteli prof. MUDr. Josefu Zámečníkovi, Ph.D. za odborné vedení a cenné rady při zpracování předkládané práce.

Dále děkuji prof. MUDr. Lukáši Robovi, CSc. a jeho spolupracovníkům z Gynekologicko-porodnické kliniky 2.LF UK a FN v Motole za spolupráci při odběru bioptického materiálu, detekci sentinelových lymfatických uzlin a klinicko-patologickou korelaci nálezů. Prof. MUDr. Lukáši Robovi, CSc. jsem navíc obzvláště vděčný za podnětnou diskuzi a četné konzultace a připomínky, které mi byly neocenitelnou pomocí v klinicky zaměřené části projektu.

Za spolupráci v molekulární detekci a typizaci lidských papilomavirů děkuji RNDr. Ruth Tachezy, Ph.D. a jejímu kolektivu z Oddělení experimentální virologie Ústavu hematologie a krevní transfuze.

V neposlední řadě děkuji laborantkám Ústavu patologie a molekulární medicíny 2.LF UK a FN v Motole za histologické zpracování bioptického materiálu a provádění imunohistochemických vyšetření. Jmenovitě bych rád poděkoval paní Vlastimile Forejtové za spolupráci v imunohistochemické dignostice prekancerózních lézí děložního hrdla.

Svým nejbližším za lásku a za krásy tohoto světa.

Autor této dizertační práce si dovoluje využít možnosti, která mu byla dána oborovou radou doktorského studijního programu *Experimentální chirurgie* 2. lékařské fakulty Univerzity Karlovy v Praze, a předkládá ji jako soubor originálních a přehledných prací opatřených komentářem. Kopie jednotlivých publikací jsou uvedeny v příloze.

SEZNAM PUBLIKACÍ V PŘÍLOZE

Příloha I. Škapa P., Zámečník J., Hamšíková E., Saláková M., Šmahelová J., Jandová K., Robová H., Rob L. a Tachezy R. (2007) Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. ***Am J Surg Pathol*** 31:1834-1843.
IF 4,144

Příloha II. Tachezy R., Šmahelová J., Saláková M., Arbyn M., Rob L., Škapa P., Jirásek T. a Hamšíková E. (2011) Human papillomavirus genotype distribution in Czech women and men with diseases etiologically linked to HPV. ***PLoS One*** 6:e21913.
IF 4,411

Příloha III. Škapa P., Robová H., Rob L. a Zámečník J. (2012) Prekancerózní léze vulvy. ***Cesk Patol*** 48:15-21.

Příloha IV. Škapa P., Pichlík T., Pluta M., Halaška M., Robová H., Rob L., Tachezy R., Zámečník J. (2014) Klasifikace vulvárních prekanceróz pohledem patologa. ***Actual Gyn*** 6:26-32.

Příloha V. Škapa P., Robová H., Rob L. a Zámečník J. (2013) $p16^{INK4a}$ immunoprofiles of squamous lesions of the uterine cervix – implications for the reclassification of atypical immature squamous metaplasia. ***Pathol Oncol Res*** 19:707-714.
IF 1,555

Příloha VI. Rob L., Charvát M., Robová H., Pluta M., Strnad P., Hrehorčák M. a Škapa P. (2007) Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. ***Int J Gynecol Cancer*** 17:304-310.
IF 1,427

Příloha VII. Rob L., Pluta M., Strand P., Hrehorčák M., Chmel R., Škapa P. a Robová H. (2008) A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. ***Gynecol Oncol*** 111:S116-120.
IF 2,614

Příloha VIII. Strnad P., Robová H., Škapa P., Pluta M., Hrehorčák M., Halaška M. a Rob L. (2008) A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. ***Gynecol Oncol*** 109:280-284.
IF 2,319

Příloha IX. Robová H., Pluta M., Hrehorčák M., Škapa P. a Rob L. (2008) High-dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. ***Int J Gynecol Cancer*** 18:1367-1371.
IF 1,425

Příloha X. Pluta M., Rob L., Charvát M., Chmel R., Halaška M., Škapa P. a Robová H. (2009) Less radical surgery than radical hysterectomy in early stage cervical cancer: A pilot study. ***Gynecol Oncol*** 113:181-184.
IF 2,614

Příloha XI. Rob L., Pluta M., Škapa P. a Robová H. (2010) Advances in fertility-sparing surgery for cervical cancer. ***Expert Rev Anticancer Ther*** 10:1101-1114.
IF 2,493

Příloha XII. Rob L., Škapa P. a Robová H. (2011) Fertility-sparing surgery in patients with cervical cancer. ***Lancet Oncol*** 12:192-200.
IF 14,470

Příloha XIII. Rob L., Robová H., Chmel R., Komár M., Halaška M. a Škapa P. (2012) Surgical options in early cervical cancer. ***Int J Hyperthermia*** 28:489-500.
IF 1,923

Příloha XIV. Robová H., Halaška M., Pluta M., Škapa P., Matěcha J., Lisý J., Rob L. (2014) Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer. ***Gynecol Oncol*** (v tisku)
IF 3,687

OBSAH

1. ÚVOD A PŘEHLED PROBLEMATIKY

1.1. Nádorová onemocnění dolního ženského pohlavního systému

Maligní nádory ženského pohlavního systému se v České republice již dlouhodobě umísťují na předních místech žebříčku incidence a mortality nádorových onemocnění. Dle posledních publikovaných údajů Ústavu zdravotnických informací a statistiky ČR (ÚZIS ČR) prezentovaných na informačním webovém portálu SVOD (Systém pro Vizualizaci Onkologických Dat, http://www.svod.cz) měly v roce 2011 mezi malignitami ženského genitálu nejvyšší incidenci nádory děložního těla (36,45 případu / 100 tis. žen), následované nádory ovaria (20,20 případu / 100 tis. žen), nádory děložního hrdla (19,13 případu / 100 tis. žen), nádory vulvy (4,06 případu / 100 tis. žen) a nádory vagíny (0,84 případu / 100 tis. žen). Více než jedna třetina maligních nádorů ženského genitálu tedy vzniká v oblasti dolního ženského pohlavního systému (vulva, vagína a děložního hrdlo). Tyto orgány tvoří komplexní anatomickou jednotku s jednotnou histologickou stavbou, vnímavostí k obdobným etiologickým faktorům a tedy i s jasně patrnou predispozicí ke vzniku podobného spektra epiteliálních prekancerózních lézí a karcinomů. Termín *dolní ženský pohlavní systém* je v česky psané literatuře používán poměrně zřídka, mnohem rozšířenější je v anglosaské literatuře v podobě *lower female genital tract* (LFGT).

1.2. Obecný koncept kancerogeneze orgánů dolního ženského pohlavního systému

Orgány LFGT jsou na povrchu převážně kryté keratinizujícím nebo nekeratinizujícím dlaždicovým epitelem, endocervikální část děložního hrdla pak cylindrickým žlázovým epitelem. Oba typy epitelu jsou vnímavé k sexuálně přenosné infekci lidským papilomavirem (HPV), která je hlavním etiologickým faktorem vedoucím ke vzniku nenádorových, prekancerózních i nádorových epiteliálních lézí v oblasti LFGT.

HPV je neobalený epiteliotropní DNA virus z čeledi Papovaviridae. Základními stavebními jednotkami virionu jsou cirkulární molekula DNA o délce 8000 párů bazí (bp) a kapsida tvořená strukturálními proteiny L1 a L2. V současnosti je známo více než 120 sérotypů HPV, které se liší nejen afinitou k jednotlivým anatomickým oblastem a typům epitelu, ale též potenciálem prekancerózní a nádorové transformace epiteliálních buněk. Nízce rizikové (LR) typy HPV, např. 6 a 11, jsou v oblasti LFGT příčinou epiteliálních proliferací charakteru condyloma acuminatum (CoA) nebo plochých kondylomatózních lézí, zatímco vysoce rizikové (HR) typy, např. 16, 18 a 45, mohou za určitých podmínek vést ke vzniku dysplastických změn směřujících až k invazivnímu dlaždicobuněčnému

karcinomu (SCC) nebo adenokarcinomu (*Munoz et al. 2006*). V procesu kancerogeneze se uplatňují především virové onkoproteiny E6 a E7, které degradují intracelulární regulační proteiny p53 a pRb, tím potlačují funkci odpovídajících tumor supresorových genů a destabilizují buněčný cyklus. Následkem je ztráta kontrolních mechanismů buněčné proliferace a nádorová transformace buňky (*Munger et al. 2004*).

Dysplastické léze dlaždicového epitelu LFGT jsou již tradičně popisovány v konceptu tzv. intraepiteliální neoplázie a dle anatomické lokalizace odpovídají vulvární (VIN), vaginální (VaIN) a cervikální (CIN) intraepiteliální neoplázii. Rozsah dysplastických změn je dle aktuální WHO klasifikace kvantifikován do tří stupňů (I, II a III) podle vertikálního rozsahu ztráty diferenciace, cytologických atypií a mitotické aktivity (*Wilkinson a Teixeira 2003*).

Klinicky důležitou vlastností HPV pozitivních prekanceróz a karcinomů dolního ženského pohlavního systému je jejich multicentrický výskyt nejen v rámci jednotlivého orgánu, ale i v rozsahu celého LFGT, kde se mohou v libovolných kombinacích rozvíjet simultánně nebo sukcesivně (*Hampl et al. 2006, van Beurden et al. 1998*). Určitou výjimku v obecném konceptu kancerogeneze orgánů LFGT představuje vulva, kde se ve výraznější míře kromě HPV asociovaných lézí vyskytují i HPV negativní prekancerózy a karcinomy. Tyto nádory vznikají odlišnou etiopatogenetickou cestou, zejména mutacemi tumor supresorového genu TP53 (*Hoevenaars et al. 2008, Toki et al. 1991, van der Avoort et al. 2006*) v terénu vulvárních dermatóz typu lichen sclerosus (LS) a lichen simplex chronicus (LSC) (*Scurry 1999*). Oba typy vulvárních dysplastických lézí lze ve většině případů jednoznačně a spolehlivě odlišit histologicky a jsou tedy klasifikovány jako samostatné diagnostické jednotky – HPV asociovaná **VIN obvyklého (klasického) typu (u-VIN)** a HPV negativní **VIN diferencovaného (simplexního) typu (d-VIN)** (*Hart 2001*). Kromě histologického vzhledu existují mezi u-VIN a d-VIN ještě další zásadní klinicko-patologické rozdíly, které jsou shrnuty v tabulce č. 1.

Původní terminologie VIN (WHO 2003) (*Wilkinson a Teixeira 2003*) vycházela z klasifikace předložené Mezinárodní společností pro studium vulvovaginálních chorob (International Society for the Study of Vulvovaginal Disease, ISSVD) v roce 1986, která reflektovala třístupňový grading u-VIN a považovala d-VIN za prekancerózu odpovídající biologickým chováním karcinomu in situ. V roce 2004 navrhla ISSVD modifikaci klasifikace VIN, ve které byl grading u-VIN zrušen, kategorie u-VIN II a u-VIN III sloučeny a jednotka u-VIN I zcela vypuštěna (tab. 2) (*Sideri et al. 2005*). Tyto změny se částečně odrážejí i v aktuální WHO klasifikaci VIN předložené v roce 2014, která do vulvární patologie zavádí koncept skvamózních intraepiteliálních lézí (SIL), dosud užívaný výlučně pro popis prekanceróz děložního hrdla (tab. 2) (*Crum et al. 2014*).

Tabulka 1.
Klinicko-patologické rozdíly dvou základních typů prekanceróz vulvy (u-VIN a d-VIN).

	VIN, obvyklý typ (u-VIN)	VIN, diferencovaný typ (d-VIN)
Celková četnost	více než 80 %	méně než 20 %
Věková predilekce	premenopauzální ženy	postmenopauzální ženy
Asociace s CIN a VaIN (multicentrická neoplázie LFGT)	ano	ne
Asociace s pohlavně přenosnými chorobami a s condylomata acuminata	ano	ne
Etiologický faktor	HR HPV	genové mutace (TP53)
Kofaktory	kouření cigaret imunosuprese	dermatózy vulvy (LS, LSC)
Tendence k multifokalitě	silná	slabá
Potenciál ke stromální invazi	slabý	silný
Imunohistochemický marker	p16 ^{INK4a}	p53
Asociovaný SCC	bazaloidní SCC warty SCC	dobře diferencovaný keratinizující SCC

VIN – vulvární intraepiteliální neoplázie, CIN – cervikální intraepiteliální neoplázie, VaIN – vaginální intraepiteliální neoplázie, LFGT – lower female genital tract (dolní ženský pohlavní systém), HR HPV – high risk human papillomavirus (vysoce rizikový typ lidského papilomaviru), LS – lichen sclerosus, LSC – lichen simplex chronicus, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom)

Tabulka 2.
Srovnávací tabulka klasifikace ISSVD 1986 (WHO 2003), modifikovaného schématu ISSVD 2004 a aktuální WHO 2014 klasifikace vulvárních prekanceróz.

ISSVD 1986 (WHO 2003)		ISSVD 2004		WHO 2014	
Histologický typ	Grade	Histologický typ	Grade	Histologický typ	Grade
VIN, obvyklý typ (u-VIN)	I	Reaktivní změny HPV infekce Plochá kondylomatózní léze	-	Low grade SIL	-
	II III	VIN, obvyklý typ (u-VIN)	-	High grade SIL	-
VIN, diferencovaný typ (d-VIN)	III	VIN, diferencovaný typ (d-VIN)	-	VIN, diferencovaný typ (d-VIN)	-

ISSVD - International Society for the Study of Vulvovaginal Disease (Mezinárodní společnost pro studium vulvovaginálních chorob), VIN – vulvární intraepiteliální neoplázie, HPV – human papillomavirus (lidský papilomavirus), SIL – squamous intraepithelial lesion (skvamózní intraepiteliální léze)

1.3. Profylaktická vakcinace proti HPV

Základní strukturální jednotkou virionu HPV je kapsidový protein L1, který je v lidském organismu schopen vyvolat tvorbu virus neutralizujících protilátek. Z této skutečnosti vychází princip profylaktické vakcinace proti HPV (*Stanley et al. 2006*). Rekombinantně získaný L1 protein vytváří za vhodných podmínek prázdné kapsidy bez virové DNA (tzv. virus-like particles, VLPs), které jsou morfologicky a antigenně prakticky identické s přírodními viriony a využívají se jako profylaktická vakcína. V současnosti jsou používány dvě očkovací látky: bivalentní HPV-16/18 L1 VLP vakcína (GlaxoSmithKline Biologicals, Rixensart, Belgium) a tetravalentní HPV-16/18/6/11 L1 VLP vakcína (Merck, West Point, Pennsylvania, USA). Komplikací při očkování proti HPV může být antigenní nepříbuznost L1 proteinů jednotlivých typů HPV. Proto byly za základ v současnosti používaných vakcín zvoleny VLPs v populaci nejčastějších HR HPV typů, které byly v případě tetravalentní vakcíny doplněny i dvěma nejčastějšími LR HPV typy. V určitém rozsahu existuje u očkovaných jedinců zkřížená protekce i proti ostatním nevakcinačním HPV typům (především HPV 31, 33 a 45), jejíž přesný rozsah je nyní mapován klinickými studiemi (*De Vincenzo et al. 2013, Malagon et al. 2012, Verdenius et al. 2013*). Situaci může dále komplikovat i rozdílná geografická frekvence jednotlivých typů HPV (*Clifford et al. 2006, Munoz et al. 2004*).

1.4. Prekancerózní léze orgánů LFGT a význam biomarkerů v diferenciální diagnostice

Histopatologická diagnóza prekancerózních lézí LFGT může být v některých případech zatížena značnou interpersonální a intrapersonální variabilitou (*McCluggage et al. 1998*). Tato problematika je nejlépe zdokumentována u dysplastických lézí děložního hrdla, kde se projevuje především v kategoriích CIN I (*Stoler a Schiffman 2001*) a CIN II (*Carreon et al. 2007*). Často může dojít také k záměně prekancerózy s benigními epiteliálními proliferacemi nebo vágně definovanými diagnostickými jednotkami s nejasným biologickým potenciálem, mezi které spadá především tzv. **atypická nezralá dlaždicobuněčná metaplázie** (atypical immature squamous metaplasia, AIM) (*Crum et al. 1983*). V neposlední řadě bývá často problematická i analýza bioptických vzorků suboptimální kvality, ať již z důvodu jejich malé velikosti anebo mechanických a termických artefaktů.

Správná histopatologická diagnóza bioptického materiálu z děložního hrdla má značný klinický význam pro volbu odpovídajícího terapeutického postupu (*Wright et al. 2007*). Většina low grade skvamózních intraepiteliálních lézí (LSIL; CIN I) spontánně regreduje, proto jsou pacientky zpravidla léčeny konzervativně a pouze klinicky sledovány. Ženy s high grade skvamózními intraepiteliálními lézemi (HSIL; CIN II a CIN III) naopak podstupují excizní terapeutické zákroky, zejména pak konizaci nebo simplexní hysterektomii. Z tohoto hlediska má nepřesná klasifikace stupně dysplázie přímý

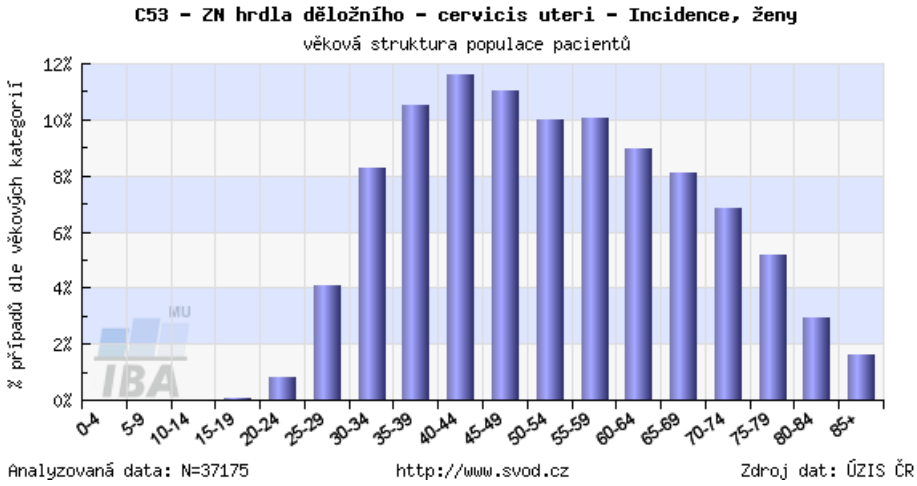
negativní dopad na léčbu pacientky. Dalším příkladem zavádějícího výsledku bioptického vyšetření je diagnóza AIM, která je pro gynekologa bezcenným údajem, neboť neodráží biologické chování léze a sdružuje pod stejným termínem zcela benigní epiteliální proliferace (reaktivní změny v dlaždicobuněčné metaplázii) a skutečné prekancerózy (CIN III).

S rozšířením imunohistochemických metod do rutinní histopatologické praxe byly identifikovány biomarkery umožňující zlepšit diferenciální diagnostiku prekancerózních lézí orgánů LFGT. V současnosti hraje pravděpodobně nejvýznamnější roli proteinový produkt tumor supresorového genu $p16^{INK4a}$, který se jako inhibitor cyklin dependentní kinázy účastní regulace buněčného cyklu a jehož exprese může být zvýšena následkem infekce HR HPV typy (*O'Neill a McCluggage 2006*). Imunohistochemicky detekovatelná patologická exprese markeru $p16^{INK4a}$ je pozorována v dysplastických lézích děložního hrdla, kde její intenzita a rozsah koreluje se stupněm dysplázie (*Keating et al. 2001, Klaes et al. 2001, Omori et al. 2007, Sano et al. 1998*). Obdobný charakter imunoexprese antigenu $p16^{INK4a}$ lze pozorovat i v prekancerózních lézích vulvy (*Santos et al. 2004*).

Cytokeratin 17 (CK 17) je dalším imunohistochemickým markerem, který je v odborné literatuře diskutován v kontextu diferenciální diagnózy a určení prognózy dysplastických lézí LFGT. CK 17 byl identifikován jako marker kmenových buněk děložního hrdla (*Martens et al. 2004*) a je konzistentně exprimován v nezralé dlaždicové metaplázii (*Smedts et al. 1992*). Dle některých pozorování bývá CK 17 imunohistochemicky negativní v prekancerózních lézích, především pak v CIN III (*Regauer a Reich 2007*). Další studie však toto pozorování nepotvrdily (*Ikeda et al. 2008, Smedts et al. 1992*) a využití markeru CK 17 se tedy stále jeví jako kontroverzní metoda, která před zařazením do rutinní praxe vyžaduje důkladnější ověření na větším počtu případů.

1.5. Fertilitu šetřící a méně radikální chirurgické výkony v terapii časných stádií karcinomu děložního hrdla

Karcinom děložního hrdla je histologicky heterogenní skupina nádorů reprezentovanou především SCC (85 %), adenokarcinomem (10 %), adenoskvamózním karcinomem a dalšími vzácnějšími histologickými typy (5 %) (*Vizcaino et al. 1998*). Z epidemiologického hlediska jde o nádor se značně nepříznivým chováním, neboť je jeho maximální incidence ve srovnání s karcinomy ostatních orgánů posunuta do mladších věkových kategorií. V populaci České republiky se téměř 24 % karcinomů děložního hrdla vyskytuje u žen do 40 let věku (ÚZIS ČR, http://www.svod.cz, obr. 1). Podobná epidemiologická situace je hlášena i z ostatních států Evropy a USA (*Arbyn et al. 2007, Watson et al. 2008*).



Obr. 1. Věková struktura pacientek se zhoubnými nádory děložního hrdla v České republice.

Tak jako u ostatních typů epitelových nádorů zahrnuje diagnostický algoritmus u pacientek s karcinomem děložního hrdla tři základní kroky, které jsou nedílnou součástí bioptického a případně i zobrazovacího a klinického vyšetření: určení typu nádoru (typing), stanovení stupně diferenciace nádoru (grading) a popis anatomického rozsahu nádoru (staging). Dále se hodnotí i přítomnost nádorové lymfangioinvaze a invaze do krevních cév, perineurální invaze, infiltrace chirurgických resekčních okrajů a další patologické změny. Anatomický rozsah nádoru je tradičně hodnocen ve stagingovém systému TNM, který je výsledkem spolupráce *Union for International Cancer Control (UICC)* a *American Joint Comittee on Cancer (AJCC)*. Klinický (cTNM) i patologický (pTNM) staging závisí na třech základních parametrech nádoru: rozsahu primárního nádoru (T), přítomnosti metastáz v regionálních lymfatických uzlinách (N) a přítomnosti vzdálených metastatických ložisek (M). Alternativou TNM klasifikace gynekologických malignit je stagingový systém předložený *International Federation of Gynecology and Obstetrics (FIGO)*. Přehled TNM klasifikace karcinomu děložního hrdla (*Sobin et al. 2009*) včetně odpovídajících FIGO stádií (*Pecorelli et al. 2009*) je uveden v tabulce č. 3.

Zvolení odpovídajícího terapeutického postupu u pacientek s karcinomem děložního hrdla se odvíjí od klinického stádia onemocnění. V časných stádiích choroby se používá primární chirurgická léčba, u vyšších stádií je nutno přistoupit k radioterapii anebo chemoterapii, kterou lze případně aplikovat i neoajuvantně. Orientační přehled primární chirurgické léčby karcinomu děložního hrdla v závislosti na stádiu onemocnění je podává tabulka č. 4. Přehled používané terminologie radikálních hysterektomií je uveden v tabulce č. 5. Ženy s minimálně invazivními karcinomy stádia IA1 bez nádorové lymfangioinvaze mohou dle současných léčebných protokolů podstoupit pouze konizaci

a ponechat si tak možnost budoucího těhotenství. Standardní chirurgická terapie karcinomu děložního hrdla stádia IA1 s lymfangioinvazí a stádia IA2 a vyšších je však neslučitelná se zachováním fertility a sestává z radikální hysterektomie typu B nebo C s pánevní lymfadenektomií anebo z radioterapie (*Holtz a Dunton 2002*).

Tabulka 3.
TNM kategorie a FIGO stádia karcinomu děložního hrdla.

TNM kategorie	FIGO stádia	Klinický nebo patologický nález
Primární nádor (T)		
Tx		primární nádor nelze hodnotit
T0		bez známek primárního nádoru
Tis		karcinom in situ (preinvazivní karcinom)
T1	**I**	nádor omezený na děložní hrdlo (šíření na děložní tělo by nemělo být zohledněno)
T1a	IA	invazivní karcinom diagnostikovaný pouze mikroskopicky; stromální invaze ≤ 5,0 mm měřená od baze epitelu a horizontální rozsah ≤ 7,0 mm
T1a1	IA1	stromální invaze ≤ 3,0 mm horizontální rozsah ≤ 7,0 mm
T1a2	IA2	stromální invaze > 3,0 mm a ≤ 5,0 mm horizontální rozsah ≤ 7,0 mm
T1b	IB	klinicky zřetelná léze omezená na děložní hrdlo nebo mikroskopická léze větší než T1a2/IA2
T1b1	IB1	klinicky zřetelná léze ≤ 4,0 cm v největším rozměru
T1b2	IB2	klinicky zřetelná léze > 4,0 cm v největším rozměru
T2	**II**	nádor se šíří mimo dělohu, ne však do stěny pánevní nebo dolní třetiny pochvy
T2a	IIA	bez šíření do parametrií
T2a1	IIA1	klinicky zřetelná léze ≤ 4,0 cm v největším rozměru
T2a2	IIA2	klinicky zřetelná léze > 4,0 cm v největším rozměru
T2b	IIB	nádor se šířením do parametrií
T3	**III**	nádor se šíří ke stěně pánevní anebo postihuje dolní třetinu pochvy anebo způsobuje hydronefrózu nebo afunkci ledviny
T3a	IIIA	nádor postihuje dolní třetinu pochvy, bez šíření ke stěně pánevní
T3b	IIIB	nádor se šíří ke stěně pánevní anebo způsobuje hydronefrózu nebo afunkci ledviny
T4	**IV**	nádor postihuje sliznici močového měchýře nebo rekta anebo se šíří mimo malou pánev
T4a	IVA	nádor postihuje sliznici močového měchýře nebo rekta
T4b	IVB	nádor se šíří mimo malou pánev
Regionální lymfatické uzliny (N)		
NX		regionální lymfatické uzliny nelze hodnotit
N0		regionální lymfatické uzliny bez metastáz
N1		metastázy v regionálních lymfatických uzlinách
Vzdálené metastázy (M)		
MX		vzdálené metastázy nelze hodnotit
M0		bez vzdálených metastáz
M1		vzdálené metastázy

Tabulka 4.
Orientační přehled primární chirurgické léčby karcinomu děložního hrdla v závislosti na stádiu onemocnění.

TNM kategorie / FIGO stádium	Další parametry	Doporučovaná chirurgická terapie
T1a1 / IA1	bez lymfangioinvaze	• konizace s histologicky negativním resekčním okrajem • hysterektomie typu A
	s lymfangioinvazí	trachelektomie s pávevní lymfadenektomií nebo hysterektomie typu B s pánevní lymfadenektomií
T1a2 / IA2		trachelektomie s pánevní lymfadenektomií nebo hysterektomie typu C s pánevní lymfadenektomií
T1b1 / IB1	nádor maximálního rozměru 2 cm	trachelektomie s pánevní lymfadenektomií
	nádor větší než 2 cm	radikální hysterektomie typu C s pánevní lymfadenektomií
T1B2 / IB2 T2a / IIA		radikální hysterektomie typu C s pánevní lymfadenektomií

Chirurgická léčba je řešena laparotomicky, laparoskopicky nebo roboticky. Pánevní lymfadenektomie je spojena s detekcí SLN.

Tabulka 5.
Revidovaná terminologie hysterektomií (*Querleu a Morrow 2008*).

Typ		Rozsah resekce
A		• paracervikální tkáně přerušeny mediálně od ureteru a laterálně od děložního hrdla • uterosakrální a vezikouterinní ligamenta přerušena těsně u dělohy • minimální resekce vagíny bez odstranění parakolpických měkkých tkání
B	B1	• paracervikální tkáně přerušeny na úrovni průchodu ureteru • parciální resekce uterosakrálních a vezikouterinních ligament • bez odstranění kaudálních hlubokých nervových plexů paracervixu • resekce minimálně 10 mm vagíny od děložního hrdla nebo od nádoru
	B2	• jako u typu B1 + odstranění laterálních pánevních lymfatických uzlin
C		• paracervikální tkáně přerušeny v úrovni vnitřních iliackých cév • uterosakrální ligamenta přerušeny u dělohy a vezikouterinní ligamenta u močového měchýře • resekce 15 - 20 mm vagíny od děložního hrdla nebo od nádoru společně s přilehlými parakolpickými měkkými tkáněmi Typ C1: se zachováním autonomní nervové inervace Typ C2: bez zachování autonomní nervové inervace
D	D1	• paracervikální tkáně přerušeny v oblasti pánevní stěny • resekce vaskulárních větví vnitřního iliackého systému s odhalením kořenů n. ischiadicus
	D2	• paracervikální tkáně přerušeny v oblasti pánevní stěny • resekce hypogastrických vaskulárních větví s přilehlými fasciemi a úseky kosterní svaloviny

Odkládání těhotenství do pozdějšího věku se v současnosti stává trendem v rozvinutých zemích, a proto může mít diagnóza karcinomu děložního hrdla dalekosáhlý dopad na reprodukční plány pacientky. S rozvojem skríningových a zobrazovacích metod zároveň dochází k posunu detekce karcinomů děložního hrdla do časných stádií a otázka alternativních chirurgických postupů k zachování fertilních funkcí se stává stále více aktuální. Efektivita fertilitu šetřících chirurgických výkonů u pacientek s karcinomem děložního hrdla se odvíjí od správně zvolených indikačních kritérií a vhodného operačního postupu. Cílem takové léčby je minimalizace recidivy nádoru (onkologické výsledky) při zachování možnosti úspěšně završené gravidity (těhotenské výsledky). Prvním prakticky používaným fertilitu šetřícím chirurgickým výkonem byla **vaginální radikální trachelektomie (VRT)**, jejíž výsledky prezentoval v roce 1994 profesor Daniel Dargent (Dargentova operace) (*Dargent et al. 1994, Dargent et al. 2000*). Onkologické výsledky tohoto postupu jsou velmi příznivé a výrazněji se neliší od radikálních výkonů (*Plante et al. 2004, Shepherd et al. 2001*). Těhotenské výsledky jsou též uspokojivé, zatížené pouze vyšším rizikem předčasného porodu (*Plante et al. 2005, Shepherd et al. 2001*). Alternativním výkonem je **abdominální radikální trachelektomie (ART)** *(Smith et al. 1997)*, jejíž radikalita resekce paracervikálních tkání se sice nejvíce blíží standardním onkologickým výkonům, ale těhotenské výsledky jsou dramaticky horší než u ostatních fertilitu šetřících operací (*Kim et al. 2010, Nishio et al. 2009*). Dalším možným přístupem především u žen s karcinomy větších rozměrů dosahujících až k horní hranici indikačních kritérií pro fertilitu šetřící výkony je využití neoadjuvantní chemoterapie (NAC) s protokoly na bázi cisplatiny (*Landoni et al. 2007, Maneo et al. 2008, Plante et al. 2006*).

Chirurgické výkony s omezenou radikalitou mohou mít opodstatnění i u žen staršího věku nebo u pacientek, které již neplánují další těhotenství. Radikální hysterektomie s pánevní lymfadenektomií v různých modifikacích mohou být totiž doprovázeny časnou a pozdní pooperační morbiditou (*Magrina et al. 1995*). Hlavní příčinou urologických, anorektálních a sexuálních komplikací bývá především extenzivní lymfadenektomie a resekce parametrií poškozující pánevní nervové pleteně (*Frumovitz et al. 2005, Kadar et al. 1983, Sood et al. 2002*). Při správně indikované individualizované terapii by část pacientek mohla těžit ze sníženého rozsahu resekčního výkonu (např. extrafasciální hysterektomie typ A místo radikálních hysterektomií typů B a C) a tedy ze snížené pooperační morbidity.

Otázkou zůstává, zda je možno ve snižování radikality výše popsaných chirurgických výkonů postupovat ještě dále a omezit rozsah resekce mediálních částí parametrií, které jsou standardně resekovány nejen při radikální hysterektomii, ale i při radikální trachelektomii. Existují důkazy, že u časných stádií karcinomu děložního hrdla s negativitou pánevních lymfatických uzlin je nádorové postižení této části parametrií vzácné a nedosahuje ani 1 % (*Covens et al. 2002, Kinney et al. 1995, Plante et al. 2004, Stegeman et al. 2007*). K výraznější redukci radikality těchto výkonů může pomoci chirurgická detekce a podrobné histopatologické vyšetření tzv. **sentinelové lymfatické uzliny (SLN)**,

která je definována jako první uzlina v přímé lymfatické drenáži primárního nádoru a jejíž stav predikuje riziko postižení lymfatických uzlin ve vyšších etážích. Peroperační detekce SLN pomocí Patentové modři nebo radiokoloidem technecia (^{99m}Tc), případně kombinací obou metod, je v současnosti experimentálně používaná v mnoha onkogynekologických centrech, která potvrzují spolehlivost tohoto postupu (*Marnitz et al. 2006, Plante et al. 2003, Rob et al. 2005*). Nejasnosti ale dosud přetrvávají ohledně efektivity a bezpečnosti peroperačního histologického vyšetření SLN (*Bats et al. 2011, Fader et al. 2008, Fanfani et al. 2004, Slama et al. 2013*). Na základě velikosti nádorového ložiska v SLN lze histopatologický nález v lymfatické uzlině klasifikovat do tří kategorií:

- *makrometastáza* (> 2 mm),
- *mikrometastáza* (0,2 mm - 2 mm),
- *izolovaná skupina nádorových buněk* (ITC; ≤ 0,2 mm).

Prognostický význam mikrometastáz je s největší pravděpodobností srovnatelný s makrometastázami (*Cibula et al. 2012, Fregnani et al. 2006, Horn et al. 2008*). Naopak přítomnost ITC v SLN má nejspíše zanedbatelný dopad na prognózu pacientky, což je nicméně ještě nutno potvrdit širšími prospektivními studiemi (*Cibula et al. 2012*).

2. OTÁZKY A CÍLE PRÁCE

2.1. HPV infekce v nenádorových lézích, prekancerózách a SCC vulvy – dopad na klasifikaci VIN a odhad efektivity profylaktické vakcinace proti HPV

Profylaktická vakcinace proti HPV infekci je primárně určena k prevenci dysplastických lézí a karcinomu děložního hrdla, nicméně v menším rozsahu může redukovat i incidenci prekanceróz a SCC vulvy. Zastoupení HPV v dysplastických lézích a SCC vulvy a frekvence zastoupení jednotlivých typů HPV se v jednotlivých studiích liší a vykazuje i geografickou variabilitu. Položili jsme si tedy otázku, jaká je situace v zastoupení HPV typů v etnicky poměrně homogenní populaci České republiky, jaký efekt profylaktické vakcinace proti HPV zde případně můžeme očekávat a v jaké míře jsou HPV profily v souladu s klasifikačními schématy VIN. Za tímto účelem jsme si vytyčili následující cíle:

- analyzovat přítomnost HPV infekce a stanovit frekvenci jednotlivých HPV typů v epiteliálních lézích, které se uplatňují při vzniku SCC vulvy u pacientek v České republice
- na základě získaných dat orientačně odhadnout případný efekt profylaktické vakcinace proti HPV
- korelovat HPV profily prekancerózních lézí vulvy s původní klasifikací VIN (ISSVD 1986), s modifikovanou terminologií ISSVD 2004 a s aktuálním schématem WHO 2014 a posoudit vhodnost a odůvodněnost změny klasifikace VIN.

2.2. Význam imunohistochemické detekce markerů p16^{INK4a} a CK 17 v diferenciální diagnostice nenádorových a prekancerózních dlaždicobuněčných lézí děložního hrdla

Položili jsme si otázku, zda by imunohistochemické stanovení exprese markerů p16^{INK4a} a CK 17 mohlo pomoci v diferenciální diagnóze dlaždicobuněčných prekancerózních lézí děložního hrdla, která je zatížena značnou interpersonální a intrapersonální variabilitou. Cílem této části práce bylo:

- definovat charakteristické p16^{INK4a} imunoprofily širokého spektra nenádorových a prekancerózních dlaždicobuněčných lézí děložního hrdla, které by mohly v praxi sloužit jako referenční údaje pro interpretaci nejasných nálezů
- demonstrovat tento přístup v praxi na příkladu skupiny lézí s nejasným prekancerózním potenciálem (AIM) a pokusit se na základě analýzy imunoexprese markerů p16^{INK4a} a CK 17 o jejich reklasifikaci do kategorií s přesněji definovaným biologickým chováním.

2.3. Význam vybraných patologických prognostických faktorů pro fertilitu šetřící a méně radikální chirurgické výkony u pacientek s časným stádiem karcinomu děložního hrdla

Protože dosud nebyly jednoznačně definovány podmínky pro provádění fertilitu šetřících a méně radikálních chirurgických výkonů, pokusili jsme se vypracovat odpovídající indikační kritéria a terapeuticko-diagnostické algoritmy. Důraz jsme kladli především na úlohu histopatologického vyšetření pro predikci rozsahu postižení parametrií, od kterého se odvíjí potřebná radikalita chirugického výkonu. Cíle této části práce byly následující:

- vypracovat a v praxi ověřit protokol peroperačního a definitivního zpracování SLN, který by bylo možno použít v reálném čase v rutinní bioptické praxi, a to především z hlediska optimalizace přínosu pro pacientku a časových, personálních a materiálních nákladů patologické laboratoře
- provést prognostickou analýzu přítomnosti nádorových depozit v SLN ve vztahu k objemu primárního nádorového ložiska
- vyhodnotit riziko patologického postižení parametrií v závislosti na objemu primárního nádorového ložiska a přítomnosti nádorových depozit v SLN
- analyzovat význam neoadjuvantní chemoterapie pro fertilitu šetřící chirurgické výkony u pacientek s hraniční velikostí primárního nádorového ložiska.

3. METODIKA

Jednotlivé metody a soubory pacientů jsou podrobně charakterizovány v příslušných publikacích v příloze této dizertační práce. Níže je uveden pouze přehled a základní principy metod používaných v našich studiích.

3.1. Zpracování bioptického materiálu

Veškerý bioptický materiál v našich studiích byl zpracován běžnými histologickými technikami. SLN určené k peroperačnímu vyšetření byly do laboratoře patologie dopraveny nativně a jejich následné zpracování je podrobně popsáno níže. Materiál zaslaný ke standardnímu zpracování byl fixován po dobu 24 hodin v 10% pufrovaném formolu (pH 7,2). Všechny větší resekáty vulvy a resekáty dělohy včetně konizátů byly ihned po odběru ještě před fixací operatérem orientovány a připevněny na podložku. U menších excizí a materiálu z lymfadenektomií nebyla orientace vzorku před fixací nutná.

3.2. Molekulární metody detekce a typizace HPV

Molekulární vyšetření k typizaci HPV jsme prováděli z archivního bioptického materiálu fixovaného ve formolu a zalitého do parafínu. Po izolaci deoxyribonukleové kyseliny (DNA) byla provedena polymerázová řetězová reakce (PCR) kontrolního genu (lidský β-globin) k potvrzení dostatečného množství DNA a nepřítomnosti inhibitorů DNA polymerázy. U vzorků vyhovující kvality následovala metodou PCR amplifikace fragmentu virového strukturálního genu L1 délky 150 bp s využitím primerů GP5+ a GP6+. Vlastní detekce a typizace HPV probíhala metodou reverzní hybridizace na membráně (reverse line blot hybridisation, RLB), která umožňuje v jedné reakci zachytit až 37 různých typů HPV. Pokud vzorek obsahoval HPV DNA, ale nedošlo k hybridizaci, proběhlo určení HPV typu pomocí sekvenace DNA.

3.3. Imunohistochemické metody

Cílem imunohistochemického vyšetření je vizualizace jaderných, cytoplazmatických i membránových antigenů v histologických řezech, které jsou hodnoceny ve světelném mikroskopu. Metoda je založena na principu vazby specifické primární protilátky se zkoumaným antigenem, který je ve tkáni demaskován a zpřístupněn různými technikami – nejčastěji působením tepla, mikrovlnného

záření nebo roztoků s různým pH, případně natrávením enzymem (např. trypsinem). Komplex antigenu a primární protilátky je v dalším kroku detekován polyklonální sekundární protilátkou s navázaným biotinem, která s epitopy primární protilátky reaguje na podkladě mezidruhové specificity. Polyklonalita sekundární protilátky vede k amplifikaci vazebných míst a k zesílení reakce. Vizualizace imunokomplexů primární a sekundární protilátky probíhá enzymaticky (např. aktivitou peroxidázy). Enzym je navázán na avidin (v praxi nejčastěji streptavidin), který vytváří vazbu s biotinem na sekundární protilátce a po následném přidání substrátu vzniká enzymatickou reakcí barevný precipitát pozorovatelný v histologickém řezu v místě vazby primární protilátky na tkáňový antigen. V našich studiích jsme imunohistochemicky analyzovali přítomnost celkem tří markerů – $p16^{INK4a}$ (klon G175-405, BD Biosciences, Franklin Lakes, USA), CK 17 (klon E3, DakoCytomation, Glostrup, Dánsko) a širokospektrého cytokeratinu CK KL1 (Immunotech, Marseille, Francie).

3.4. Klinická a zobrazovací vyšetření pacientek

Součástí předoperačního stagingu pacientek byla kromě fyzikálního vyšetření a dalších standardních metod i expertní volumometrie nádoru prováděná transvaginálním ultrazvukovým vyšetřením (8 MHz sonda, Acuson Sequoia 512, Siemens, Malvern, USA) a magnetickou rezonancí o síle magnetického pole 1,5 T (Gyroscan ACS-15NT Power Track 1000, Philips, DA Best, Nizozemí) s užitím T2 vážených snímků v turbo spin echo módu (TSE).

Po operačním výkonu byly pacientky dispenzarizovány v rámci standardních onkologických protokolů, v průběhu periodických prohlídek byly podrobeny rutinnímu fyzikálnímu vyšetření a v případě potřeby byla provedena příslušná zobrazovací vyšetření.

3.5. Peroperační chirurgická detekce SLN

K identifikaci SLN jsme použili krátký protokol, který nezahrnoval předoperační lymfoscintigrafii. SLN byly detekovány přímo během chirurgického výkonu dle dříve publikovaného postupu (*Rob et al. 2005*) pomocí kombinované aplikace Patentové modři (Patentblau V 2,5 %, BYK Gulden, Konstanz, Německo nebo Bleu Patenté V 2,5 %, Guerbert, Roissy, Francie) a lidského koloidního albuminu radioaktivně značeného 20 MBq ^{99m}Tc s velikostí částic 100 - 600 nm (SentiScint, Medi-Radiopharma Ltd, Érd, Maďarsko). Obě farmaka byla před podáním naředěna v poměru 2 ml aktivní látky na 2 ml fyziologického roztoku. Po uvedení pacientky do anestezie byl ^{99m}Tc radiokoloid aplikován vaginálním přístupem velmi pomalu (5 - 8 sekund) peritumorózně do čtyřech kvadrantů děložního hrdla, nikoliv však přímo do nádoru. Bezprostředně poté byla do okolí nádoru vaginálně podána Patentová modř stejným způsobem jako při předchozí aplikaci ^{99m}Tc radiokoloidu. S časovým

odstupem 10 - 15 minut po aplikaci Patentové modři bylo chirurgicky otevřeno retroperitoneum. Chirurgický přístup do malé pánve umožnil přímou vizualizaci modře se barvících lymfatických kanálů a lymfatických uzlin. Radioaktivita byla následně detekována laparoskopickou nebo ruční laparotomickou gamma sondou (Neoprobe, Johnson & Johnson, USA). Jako SLN byly identifikovány všechny lymfatické uzliny nejbližší k nádoru, které byly v čase detekce modře zbarvené a vykazovaly radioaktivitu. Po exstirpaci SLN byla radioaktivita ještě jednou ověřena na resekátu, který byl následně odeslán k peroperačnímu histologickému vyšetření.

Pro statistické zhodnocení úspěšnosti peroperační chirurgické detekce SLN jsme vycházeli z faktu, že lymfatická drenáž děložního hrdla je oboustranná a že SLN jsou tedy lokalizovány pravostranně i levostranně (*Rob et al. 2005*). Na tomto základě jsme definovali následující parametry:

- *selhání detekce* – počet pacientek, u kterých nebylo možno SLN identifikovat ani na jedné straně
- *jednostranná detekce* – počet pacientek, u kterých se podařilo SLN identifikovat pouze jednostranně
- *úspěšnost detekce (detection rate, DR)* – percentuální poměr pacientek s úspěšně detekovanou SLN (jednostranně nebo oboustranně) k celkovému počtu pacientek

$$DR\ (\%) = \frac{\text{celkový počet pacientek – selhání detekce}}{\text{celkový počet pacientek}}$$

- *stranově specifická úspěšnost detekce (side specific detection rate, SSDR)* – percentuální poměr počtu levých a pravých stran s úspěšně detekovanou SLN k celkovému počtu levých a pravých stran.

$$SSDR\ (\%) = \frac{\text{2 x celkový počet pacientek – (2 x selhání detekce + jednostranná detekce)}}{\text{2 x celkový počet pacientek}}$$

3.6. Histopatologické zpracování SLN a parametrií

Po zaslání nefixovaného materiálu na pracoviště patologie byla makroskopicky patrná SLN vypreparována z okolní tukové tkáně, podélně rozpůlena a každá část byla zpracována samostatně. SLN menší velikosti, které nebylo možno identifikovat prostým okem, byly ponechány v kontinuitě s přilehlými tkáněmi. Peroperační vyšetření materiálu probíhalo standardizovaným protokolem, který

sestává ze zamražení tkáně v tekutém dusíku a zhotovení 3 až 5 sériových řezů o tloušťce 4 μm, bez hlubšího prokrajování materiálu. Tkáňové řezy byly barveny Harrisovým hematoxylinem a eozinem.

Po peroperačním vyšetření byl materiál rozmražen a fixován po dobu 24 hodin v 10% pufrovaném formolu (pH 7,2). Následně byly SLN zpracovány běžnými histologickými technikami a zality do parafínových bloků, které byly sériově prokrajovány v intervalu 250 μm a z každé úrovně byl zhotoven jeden řez barvený hematoxylin-eozinem a další řez k imunohistochemickému vyšetření širokospektrou protilátkou proti cytokeratinům CK KL1. Interval 250 μm mezi jednotlivými úrovněmi řezů je považován za optimální kompromis mezi efektivitou záchytu nádorového postižení SLN a materiálních, časových a personálních nároků na laboratoře patologie (*Cserni 2004*).

Na základě maximálního rozměru nádorového ložiska v histologickém řezu bylo případné postižení SLN klasifikováno podle výše uvedeného schématu jako makrometastáza (> 2 mm), mikrometastáza (0,2 – 2 mm) nebo ITC (≤ 0,2 mm). K měření mikroskopických vzdáleností byl použit program QuickPHOTO MICRO 2.3 (PROMICRA, Praha). Přítomnost ITC v SLN byla v souladu se závaznými pravidly TNM klasifikace (*Sobin et al. 2009*) interpretována jako histologická negativita lymfatické uzliny.

Parametria zaslaná spolu s resekátem dělohy byla stranově orientována a po změření velikosti byla na jejich povrch aplikována tuš k identifikaci chirurgických resekčních okrajů v histologických řezech. Následně byla parametria odstřižena těsně v odstupu od děložního hrdla. Poté byla parametria v dlouhé ose prokrajována v sériových řezech v intervalu 5 mm. Histologicky byl vždy zpracován první řez v sekvenci odpovídající úponu parametrií k děložnímu hrdlu, dále laterální úsek parametrií v největší vzdálenosti od děložního hrdla a jeden náhodný řez přibližně z poloviny šíře parametria. Samostatně byla zpracována i všechna makroskopicky a palpačně suspektní ložiska anebo lymfatické uzliny.

3.7. Statistické metody

Pro hodnocení rozdílů v mediánech věku jednotlivých skupin pacientek jsme použili nepárový t-test s Welchovou korekcí, případně analýzu rozptylu jednoduchého třídění (ANOVA test). Kontingenční tabulky jsme analyzovali standardním chi-kvadrát testem (eventuálně s Yatesovou korekcí) nebo Fisherovým exaktním testem. Všechny výpočty jsme prováděli pomocí statistického programu GraphPad InStat (verze 3.06) (GraphPad Software, San Diego, USA). Všechny testy byly oboustranné a hodnoty významnosti $p < 0,05$ jsme považovali za statisticky signifikantní. Používali jsme 95% interval spolehlivosti.

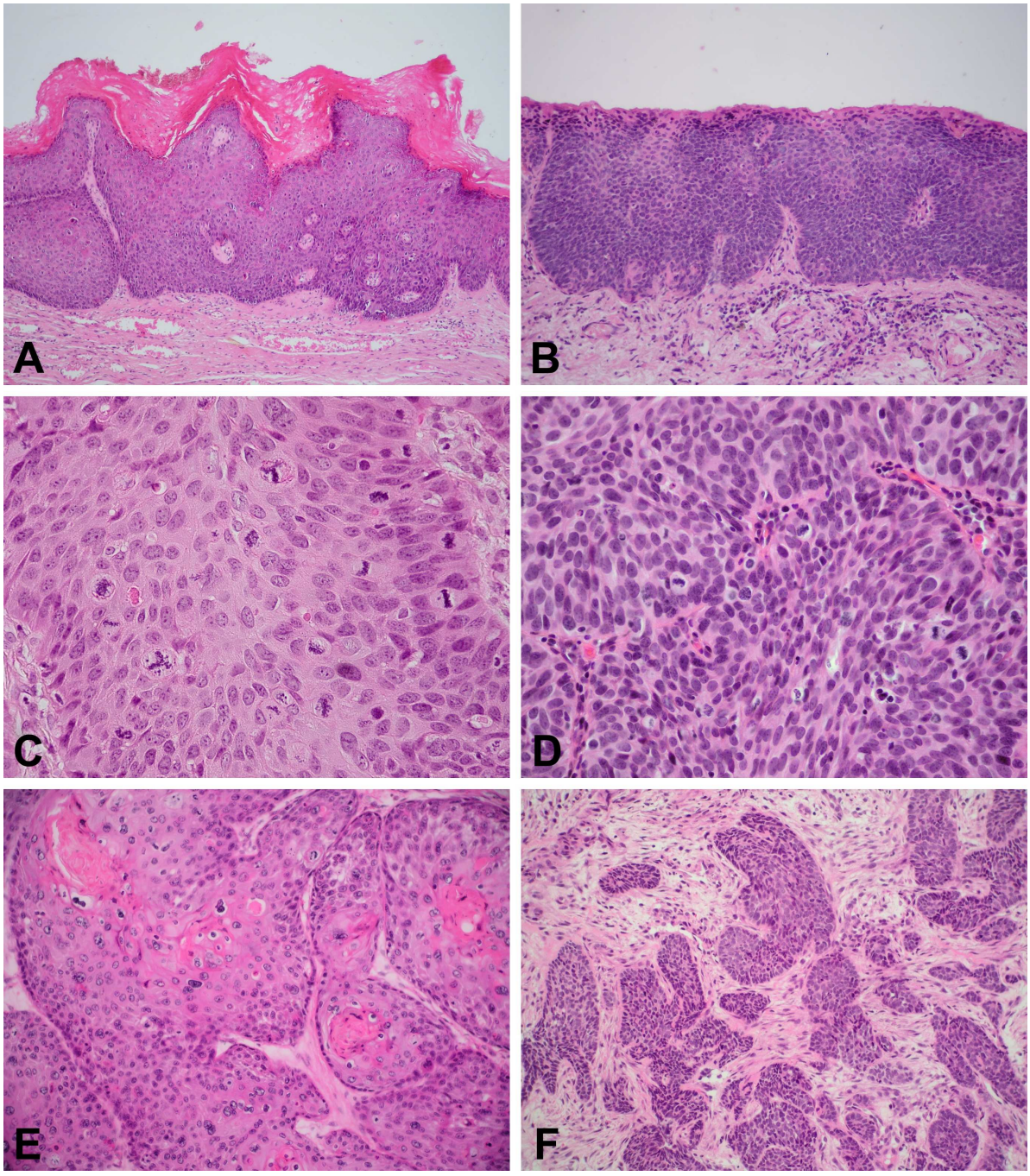
4. VÝSLEDKY A DISKUZE

4.1. HPV infekce v nenádorových lézích, prekancerózách a SCC vulvy – dopad na klasifikaci VIN a odhad efektivity profylaktické vakcinace proti HPV

Cílem dvou našich studií (**přílohy I a II**) a navazujících přehledových článků (**přílohy III a IV**) bylo definovat prevalenci jednotlivých typů HPV v nenádorových, prekancerózních a nádorových dlaždicobuněčných lézích vulvy a odhadnout efektivitu profylaktické vakcinace proti HPV v populaci České republiky. Dalším cílem našeho projektu byla korelace získaných HPV profilů jednotlivých typů vulvárních lézí s tradiční klasifikací VIN (ISSVD 1986) a s návrhem na její modifikaci (ISSVD 2004).

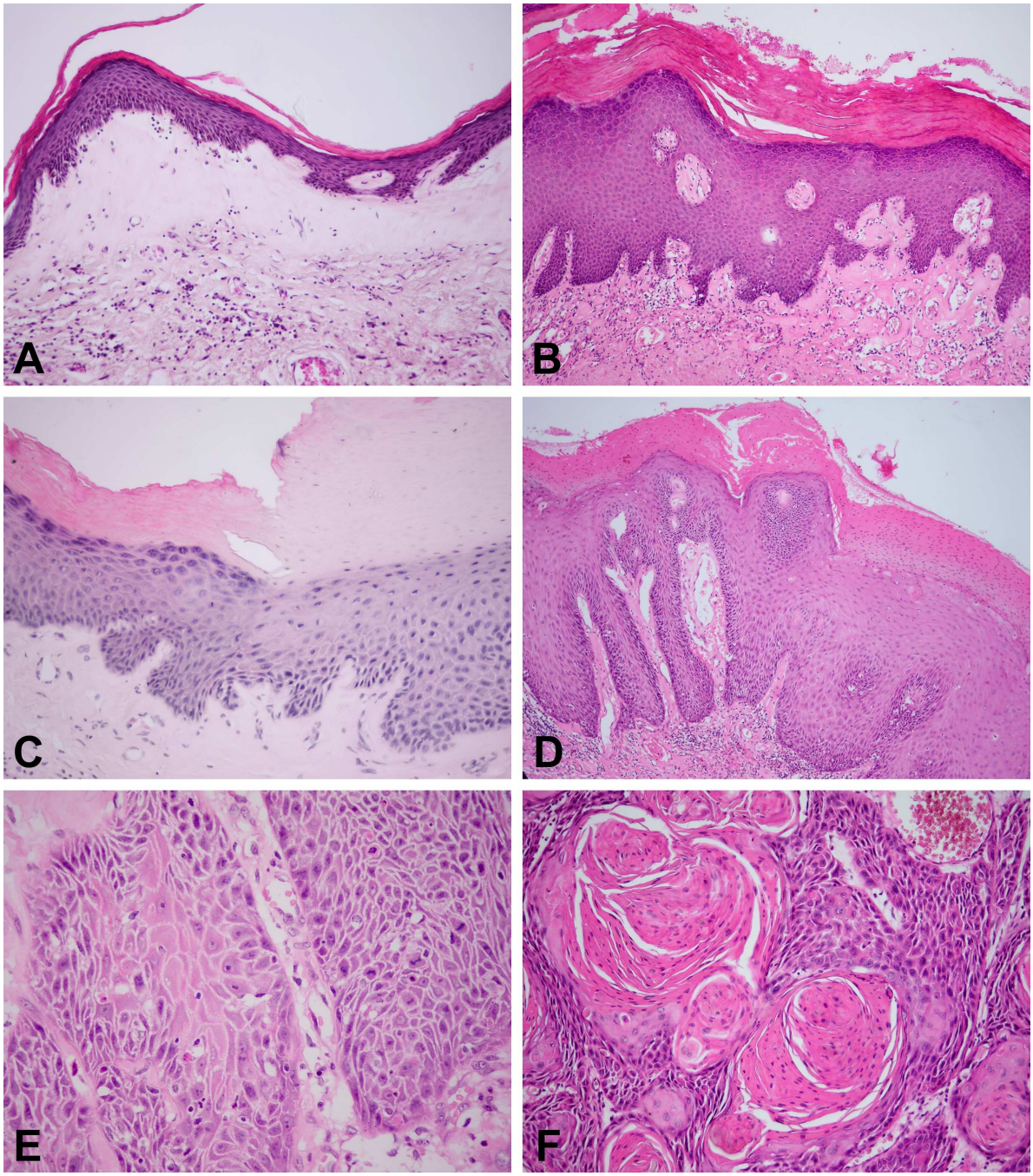
Do studie jsme zařadili celkem 269 dlaždicobuněčných lézí vulvy (obr. 2 a obr. 3) – LS (n = 35), LSC (n = 14), CoA (n = 57), u-VIN I (n = 4), u-VIN II (n = 12), u-VIN III (n = 66), d-VIN (n = 12) a SCC (n = 69), u kterých byla testována přítomnost HPV a stanoveno spektrum typů HPV metodou reverzní hybridizace (RLB), případně sekvenace DNA. Detekované typy HPV byly rozděleny na nízce rizikové (LR HPV), vysoce rizikové (HR HPV), pravděpodobně vysoce rizikové (pHR HPV) a HPV typy s neurčeným rizikem (UR HPV). K rozčlenění jednotlivých HPV typů dle rizikovosti pro lidský organismus jsme využili data předchozí epidemiologické studie (*Munoz et al. 2006*).

Celkem jsme v naší sestavě detekovali 15 HPV typů. Ve skupině HR HPV převažovaly HPV 16, 33 a 45, zatímco mezi LR HPV typy byly nejčastější HPV 6 a 11. Vysoká frekvence HPV pozitivity byla dle očekávání zastižena v CoA a ve všech stupních u-VIN, dále bylo HPV pozitivních 42,0 % SCC (obr. 4). Ve skupině CoA dominovalo zastoupení LR HPV typů, v u-VIN a SCC se naopak vyskytovaly převážně HR HPV (obr. 5). Spektrum HPV typů bylo nejširší ve skupině CoA a u-VIN II a podobně i výskyt lézí infikovaných více HPV typy byl v těchto dvou kategoriích nejvyšší. Při analýze věku pacientek jsme pozorovali signifikantní rozdíl mezi věkovými mediány skupiny u-VIN a d-VIN, mezi HPV pozitivními SCC a HPV negativními SCC, ale také mezi ženami s diagnózou u-VIN II a u-VIN III (obr. 6).



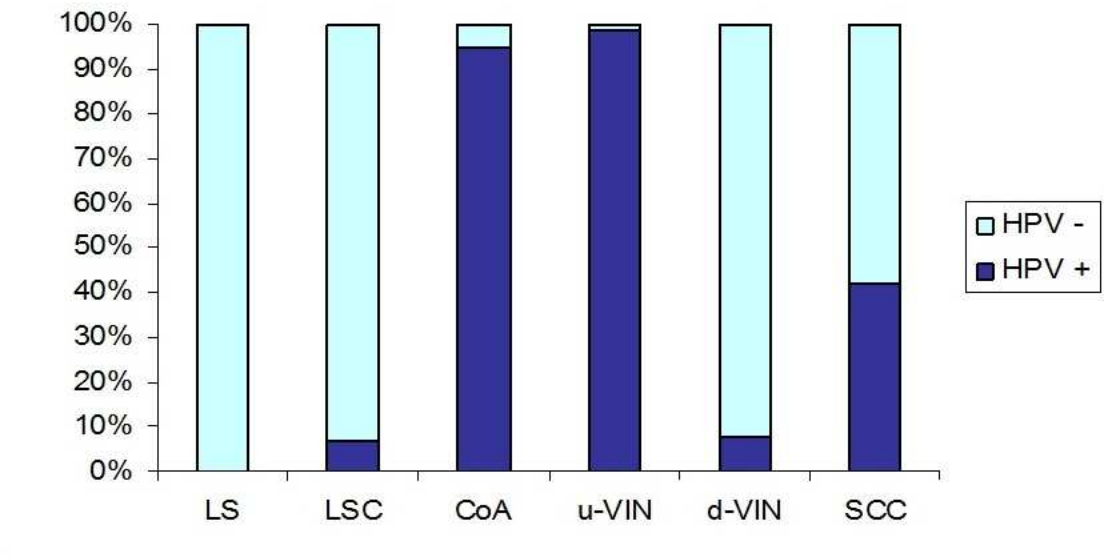
Obr. 2. Prekancerózní léze vulvy a SCC asociované s HPV infekcí.
A – u-VIN III, warty typ (HE, 40x); **B** – u-VIN III, bazaloidní typ (HE, 100x); **C** – u-VIN III, warty typ (HE, 400x); **D** – u-VIN III, bazaloidní typ (HE, 400x); **E** – SCC, warty typ (HE, 200x); **F** – SCC, bazaloidní typ (HE, 100x).

u-VIN – vulvární intraepiteliální neoplázie obvyklého typu, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom), HE – hematoxylin-eozin



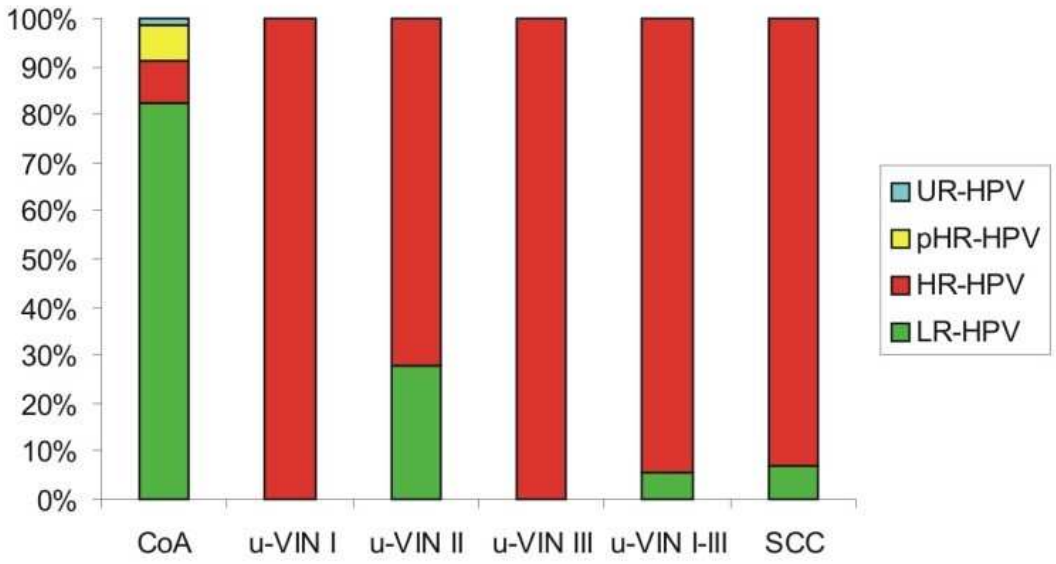
Obr. 3. Dlaždicobuněčné léze vulvy sdružené s HPV negativní cestou karcinogeneze.
A – LS (HE, 100x); **B** – LSC (HE, 40x); **C** – přechodová zóna mezi LSC a d-VIN (HE, 200x);
D – d-VIN (HE, 40x); **E** – d-VIN (HE, 400x); **F** – SCC keratinizující typ (HE, 200x).

LS – lichen sclerosus, LSC – lichen simplex chronicus, d-VIN - vulvární intraepiteliální neoplázie diferencovaného typu, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom), HE – hematoxylin-eozin



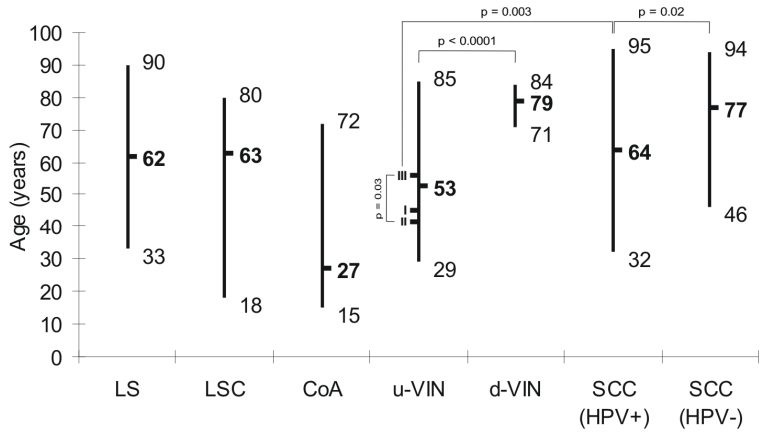
Obr. 4. Prevalence HPV v nenádorových, prekancerózních a nádorových dlaždicobuněčných lézích vulvy.

HPV – lidský papilomavirus, LS – lichen sclerosus, LSC – lichen simplex chronicus, CoA – condyloma acuminatum, u-VIN – vulvární intraepiteliální neoplázie obvyklého typu, d-VIN - vulvární intraepiteliální neoplázie diferencovaného typu, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom)



Obr. 5. Spektrum rizikových skupin HPV v HPV asociovaných lézích vulvy.

HPV – lidský papilomavirus, CoA – condyloma acuminatum, u-VIN – vulvární intraepiteliální neoplázie obvyklého typu, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom), LR HPV – nízce rizikový typ lidského papilomaviru, HR HP – vysoce rizikový typ lidského papilomavirus, pHR HPV – pravděpodobně vysoce rizikový typ lidského papilomaviru, UR HPV – typ lidského papilomaviru s neurčeným rizikem



Obr. 6. Věková struktura pacientek s dlaždicobuněčnými lézemi vulvy s vyznačením minimálního a maximálního věku a věkového mediánu. Schematicky jsou znázorněny i mediány věku skupin u-VIN I, II a III. U statisticky významných rozdílů jsou uvedeny odpovídající hodnoty *p*.

HPV – lidský papilomavirus, LS – lichen sclerosus, LSC – lichen simplex chronicus, CoA – condyloma acuminatum, u-VIN – vulvární intraepiteliální neoplázie obvyklého typu, d-VIN - vulvární intraepiteliální neoplázie diferencovaného typu, SCC – squamous cell carcinoma (dlaždicobuněčný karcinom)

4.1.1. Diskuze

Prevalence HPV pozitivity v jednotlivých kategoriích dlaždicobuněčných vulvárních lézí je v naší studii plně v souladu s modelem dvou nezávislých cest vzniku SCC vulvy (*Hoevenaars et al. 2008, Toki et al. 1991, van der Avoort et al. 2006*). Prokázali jsme, že vulvární dermatózy (LS a LSC), d-VIN a většina SCC vulvy nejsou asociovány s HPV infekcí. Dále jsme potvrdili, že histopatologická diagnóza u-VIN a d-VIN dobře koreluje s experimentálně zjištěným HPV profilem léze. Důležitým nálezem u diagnostických jednotek CoA a u-VIN II byla tendence k simultánní infekci více než jedním typem HPV. V případě CoA to není překvapující zjištění, neboť CoA jsou považovány za polyklonální epiteliální proliferace bez maligního potenciálu (*Vandepapeliere et al. 2005*). u-VIN III a SCC jsou naopak definovány jako monoklonální léze (*Rosenthal et al. 2002*) s integrovaným genomem jednoho transkripčně aktivního HPV (*Wentzensen et al. 2004*), což se v naší studii odrazilo minimální frekvencí výskytu koinfekcí různými typy HPV v těchto kategoriích. Naše data proto naznačují, že u-VIN II je heterogenní diagnostická jednotka zahrnující jak typické prekancerózní léze, tak i nenádorové epiteliální proliferace obsahující HPV typy, které nenacházíme v u-VIN III a SCC. Kategorie u-VIN II a u-VIN III se navíc signifikantně liší věkovou strukturou pacientek. Proto by dle našeho názoru měla být u-VIN II považována za lézi s nejednoznačným prekancerózním potenciálem, podobně jako je v současnosti vnímána CIN II (*Moscicki et al. 2006, Snijders et al. 2006, Zuna et al. 2004*). Dalším důležitým poznatkem vyplývajícím z naší studie je vysoká frekvence HR HPV infekce ve skupině

u-VIN I, kterou prokázala i další studie (*Srodon et al. 2006*).

Na základě výše uvedených faktů se domníváme, že modifikované klasifikační schéma VIN (ISSVD 2004) může být po sloučení kategorií u-VIN II a u-VIN III a zrušení diagnostické jednotky u-VIN I zavádějící a nemusí jednoznačně korelovat se skutečným prekancerózním potenciálem léze. Tento problém částečně řeší aktuální klasifikace WHO 2014 zanesením kategorie LSIL; nicméně skupiny u-VIN II a u-VIN III mají být na základě jejího doporučení zahrnuty do společné jednotky HSIL a heterogenita u-VIN II tak není nijak zohledněna.

Zastoupení jednotlivých typů HPV se v naší sestavě mírně liší od části dalších studií, a to především v užším spektru HPV typů v SCC a dále v nižší prevalenci HPV 16 a vyšším zastoupení HPV 33 v u-VIN III a SCC (*Srodon et al. 2006, van Beurden et al. 1998*). Výjimkou je pouze studie z blízkého regionu Německa (*Hampl et al. 2006*), což je v souladu s již dříve popisovanou geografickou variabilitou výskytu jednotlivých typů HPV (*Clifford et al. 2006*). V u-VIN a SCC jsme detekovali i přítomnost HPV 45. Vzhledem ke skutečnosti, že žádná z dosud volně dostupných HPV vakcín neobsahuje VLP HPV 33 a HPV 45, může být efektivita profylaktického očkování proti HPV a tím i karcinomu vulvy v populaci České republiky nižší než v ostatních regionech. Současná data však naznačují zkříženou protektivitu vakcinací získaných protilátek proti nevakcinačním typům HPV (především HPV 31, 33 a 45), jejíž přesný rozsah je nyní mapován klinickými studiemi (*De Vincenzo et al. 2013, Malagon et al. 2012, Verdenius et al. 2013*).

4.2. Význam imunohistochemické detekce markerů p16^{INK4a} a CK 17 v diferenciální diagnostice nenádorových a prekancerózních dlaždicobuněčných lézí děložního hrdla

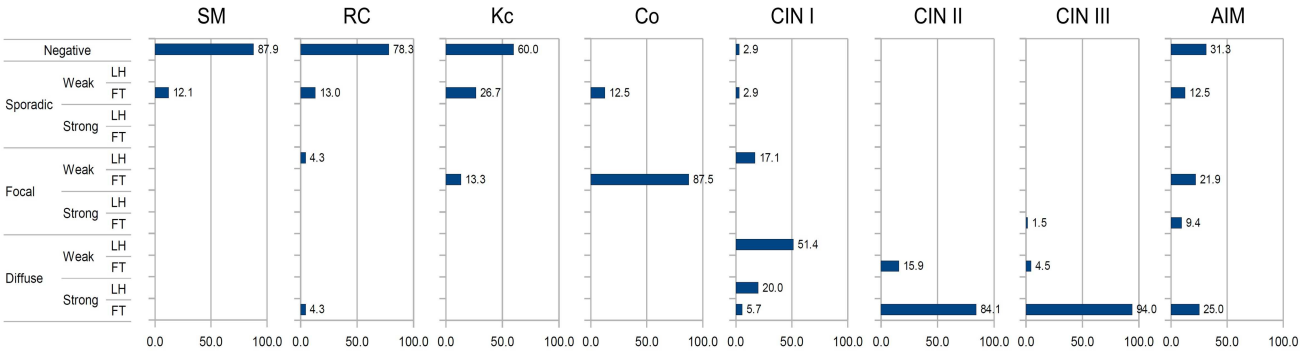
V naší další studii (**příloha V**) věnované prekancerózám LFGT jsme se věnovali možnostem zpřesnění histopatologické diagnostiky dlaždicobuněčných lézí děložního hrdla s využitím imunohistochemických metod k diferenciální diagnóze nejednoznačných nálezů. Primárním cílem projektu bylo co nejpřesněji definovat charakteristické imunoprofily jednotlivých kategorií CIN a zpřesnit tak možnosti jejich histopatologické diagnózy, která je zatížena značnou interpersonální i intrapersonální variabilitou. Dalším cílem studie bylo porovnat získané imunoprofily známých a jednoznačně charakterizovaných lézí s rozsahem imunoexprese u dlaždicobuněčných proliferací s vágně definovanými histopatologickými kritérii anebo s nejasným prekancerózním potenciálem. Typickou ukázkou takové léze je především AIM, která může mikroskopicky imitovat CIN III. Na základě literárních údajů se v této problematice jako nejvhodnější jevily protilátky proti dvěma antigenům – regulátoru buněčného cyklu p16^{INK4a} a cytoplazmatickým intermediárním filamentům cytokeratinu 17 (CK 17).

Do sestavy jsme zařadili 295 konizátů děložního hrdla s dlaždicobuněčnými lézemi, které jsme

klasifikovali do 8 kategorií – dlaždicobuněčná metaplázie (n = 33), reaktivní změny (n = 23), koilocytóza (n = 15), plochý kondylom (n = 8), CIN I (n = 35), CIN II (n = 82), CIN III (n = 67) a AIM (n = 32). Všechny léze jsme vyšetřili imunohistochemicky protilátkou proti antigenu $p16^{INK4a}$ a případy AIM navíc ještě protilátkou proti CK 17. Léze se známým biologickým potenciálem jsme sdružili do 3 základních skupin – **metaplastický fenotyp** (dlaždicobuněčná metaplázie a reaktivní změny), **LSIL/HPV fenotyp** (koilocytóza, plochý kondylom a CIN I) a **HSIL fenotyp** (CIN II a CIN III) a jejich typický imunoprofil jsme použili pro reklasifikaci lézí ze skupiny AIM. Vycházeli jsme z předpokladu, že zařazení dlaždicobuněčné léze děložního hrdla do jedné z těchto tří základních skupin je dostačující informací pro ošetřujícího lékaře ke zvolení vhodného terapeutického přístupu k pacientce. Na základě aktuálních protokolů jsou totiž pacientky s LSIL zpravidla léčeny konzervativně a pouze klinicky sledovány, zatímco ženy s HSIL naopak podstupují excizní terapeutické zákroky, zejména pak konizaci nebo simplexní hysterektomii (*Wright et al. 2007*).

V naší sestavě jsme zaznamenali celkem 13 možných $p16^{INK4a}$ imunoprofilů. Kromě jednoznačně definovaného negativního nálezu, mohlo imunohistochemické vyšetření vykazovat různé obrazy pozitivity, které jsme klasifikovali na základě horizontálního rozsahu (sporadická, fokální a difuzní pozitivita), vertikálního rozsahu (pozitivita dolní poloviny epitelu a celé šíře epitelu) a intenzity barvení (slabá a silná pozitivita). Kombinací různých variant horizontálního a vertikálního rozsahu pozitivity a intenzity barvení vzniklo dalších dvanáct $p16^{INK4a}$ imunoprofilů (obr. 7 a 8).

Negativita $p16^{INK4a}$ převažovala v kategorii dlaždicobuněčné metaplázie, reaktivních změn a koilocytózy. Všechny léze ze skupiny CIN II a CIN III, téměř všechny případy CIN I pouze s jedinou výjimkou a všechny ploché kondylomy vykazovaly zvýšenou expresi $p16^{INK4a}$, jejich imunoprofily se však vzájemně lišily intenzitou a horizontální a vertikální distribucí pozitivity. Charakteristickým rysem metaplastického fenotypu byla $p16^{INK4a}$ negativita. LSIL/HPV fenotyp vykazoval výrazně heterogenní expresi $p16^{INK4a}$, všechny léze s difúzní pozitivitou v dolní polovině epitelu jakékoliv intenzity však spadaly do kategorie CIN I. HSIL fenotyp byl jasně definován především difúzní silnou pozitivitou v celé šíři epitelu.



Obr. 7. Přehled zastoupení 13 možných p16^{INK4a} imunoprofilů v jednotlivých kategoriích dlaždicobuněčných lézí děložního hrdla.

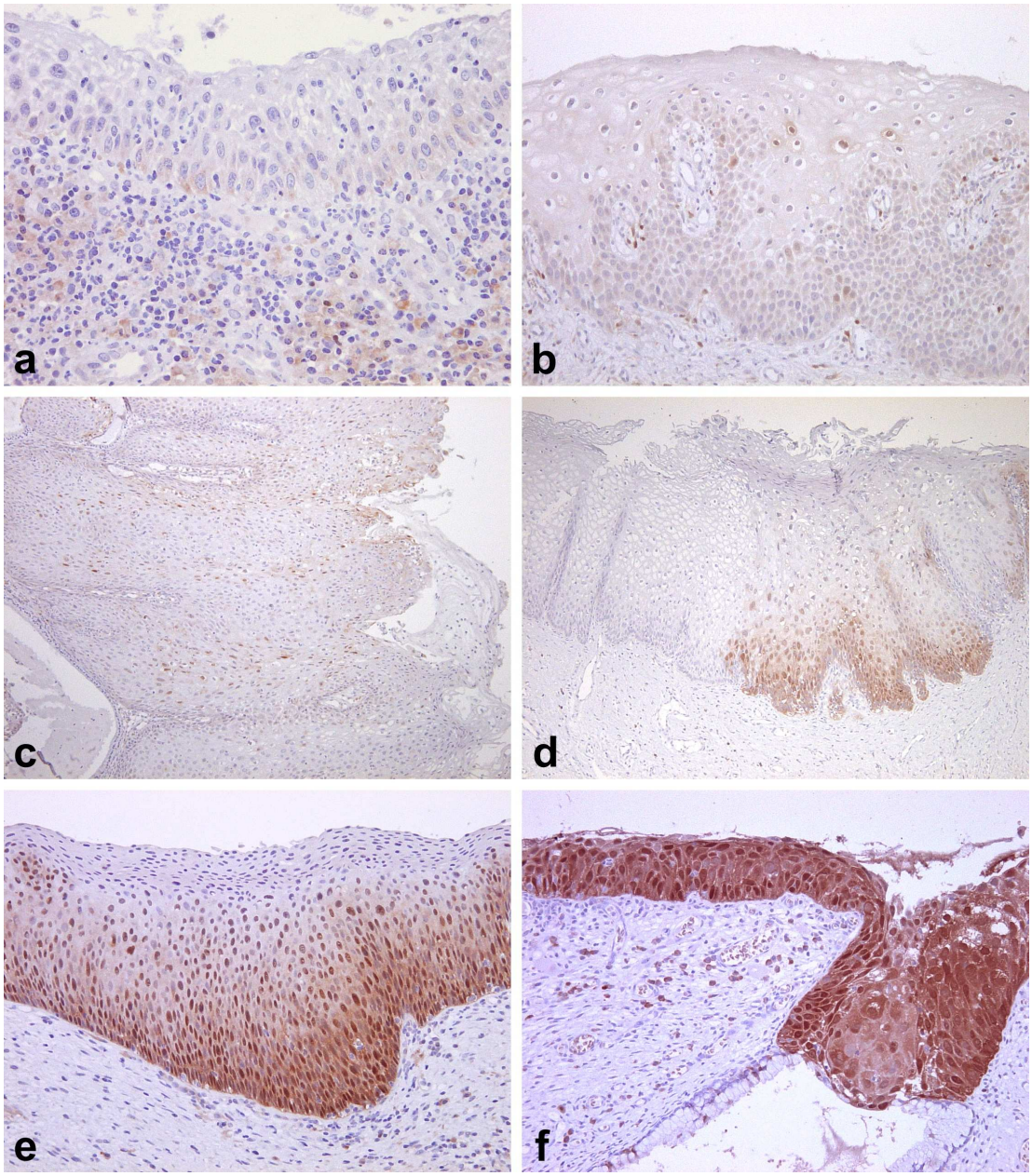
SM – dlaždicobuněčná metaplázie, RC – reaktivní změny, Kc – koilocytóza, Co – plochý kondylom, CIN – cervikální intraepiteliální neoplázie, AIM – atypical immature squamous metaplasia (atypická nezralá dlaždicová metaplázie), LH – lower half (dolní polovina epitelu), FT – full thickness (celá šíře epitelu)

Ve skupině AIM bylo 68,8 % případů p16^{INK4a} pozitivních s celkem 5 různými imunoprofily včetně negativity. Na základě jejich porovnání s výše popsanými referenčními skupinami spadá přibližně jedna třetina případů AIM mezi léze s metaplastickým fenotypem, jedna třetina do skupiny LSIL/HPV a jedna třetina odpovídá HSIL. Všechny případy AIM metaplastického a LSIL/HPV fenotypu exprimovaly CK 17 difúzně, naopak u AIM s HSIL fenotypem lehce převažovala pouze fokální pozitivita CK 17, žádná léze však nebyla zcela negativní (tab. 6).

Tabulka 6.
Přehled imunoexprese CK 17 v jednotlivých skupinách AIM stratifikovaných na základě jejich p16^{INK4a} imunoprofilů a klinicky relevantních fenotypů

p16^{INK4a} imunoprofil	Fenotyp	CK 17 imunoexprese	
		Difúzní	Fokální
Negativita	Metaplastický	10/10	-
Sporadická slabá pozitivita v celé šíři epitelu	LSIL/HPV	2/2	-
Fokální slabá pozitivita v celé šíři epitelu	LSIL/HPV	9/9	-
Fokální silná pozitivita v celé šíři epitelu	HSIL	-	2/2
Difuzní silná pozitivita v celé šíři epitelu	HSIL	4/8	4/8

CK 17 – cytokeratin 17, AIM – atypical immature squamous metaplasia (atypická nezralá dlaždicová metaplázie), LSIL – low grade squamous intraepithelial lesion (low grade skvamózní intraepiteliální léze), HSIL - high grade squamous intraepithelial lesion (high grade skvamózní intraepiteliální léze), HPV – human papillomavirus (lidský papilomavirus)



Obr. 8. Příklady p16^{INK4a} imunoprofilů u různých typů dlaždicobuněčných lézí děložního hrdla.

a – negativita v metaplastickém dlaždicovém epitelu s reaktivními změnami (200x), **b** – sporadická, slabá pozitivita v celé šíři metaplastického dlaždicového epitelu s koilocytózou (200x), **c** – fokální, slabá pozitivita v celé šíři epitelu v plochém kondylomu (100x), **d** – ostrý přechod mezi metaplastickým dlaždicovým epitelem a CIN I s difúzní, slabou pozitivitou v dolní polovině epitelu (100x), **e** – difúzní, silná pozitivita zasahující do horní poloviny epitelu v CIN II (200x), **f** – difúzní, silná pozitivita v celé šíři epitelu v CIN III (200x).

CIN – cervikální intraepiteliální neoplázie

4.2.1. Diskuze

V naší studii jsme potvrdili, že imunohistochemicky prokázaná difúzní p16^{INK4a} pozitivita je silně asociována s přítomností dysplastických změn v dlaždicobuněčné lézi děložního hrdla. V kategorii CIN I byla difúzní pozitivita převážně slabá a limitovaná na dolní polovinu epitelu, zatímco u CIN II a CIN III převažovala silná pozitivita v celé šíři epitelu. Podobné závěry byly prezentovány i v dalších studiích (*Keating et al. 2001, Klaes et al. 2001, Omori et al. 2007, Sano et al. 1998*). Pouze fokální exprese p16^{INK4a} je charakteristická pro dlaždicobuněčné léze děložního hrdla asociované s HPV infekcí bez prekancerózního potenciálu nebo s minimálním rizikem progrese jako jsou například ploché kondylomy, koilocytóza a některé případy CIN I (*Klaes et al. 2001, Sano et al. 1998*). Ostatní dlaždicové afekce zcela benigního chování jako je dlaždicobuněčná metaplázie nebo reaktivní změny mohou vykazovat slabou sporadickou p16^{INK4a} pozitivitu (*Keating et al. 2001, Klaes et al. 2001, Sano et al. 1998*). V naší sestavě jsme také zaznamenali některé z lézí z kategorie CIN I (5,7 %), jejichž p16^{INK4a} imunoprofil odpovídal HSIL fenotypu a které by mohly reprezentovat podskupiny CIN I s rizikem progrese do vyššího stupně dysplázie (*Hariri a Oster 2007, Negri et al. 2004, Wang et al. 2004*).

Na základě imunoexprese p16^{INK4a} jsme v našem souboru překlasifikovali přibližně jednu třetinu případů AIM na HSIL, což je v souladu s dosud publikovanými výsledky, i když se poměr reklasifikovaných lézí v jednotlivých studiích výrazně liší (19 % až 65 %) (*Duggan et al. 2006, Iaconis et al. 2007, Regauer a Reich 2007*). Někteří autoři v této indikaci doporučují i imunohistochemické vyšetření CK 17, které má poskytnout inverzní výsledek ve srovnání s průkazem p16^{INK4a} (*Regauer a Reich 2007*). Naše data však nepotvrzují dostatečnou spolehlivost a jednoznačnost detekce CK 17 ve srovnání s p16^{INK4a} imunohistochemií. K podobným závěrům došly i některé další studie (*Ikeda et al. 2008, Smedts et al. 1992*) a p16^{INK4a} proto i nadále zůstává jediným spolehlivým imunohistochemickým markerem k diferenciální diagnostice dlaždicobuněčných lézí děložního hrdla.

4.3. Význam vybraných patologických prognostických faktorů pro fertilitu šetřící a méně radikální chirurgické výkony u pacientek s časným stádiem karcinomu děložního hrdla

4.3.1. Přehled souboru pacientek, úspěšnost chirurgické detekce SLN a korelace peroperačního a definitivního histopatologického zpracování SLN

Pro zhodnocení možnosti provedení fertilitu šetřících a méně radikálních chirurgických výkonů u pacientek s karcinomem děložního hrdla jsme sestavili a postupně doplňovali soubor celkem 395

žen s různou velikostí a rozsahem nádoru děložního hrdla, který jsme dále analyzovali především z hlediska rizika postižení SLN a patologického postižení parametrií. Jednotlivé závěry s odpovídajícími publikačními výstupy **(přílohy VI – XIII**) budou diskutovány na následujících stranách.

Za účelem statistické analýzy jsme soubor pacientek rozdělili do 3 základních skupin (tab. 7):

- **skupina A** – maximální rozměr nádoru do 20 mm a infiltrace méně než do 1/2 šíře stromatu děložního hrdla
- **skupina B** – maximální rozměr nádoru 20 mm až 30 mm a infiltrace méně než do 2/3 šíře stromatu děložního hrdla
- **skupina C** – maximální rozměr nádoru větší než 30 mm a infiltrace minimálně do 2/3 šíře stromatu děložního hrdla.

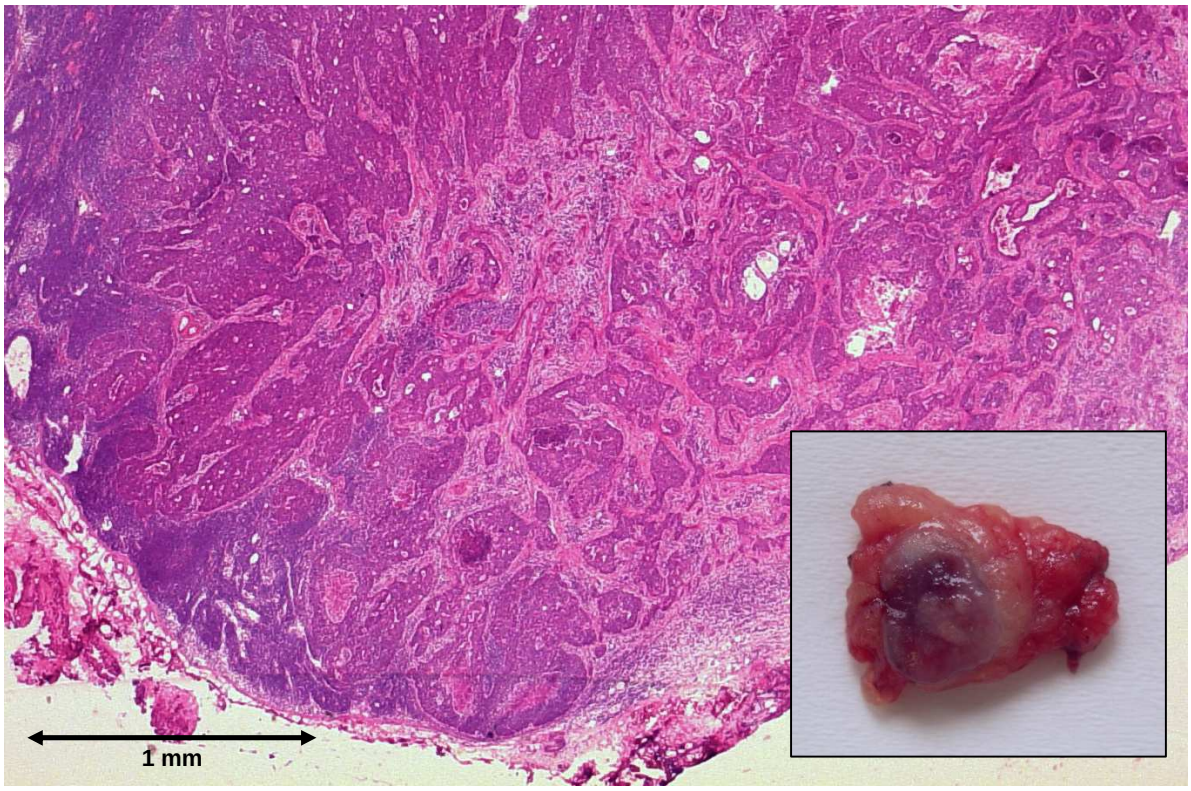
Tabulka č. 7 ukazuje trend závislosti objemu primárního nádoru a rozsahu infiltrace stromatu na detekční efektivitu jak v parametru celkové úspěšnosti detekce SLN (DR), tak i stranově specifické úspěšnosti detekce SLN (SSDR). Tyto rozdíly jsou statisticky významné pro DR (χ^2 pro trend = 10,121; p = 0,0015) a podobně i pro SSDR (χ^2 pro trend = 16,220; p = 0,0001). Nejspolehlivější pro klinickou praxi je tedy detekce SLN u časných stádií nádorů skupiny A, tedy u lézí velikosti pod 20 mm s infiltrací méně než 1/2 šíře stromatu děložního hrdla. U skupiny pacientek s nádory skupiny A naše data dále dokládají nižší riziko nádorového postižení SLN a to nejen formou makrometastáz a mikrometastáz, ale i ITC. Závěry peroperačního vyšetření SLN dobře korelovaly s nálezy při definitivním zpracování uzliny sériovým prokrajováním a imunohistochemickým vyšetřením. Falešná pozitivita SLN nebyla v našem souboru pozorována a případy falešné negativity se vyskytovaly ojediněle. U falešně negativních SLN odpovídala ve všech případech nádorová depozita mikrometastázám nebo ITC, všechny makrometastázy byly identifikovány již peroperačním vyšetřením. V některých případech byly peroperačně detekovány i ITC.

Tabulka 7.
Přehled a charakteristika souboru pacientek, u kterých byla provedena detekce a histopatologické zpracování SLN. Pacientky jsou rozděleny do skupin podle velikosti a rozsahu primárního nádoru.

	A	B	C
Počet pacientek	199	89	107
Počet SLN	567	269	278
Selhání detekce SLN (počet pacientek)	3	5	10
Jednostranná detekce SLN (počet pacientek)	14	9	11
Detekce na pacientku (DR)	98,5 %	94,4 %	90,7 %
Detekce na stranu (SSDR)	95,0 %	89,3 %	85,5 %
Průměrný počet SLN na pacientku	2,9	3,2	2,9
Pacientky s pozitivitou SLN	19 (9,6 %)	15 (16,9 %)	18 (16,8 %)
– makrometastáza	13	11	13
– mikrometastáza	6	4	5
Pacientky s ITC v SLN	5 (2,5 %)	5 (5,6 %)	4 (3.7 %)
Falešná negativita SLN	0	1 (0,4 %)	1 (0,4 %)
Falešná pozitivita SLN	0	0	0

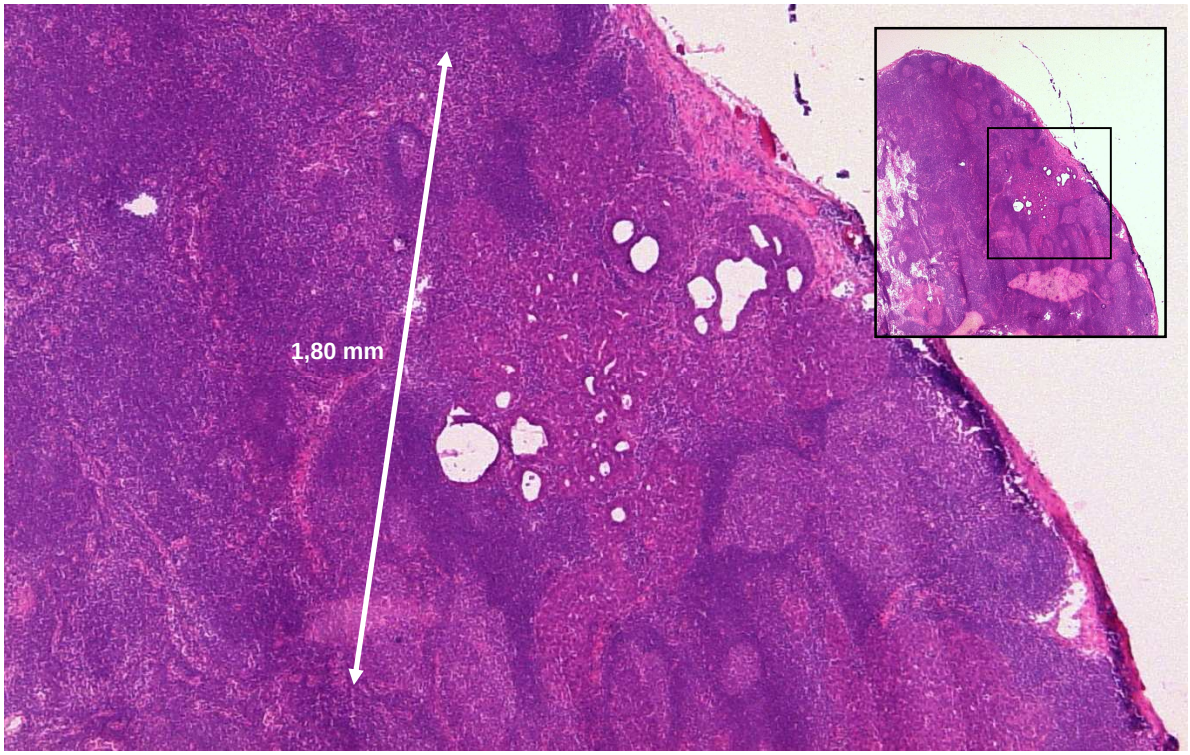
SLN – sentinel lymph node (sentinelová lymfatická uzlina), DR – detection rate (úspěšnost detekce), SSDR – side specific detection rate (stranově specifická úspěšnost detekce), ITC – isolated tumor cells (izolované nádorové buňky)

Možnosti peroperačního vyšetření SLN vyjadřují obrázky č. 9 až 12, které zobrazují celkem 3 SLN od pacientky s adenoskvamózním karcinomem děložního hrdla, grade 2, pT1b1. V každé SLN byl peroperačně zastižen jiný typ nádorového depozita. SLN č. 1 obsahovala 1 makrometastázu viditelnou již pouhým okem (obr. 9), v SLN č. 2 bylo několik mikrometastáz různé velikosti (obr. 10 a obr. 11) a v SLN č. 3 byly identifikovány ITC (obr. 12). Další případ pacientky s diagnózou SCC děložního hrdla, grade 3 již ukazuje záchyt ITC v SLN během sériového prokrajování a imunohistochemického vyšetření (obr. 13). Všechna nádorová depozita v SLN včetně ITC bylo v našem souboru možno identifikovat již z kvalitně provedených histologických řezů v základním barvením hematoxylinem-eozinem. Imunohistochemické vyšetření proto nepřineslo další zlepšení záchytu patologického postižení SLN.



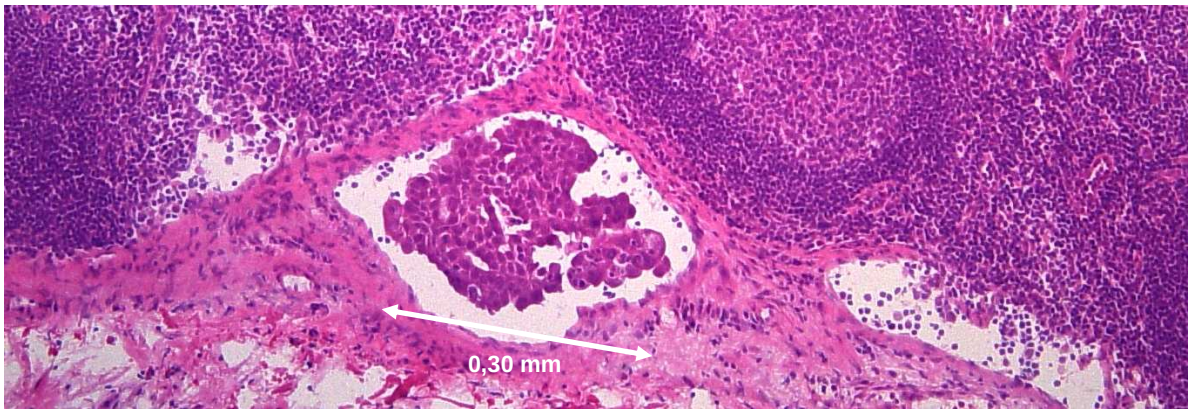
Obr. 9. Adenoskvamózní karcinom děložního hrdla, grade 2. Makrometastáza v SLN č. 1 (viditelná již při makroskopickém hodnocení resekátu), peroperační vyšetření. HE, zvětšení 20x.

SLN – sentinel lymph node (sentinelová lymfatická uzlina), HE – hematoxylin-eozin



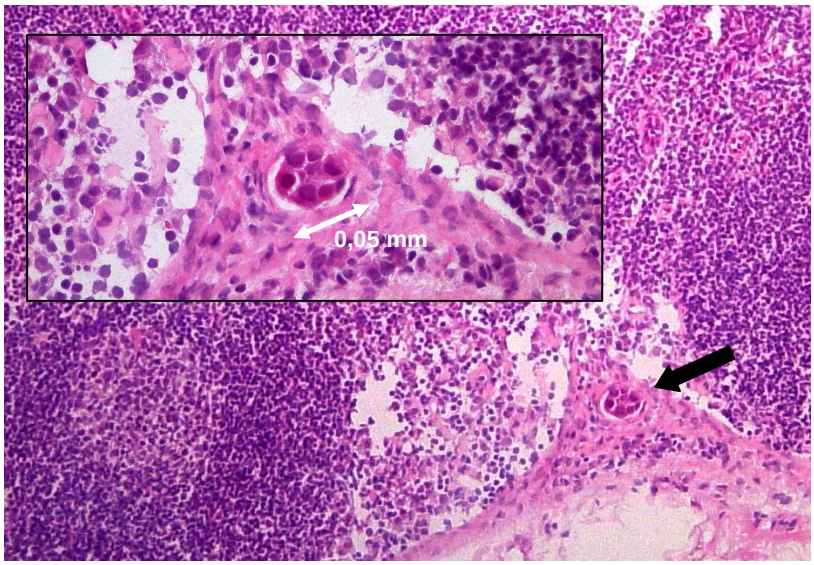
Obr. 10. Adenoskvamózní karcinom děložního hrdla, grade 2. Mikrometastáza v SLN č. 2, peroperační vyšetření, HE, zvětšení 40x.

SLN – sentinel lymph node (sentinelová lymfatická uzlina), HE – hematoxylin-eozin



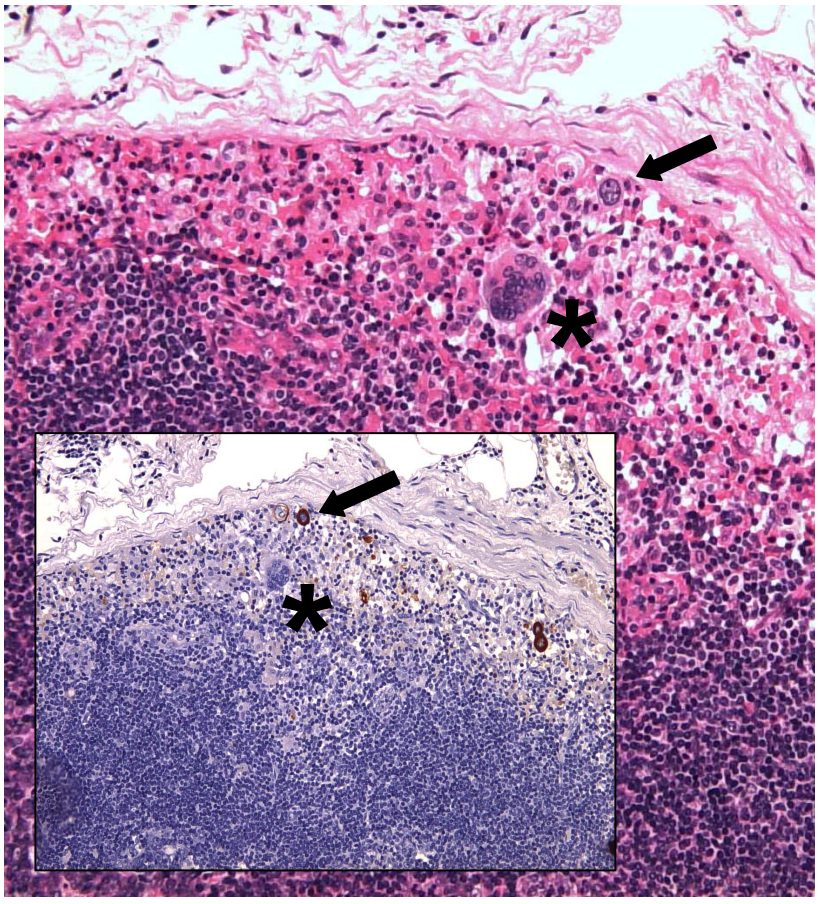
Obr. 11. Adenoskvamózní karcinom děložního hrdla, grade 2. Mikrometastáza v SLN č. 2, peroperační vyšetření, HE, zvětšení 100x.

SLN – sentinel lymph node (sentinelová lymfatická uzlina), HE – hematoxylin-eozin



Obr. 12. Adenoskvamózní karcinom děložního hrdla, grade 2. ITC (šipka) v SLN č. 2, peroperační vyšetření. HE, zvětšení 100x a 400x.

SLN – sentinel lymph node (sentinelová lymfatická uzlina), ITC – isolated tumor cells (izolované nádorové buňky), HE – hematoxylin-eozin



Obr. 13. Dlaždicobuněčný karcinom děložního hrdla, grade 3. ITC v periferním splavu SLN (šipka), ve kterém je dále zastižena reaktivní zánětlivá celulizace včetně vícejaderných histiocytů (hvězdička). HE a imunohistochemické vyšetření širokospektrou protilátkou proti cytokeratinům CK KL1 (vložený snímek), zvětšení 100x a 200x.

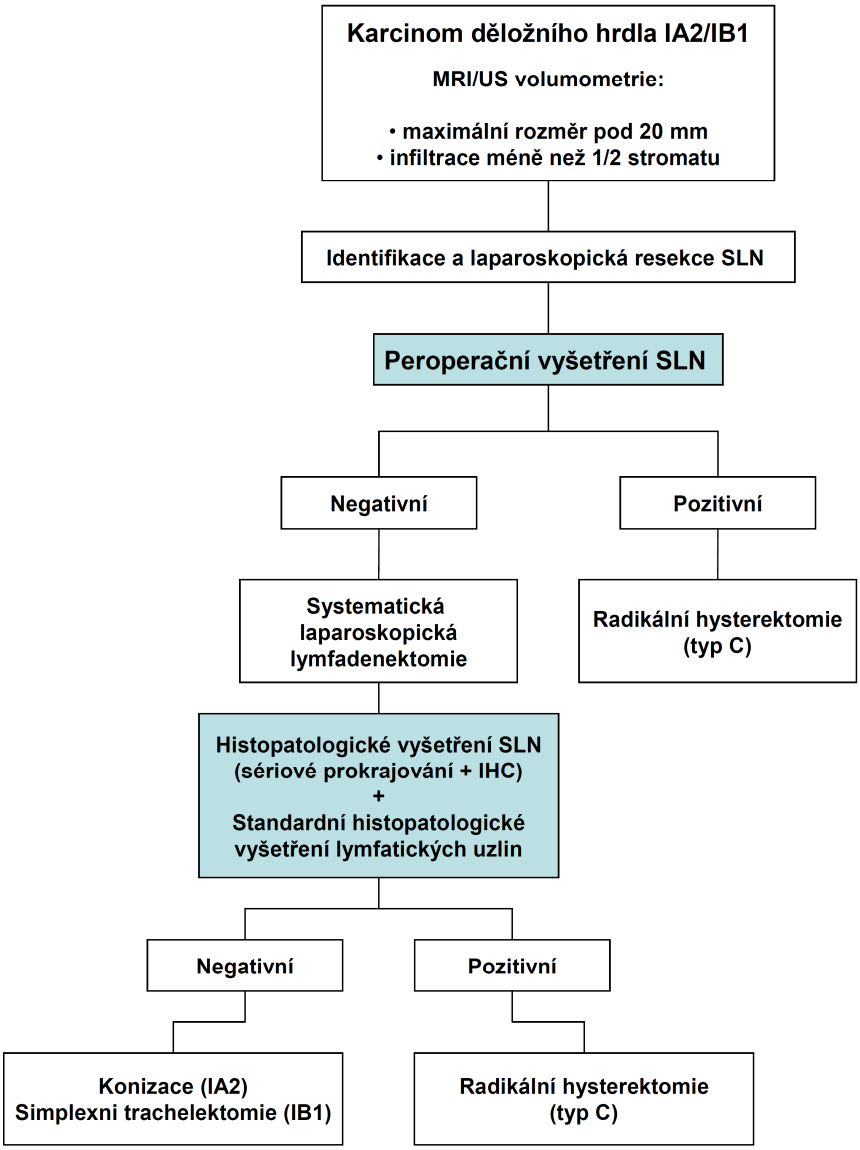
SLN – sentinel lymph node (sentinelová lymfatická uzlina), ITC – isolated tumor cells (izolované nádorové buňky), HE – hematoxylin-eozin

4.3.2. Fertilitu šetřící chirurgické výkony s omezenou resekcí mediálních částí parametrií (LAP-I protokol)

Ve dvou projektech (**přílohy VI a VII**) jsme demonstrovali proveditelnost a bezpečnost nového fertilitu šetřícího chirurgického postupu – laparoskopické lymfadenektomie s identifikací SLN a s následnou konizací nebo simplexní trachelektomií. Inovací tohoto výkonu ve srovnání s VRT a ART je omezení resekce mediálních částí parametriií, což by mohlo vést ke snížení morbidity pacientek a ke zlepšení těhotenských výsledků. Cílem těchto studií bylo též ověření onkologických výsledků a jejich srovnání s efektivitou ostatních fertilitu šetřících výkonů.

Podmínkou zařazení do projektu byl požadavek pacientky na zachování fertility, z medicínských kritérií pak dále velikost nádoru menší než 20 mm v největším rozměru a infiltrace méně než 1/2 šíře stromatu děložního hrdla zjištěná předoperační ultrasonografickou volumometrií a magnetickou rezonancí (skupina A). Obě studie byly založeny na dvoudobém diagnosticko-terapeutickém algoritmu, který jsme nazvali LAP-I protokol (obr. 14). V LAP-I protokolu závisí rozsah chirurgického výkonu v první době na peroperačním vyšetření SLN a ve druhé době po týdenním odstupu na výsledcích definitivního histopatologického zpracování SLN a ostatních lymfatických uzlin.

Do iniciální studie (**příloha VI**) bylo zařazeno celkem 26 žen s časným stádiem karcinomu děložního hrdla (stádia IA2 a IB1). Nádorová depozita v SLN byla peroperační biopsií zastižena u 4 žen (15,4 %), u nichž byla následně provedena radikální hysterektomie. U ostatních pacientek byl výkon omezen pouze na laparoskopickou pánevní lymfadenektomii. Definitivní histopatologické vyšetření neodhalilo v žádné další lymfatické uzlině včetně SLN metastatické postižení, a proto mohlo být u 15 pacientek dle protokolu přistoupeno k simplexní trachelektomii a u 7 pacientek ke konizaci. Z onkologického pohledu se LAP-I protokol jeví jako bezpečný postup. Lokální recidiva nádoru byla diagnostikována pouze u jedné pacientky v oblasti istmu dělohy 14 měsíců po operaci. LAP-I protokol je efektivní i z hlediska možnosti budoucí koncepce. U pacientek zařazených do studie jsme zaznamenali celkem 15 těhotenství u 11 žen a z toho celkem 8 porodů u 7 žen. Jeden porod byl předčasný, a to ve 24. týdnu těhotenství, dalšími komplikacemi byly 4 spontánní potraty a 1 ektopická gravidita.



Obr. 14. Diagnosticko-terapeutický algoritmus LAP-I protokolu.

MRI – magnetická rezonance, US – ultrasonografie, SLN – sentinel lymph node (sentinelová lymfatická uzlina), IHC – imunohistochemické vyšetření

Výše popsaný soubor pacientek se v následující studii (**příloha VI**) podařilo rozšířit na 40 žen. Kromě 10 pacientek ve stádiu IA2 a 27 pacientek ve stádiu IB1 jsme do souboru zařadili i 3 pacientky

ve stádiu IA1 s nádorovou lymfangioinvazí. Nádorová depozita v SLN byla v rozšířeném souboru peroperačně zastižena u 6 žen (15,0 %). V definitivním histologickém vyšetření byly všechny ostatní lymfatické uzliny včetně SLN negativní a proto mohlo 34 pacientek podstoupit fertilitu šetřící chirurgický výkon. V průběhu onkologického sledování pacientek, které trvalo průměrně 47 měsíců, došlo k recidivě nádoru pouze u jedné pacientky (viz výše). Do doby publikace studie proběhlo celkem 23 těhotenství u 17 žen a z toho 12 úspěšných porodů u 11 pacientek. Počet předčasných porodů se oproti předchozí studii nezvýšil.

4.3.3. Rozsah postižení SLN a parametrií u pacientek s časnými stádii karcinomu děložního hrdla

Diagnosticko-terapeutický LAP-I protokol představený v předchozích studiích (**přílohy VI a VII**) vychází z postulátu podpořeného literárními údaji (*Covens et al. 2002, Plante et al. 2004, Stegeman et al. 2007*), že patologické postižení parametrií je u časných stádií karcinomu děložního hrdla vzácné, a proto mohou pacientky profitovat z omezeného rozsahu resekce mediálních částí parametrií. Tzv. patologické postižení parametrií (PPP) je definováno jako přítomnost pozitivní parametriální lymfatické uzliny nebo jako nekontinuální šíření nádoru v parametriích (např. formou nádorové lymfangioinvaze).

Výše zmíněný předpoklad jsme podpořili simultánně prováděnou prospektivní studií **(příloha VIII**), kde jsme experimentálně zjišťovali rozsah a distribuci nádorově změněných SLN i dalších lymfatických uzlin a patologického postižení parametrií u pacientek s karcinomy děložního hrdla stádia IA2 a karcinomů stádia IB1 menší velikosti, které nepronikají hlouběji než do 2/3 šíře stromatu děložního hrdla. Kritéria k zařazení do studie splňovalo 158 pacientek, které jsme za účelem porovnání s předchozími výsledky rozdělili do dvou již dříve definovaných skupin A (91 pacientek) a B (67 pacientek). Detekce a peroperační vyšetření SLN probíhaly standardním způsobem jako ve výše popsaném LAP-I protokolu. Následně byla u všech pacientek provedena radikální hysterektomie s resekcí parametrií.

V obou skupinách pacientek bylo PPP a metastatické postižení nesentinelových lymfatických uzlin limitováno výlučně na pacientky s nádorovými depozity v SLN (tab. 8). Celkem bylo v obou skupinách diagnostikováno 25 pacientek s metastatickým postižením SLN, z této skupiny bylo PPP zastiženo v 7 případech (28,0 %) a metastázy v nesentinelových lymfatických uzlinách v 8 případech (32,0 %). PPP mělo nejčastěji formu lymfangioinvaze (3 případy), dále nádorových depozit v parametriálních SLN (2 případy) a nesentinelových lymfatických uzlinách (2 případy). Dalším zajímavým poznatkem je i skutečnost, že pouze 4,2 % SLN se vyskytuje v mediálních částech parametrií. Tento fakt je v souladu s našimi předchozími výsledky (*Rob et al. 2005*).

Tabulka 8.
Patologické postižení parametrií a nesentinelových lymfatických uzlin v závislosti na stavu SLN u jednotlivých skupin pacientek.

	Počet pacientek (%)	PPP	Postižení nesentinelových lymfatických uzlin
Skupina A (91 pacientek)			
SLN negativní	80 (87,9 %)	80 negativní (100,0 %)	80 negativní (100,0 %)
SLN pozitivní	11 (12,1 %)	3 pozitivní (27,3 %)	3 pozitivní (27,3 %)
Skupina B (67 pacientek)			
SLN negativní	53 (79,1 %)	53 negativní (100,0 %)	53 negativní (100,0 %)
SLN pozitivní	14 (20.9 %)	4 pozitivní (28,6 %)	5 pozitivní (35,7 %)

SLN – sentinel lymph node (sentinelová lymfatická uzlina), PPP – patologické postižení parametrií

4.3.4. Využití neoadjuvantní chemoterapie pro fertilitu šetřící chirurgické výkony u časných stádií karcinomu děložního hrdla (LAP-III protokol)

Dalším krokem našeho výzkumu (**přílohy VII a IX**) bylo ověření proveditelnosti a zhodnocení onkologických a těhotenských výsledků fertilitu šetřících výkonů u pacientek s nádory, jejichž rozsah či velikost přesahují horní kritéria pro zařazení do protokolu LAP-I (nádor velikosti nad 20 mm anebo s invazí pohybující se v rozsahu 1/2 až 2/3 šíře stromatu děložního hrdla). Tato skupina žen není vhodná k primární fertilitu šetřící chirurgické léčbě, protože rozsah resekce by nedovolil zachovat dostatečný objem nádorem nepostiženého stromatu děložního hrdla nutný k úspěšnému dokončení těhotenství. Základním principem modifikovaného léčebného přístupu je v této situaci především snaha o předoperační zmenšení primárního nádoru, kterého lze dosáhnout pomocí neoadjuvantní chemoterapi (NAC). Za tímto účelem jsme vypracovali LAP-III protokol založený na iniciální léčbě vysokodávkovanou NAC na bázi cisplatiny, všechny další diagnosticko-terapeutické kroky jsou shodné s protokolem LAP-I.

Do pilotní sestavy (**příloha IX**) jsme zařadili celkem 5 pacientek a tento soubor jsme prezentovali formou kazuistických sdělení. V další publikaci (**příloha VII**) jsme rozšířili počet pacientek na 9 a demonstrovali prvotní onkologické a těhotenské výsledky. Z onkologického hlediska došlo po NAC k významné regresi primárního nádoru. Celkem 3 pacientky byly bez histopatologicky prokazatelného nádorového rezidua, u 4 pacientek bylo biopticky prokázáno mikroskopické reziduum velikosti do 2 mm, u 1 pacientky makroskopické reziduum největšího rozměru 13 mm a u 1 pacientky

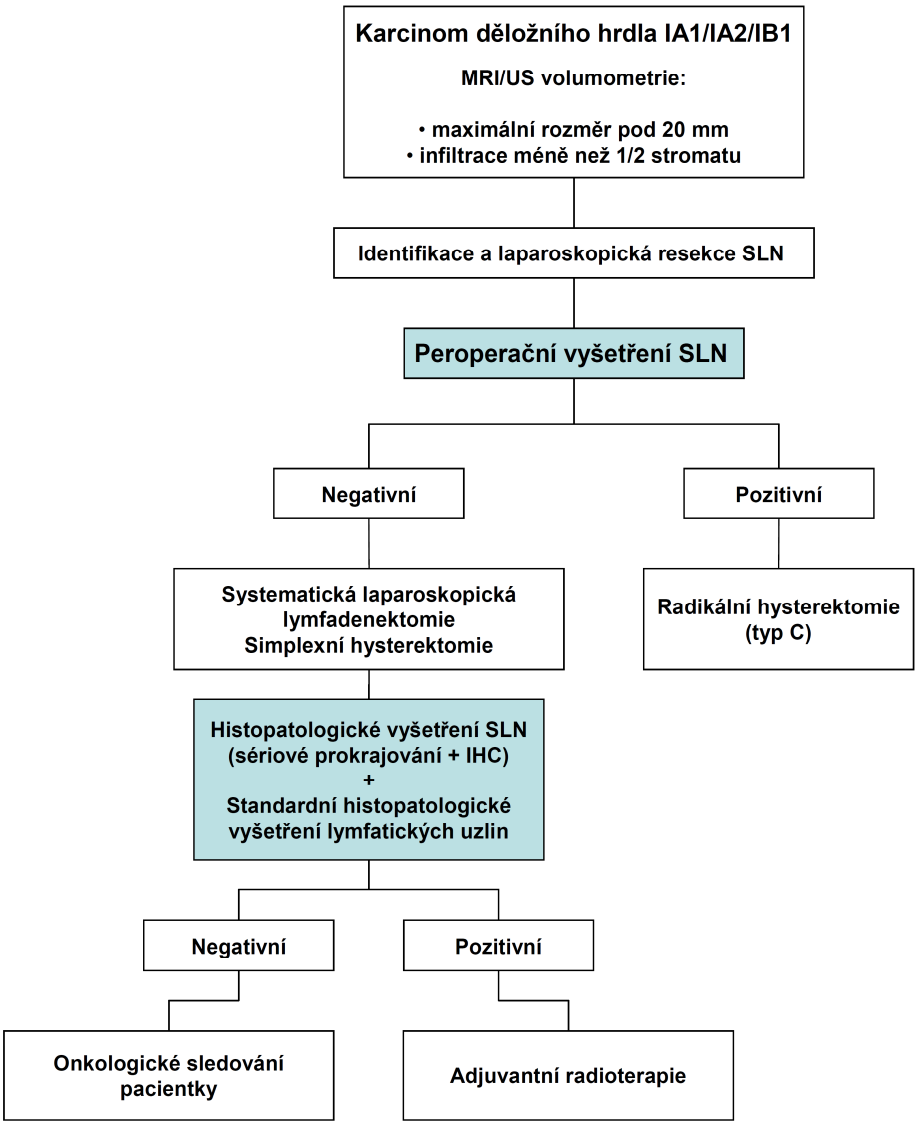
zasahovala reziduální nádorová infiltrace do kraniálního chirurgického okraje trachelektomie. Reprodukční schopnost byla zachována u 7 pacientek a v době publikace jsem zaznamenali již 1 porod zdravého dítěte a 2 probíhající těhotenství. Za poměrně krátký interval onkologického sledování pacientek nedošlo k žádné recidivě nádoru. Prospektivní studii jsme v následujících letech rozšířili až na 28 pacientek (**příloha XIV**). Po aplikaci NAC nebyl reziduální nádor zastižen celkem u 6 pacientek (21,4 %), mikroskopické reziduum menší než 3 mm bylo zastiženo u 11 žen (39,3 %) a makroskopické reziduum bylo diagnostikováno též u 11 pacientek (39,3 %). Fertilitu se podařilo zachovat u 20 žen (71,4 %), ze kterých 10 žen otěhotnělo (50,0 %) a 8 z nich porodilo celkem 10 dětí. Recidivu nádoru jsme ve skupině pacientek po fertilitu zachovávajícím výkonu zaznamenali ve 4 případech (20,0 %), z toho lokální recidiva v oblasti děložního hrdla byla diagnostikována u 3 pacientek a metastatické postižení ovaria u 1 pacientky. Celkem 2 ženy s recidivou nádoru onemocnění podlehly (10 %).

4.3.5. Méně radikální chirurgické výkony v terapii časných stádií karcinomu děložního hrdla

V jedné z našich předchozích studií (**příloha VIII**) jsme demonstrovali minimální riziko PPP u pacientek s časným stádiem karcinomu děložního hrdla a s negativitou SLN. V našem dalším projektu (**příloha X**) jsme navázali na předchozí výsledky a posuzovali bezpečnost a onkologické výsledky limitované resekce mediálních částí parametrií u žen různých věkových kategorií s časnými stádii karcinomu děložního hrdla. Do studie bylo zařazeno celkem 60 pacientek – 3 pacientky ve stádiu IA1 s nádorovou lymfangioinvazí, 11 pacientek ve stádiu IA2 a 46 pacientek ve stádiu IB1. Postupovali jsme standardizovaným způsobem na základě modifikovaného diagnosticko-terapeutického algoritmu LAP-II, který vychází z LAP-I protokolu (obr. 15). Na rozdíl od klasických léčebných postupů podstoupily pacientky ve studii pouze vaginální laparoskopicky asistovanou anebo laparoskopickou simplexní hysterektomii místo radikální hysterektomie. V případě pozitivního nálezu v lymfatických uzlinách při definitivním zpracování byla indikována adjuvantní radioterapie.

SLN byly úspěšně identifikovány u všech pacientek a jejich pozitivita byla prokázána u 5 pacientek (8,3 %). U 3 žen byly v SLN peroperačně zastiženy metastázy velikosti 5 mm, 8 mm a 10 mm, u dalších 2 pacientek bylo peroperační vyšetření SLN falešně negativní a definitivní zpracování odhalilo nádorová depozita z kategorie mikrometastáz a ITC. Žádné další metastázy v nesentinelových lymfatických uzlinách nebyly v průběhu studie zastiženy. U prvních 25 pacientek s negativitou SLN byla provedena lymfadenektomie standardního rozsahu a v této skupině jsme zaznamenali vznik pooperačního lymfedému ve 12 % případů a lokalizované dilatace lymfatických cév (tzv. lymfocyst) u 24 % pacientek. U dalších 32 žen s negativní SLN byla ve druhé polovině studie radikalita lymfadenektomie omezena a incidence lymfocyst klesla na 6,3 %, vznik lymfedému jsme

v této skupině klinicky nediagnostikovali. Průměrná doba onkologického sledování pacientek byla 47 měsíců a během tohoto období nedošlo u žádné z pacientek, včetně žen s pozitivitou SLN, k recidivě nádoru.



Obr. 15. Diagnosticko-terapeutický algoritmus LAP-II protokolu.

MRI – magnetická rezonance, US – ultrasonografie, SLN – sentinel lymph node (sentinelová lymfatická uzlina), IHC – imunohistochemické vyšetření

4.3.6. Diskuze

Z hlediska optimálních onkologických a těhotenských výsledků mají fertilitu šetřící chirurgické výkony opodstatnění pouze v časném stádiu karcinomu děložního hrdla běžných histologických typů.

Výjimkou jsou karcinomy s neuroendokrinní diferenciací, u kterých je vysoká tendence nádoru k lymfogenní a hematogenní propagaci, a neradikální chirurgický výkon proto v tomto případě nelze vnímat jako onkologicky bezpečný. Základem správné indikace k fertilitu šetřící operaci je kromě bioptického ověření nádorového procesu především přesné předoperační určení velikosti nádoru ultrazvukovou volumometrií a magnetickou rezonancí (*Chou et al. 1997, Sahdev et al. 2005, Wagenaar et al. 2001*), včetně určení rozsahu infiltrace stromatu děložního hrdla. Většina onkogynekologických center používá jako limitující indikační kritérium maximální velikost nádoru do 20 mm (*Bernardini et al. 2003, Covens et al. 1999, Plante et al. 2004, Shepherd et al. 2001, Schlaerth et al. 2003*). Dalším indikačním kritériem je hloubka nádorové infiltrace maximálně do 1/2 šíře stromatu děložního hrdla, neboť chirurgický výkon by měl zachovat dostatečný lem zdravé tkáně pro dosažení uspokojujících těhotenských výsledků.

V našich studiích jsme potvrdili vysokou spolehlivost chirurgické detekce SLN, která byla efektivnější u nádorů nižšího objemu a s menším rozsahem infiltrace stromatu děložního hrdla. Tuto skutečnost lze nejspíše vysvětlit blokádou lymfatických cév nádorovými buňkami při masivnější lymfangioinvazi u nádorů větší velikosti. Jistou úlohu mohou také hrát regresivní změny ve větších nádorech charakteru nekrózy nebo reaktivní fibroprodukce v okolí. Pacientky s nejmenšími nádory (maximální rozměr pod 20 mm a infiltrace méně než 1/2 šíře stromatu děložního hrdla) měly též nejnižší riziko nádorových depozit v SLN. Spolehlivost peroperačního bioptického vyšetření SLN se v našem souboru pacientek jeví jako vysoká. Falešně negativní SLN obsahovaly pouze mikrometastázy nebo ITC, všechny makrometastázy se nám podařilo zachytit již peroperačně. Literární údaje o spolehlivosti peroperačního zpracováni SLN jsou nicméně poměrně heterogenní (*Bats et al. 2011, Fader et al. 2008, Fanfani et al. 2004, Slama et al. 2013*), což odráží variabilitu používaných protokolů pro peroperační a definitivní zpracování SLN a podtrhuje potřebu jejich standardizace.

Resekce parametrií a pánevní lymfadenektomie jsou hlavními příčinami vzniku pozdních pooperačních komplikací (*Frumovitz et al. 2005, Kadar et al. 1983, Magrina et al. 1995, Sood et al. 2002*). Výrazné zlepšení možnosti detekce nádorových depozit v nejrizikovějších lymfatických uzlinách při současném snížení radikality resekce lymfatických uzlin přinesl koncept SLN (*Altgassen et al. 2008, Marnitz et al. 2006, Plante et al. 2003, Rob et al. 2005*). Z dosud dostupných studií vyplývá, že SLN bývá detekována velmi vzácně (méně než v 5 % případů) v mediálních částech laterálních parametrií (*Covens et al. 2002, Rob et al. 2005*) a že nádorové postižení této části parametrií je raritní (pod 1 %) u karcinomů maximální velikosti pod 20 mm a s infiltrací méně než 1/2 šíře stromatu děložního hrdla (*Covens et al. 2002, Kinney et al. 1995, Plante et al. 2004, Stegeman et al. 2007*).

Naše prospektivní studie (**příloha VIII**) potvrdila výše uvedená data a navíc jako první kvantifikovala riziko nádorového postižení parametrií v závislosti na stavu SLN. Ve skupině pacientek

s malým karcinomem (maximální rozměr pod 20 mm a infiltrace méně než 1/2 šíře stromatu děložního hrdla) spadajícího do indikačních kritérií k fertilitu šetřící operaci bylo celkové riziko patologického postižení parametrií 3,3 %, avšak u pacientek s pozitivní SLN se zvýšilo na 27,3 %. Podobně ve skupině žen s větším nádorem (maximální rozměr 20 mm až 30 mm, invaze do 2/3 šíře stromatu děložního hrdla) jsme pozorovali celkové riziko patologického postižení parametrií 6,0 %, ale při pozitivitě SLN až 28,6 %. Obdobnou korelaci jsme prokázali i mezi pozitivitou SLN a frekvencí nádorových depozit v nesentinelových lymfatických uzlinách. Naše data podtrhují význam vyšetření SLN jako prognostického faktoru rozsahu nádorového postižení v časných stádiích karcinomu děložního hrdla.

Výše uvedené poznatky jsme v našich studiích uplatnili při fertilitu šetřících chirurgických výkonech u pacientek s karcinomy děložního hrdla stádia IA1 s nádorovou lymfangioinvazí, IA2 a IB1 (**přílohy VI a VII**) v rámci protokolu LAP-I. Simplexní trachelektomie nebo konizace bez resekce mediálních částí parametrií se ukázaly být onkologicky vysoce efektivními metodami u pacientek s negativními SLN. Recidiva nádoru byla u onkologicky sledovaných pacientek zaznamenána pouze v jednom případě (2,9 %). Ve srovnání s ostatními fertilitu šetřícími výkony jako je např. ART (*Kim et al. 2010, Nishio et al. 2009*) má protokol LAP-I velmi dobré těhotenské výsledky a minimum pooperačních komplikací.

Určitou šanci na zachování fertility mohou mít i pacientky s karcinomy děložního hrdla, jejichž velikost přesahuje běžná indikační kritéria pro fertilitu šetřící operaci (nádor velikosti nad 20 mm anebo s invazí pohybující se v rozsahu 1/2 až 2/3 šíře stromatu děložního hrdla). V těchto případech lze aplikovat NAC, která je v současnosti běžně používána v terapii stádia IB2 karcinomu děložního hrdla. Redukce objemu nádoru působením NAC může umožnit provedení méně radikálního operačního výkonu, a tím zachování dostatečného objemu stromatu děložního hrdla (*Kobayashi et al. 2006, Landoni et al. 2007, Maneo et al. 2008, Plante et al. 2006*). V našich pracích (**přílohy VII, IX a XIV**) jsme potenciál NAC demonstrovali ve formě LAP-III protokolu na celkem 28 pacientkách s povzbudivými onkologickými i těhotenskými výsledky.

Možnosti méně radikálních chirurgických postupů je možno rozšířit i na ženy s časným karcinomem děložního hrdla, které nepožadují zachování fertility. Nutnost a rozsah resekce parametrií při radikální hysterektomii u těchto pacientek byla v poslední letech intenzivně diskutována (*Covens et al. 2002, Kinney et al. 1995, Landoni et al. 2001, Magrina et al. 1995, Plante a Roy 2001, Wright et al. 2007*). Četné studie navíc potvrdily korelaci mezi pozitivitou pánevních lymfatických uzlin, velikostí nádoru nebo rozsahem infiltrace stromatu děložního hrdla a nádorovým postižením parametrií (*Covens et al. 2002, Kinney et al. 1995, Magrina et al. 1995, Wright et al. 2007*). V jedné z našich studií (**příloha X**) jsme dosáhli uspokojujících onkologických výsledků simplexní hysterektomie a méně radikální lymfadenektomie u pacientek s časným stádiem karcinomu děložního

hrdla. Podmínkou provedení chirurgického výkonu s limitovanou radikalitou je negativita SLN, kterou považujeme za nejvýznamnější prognostický faktor. Na základě našich předchozích poznatků je radikální operace u pacientek s pozitivní SLN plně indikována, protože riziko nádorového postižení parametrií přesahuje 27 % (**příloha VIII**). V naší studii jsme také zaznamenali snížený výskyt nežádoucích pooperačních komplikací vyplývající z omezené radikality lymfadenektomie při zachování optimálních onkologických výsledků.

Naše zkušenosti s fertilitu šetřícími výkony u pacientek s časným stádiem karcinomu děložního hrdla jsme recentně shrnuli ve 3 přehledových článcích (**přílohy XI, XII a XIII**), kde jsme podrobně diskutovali chirurgickou i histopatologickou problematiku.

5. ZÁVĚRY

5.1. HPV infekce v nenádorových lézích, prekancerózách a SCC vulvy – dopad na klasifikaci VIN a odhad efektivity profylaktické vakcinace proti HPV

- Z hlediska HPV profilu lze rozdělit dlaždicobuněčné léze vulvy na skupinu HPV negativní (LS, LSC, d-VIN a většina SCC), skupinu obsahující LR HPV typy (CoA) a skupinu obsahující HR HPV typy (všechny stupně u-VIN a část SCC). Rozložení HPV pozitivity ve vulvárních dlaždicobuněčných proliferacích je v souladu s teoretickým modelem dvou nezávislých cest vzniku SCC vulvy.
- Modifikovaná klasifikace VIN (ISSVD 2004) a aktuální klasifikace WHO 2014 vedou ke zjednodušení terminologie vulvárních prekaceróz a usnadní tak práci patologa. U heterogenních skupin vulvárních lézí s variabilním HPV profilem a biologickým chováním (u-VIN I a u-VIN II) však mohou být v některých případech zavádějící při odhadu prognózy pacientky.
- Při hodnocení efektivity profylaktické vakcinace v populaci České republiky je nutno vzít v úvahu především fakt, že očkování ovlivňuje pouze incidenci HPV asociovaných prekanceróz a SCC. Dále je nutno zvážit jistou geografickou variabilitu, která se v našem regionu projevuje především nižší prevalencí HPV 16 a vyšším zastoupením HPV 33. Úspěšnost očkování se proto bude odvíjet též od rozsahu zkřížené protektivity s vakcinačními typy HPV.

5.2. Význam imunohistochemické detekce markerů p16^{INK4a} a CK 17 v diferenciální diagnostice nenádorových a prekancerózních dlaždicobuněčných lézí děložního hrdla

- Dlaždicobuněčné proliferace děložního hrdla mají charakteristické p16^{INK4a} imunoprofily, které lze využít jako pomocnou metodu při histopatologickém vyšetření. Difúzní p16^{INK4a} pozitivita je silně asociována s přítomností dysplastických změn. Silná intenzita reakce společně s vertikálním posunem pozitivity do horní poloviny epitelu pak svědčí pro high grade dysplázii (CIN II a CIN III).
- Imunohistochemické vyšetření markeru p16^{INK4a} lze využít k reklasifikaci dlaždicobuněčných proliferací s nejasným nebo obtížně interpretovatelným prekancerózním potenciálem jako je např. AIM, kterou je možno rozčlenit do tří klinicky relevantních kategorií – třetina lézí ze

skupiny AIM odpovídá HSIL, třetina spadá do kategorie LSIL/HPV a třetina odpovídá pouze nezralé dlaždicové metaplázii.

- Diagnostický přínos imunohistochemického vyšetření CK 17 je ve srovnání se stanovením markeru $p16^{INK4a}$ nízký.

5.3. Význam vybraných patologických prognostických faktorů pro fertilitu šetřící a méně radikální chirurgické výkony u pacientek s časným stádiem karcinomu děložního hrdla

- Peroperační chirurgická detekce a histopatologické vyšetření SLN s následným sériovým prokrajováním a imunohistochemickou analýzou je ve formě algoritmu vypracovaného naším týmem bezpečnou metodou, která umožňuje již peroperačně zachytit makrometastázy a většinu větších mikrometastáz. Ve formě LAP-I protokolu lze tento postup využít pro fertilitu šetřící a méně radikální chirurgické výkony u pacientek s časným stádiem karcinomu děložního hrdla.
- Riziko nádorových depozit v SLN roste se zvětšujícím se objemem nádorové tkáně a s hloubkou infiltrace stromatu děložního hrdla. Nejnižší výskyt nádorového postižení SLN byl zaznamenán ve skupině pacientek s maximálním rozměrem nádoru pod 20 mm a s maximální hloubkou stromální invaze do 1/2 šíře stromatu děložního hrdla.
- Analogicky k předchozímu bodu roste riziko nádorového postižení parametrií se zvětšujícím se objemem nádorové tkáně a s hloubkou infiltrace stromatu děložního hrdla a radikálně se zvyšuje při metastatickém postižení SLN. Nepřítomnost nádorových depozit v SLN je proto významným a klinicky relevantním prognostickým faktorem, na jehož podkladě lze snižovat radikalitu onkologických operací časných stádií karcinomu děložního hrdla.
- Výše popsaný princip fertilitu šetřících a méně radikálních chirurgických výkonů lze po předchozí neoadjuvantní chemoterapii s úspěchem použít ve formě LAP-III protokolu i u pacientek s pro tyto výkony hraniční velikostí karcinomu děložního hrdla.

6. SOUHRN

Cílem předkládané dizertační práce je představit moderní možnosti histopatologického vyšetření včetně imunohistochemických a molekulárních metod pro diagnostiku, prevenci a inovativní chirurgické přístupy v terapii prekancerózních lézí a maligních nádorů dolního ženského pohlavního systému (vulva, děložní hrdlo).

V *první části* dizertační práce jsme se zaměřili na zastoupení jednotlivých typů lidského papilomaviru (HPV) v nenádorových, prekancerózních a nádorových dlaždicobuněčných lézích vulvy, na odhad efektivity profylaktické vakcinace proti HPV a na zhodnocení účelnosti modifikace klasifikačního schématu vulvárních intraepiteliálních neoplázií (VIN). Analyzovali jsme spektrum typů HPV v jednotlivých histologických kategoriích vulvárních lézí a potvrdili správnost konceptu dvou nezávislých cest karcinogeneze v oblasti vulvy - HPV asociované a HPV negativní. Identifikovali jsme několik heterogenních skupin prekanceróz vulvy s variabilními HPV profily, u kterých může být aplikace nové revidované klasifikace VIN zavádějící pro odhad jejich biologického chování. Poukázali jsme též na fakt, že se úspěšnost profylaktického očkování proti HPV bude odvíjet od rozsahu zkřížené protektivity proti nevakcinačním typům HPV, které se v populaci České republiky ve zvýšené míře vyskytují ve srovnání s ostatními geografickými regiony.

Druhou část dizertační práce jsme věnovali hodnocení významu imunohistochemické detekce markerů $p16^{INK4a}$ a CK 17 pro diferenciální diagnostiku dlaždicobuněčných proliferací děložního hrdla. Identifikovali jsme charakteristické $p16^{INK4a}$ imunoprofily lézí o dobře definovaném prekancerózním potenciálu a potvrdili, že difuzní $p16^{INK4a}$ pozitivita je silně asociována s přítomností dysplastických změn a že silná intenzita reakce společně s posunem pozitivity do povrchových partií epitelu svědčí pro high grade skvamózní intraepiteliální lézi (HSIL). Na tomto podkladě jsme léze s nejistým prekancerózním potenciálem dříve diagnostikované jako atypická nezralá dlaždicobuněčná metaplázie (AIM) reklasifikovali do tří proporcionálních skupin, které biologickou povahou odpovídají HSIL, low grade skvamózní intraepiteliální lézi a nezralé dlaždicové metaplázii. Diagnostická kategorie AIM by proto již v současnosti měla být považována za obsolentní. Dále jsme poukázali na nízký diagnostický přínos imunohistochemického vyšetření CK 17 ve srovnání se stanovením markeru $p16^{INK4a}$.

Ve *třetí části* dizertační práce jsme posuzovali indikační kritéria a terapeuticko-diagnostické algoritmy pro provádění fertilitu šetřících a méně radikálních chirurgických výkonů u pacientek s časným stádiem karcinomu děložního hrdla. Používali jsme techniky peroperační chirurgické detekce a peroperačního histopatologického vyšetření sentinelové lymfatické uzliny (SLN) s následným sériovým prokrajováním a imunohistochemickou analýzou. Prokázali jsme, že riziko

nádorového postižení SLN roste se zvětšujícím se objemem nádorové tkáně a s hloubkou infiltrace stromatu děložního hrdla. Analogicky roste i riziko nádorového postižení parametrií, které se navíc dramaticky zvyšuje při metastatickém postižení SLN. Negativita SLN je proto významným a klinicky relevantním prognostickým faktorem, na jehož podkladě lze snižovat radikalitu chirurgických výkonů užívaných v léčbě časných stádií karcinomu děložního hrdla. Obdobný terapeutický přístup lze po předchozí neoadjuvantní chemoterapii s úspěchem použít i u pacientek s hraniční velikostí karcinomu děložního hrdla, u kterých by primární fertilitu šetřící operace nebo méně radikální chirurgický výkon nebyly dříve indikovány.

7. ABSTRACT

The aim of the presented thesis is to introduce modern procedures of histopathological analysis including immunohistochemical and molecular methods for the diagnosis, prevention and innovative surgical approaches for the therapy of precancerous lesions and malignant tumors of the lower female genital tract (vulva, uterine cervix).

In the *first part* of the thesis, we focused on the prevalence of human papillomavirus (HPV) types in non-precancerous, precancerous and neoplastic squamous cell lesions of the vulva. We aimed at estimating the efficacy of prophylactic HPV vaccination as well as at evaluating the usefulness of the modified classification scheme of vulvar intraepithelial neoplasia (VIN). We analyzed the spectrum of HPV types in particular histological categories of vulvar lesions and confirmed the justification of the concept of two independent pathways of vulvar carcinogenesis - HPV associated and HPV negative. We identified several heterogeneous groups of precancerous vulvar lesions with variable HPV profiles, for which the application of the new revised VIN classifications may be misleading for the estimation of their biological behavior. We also pointed out the fact that the efficacy of the prophylactic HPV vaccination will depend on the extent of cross-protection against the non-vaccine types of HPV, which are more prevalent in the Czech Republic in comparison with other geographic regions.

The *second part* of the thesis was dedicated to the evaluation of the impact of the $p16^{INK4a}$ and CK 17 immunohistochemistry on the differential diagnosis of squamous cell proliferations of the uterine cervix. We identified the characteristic $p16^{INK4a}$ immunoprofiles of different lesions with well defined precancerous potential and confirmed that the diffuse $p16^{INK4a}$ positivity is significantly associated with the presence of dysplastic changes. The strong intensity of the reaction together with the extension of positivity into the superficial layers of the epithelium is indicative for the high grade squamous intraepithelial lesion (HSIL). Based on that, we reclassified lesions with the uncertain precancerous potential previously diagnosed as an atypical immature squamous metaplasia (AIM) into the three proportional groups, which corresponded to HSIL, low grade squamous intraepithelial lesions and immature squamous metaplasia. Thus, the diagnostic category of AIM should be considered as obsolete. Furthermore, we addressed the low diagnostic contribution of CK 17 immunohistochemistry in comparison with $p16^{INK4a}$.

In the *third part* of the thesis we considered the indication criteria and therapeutic-diagnostic algorithms for the fertility sparing and less radical surgery in patients with the early stage of carcinoma of the uterine cervix. We used different techniques of intraoperative surgical detection and frozen section analysis of sentinel lymph nodes (SLN) followed by subsequent serial sectioning and

immunohistochemical analysis. We demonstrated that the risk of the tumor dissemination into the sentinel lymph node increases with the growing volume of the tumor and with the depth of infiltration into the stromal tissue of the uterine cervix. The risk of tumor progression into the parametria grows analogously and it dramatically increases with the presence of metastatic deposits in SLN. Negativity of SLN can therefore be considered as a significant and clinically relevant prognostic factor, which may be exploited for the reduction of the radicality of surgical therapy in early stages of carcinoma of the uterine cervix. Similar therapeutic approach might be used after neoadjuvant chemotherapy also in patients with the borderline sized tumors, in which the primary fertility sparing surgery or less radical therapy were not indicated previously.

8. SEZNAM POUŽITÉ LITERATURY

Altgassen C, Hertel H, Brandstadt A, Kohler C, Durst M a Schneider A (2008) Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO Study Group. *J Clin Oncol* 26:2943-2951.

Arbyn M, Autier P a Ferlay J (2007) Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann Oncol* 18:1423-1425.

Bats AS, Buenerd A, Querleu D, Leblanc E, Darai E, Morice P, Marret H, Gillaizeau F, Mathevet P a Lecuru F (2011) Diagnostic value of intraoperative examination of sentinel lymph node in early cervical cancer: a prospective, multicenter study. *Gynecol Oncol* 123:230-235.

Bernardini M, Barrett J, Seaward G a Covens A (2003) Pregnancy outcomes in patients after radical trachelectomy. *Am J Obstet Gynecol* 189:1378-1382.

Carreon JD, Sherman ME, Guillén D, Solomon D, Herrero R, Jerónimo J, Wacholder S, Rodríguez AC, Morales J, Hutchinson M, Burk RD a Schiffman M (2007) CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. *Int J Gynecol Pathol* 26:441-446.

Cibula D, Abu-Rustum NR, Dusek L, Zikan M, Zaal A, Sevcik L, Kenter GG, Querleu D, Jach R, Bats AS, Dyduch G, Graf P, Klat J, Lacheta J, Meijer CJ, Mery E, Verheijen R a Zweemer RP (2012) Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. *Gynecol Oncol* 124:496-501.

Clifford G, Franceschi S, Diaz M, Munoz N a Villa LL (2006) Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* 24 Suppl 3:S3/26-34.

Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, Colgan T, Chapman W a Shaw P (2002) How important is removal of the parametrium at surgery for carcinoma of the cervix? *Gynecol Oncol* 84:145-149.

Covens A, Shaw P, Murphy J, DePetrillo D, Lickrish G, Laframboise S a Rosen B (1999) Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA-B carcinoma of the cervix? *Cancer* 86:2273-2279.

Crum CP, Egawa K, Fu YS, Lancaster WD, Barron B, Levine RU, Fenoglio CM a Richart RM (1983) Atypical immature metaplasia (AIM). A subset of human papilloma virus infection of the cervix. *Cancer* 51:2214-2219.

Crum CP, Herrington CS, McCluggage WG, Regauer S a Wilkinson EJ (2014) WHO classification of tumours of female reproductive organs. Lyon, IARC.

Cserni G (2004) A model for determining the optimum histology of sentinel lymph nodes in breast cancer. *J Clin Pathol* 57:467-471.

Dargent D, Brun JL, Roy M, Mathevet P a Remy I (1994) La trachélectomie élargie (TE). Une alternative à l'hystérectomie radicale dans le traitement des cancers infiltrants dévellopés sur la face externe du col utérine. *J Obstet Gynecol* 2:292-295.

Dargent D, Martin X, Sacchetoni A a Mathevet P (2000) Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. *Cancer* 88:1877-1882.

De Vincenzo R, Ricci C, Conte C a Scambia G (2013) HPV vaccine cross-protection: Highlights on additional clinical benefit. *Gynecol Oncol* 130:642-651.

Duggan MA, Akbari M a Magliocco AM (2006) Atypical immature cervical metaplasia: immunoprofiling and longitudinal outcome. *Hum Pathol* 37:1473-1481.

Fader AN, Edwards RP, Cost M, Kanbour-Shakir A, Kelley JL, Schwartz B, Sukumvanich P, Comerci J, Sumkin J, Elishaev E a Rohan LC (2008) Sentinel lymph node biopsy in early-stage cervical cancer: utility of intraoperative versus postoperative assessment. *Gynecol Oncol* 111:13-17.

Fanfani F, Ludovisi M, Zannoni GF, Distefano M, Fagotti A, Ceccaroni M, Mancuso S a Scambia G (2004) Frozen section examination of pelvic lymph nodes in endometrial and cervical cancer: accuracy in patients submitted to neoadjuvant treatments. *Gynecol Oncol* 94:779-784.

Fregnani JH, Latorre MR, Novik PR, Lopes A a Soares FA (2006) Assessment of pelvic lymph node micrometastatic disease in stages IB and IIA of carcinoma of the uterine cervix. *Int J Gynecol Cancer* 16:1188-1194.

Frumovitz M, Sun CC, Schover LR, Munsell MF, Jhingran A, Wharton JT, Eifel P, Bevers TB, Levenback CF, Gershenson DM a Bodurka DC (2005) Quality of life and sexual functioning in cervical cancer survivors. *J Clin Oncol* 23:7428-7436.

Hampl M, Sarajuuri H, Wentzensen N, Bender HG a Kueppers V (2006) Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. *Obstet Gynecol* 108:1361-1368.

Hariri J a Oster A (2007) The negative predictive value of p16INK4a to assess the outcome of cervical intraepithelial neoplasia 1 in the uterine cervix. *Int J Gynecol Pathol* 26:223-228.

Hart WR (2001) Vulvar intraepithelial neoplasia: historical aspects and current status. *Int J Gynecol Pathol* 20:16-30.

Hoevenaars BM, van der Avoort IA, de Wilde PC, Massuger LF, Melchers WJ, de Hullu JA a Bulten J (2008) A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell carcinoma. *Int J Cancer* 123:2767-2773.

Holtz DO a Dunton C (2002) Traditional management of invasive cervical cancer. *Obstet Gynecol Clin North Am* 29:645-657.

Horn LC, Hentschel B, Fischer U, Peter D a Bilek K (2008) Detection of micrometastases in pelvic lymph nodes in patients with carcinoma of the cervix uteri using step sectioning: Frequency, topographic distribution and prognostic impact. *Gynecol Oncol* 111:276-281.

Chou CY, Hsu KF, Wang ST, Huang SC, Tzeng CC a Huang KE (1997) Accuracy of three-dimensional ultrasonography in volume estimation of cervical carcinoma. *Gynecol Oncol* 66:89-93.

Iaconis L, Hyjek E, Ellenson LH a Pirog EC (2007) p16 and Ki-67 immunostaining in atypical immature squamous metaplasia of the uterine cervix: correlation with human papillomavirus detection. *Arch Pathol Lab Med* 131:1343-1349.

Ikeda K, Tate G, Suzuki T a Mitsuya T (2008) Coordinate expression of cytokeratin 8 and cytokeratin 17 immunohistochemical staining in cervical intraepithelial neoplasia and cervical squamous cell carcinoma: an immunohistochemical analysis and review of the literature. *Gynecol Oncol* 108:598-602.

Kadar N, Saliba N a Nelson JH (1983) The frequency, causes and prevention of severe urinary dysfunction after radical hysterectomy. *Br J Obstet Gynaecol* 90:858-863.

Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade BJ, Sun D, Duensing S, Sheets EE, Munger K a Crum CP (2001) Ki-67, cyclin E, and p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. *Am J Surg Pathol* 25:884-891.

Kim JH, Park JY, Kim DY, Kim YM, Kim YT a Nam JH (2010) Fertility-sparing laparoscopic radical

trachelectomy for young women with early stage cervical cancer. *Br J Obstet Gynaecol* 117:340-347.

Kinney WK, Hodge DO, Egorshin EV, Ballard DJ a Podratz KC (1995) Identification of a low-risk subset of patients with stage IB invasive squamous cancer of the cervix possibly suited to less radical surgical treatment. *Gynecol Oncol* 57:3-6.

Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, Dallenbach-Hellweg G, Schmidt D a von Knebel Doeberitz M (2001) Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer* 92:276-284.

Kobayashi Y, Akiyama F a Hasumi K (2006) A case of successful pregnancy after treatment of invasive cervical cancer with systemic chemotherapy and conization. *Gynecol Oncol* 100:213-215.

Landoni F, Maneo A, Cormio G, Perego P, Milani R, Caruso O a Mangioni C (2001) Class II versus class III radical hysterectomy in stage IB-IIA cervical cancer: a prospective randomized study. *Gynecol Oncol* 80:3-12.

Landoni F, Parma G, Peiretti M, Zanagnolo V, Sideri M, Colombo N a Maggioni A (2007) Chemo-conization in early cervical cancer. *Gynecol Oncol* 107:S125-126.

Magrina JF, Goodrich MA, Weaver AL a Podratz KC (1995) Modified radical hysterectomy: morbidity and mortality. *Gynecol Oncol* 59:277-282.

Malagon T, Drolet M, Boily MC, Franco EL, Jit M, Brisson J a Brisson M (2012) Cross-protective efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect Dis* 12:781-789.

Maneo A, Chiari S, Bonazzi C a Mangioni C (2008) Neoadjuvant chemotherapy and conservative surgery for stage IB1 cervical cancer. *Gynecol Oncol* 111:438-443.

Marnitz S, Kohler C, Bongardt S, Braig U, Hertel H a Schneider A (2006) Topographic distribution of sentinel lymph nodes in patients with cervical cancer. *Gynecol Oncol* 103:35-44.

Martens JE, Arends J, Van der Linden PJ, De Boer BA a Helmerhorst TJ (2004) Cytokeratin 17 and p63 are markers of the HPV target cell, the cervical stem cell. *Anticancer Res* 24:771-775.

McCluggage WG, Walsh MY, Thornton CM, Hamilton PW, Date A, Caughley LM a Bharucha H (1998) Inter- and intra-observer variation in the histopathological reporting of cervical squamous intraepithelial lesions using a modified Bethesda grading system. *Br J Obstet Gynaecol* 105:206-210.

Moscicki AB, Schiffman M, Kjaer S a Villa LL (2006) Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine* 24 Suppl 3:S42-51.

Munger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, Grace M a Huh K (2004) Mechanisms of human papillomavirus-induced oncogenesis. *J Virol* 78:11451-11460.

Munoz N, Bosch FX, Castellsague X, Diaz M, de Sanjose S, Hammouda D, Shah KV a Meijer CJ (2004) Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer* 111:278-285.

Munoz N, Castellsague X, de Gonzalez AB a Gissmann L (2006) Chapter 1: HPV in the etiology of human cancer. *Vaccine* 24 Suppl 3:S3/1-10.

Negri G, Vittadello F, Romano F, Kasal A, Rivasi F, Girlando S, Mian C a Egarter-Vigl E (2004) p16INK4a expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. *Virchows Arch* 445:616-620.

Nishio H, Fujii T, Kameyama K, Susumu N, Nakamura M, Iwata T a Aoki D (2009) Abdominal radical trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer in a

series of 61 women. *Gynecol Oncol* 115:51-55.

O'Neill CJ a McCluggage WG (2006) p16 expression in the female genital tract and its value in diagnosis. *Adv Anat Pathol* 13:8-15.

Omori M, Hashi A, Nakazawa K, Yuminamochi T, Yamane T, Hirata S, Katoh R a Hoshi K (2007) Estimation of prognoses for cervical intraepithelial neoplasia 2 by p16INK4a immunoexpression and high-risk HPV in situ hybridization signal types. *Am J Clin Pathol* 128:208-217.

Pecorelli S, Zigliani L a Odicino F (2009) Revised FIGO staging for carcinoma of the cervix. *Int J Gynaecol Obstet* 105:107-108.

Plante M, Lau S, Brydon L, Swenerton K, LeBlanc R a Roy M (2006) Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage IB1 cervical cancer: case report. *Gynecol Oncol* 101:367-370.

Plante M, Renaud MC, Francois H a Roy M (2004) Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An updated series of 72 cases and review of the literature. *Gynecol Oncol* 94:614-623.

Plante M, Renaud MC, Hoskins IA a Roy M (2005) Vaginal radical trachelectomy: a valuable fertility-preserving option in the management of early-stage cervical cancer. A series of 50 pregnancies and review of the literature. *Gynecol Oncol* 98:3-10.

Plante M, Renaud MC, Tetu B, Harel F a Roy M (2003) Laparoscopic sentinel node mapping in early-stage cervical cancer. *Gynecol Oncol* 91:494-503.

Plante M a Roy M (2001) New approaches in the surgical management of early stage cervical cancer. *Curr Opin Obstet Gynecol* 13:41-46.

Querleu D a Morrow CP (2008) Classification of radical hysterectomy. *Lancet Oncol* 9:297-303.

Regauer S a Reich O (2007) CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). *Histopathology* 50:629-635.

Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D a Hrehorcak M (2005) Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. *Gynecol Oncol* 98:281-288.

Rosenthal AN, Ryan A, Hopster D a Jacobs IJ (2002) Molecular evidence of a common clonal origin and subsequent divergent clonal evolution in vulval intraepithelial neoplasia, vulval squamous cell carcinoma and lymph node metastases. *Int J Cancer* 99:549-554.

Sahdev A, Jones J, Shepherd JH a Reznek RH (2005) MR imaging appearances of the female pelvis after trachelectomy. *Radiographics* 25:41-52.

Sano T, Oyama T, Kashiwabara K, Fukuda T a Nakajima T (1998) Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 153:1741-1748.

Santos M, Montagut C, Mellado B, Garcia A, Ramon y Cajal S, Cardesa A, Puig-Tintore LM a Ordi J (2004) Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. *Int J Gynecol Pathol* 23:206-214.

Scurry J (1999) Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. *Int J Gynecol Cancer* 9:89-97.

Shepherd JH, Mould T a Oram DH (2001) Radical trachelectomy in early stage carcinoma of the

cervix: outcome as judged by recurrence and fertility rates. *Bjog* 108:882-885.

Schlaerth JB, Spirtos NM a Schlaerth AC (2003) Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. *Am J Obstet Gynecol* 188:29-34.

Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, Haefner H a Neill S (2005) Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med* 50:807-810.

Slama J, Dundr P, Dusek L a Cibula D (2013) High false negative rate of frozen section examination of sentinel lymph nodes in patients with cervical cancer. *Gynecol Oncol* 129:384-388.

Smedts F, Ramaekers F, Troyanovsky S, Pruszczynski M, Robben H, Lane B, Leigh I, Plantema F a Vooijs P (1992) Basal-cell keratins in cervical reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. *Am J Pathol* 140:601-612.

Smith JR, Boyle DC, Corless DJ, Ungar L, Lawson AD, Del Priore G, McCall JM, Lindsay I a Bridges JE (1997) Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. *Br J Obstet Gynaecol* 104:1196-1200.

Snijders PJ, Steenbergen RD, Heideman DA a Meijer CJ (2006) HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J Pathol* 208:152-164.

Sobin LH, Gospodarowicz MK a Wittekind C (2009) TNM classification of malignant tumours. Chichester, John Wiley & Sons Ltd.

Sood AK, Nygaard I, Shahin MS, Sorosky JI, Lutgendorf SK a Rao SS (2002) Anorectal dysfunction after surgical treatment for cervical cancer. *J Am Coll Surg* 195:513-519.

Srodon M, Stoler MH, Baber GB a Kurman RJ (2006) The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 30:1513-1518.

Srodon M, Stoler MH, Baber GB a Kurman RJ (2006) The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 30:1513-1518.

Stanley M, Lowy DR a Frazer I (2006) Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. *Vaccine* 24 Suppl 3:S3/106-113.

Stegeman M, Louwen M, van der Velden J, ten Kate FJ, den Bakker MA, Burger CW a Ansink AC (2007) The incidence of parametrial tumor involvement in select patients with early cervix cancer is too low to justify parametrectomy. *Gynecol Oncol* 105:475-480.

Stoler MH a Schiffman M (2001) Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. *JAMA* 285:1500-1505.

Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW a Shah KV (1991) Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol* 10:107-125.

van Beurden M, ten Kate FW, Tjong AHSP, de Craen AJ, van der Vange N, Lammes FB a ter Schegget J (1998) Human papillomavirus DNA in multicentric vulvar intraepithelial neoplasia. *Int J Gynecol Pathol* 17:12-16.

van der Avoort IA, Shirango H, Hoevenaars BM, Grefte JM, de Hullu JA, de Wilde PC, Bulten J, Melchers WJ a Massuger LF (2006) Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. *Int J Gynecol Pathol* 25:22-29.

Vandepapeliere P, Barrasso R, Meijer CJ, Walboomers JM, Wettendorff M, Stanberry LR a Lacey CJ (2005) Randomised controlled trial of an adjuvanted human papillomavirus (HPV) type 6 L2E7 vaccine: infection of external anogenital warts with multiple HPV types and failure of therapeutic vaccination. *J Infect Dis* 192:2099-2107.

Verdenius I, Groner JA a Harper DM (2013) Cross protection against HPV might prevent type replacement. *Lancet Infect Dis* 13:195.

Vizcaino AP, Moreno V, Bosch FX, Munoz N, Barros-Dios XM a Parkin DM (1998) International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. *Int J Cancer* 75:536-545.

Wagenaar HC, Trimbos JB, Postema S, Anastasopoulou A, van der Geest RJ, Reiber JH, Kenter GG, Peters AA a Pattynama PM (2001) Tumor diameter and volume assessed by magnetic resonance imaging in the prediction of outcome for invasive cervical cancer. *Gynecol Oncol* 82:474-482.

Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS a Wallin KL (2004) Predictive significance of the alterations of p16INK4A, p14ARF, p53, and proliferating cell nuclear antigen expression in the progression of cervical cancer. *Clin Cancer Res* 10:2407-2414.

Watson M, Saraiya M, Benard V, Coughlin SS, Flowers L, Cokkinides V, Schwenn M, Huang Y a Giuliano A (2008) Burden of cervical cancer in the United States, 1998-2003. *Cancer* 113:2855-2864.

Wentzensen N, Vinokurova S a von Knebel Doeberitz M (2004) Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res* 64:3878-3884.

Wilkinson EJ a Teixeira MR (2003) Pathology and genetics of tumours of the breast and female genital organs. Lyon, IARC Press.

Wright JD, Grigsby PW, Brooks R, Powell MA, Gibb RK, Gao F, Rader JS a Mutch DG (2007) Utility of parametrectomy for early stage cervical cancer treated with radical hysterectomy. *Cancer* 110:1281-1286.

Wright TC, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ a Solomon D (2007) 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *Am J Obstet Gynecol* 197:340-345.

Zuna RE, Allen RA, Moore WE, Mattu R a Dunn ST (2004) Comparison of human papillomavirus genotypes in high-grade squamous intraepithelial lesions and invasive cervical carcinoma: evidence for differences in biologic potential of precursor lesions. *Mod Pathol* 17:1314-1322.

9. SEZNAM POUŽITÝCH ZKRATEK

AIM	atypical immature squamous metaplasia *(atypická nezralá dlaždicová metaplázie)*
ART	abdominální radikální trachelektomie
CIN	cervikální intraepiteliální neoplázie
CK 17	cytokeratin 17
CoA	condyloma acuminatum
d-VIN	vulvární intraepiteliální neoplázie diferencovaného typu
DR	detection rate *(úspěšnost detekce SLN)*
HE	hematoxylin-eozin
HPV	human papillomavirus *(lidský papilomavirus)*
HR HPV	high risk human papillomavirus *(vysoce rizikový typ lidského papilomaviru)*
HSIL	high grade squamous intraepithelial lesion *(high grade skvamózní intraepiteliální léze)*
ISSVD	International Society for the Study of Vulvovaginal Disease *(Mezinárodní společnost pro studium vulvovaginálních chorob)*
ITC	isolated tumor cells *(izolované nádorové buňky)*
LFGT	lower female genital tract *(dolní ženský pohlavní systém)*
LR HPV	low risk human papillomavirus *(nízce rizikový typ lidského papilomaviru)*
LS	lichen sclerosus
LSC	lichen simplex chronicus
LSIL	low grade squamous intraepithelial lesion *(low grade skvamózní intraepiteliální léze)*
NAC	neoadjuvant chemotherapy *(neoadjuvantní chemoterapie)*
PPP	patologické postižení parametrií
SCC	squamous cell carcinoma *(dlaždicobuněčný karcinom)*
SIL	squamous intraepithelial lesion *(skvamózní intraepiteliální léze)*
SLN	sentinel lymph node *(sentinelová lymfatická uzlina)*
SSDR	side specific detection rate *(stranově specifická úspěšnost detekce SLN)*
u-VIN	vulvární intraepiteliální neoplázie obvyklého typu
VaIN	vaginální intraepiteliální neoplázie

VIN	vulvární intraepiteliální neoplázie
VLP	virus-like particle *(částice připomínající virus)*
VRT	vaginální radikální trachelektomie
WHO	World Health Organization (*Světová zdravotnická organizace*)

10. SEZNAM PUBLIKACÍ AUTORA

10.1. Publikace se vztahem k tématu dizertační práce v časopisech s IF

1. Robová H., Halaška M., Pluta M., Škapa P., Matěcha J., Lisý J., Rob L. (2014) Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer. ***Gynecol Oncol*** (v tisku)
   IF 3,687
2. Škapa P., Robová H., Rob L. a Zámečník J. (2013) $p16^{INK4a}$ immunoprofiles of squamous lesions of the uterine cervix – implications for the reclassification of atypical immature squamous metaplasia. ***Pathol Oncol Res*** 19:707-714.
   IF 1,555
3. Rob L., Robová H., Chmel R., Komár M., Halaška M. a Škapa P. (2012) Surgical options in early cervical cancer. ***Int J Hyperthermia*** 28:489-500.
   IF 1,923
4. Rob L., Škapa P. a Robová H. (2011) Fertility-sparing surgery in patients with cervical cancer. ***Lancet Oncol*** 12:192-200.
   IF 14,470
5. Tachezy R., Šmahelová J., Saláková M., Arbyn M., Rob L., Škapa P., Jirásek T. a Hamšíková E. (2011) Human papillomavirus genotype distribution in Czech women and men with diseases etiologically linked to HPV. ***PLoS One*** 6:e21913.
   IF 4,411
6. Rob L., Pluta M., Škapa P. a Robová H. (2010) Advances in fertility-sparing surgery for cervical cancer. ***Expert Rev Anticancer Ther*** 10:1101-1114.
   IF 2,493
7. Pluta M., Rob L., Charvát M., Chmel R., Halaška M., Škapa P. a Robová H. (2009) Less radical surgery than radical hysterectomy in early stage cervical cancer: A pilot study. ***Gynecol Oncol*** 113:181-184.
   IF 2,614
8. Rob L., Pluta M., Strand P., Hrehorčák M., Chmel R., Škapa P. a Robová H. (2008) A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. ***Gynecol Oncol*** 111:S116-120.
   IF 2,614

9. Robová H., Pluta M., Hrehorčák M., Škapa P. a Rob L. (2008) High-dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. ***Int J Gynecol Cancer*** 18:1367-1371.
   IF 1,425
10. Strnad P., Robová H., Škapa P., Pluta M., Hrehorčák M., Halaška M. a Rob L. (2008) A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. ***Gynecol Oncol*** 109:280-284.
    IF 2,319
11. Škapa P., Zámečník J., Hamšíková E., Saláková M., Šmahelová J., Jandová K., Robová H., Rob L. a Tachezy R. (2007) Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. ***Am J Surg Pathol*** 31:1834-1843.
    IF 4,144
12. Rob L., Charvát M., Robová H., Pluta M., Strnad P., Hrehorčák M. a Škapa P. (2007) Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. ***Int J Gynecol Cancer*** 17:304-310.
    IF 1,427

10.2. Publikace se vztahem k tématu dizertační práce v časopisech bez IF

1. Škapa P., Pichlík T., Pluta M., Halaška M., Robová H., Rob L., Tachezy R., Zámečník J. (2014) Klasifikace vulvárních prekanceróz pohledem patologa. ***Actual Gyn*** 6:26-32.
2. Škapa P., Robová H., Rob L. a Zámečník J. (2012) Prekancerózní léze vulvy. ***Cesk Patol*** 48:15-21.

10.3. Publikace bez vztahu k tématu dizertační práce v časopisech s IF

1. Robová H., Rob L., Halaška M., Pluta M., Škapa P., Strnad P., Lisý J., Komár M. (2013) High-dose density neoadjuvant chemotherapy in bulky IB cervical cancer. ***Gynecol Oncol*** 128:49-53.
   IF 3,888
2. Robová H., Rob L., Halaška M., Pluta M., Škapa P. (2013) Current status of sentinel lymph node mapping in the management of endometrial cancer. ***Expert Rev Anticancer Ther*** 13:55-61.
   IF 2,652

3. Vasovčák P., Křepelová A., Menigatti M, Puchmajerová A., Škapa P, Augustiňáková A., Amann G., Wernstedt A., Jiřičný J., Marra G., Wimmer K. (2012) Unique mutational profile associated with a loss of TDG expression in the rectal cancer of a patient with a constitutional PMS2 deficiency. ***DNA Repair*** 11:616-623.
   IF 4,293
4. Vasovčák P., Pavlíková K., Sedláček Z., Škapa P., Kouda M., Hoch J., Křepelová A. (2011) Molecular genetic analysis of 103 sporadic colorectal tumours in Czech patients. ***PloS One*** 6:e24114.
   IF 4,411
5. Robová H., Halaška M., Pluta M., Škapa P., Strnad P., Lisý J., Rob L. (2010) The role of neoadjuvant chemotherapy and surgery in cervical cancer. ***Int J Gynecol Cancer*** 20:S42-46.
   IF 2,179
6. Robová H., Charvát M., Strnad P., Hrehorčák M., Táborská K., Škapa P., Rob L. (2009) Lymphatic mapping in endometrial cancer: comparison of hysteroscopic and subserosal injection and the distribution of sentinel lymph nodes. ***Int J Gynecol Cancer*** 19:391-394.
   IF 1,425
7. Hyršl L., Závada J., Závadová Z., Kawaciuk I., Veselý S., Škapa P. (2009) Soluble form of carbonic anhydrase IX (CAIX) in transitional cell carcinoma of urinary tract. ***Neoplasma*** 56:298-302.
   IF 1,179
8. Hájek P., Alan D., Vejvoda J., Linhartová K., Škapa P., Hajsmannová Z., Veselka J. (2009) Treatment of a large left main coronary artery thrombus by aspiration thrombectomy. ***J Thromb Thrombolysis*** 27:352-354.
   IF 1,432
9. Škapa P., Hyršl L., Závada J., Soukup J., Zámečník J. (2008) Carbonic anhydrase IX expression in clear cell renal cell carcinomas negatively correlates with the proportion of the granular cell component. ***J Clin Oncol*** 26:3809-3811.
   IF 15,484
10. Rob L., Robová H., Pluta M., Strnad P., Kačírek J., Škapa P., Táborská K. (2007) Further data on sentinel lymph node mapping in vulvar cancer by blue dye and radiocolloid Tc99. ***Int J Gynecol Cancer*** 17:147-153.
    IF 1,427

10.4. Publikace bez vztahu k tématu dizertační práce v časopisech bez IF

1. Rob L., Robová H., Chmel R., Škapa P. (2012) Gynekologické prekancerózy z pohledu klinika dnes a zítra. ***Cesk Patol*** 48:9-14.
2. Strnad P., Rob L., Škapa P., Stankušová H., Michalová K., Chod J. (2008) Klinický význam mikrometastáz karcinomu prsu v sentinelové uzlině. ***Ceska Gynekol*** 73:360-364.
3. Robová H., Rob L., Pluta M., Kačírek J., Slavík V., Škapa P., Hamšíková E., Tachezy R. (2007) Progrese a regrese low grade intraepiteliálních skvamózních neoplazií v závislosti na přítomnosti HPV HR virů. ***Ceska Gynekol*** 72:347-350.
4. Rob L., Robová H., Pluta M., Strnad P., Kačírek J., Chmel R., Teslík L., Škapa P., Táborská K. (2006) Detekce sentinelových uzlin u karcinomu vulvy – metody a technika. ***Ceska Gynekol*** 71:298-301.
5. Rob L., Charvát M., Robová H., Pluta M., Strnad P., Hrehorčák M., Kačírek J., Chmel R., Škapa P., Táborská K. (2006) Fertilitu zachovávající operace u časného stadia karcinomu děložního hrdla dnes a zítra. ***Ceska Gynekol*** 71:302-307.
6. Umlaufová D., Škapa P., Hoch J., Prausová J. (2009) Incidence a prognostický význam mezorektálních extranodálních nádorových depozit u pacientů s karcinomem rekta po neoadjuvantní terapii. ***Rozhl Chir*** 88:326-329.
7. Klapková E., Průša R., Kukačka J., Kotaška K., Škapa P. (2007) Unexpected high serum level of CA 125 in male patient suffering from metastatic papillary carcinoma: a case report. ***Klin Biochem Metab*** 15:155–156.
8. Hoch J., Škapa P., Jech Z. (2005) The role of surgery for colorectal cancer in the era of effective neoadjuvant radiochemotherapy. ***Proktologia*** 6 Suppl 1:14-17.

11. PŘÍLOHY

Příloha I. Škapa P., Zámečník J., Hamšíková E., Saláková M., Šmahelová J., Jandová K., Robová H., Rob L. a Tachezy R. (2007) Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. ***Am J Surg Pathol*** 31:1834-1843.
IF 4,144

Příloha II. Tachezy R., Šmahelová J., Saláková M., Arbyn M., Rob L., Škapa P., Jirásek T. a Hamšíková E. (2011) Human papillomavirus genotype distribution in Czech women and men with diseases etiologically linked to HPV. ***PLoS One*** 6:e21913.
IF 4,411

Příloha III. Škapa P., Robová H., Rob L. a Zámečník J. (2012) Prekancerózní léze vulvy. ***Cesk Patol*** 48:15-21.

Příloha IV. Škapa P., Pichlík T., Pluta M., Halaška M., Robová H., Rob L., Tachezy R., Zámečník J. (2014) Klasifikace vulvárních prekanceróz pohledem patologa. ***Actual Gyn*** 6:26-32.

Příloha V. Škapa P., Robová H., Rob L. a Zámečník J. (2013) $p16^{INK4a}$ immunoprofiles of squamous lesions of the uterine cervix – implications for the reclassification of atypical immature squamous metaplasia. ***Pathol Oncol Res*** 19:707-714.
IF 1,555

Příloha VI. Rob L., Charvát M., Robová H., Pluta M., Strnad P., Hrehorčák M. a Škapa P. (2007) Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. ***Int J Gynecol Cancer*** 17:304-310.
IF 1,427

Příloha VII. Rob L., Pluta M., Strand P., Hrehorčák M., Chmel R., Škapa P. a Robová H. (2008) A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. ***Gynecol Oncol*** 111:S116-120.
IF 2,614

Příloha VIII. Strnad P., Robová H., Škapa P., Pluta M., Hrehorčák M., Halaška M. a Rob L. (2008) A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. ***Gynecol Oncol*** 109:280-284.
IF 2,319

Příloha IX. Robová H., Pluta M., Hrehorčák M., Škapa P. a Rob L. (2008) High-dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. ***Int J Gynecol Cancer*** 18:1367-1371.
IF 1,425

Příloha X. Pluta M., Rob L., Charvát M., Chmel R., Halaška M., Škapa P. a Robová H. (2009) Less radical surgery than radical hysterectomy in early stage cervical cancer: A pilot study. ***Gynecol Oncol*** 113:181-184.
IF 2,614

Příloha XI. Rob L., Pluta M., Škapa P. a Robová H. (2010) Advances in fertility-sparing surgery for cervical cancer. ***Expert Rev Anticancer Ther*** 10:1101-1114.
IF 2,493

Příloha XII. Rob L., Škapa P. a Robová H. (2011) Fertility-sparing surgery in patients with cervical cancer. ***Lancet Oncol*** 12:192-200.
IF 14,470

Příloha XIII. Rob L., Robová H., Chmel R., Komár M., Halaška M. a Škapa P. (2012) Surgical options in early cervical cancer. ***Int J Hyperthermia*** 28:489-500.
IF 1,923

Příloha XIV. Robová H., Halaška M., Pluta M., Škapa P., Matěcha J., Lisý J., Rob L. (2014) Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer. ***Gynecol Oncol*** (v tisku)
IF 3,687

Příloha I.

Škapa P., Zámečník J., Hamšíková E., Saláková M., Šmahelová J., Jandová K., Robová H., Rob L. a Tachezy R. (2007) Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. ***Am J Surg Pathol*** 31:1834-1843.
IF 4,144

Human Papillomavirus (HPV) Profiles of Vulvar Lesions

Possible Implications for the Classification of Vulvar Squamous Cell Carcinoma Precursors and for the Efficacy of Prophylactic HPV Vaccination

Petr Skapa, MD, Josef Zamecnik, MD, PhD,* Eva Hamsikova, MSc,† Martina Salakova, MSc, PhD,† Jana Smahelova, MSc,† Kristyna Jandova, MD,‡ Helena Robova, MD,‡ Lukas Rob, MD, PhD,‡ and Ruth Tachezy, MSc, PhD†*

Abstract: The term vulvar intraepithelial neoplasia (VIN) introduced in 1986 incorporates 3 grades of usual VIN (u-VIN I-III) and the differentiated VIN (d-VIN). Although u-VIN is etiologically associated with the human papillomavirus (HPV) infection, d-VIN represents an alternative HPV negative pathway of vulvar carcinogenesis. In 2004, the u-VIN I category was abandoned and u-VIN II and III were merged. Further, an alternative Bethesda-like terminology scheme presenting the term vulvar intraepithelial lesion was proposed recently. To analyze the impact of HPV profiles of vulvar precancerous lesions for their classification and to assess the presumable efficacy of the prophylactic HPV vaccination, 269 vulvar excisions representing lichen sclerosus, lichen simplex chronicus, condylomata acuminata, d-VIN, all grades of u-VIN and squamous cell carcinomas were subjected to the HPV typing by use of GP5+/6+ polymerase chain reaction and reverse line blot hybridization. The results showed different HPV profiles, and also differing frequency of multiple-type HPV infection and the age structure in patients with u-VIN II and III. The biologic heterogeneity within the u-VIN II category was also demonstrated. u-VIN I was distinguished as a rare disorder associated with high-risk HPV infection. We conclude that the original VIN terminology proposed in 1986 seems to be appropriate for the classification of vulvar squamous dysplastic lesions. The spectrum of HPV types found in vulvar squamous cell carcinomas indicates that the efficacy of HPV vaccination in preventing vulvar cancer might be diminished in the studied population, because the recently developed prophylactic vaccines are targeted against a limited number of HPV types.

From the Departments of *Pathology and Molecular Medicine; ‡Obstetrics and Gynecology, Charles University, 2nd Medical School, Faculty Hospital Motol; and †Department of Experimental Virology, National Reference Laboratory for Papillomaviruses, Institute of Hematology and Blood Transfusion, Prague, Czech Republic.

Supported by the grant NR/8852-3 from the Ministry of Health of the Czech Republic and by the research project VZ FNM 00000064203.

Reprints: Petr Skapa, MD, Department of Pathology and Molecular Medicine, Charles University, 2nd Medical School, Faculty Hospital Motol, V Uvalu 84, 150 06 Prague 5, Czech Republic (e-mail: petr.skapa@lfmotol.cuni.cz).

Key Words: vulvar diseases, intraepithelial neoplasms, papillomavirus infections, classification, HPV vaccines

(*Am J Surg Pathol* 2007;31:1834–1843)

Squamous precancerous lesions and invasive squamous cell carcinomas (SCCs) of the lower female genital tract are etiologically linked to human papillomavirus (HPV) infection and appertain to the complex of the multicentric lower genital tract squamous neoplasia.[24] Unlike in uterine cervix, the majority of vulvar SCCs are HPV DNA negative[15,21,22,26] with TP53 gene mutations emerging in the environment of vulvar dermatoses such as lichen sclerosus (LS) and lichen simplex chronicus (LSC) as the probable main etiologic factor.[14]

On the basis of histologic features, vulvar intraepithelial neoplasia (VIN) has been subdivided into the usual (u-VIN) and differentiated (d-VIN) types.[5] u-VIN is associated with HPV and occurs preferentially in young women at reproductive age. It has been further assorted into warty, basaloid, and mixed subtypes and subclassified into 3 grades (u-VIN I, II, and III). d-VIN affects mainly postmenopausal women and behaves as a carcinoma in situ with a considerable risk of progression into invasive, mostly well differentiated and keratinizing HPV negative SCC.[1]

Primarily because of both the low interobserver and intraobserver reproducibility of the u-VIN I category[11] and the doubts about its precancerous potential, the classification proposed by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 1986,[29] and subsequently adopted by World Health Organization,[30] was modified in 2004.[16] Further, an alternative Bethesda-like terminology scheme introducing the term vulvar intraepithelial lesion (VIL) was proposed in 2005[7] (summarized in Table 1). To contribute to the current discussion concerning the validity of the newly introduced classifications of vulvar squamous precancerous lesions, we correlated histopathologic and virologic features of vulvar non-neoplastic [LS, LSC, condylomata acuminata (CoA)], precancerous (VIN) and neoplastic (SCC) lesions

in a larger series of cases. The presented HPV profiles of particular vulvar lesions might be of value for the estimation of the efficacy of HPV immunization programs, because the recently developed prophylactic vaccines are targeted against a limited spectrum of HPV types.[19]

MATERIALS AND METHODS

Histopathologic Analysis and Case Selection

Patients enrolled into this study were surgically treated for vulvar LS, LSC, CoA, VIN, and SCC in the Faculty Hospital Motol, Prague, Czech Republic in the period from 2000 to 2004. All specimens were taken by the laser skinning vulvectomy or cold knife excision, fixed in 10% buffered formalin immediately after removal, embedded in paraffin and processed according to the standard histopathologic procedures with the hematoxylin-eosin staining.

Particular categories of lesions (LS, LSC, CoA, VIN, and SCC) were defined according to the criteria described in detail in Table 2 and exemplified in Figure 1. Every single vulvar excision obtained from a particular patient during the course of the study and also all u-VIN lesions of different grades detected in synchronous multifocal lesions were included. The total of 269 different vulvar lesions from 172 patients were finally enrolled into the study. The spectrum and counts of particular lesions are summarized in Table 3.

Representative paraffin blocks selected for HPV DNA detection were cut according to the following procedure: The first and the fourth sections (both 4-μm thick) were histopathologically processed and stained with hematoxylin-eosin for the evaluation of a sufficient area of the assessed lesion (> 10% of the whole tissue surface). The second and the third section (each of 20-μm thick) were transferred into the sterile Eppendorf tube for the molecular analysis. The cross-contamination of specimens by viral DNA during the cutting was prevented by cleaning the knife of the microtome with the ethanol-impregnated swab. For the detection of a potential contamination, an empty paraffin block was used as a negative control in each series.

DNA Extraction

Paraffin was removed with xylene and DNA was extracted by incubation with proteinase K (Sigma, St Louis, MO) at final concentration 200 μg/mL in lysis buffer (50 mM Tris-HCl, pH 8; 5 mM EDTA, pH 8; 1% Tween 20) for 2 hours at 55°C. Proteinase K was inactivated at 95°C for 10 minutes and all samples were consequently phenol/chloroform-extracted and stored at −20°C. For every 9 samples, 1 negative control was included in the process of DNA preparation (lysis buffer with proteinase K and no paraffin).

Polymerase Chain Reaction of the Control Gene

A fragment of the human β-globin gene was amplified with primers PC 03 (5′ACACAACTGTGT

TABLE 1. The Comparative Table Between the ISSVD 1986 Classification, ISSVD 2004 Modified Scheme, and Bethesda-like Terminology (2005)

ISSVD 1986		ISSVD 2004		Bethesda-like (2005)	
Histologic type	Grade	Histologic type	Grade	Histologic type	VIL terminology
-	-	-	-	Exophytic condyloma, Classic flat condyloma	Low-grade VIL
VIN, usual type	I	-	-	Non-classic flat condyloma (discrete epithelial hyperplasia, u-VIN I)	
	II	VIN, usual type	NA	VIN, usual type	High-grade VIL
	III				
VIN, differentiated type	III	VIN, differentiated type	NA	VIN, differentiated type	
-	-	-	-	LS with acanthosis, Verruciform LSC Vulvar acanthosis with altered differentiation	Vulvar intraepithelial alterations not classified as VIL but deserving follow-up

NA indicates not applicable.

TABLE 2. Histopathologic Features of Vulvar Lesions Analyzed in the Study

Category		Histopathologic criteria*
Lichen sclerosus (LS)		- Epidermal atrophy with hyperkeratosis - Edema and hyalinization of the papillary dermis - Lymphocytic infiltrate in the superficial corium
Lichen simplex chronicus (LSC)		- Epidermal hyperplasia with elongation of rete ridges - Acanthosis, hypergranulosis and hyperkeratosis - Fibrosis of the papillary dermis with lymphocytic infiltrate - Hyalinization of the superficial corium in case of LSC superimposed on LS
Condyloma acuminatum (CoA)		- Verruciform architecture with dense fibrovascular core - Acanthosis, hyperkeratosis and parakeratosis - Koilocytosis without cytological atypia
Vulvar intraepithelial neoplasia of the usual type (u-VIN)	Warty	- Condylomatous growth pattern - Acanthosis, hyperkeratosis and parakeratosis - Signs of HPV infection (koilocytotic atypia and multinucleation)
	Basaloid	- Flat lesion lacking hyperkeratosis and parakeratosis - Small dysplastic keratinocytes resembling epidermal basal cells without manifestations of HPV cytopathic effect
	Mixed	- Lesion with overlap or mixed warty/basaloid features
Vulvar intraepithelial neoplasia of the differentiated type (d-VIN)		- Acanthosis with elongation of rete ridges and parakeratosis - Atypical cells confined to the basal parts of epidermis with mitotic activity and aberrant keratinization - Suprabasilar areas composed of abnormally differentiated keratinocytes with an abundant eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli - Absence of signs of HPV infection
Squamous cell carcinoma (SCC)		- Commonly used histopathological criteria (malignant tumor with squamous differentiation, cellular atpia and invasive growth) - All common subtypes of the vulvar SCC (keratinizing, warty, basaloid and verrucous carcinomas) were included

* ISSVD 1986[29] and World Health Organization terminology[30] were used for the classification and grading of VIN and SCC, respectively.

TCACTAGC 3′) and PC 04 (5′CAACTTCATCCACG TTCACC 3′).[13] Positive β-globin amplification proved that the sample contained enough DNA and that no polymerase chain reaction (PCR) inhibitors were present. Fifty microliters of the reaction mixture contained 1 × concentrated reaction buffer (Fermentas, Vilnius, Lithuania), 4.0 mmol/L of $MgCl_2$, 0.2 mmol of dNTPs, 0.05 pmol of each primer (PC 03 and PC 04), and 2.5 U Taq-polymerase (Fermentas). After initial denaturation for 5 minutes at 95°C, each of the 40 cycles consisted of denaturation for 1 minute at 95°C, primer annealing for 2 minutes at 55°C, and chain elongation for 2 minutes at 72°C. Finally, there was an extra incubation for 3 minutes at 72°C.

HPV Detection and Typing by GP5+/GP6+BIO Reverse Line Blot Hybridization

The presence and typing of the HPV DNA in samples were determined using the reverse line blot hybridization (RLB).[25] This method enables the detection and typing of 37 different HPV types in a single assay. The HPV detection was performed in a PCR thermocycler PTC 200 (MJ Research, Inc, Waltham, MA) by the PCR with primer GP5+ and 5′-end biotin labeled GP6+ primer, which amplified the 150 bp long fragment of L1 gene. The PCR was performed for 40 cycles and the biotinylated PCR product was hybridized with the oligonucleotide probes labeled with the 5′-terminal amino group. These probes were covalently linked to an activated negatively charged Biodyne C membrane. After washing, the membrane was incubated for 60 minutes at 42°C with peroxidase-labeled streptavidin conjugate. For the chemiluminescent detection of hybridizing DNA, the membrane was incubated in ECL detection liquid (Amersham Biosciences, Uppsala, Sweden) and exposed to LumiFilm (Roche, Indianapolis, IN) for 5 minutes.

HPV Typing by a Nucleotide DNA Sequencing

Samples that did not hybridize on the RLB but revealed a clear band on the agarose gel were subjected to

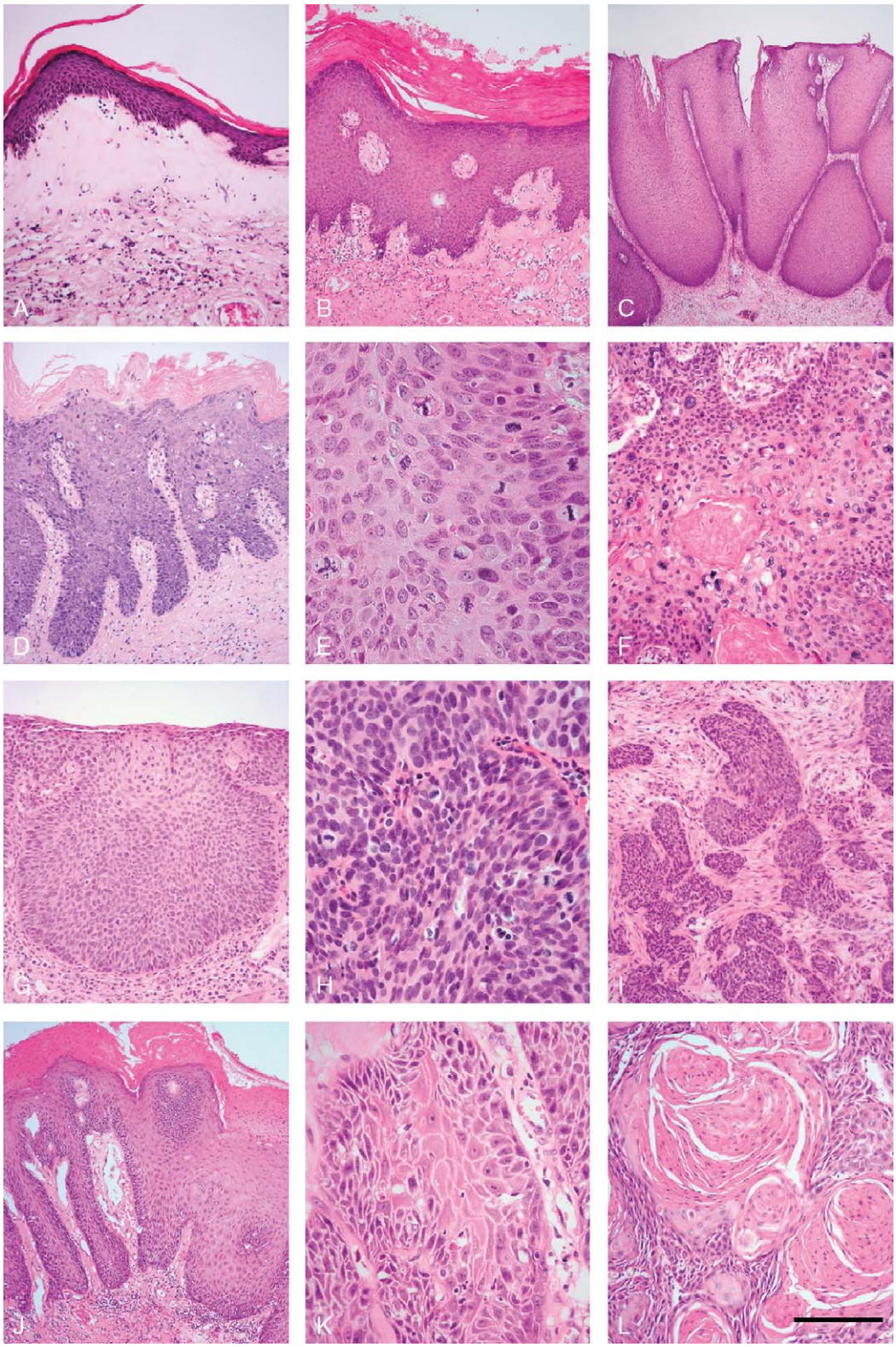


FIGURE 1. Panel exemplifying histologic features of vulvar lesions included in the study. For the detailed description see Table 2. A, LS; B, LSC; C, CoA; D and E, u-VIN, warty subtype, grade III; F, warty SCC; G and H, u-VIN, basaloid subtype, grade III; I, basaloid SCC; J and K, d-VIN; L, keratinizing SCC; hematoxylin-eosin stain; scale bar: 500 μm (B, C, J); 200 μm (A, D, G); 100 μm (F, I, L); and 50 μm (E, H, K).

TABLE 3. Data Characterizing Patients, Lesions and Their HPV Profiles

Type of lesion			Number of patients		Number of lesions		Median age (years)		HPV positive (%)		Prevalence of HPV categories detected in HPV positive lesions (%)							
											LR-HPV		HR-HPV		pHR-HPV		UR-HPV	
LS			32		35		62.0		-		-		-		-		-	
LSC			12		14		63.0		7.1		-		100.0		-		-	
CoA			55		57		27.0		94.7		96.3		11.1		9.3		1.9	
VIN	u-VIN	I	4	46	4	82	44.5	53.0	100.0	98.8	-	4.9	100.0	97.6	-	-	-	-
		II	9		12		41.5		100.0		33.3		91.7		-		-	
		III	33		66		56.0		98.5		-		100.0		-		-	
	d-VIN		6		12		79.0		8.3		-		100.0		-		-	
	SCC		46		69		75.0		42.0		6.9		93.1		-		-	

the DNA nucleotide sequencing to determine the exact HPV type. Fifty microliters of the PCR product were cut out from the 2% agarose gel (NuSieve GTG agarose, FMC BioProducts, Rockland, ME), purified using a Lego Kit (Top-Bio, Prague, Czech Republic) and sequenced with the BigDye Terminator Primer Cycle Sequencing kit (Applied Biosystems, Foster City, CA). The analysis was carried out using the automatic ABI PRISM 310 sequencing system (Applied Biosystems) and the sequences were analyzed by Chromas software and evaluated by BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

Epidemiologic Classification of Detected HPV Types

Detected HPV types were stratified into low-risk (LR), high-risk (HR), probably high-risk (pHR), and undetermined-risk (UR) groups according to the recent classification proposed by Munoz et al[9] (Table 4).

Statistical Analysis

Statistical calculations were performed using InStat 3.06 statistical software (GraphPad Software, San Diego, CA). The median age of particular groups of patients were compared by unpaired *t* test with Welch correction. Two-tail P value < 0.05 was considered significant.

RESULTS

Data describing the numerical distribution and HPV profiles of the studied lesions are shown in Table 3. u-VIN represented 87.2% and d-VIN 12.8% of all VIN lesions. The coincidence of both these disorders in a single patient was not detected in our series. In all, 24.2% of u-VIN and 83.3% of d-VIN were associated with a preceding, simultaneous or subsequent vulvar SCC.

HPV Profiles of Vulvar Lesions

Fifteen HPV types from the 37 investigated ones were identified in our specimens by the virologic analysis and their frequency in HPV positive lesions is given in Table 5 and Figure 2. High prevalence of HPV DNA was found in specimens of u-VIN (98.8%) and CoA (94.7%) being significantly lower in SCC (42.0%). LR-HPV types were identified in 96.3% of HPV positive CoA, whereas 97.6% of HPV positive u-VIN and 93.1% of HPV positive SCC harbored HR-HPV types. The spectrum of detected HPV types was broader in CoA and u-VIN II than in u-VIN I, u-VIN III, and SCC. The most frequently detected HPV types in particular HPV groups were HPV 16, 33, and 45 that represented 93.4% of the HR-HPV group and HPV 6 and 11 comprising 83.8% of the LR-HPV group. HPV 16, 33, and 45 were the only HR-HPV types found in vulvar SCCs. Both groups of LSC and d-VIN contained only one HPV 16 positive case. All lesions classified as LS were HPV DNA negative.

The majority of HPV positive samples (n = 149; 89.8%) contained only one HPV type, but various combinations were detected in the remaining cases with a maximum of 4 different HPV types in a single lesion (2 cases of CoA) (Fig. 3). Multiple infections were more

TABLE 4. The Epidemiologic Classification of HPV Types

Group	HPV type
Low-risk HPV	6, 11, 13, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89
High-risk HPV	16, 18, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59
Probably high-risk HPV	26, 53, 66, 68, 73, 82
Undetermined-risk HPV	2a, 3, 7, 10, 27, 28, 29, 30, 32, 34, 55, 57, 62, 67, 69, 71, 74, 77, 83, 84, 85, 86, 87, 90, 91

Modified according to Munoz et al.[9]

frequent in u-VIN II (41.7%) and CoA (17.5%), but were rare in u-VIN III (3%) and they were not observed in u-VIN I and SCC. The proportion of particular HPV risk groups in multiple-type infected lesions is shown in Figure 4. Forty percent of multiple-type infected CoA contained HR-HPV DNA and 60% of multiple-type infected u-VIN II contained LR-HPV sequences. Only the HR-HPV types were identified in multiple-type infected u-VIN III. pHR-HPV (HPV 26 and 73) and UR-HPV (HPV 84) were found exclusively in CoA harboring multiple HPV types.

Age Predilection of Vulvar Lesions

The age distribution of particular patient subgroups is depicted in Figure 5 and the age spectrum of women according to the HPV profile of their vulvar lesions is shown in Figure 6. The statistical analysis revealed that there was a significant difference in the age distribution between the patients with u-VIN (median age, 53 y) and d-VIN (median age, 79 y) ($P < 0.0001$) and also between those with HPV positive SCC (median age, 64 y) and HPV negative SCC (median age, 77 y) ($P = 0.02$). The interval between the median age of u-VIN III group (median age, 56 y) and the group of patients with HPV positive SCC was 8 years and such a difference was statistically significant ($P = 0.003$). On the other hand, the median age of women with d-VIN and patients affected by HPV negative SCC were far closer and differed by 2 years only. The median age of patients with u-VIN II and u-VIN III were significantly different by 14.5 years ($P = 0.03$), in contrast to the difference between the median age of u-VIN I and u-VIN II groups.

The median age of women with multiple-type infected CoA (median age, 20.5 y) and u-VIN II (median age, 32 y) was lower than the median age of patients with single-type HPV containing CoA (median age, 29 y) and u-VIN II (median age, 51 y). In case of CoA group only, this difference was statistically significant ($P = 0.01$).

DISCUSSION

HPV Profiles of Vulvar Lesions

Results of our study confirmed that CoA, u-VIN, and a proportion of SCCs contain DNA sequences of HPV. While LR-HPV types dominated in the CoA group, SCC and all 3 grades of u-VIN showed association with HR-HPV types. The group of SCC in our population was strongly linked to HPV 16, 33, and 45, whereas HPV 18, 31, and others that occur worldwide in the SCC of the uterine cervix[2] were not detected in the vulvar SCC in our series. Except from one HPV prevalence study of vulvar lesions performed in a geographically neighboring region,[4] we also found a lower prevalence of HPV 16 and a higher frequency of HPV 33 in u-VIN III and SCC in comparison with other studies, which also used highly sensitive protocols of HPV detection and typing.[15,18,24] Accordingly, the geographical variability in the distribution of HPV types was observed previously in the cervical precancerous and neoplastic lesions.[2] The efficacy of prophylactic HPV vaccines in the prevention of vulvar disorders might be, therefore, reduced in our population because neither the recently developed bivalent HPV-16/18 VLP vaccine nor the quadrivalent HPV-6/11/16/18 VLP vaccine is targeted against HPV 33 or 45.[19] Whether the potential cross-neutralizing antibodies possess the protection also against other HPV types remains still unclear.[19]

HPV Negative Vulvar Lesions

In our study, HPV negative lesions were exemplified by vulvar dermatoses (LS and LSC), d-VIN, and by the majority of SCCs, which is in concordance with the proposed model of the 2 independent pathways of the vulvar carcinogenesis. Only 1 case of LSC and d-VIN was HR-HPV (HPV 16) positive and 2 cases of keratinizing SCC in postmenopausal women contained LR-HPV types (HPV 6 and 42). No histologic signs of HPV infection were observed in these 4 lesions. Although occasional association of HPV 6 and 11 with anal, vulvar, and penile SCC was also rarely described,[6] the asymptomatic presence of HPV DNA was noted in various normal human tissues[10,20] and we consider the HPV positivity in these cases as such coincidental HPV infection.

In our study, the u-VIN and d-VIN differed by their histopathologic pattern, HPV profiles, and age of the patients. The frequency of d-VIN in our series (12.8%) is congruent with previous observations, in which this entity was revealed in 2%[23] to 16%[3] of all VIN cases. Although HPV negative SCC prevailed in the group of vulvar SCC, the frequency of d-VIN was paradoxically lower when compared with the incidence of u-VIN. The discrepancy could be explained by the discrete macroscopic appearance of d-VIN resembling vulvar dermatoses and by the lower frequency of gynecologic examinations in postmenopausal women that result in a possible underdiagnosis of this disorder. Another important factor

TABLE 5. The Spectrum of HPV Types Detected in HPV Positive Vulvar Lesions

Type of lesion		Number of HPV positive lesions	LR-HPV (%)							HR-HPV (%)													pHR-HPV (%)			UR-HPV (%)	
			6	11	42	43	81	Total		16		18		33		45		51		56		59		Total	26	73	Total	84	Total
LS		0	-	-	-	-	-	-		-		-		-		-		-		-		-		-	-	-	-	-	-
LSC		1	-	-	-	-	-	-		100.0		-		-		-		-		-		-		100.0	-	-	-	-	-
CoA		54	63.8	14.5	2.9	-	1.4	82.6		2.9		-		2.9		1.4		1.4		-		-		8.7	2.9	4.3	7.3	1.4	1.4
VIN	u-VIN I	4	-	-	-	-	-	-	-	50.0	-	-	25.0	-	25.0	-	3.4	-	-	-	5.6	-	1.1	100.0	-	-	-	-	-
	u-VIN II	12	5.6	5.6	1.1	11.1	-	27.8	5.6	38.9	70.8	11.1	3.4	16.7	14.6	-	3.4	-	-	5.6	1.1	-	1.1	72.2	94.4	-	-	-	-
	u-VIN III	65	-	-	-	-	-	-		80.6		1.5		13.4		3.0		-		-		1.5		100.0		-	-	-	-
d-VIN		1	-	-	-	-	-	-		100.0		-		-		-		-		-		-		100.0	-	-	-	-	-
SCC		29	3.4	-	3.4	-	-	6.9		65.5		-		20.7		6.9		-		-		-		93.1	-	-	-	-	-

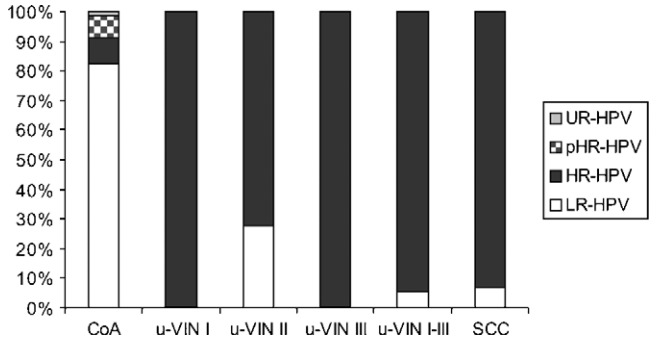


FIGURE 2. A spectrum of HPV risk groups detected in HPV associated vulvar lesions.

might be the short preinvasive interval of d-VIN leading to a decreased rate of clinical detection before the stromal invasion appears.[31] Our results support this view through the proximity of the median age of patients affected by d-VIN and HPV negative SCC in contrast to those with corresponding HPV positive lesions. In addition, the d-VIN group in our series revealed a stronger association with the preceding, simultaneous or subsequent SCC occurring in a single patient during the 5-year interval of the study than observed in the u-VIN III group.

Importantly, our data showed that the pure histopathologic assessment of the vulvar squamous precancerous lesions correlates well with their HPV status, that it is sufficient to distinguish HPV positive from HPV negative lesions and that such stratification is of a clinical value.

The Multiple-type HPV Infection

In the CoA and u-VIN II groups, we observed a tendency to the multiple-type HPV infection and detected the largest spectrum of HPV types involved. Multiple-type infected cases frequently contained HPV types unexpected for the nature of the assessed lesion (LR-HPV in 60% of u-VIN II and HR-HPV in 40% of CoA). The high rate of multiple HPV type infections and the coinfections with HR-HPV types was described in CoA previously[27] and it seems to represent a common feature of these lesions with a possible impact on the efficacy of the therapeutic HPV vaccination.[27] CoA are generally

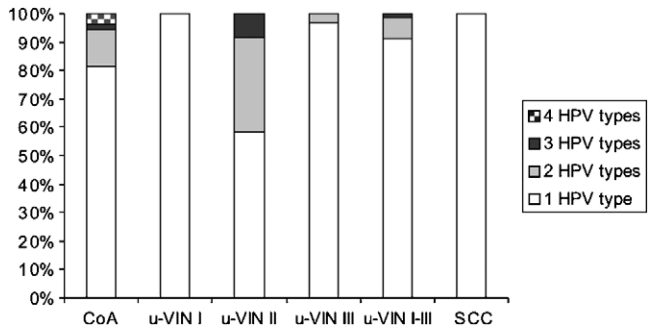


FIGURE 3. A spectrum of single and multiple HPV type infected lesions in HPV associated vulvar disorders.

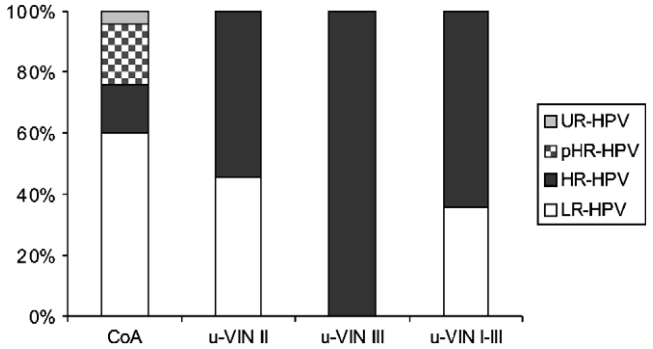


FIGURE 4. A spectrum of HPV risk groups detected in multiple-type infected vulvar lesions.

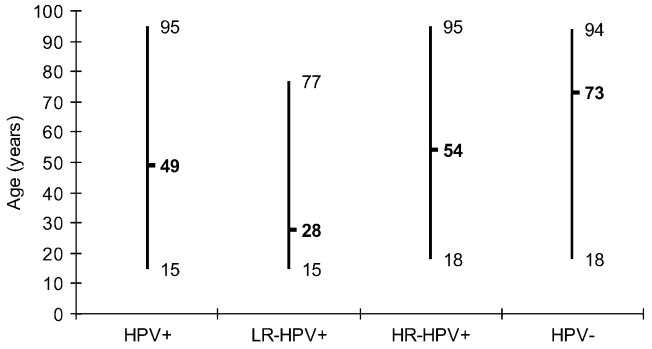


FIGURE 6. Age distribution of patients according to the HPV profile of their vulvar lesions. The minimal, maximal, and median ages are outnumbered.

assumed to be polyclonal epithelial proliferations lacking neoplastic properties, which may harbor more HPV types. On the other hand, u-VIN III and SCC are believed to be monoclonal disorders[12] defined by a single cell line proliferation with an integrated genome of one transcriptionally active HR-HPV type.[28] It corresponds with the low rate of multiple-type HPV infections detected in u-VIN III (3%) and none in SCC in our series. However, it contrasts with the 41.7% of u-VIN II being infected with multiple HPV types. The obvious difference between u-VIN II and u-VIN III became more apparent when focusing only on HPV types associated with SCC in the given population (HPV 16, 33, and 45). These types were found in 98.5% of u-VIN III (all of HPV positive cases), but only in 83.3% of u-VIN II. It may indicate that approximately 15% of u-VIN II lesions, containing HPV types not detected in u-VIN III and lacking HPV types strongly associated with u-VIN III, will probably not progress to u-VIN III. Moreover, patients affected by u-VIN II and u-VIN III represented 2 distinct groups significantly differing in their median age of presentation by 14.5 years.

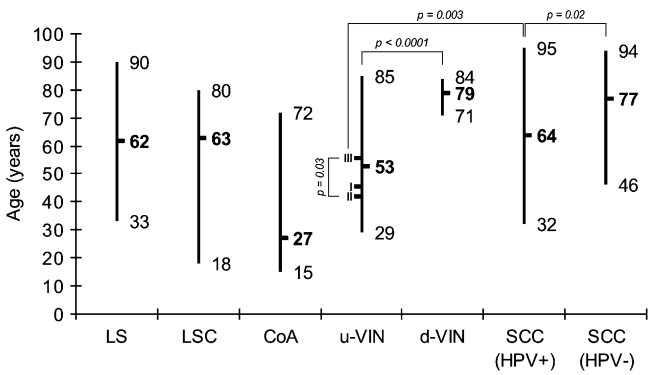


FIGURE 5. Age distribution of patient groups with vulvar lesions. Minimal, maximal, and median ages are outnumbered and median ages of the u-VIN grades (u-VIN I, II, and III) are schematically depicted. Statistically significant differences between particular patient groups and corresponding *P* values are shown. I indicates u-VIN I; II, u-VIN II; III, u-VIN III.

The lower median age of patients with multiple-type infected CoA and u-VIN II implicates that these lesions might evolve in a short interval after the infection before the viral clearance process and clonal selection.

Implications for the Classification of Vulvar Squamous Precancerous Lesions

Both the recently proposed classifications of vulvar squamous dysplastic lesions (ISSVD 2004 and Bethesda-like) merged the u-VIN II and u-VIN III into a single category of u-VIN or high-grade vulvar intraepithelial lesion (HG-VIL). The terms u-VIN (according to the ISSVD 2004) and HG-VIL should be analogous to the high-grade squamous intraepithelial lesions of the uterine cervix, which encompass 2 grades of cervical intraepithelial neoplasia (CIN II and CIN III). However, CIN II and cervical high-grade squamous intraepithelial lesions were recently recognized as a heterogeneous groups of lesions with a different biologic potential, a varying tendency to the regression and a differing spectrum of HPV types involved.[8,17,32] In our study, we obtained data indicating similar differences in age characteristics and HPV profiles of u-VIN II and u-VIN III, suggesting a biologic heterogeneity of these lesions. The u-VIN II seems to incorporate the spectrum of either genuine high-grade dysplastic lesions (older patients with single HPV type infected lesions) or HPV associated epithelial proliferations with a minimal or no precancerous potential resembling CoA (younger patients with multiple HPV type infected lesions). Merging of u-VIN II with u-VIN III into one category (u-VIN or HG-VIL) is misleading because of the formation of a wide group of lesions with a various biologic nature and brings a potential risk of overtreatment. As the subtypes of u-VIN II are not reliably distinguishable histologically, the entire category should be regarded as a group of lesions of uncertain precancerous potential rather than high-grade dysplastic lesions only.

A disagreement between the modified terminologic systems remains in the concept of low-grade dysplastic vulvar lesions. While the u-VIN I category was abandoned in the ISSVD 2004 classification, Bethesda-like scheme expanded the term of low-grade VIL with the

CoA. Our data demonstrated that u-VIN I is a rare category of vulvar lesions representing only 4.9% of u-VIN. All cases of u-VIN I in our series contained HR-HPV types and were detected in patients presenting previous, simultaneous, or subsequent u-VIN III. We found 1 case of u-VIN I in a patient with a previous HPV positive vulvar SCC and no multiple-type HPV infection was identified in this group. In u-VIN I, the high frequency of HR-HPV (42%) was recently demonstrated.[18] Unfortunately, our results are limited by a lower number of the cases studied so far and an extended research will be desirable to address this issue.

On the basis of our data, we suggest that u-VIN I should be retained in the terminology system to prevent forming a diagnostical window, but it should not merge with the CoA into one category because of the different HPV profiles and frequent coincidence of u-VIN I with u-VIN III. Likewise, in the u-VIN II group, it is difficult to decide histologically whether the alteration of u-VIN I appearance represents a true dysplastic lesion or merely a manifestation of HPV infection. To enhance the reproducibility of the u-VIN I category, the u-VIN I as a HPV-related disorder should not be confused with vulvar dermatoses or inflammatory conditions. Although features of these lesions might include reactive basal atypia and moderate mitotic activity, the signs of the HPV infection including koilocytosis, prominent nuclear enlargement, or multinucleation are absent. Identically, the manifestation of HPV cytopathic effect is not seen in d-VIN, which may be potentially mistaken for u-VIN I owing to the similar distribution of cytologic atypia and mitotic figures in the basal parts of the squamous epithelium.

CONCLUSIONS

We performed a correlation study between the histologic features and the HPV profile of vulvar non-neoplastic, precancerous, and neoplastic squamous lesions using a high sensitive protocol of HPV detection and typing. Our results indicate that both the revised terminologic systems (ISSVD 2004 and Bethesda-like) might be misleading in the diagnosis and management of vulvar dysplastic lesions owing to both the biologic heterogeneity of the u-VIN II category and the discrepancies in HPV profiles and age of patients with u-VIN II and u-VIN III. Therefore, we suggest that the ISSVD 1986 terminology should be retained as the classification scheme of vulvar dysplastic lesions with respect to the fact that u-VIN II category encompasses epithelial alterations of histologically unpredictable precancerous potential. Moreover, u-VIN I should be perceived as a rare condition occurring mostly in patients with other HPV associated lesions of the vulva or other parts of the lower female genital tract.

Differences in the HPV profiles of vulvar and cervical precancerous and neoplastic squamous lesions and the geographical variability in the distribution of HPV types in vulvar disorders were demonstrated. The increased prevalence of HPV 33 and the decreased frequency of HPV 16 and 18 detected in vulvar lesions investigated in this study might influence the efficacy of the prophylactic HPV vaccination in preventing vulvar cancer, because both the recently developed vaccines are targeted primarily against HPV 16 and 18.

REFERENCES

1. Chiesa-Vottero A, Dvoretsky PM, Hart WR. Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosus. *Am J Surg Pathol*. 2006;30:310–318.
2. Clifford G, Franceschi S, Diaz M, et al. Chapter 3: HPV-type distribution in women with and without cervical neoplastic diseases. *Vaccine*. 2006;24(suppl 3):26–34.
3. Friedrich EG, Wilkinson EJ, Fu YS. Carcinoma in-situ of the vulva: a continuing challenge. *Am J Obstet Gynecol*. 1980;136:830–843.
4. Hampl M, Sarajuuri H, Wentzensen N, et al. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. *Obstet Gynecol*. 2006;108:1361–1368.
5. Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. *Int J Gynecol Pathol*. 2001;20:16–30.
6. Lacey CJ, Lowndes CM, Shah KV. Chapter 4: burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine*. 2006;24(suppl 3):35–41.
7. Medeiros F, Nascimento AF, Crum CP. Early vulvar squamous neoplasia: advances in classification, diagnosis, and differential diagnosis. *Adv Anat Pathol*. 2005;12:20–26.
8. Moscicki AB, Schiffman M, Kjaer S, et al. Chapter 5: updating the natural history of HPV and anogenital cancer. *Vaccine*. 2006; 24(suppl 3):42–51.
9. Munoz N, Castellsague X, de Gonzales AB, et al. Chapter 1: HPV in the etiology of human cancer. *Vaccine*. 2006;24(suppl 3):1–10.
10. Perez LO, Abba MC, Laguens RM, et al. Analysis of adenocarcinoma of the colon and rectum: detection of human papillomavirus (HPV) DNA by polymerase chain reaction. *Colorectal Dis*. 2005; 7:492–495.
11. Preti M, Mezzetti M, Robertson C, et al. Inter-observer variation in histopathological diagnosis and grading of vulvar intraepithelial neoplasia: results of an European collaborative study. *Br J Obstet Gynaecol*. 2000;107:594–599.
12. Rosenthal AN, Ryan A, Hopster D, et al. Molecular evidence of a common clonal origin and subsequent divergent clonal evolution in vulval intraepithelial neoplasia, vulval squamous cell carcinoma and lymph node metastases. *Int J Cancer*. 2002;99:549–554.
13. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 1985;230:1350–1354.
14. Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. *Int J Gynecol Cancer*. 1999;9:89–97.
15. Scurry J, Flowers L, Wistuba I, et al. Human papillomavirus, lichen sclerosis and vulvar squamous cell carcinoma. *Int J Gynecol Cancer*. 1998;8:298–306.
16. Sideri M, Jones RW, Wilkinson EJ, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med*. 2005;50:807–810.
17. Snijders PJ, Steenbergen RD, Heideman DA, et al. HPV-mediated cervical carcinogenesis: concepts and clinical implications. *J Pathol*. 2006;208:152–164.
18. Srodon M, Stoler MH, Baber GB, et al. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol*. 2006;30:1513–1518.
19. Stanley M, Lowy DR, Frazer I. Chapter 12: prophylactic HPV vaccines: underlying mechanisms. *Vaccine*. 2006;24(suppl 3):106–113.
20. Svec A, Mikyskova I, Hes O, et al. Human papillomavirus infection of the epididymis and ductus deferens: an evaluation by nested polymerase chain reaction. *Arch Pathol Lab Med*. 2003;127: 1471–1474.

21. Toki T, Kurman RJ, Park JS, et al. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol*. 1991;10:107–125.
22. Trimble CL, Hildesheim A, Brinton LA, et al. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet Gynecol*. 1996;87:59–64.
23. van Beurden M, ten Kate FJ, Smits HL, et al. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. *Cancer*. 1995;75:2879–2884.
24. van Beurden M, ten Kate FW, Tjong AHSP, et al. Human papillomavirus DNA in multicentric vulvar intraepithelial neoplasia. *Int J Gynecol Pathol*. 1998;17:12–16.
25. van den Brule AJ, Pol R, Fransen-Daalmeijer N, et al. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol*. 2002;40:779–787.
26. van der Avoort IA, Shirango H, Hoevenaars BM, et al. Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. *Int J Gynecol Pathol*. 2006;25:22–29.
27. Vandepapeliere P, Barrasso R, Meijer CJ, et al. Randomised controlled trial of an adjuvanted human papillomavirus (HPV) type 6 L2E7 vaccine: infection of external anogenital warts with multiple HPV types and failure of therapeutic vaccination. *J Infect Dis*. 2005;192:2099–2107.
28. Wentzensen N, Vinokurova S, von Knebel Doeberitz M. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res*. 2004;64:3878–3884.
29. Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD terminology committee. *J Reprod Med*. 1986;31:973.
30. Wilkinson EJ, Teixeira MR. Epithelial tumours of the vulva. In: Tavassoli FA, Devilee P, eds. *Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Lyon, PA: IARC Press; 2003:316–325.
31. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol*. 2000;24:429–441.
32. Zuna RE, Allen RA, Moore WE, et al. Comparison of human papillomavirus genotypes in high-grade squamous intraepithelial lesions and invasive cervical carcinoma: evidence for differences in biologic potential of precursor lesions. *Mod Pathol*. 2004;17:1314–1322.

Příloha II.

Tachezy R., Šmahelová J., Saláková M., Arbyn M., Rob L., Škapa P., Jirásek T. a Hamšíková E. (2011) Human papillomavirus genotype distribution in Czech women and men with diseases etiologically linked to HPV. ***PLoS One*** 6:e21913.

IF 4,411

OPEN ACCESS Freely available online

PLoS one

Human Papillomavirus Genotype Distribution in Czech Women and Men with Diseases Etiologically Linked to HPV

Ruth Tachezy[1]*, Jana Smahelova[1], Martina Salakova[1], Marc Arbyn[2], Lukas Rob[3], Petr Skapa[4], Tomas Jirasek[5], Eva Hamsikova[1]

1 Institute of Hematology and Blood Transfusion, Department of Experimental Virology, Prague, Czech Republic, **2** Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium, **3** Department of Obstetrics and Gynecology, Second Faculty of Medicine, Charles University, Prague, Czech Republic, **4** Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University, Prague, Czech Republic, **5** Department of Pathology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Abstract

Background: The HPV prevalence and genotype distribution are important for the estimation of the impact of HPV-based cervical cancer screening and HPV vaccination on the incidence of diseases etiologically linked to HPVs. The HPV genotype distribution varies across different geographical regions. Therefore, we investigated the type-specific HPV prevalence in Czech women and men with anogenital diseases.

Methods: We analyzed 157 squamous cell carcinoma samples, 695 precancerous lesion samples and 64 cervical, vulvar and anal condylomata acuminate samples. HPV detection and typing were performed by PCR with GP5+/6+ primers, reverse line blot assay and sequencing.

Results: Thirty different HPV genotypes were detected in our study, HPV 16 being the most prevalent type both in precancerous lesions (45%) and squamous cell carcinomas (59%). In benign lesions, HPV 6 (72%) was the most common type. Altogether, 61% of carcinoma samples and 43% of precancerous lesion samples contained HPV 16 and/or 18. The presence of HPV types related to the vaccinal ones (HPV 31, 45, 33, 52, 58) were detected in 16% of carcinoma samples and 18% of precancerous lesion samples. HPV 16 and/or 18 were present in 76% of cervical cancer samples, 33% of CIN1, 43% CIN2 and 71% of CIN3 samples. HPV types 6 and/or 11 were detected in 84% samples of condylomata acuminate samples.

Conclusions: The prevalence of vaccinal and related HPV types in patients with HPV-associated diseases in the Czech Republic is very high. We may assume that the implementation of routine vaccination against HPV would greatly reduce the burden of HPV-associated diseases in the Czech Republic.

Citation: Tachezy R, Smahelova J, Salakova M, Arbyn M, Rob L, et al. (2011) Human Papillomavirus Genotype Distribution in Czech Women and Men with Diseases Etiologically Linked to HPV. PLoS ONE 6(7): e21913. doi:10.1371/journal.pone.0021913

Editor: Christopher B. Buck, National Cancer Institute, United States of America

Received March 9, 2011; **Accepted** June 8, 2011; **Published** July 13, 2011

Funding: This work was supported by grant NS 10/656-3/2009 from the Ministry of Health of the Czech Republic). M. Arbyn was supported by the European Commission (DG of SANCO, Luxembourg, Grand-Duchy of Luxembourg), through the ECCG project (European Cooperation on development and implementation of Cancer screening and prevention Guidelines, IARC, Lyon, France) and the 7th Framework Programme of DG Research of the European Commission through the PREHDICT project (grant No. 242061, coordinated by the Vrije Universiteit Amsterdam, the Netherlands). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and RT, LR, and EH have the following conflicts: membership in the Czech GSK advisory board for the prevention of cervical carcinoma. MA had membership in the Belgian advisory board up to 2008 for Cervarix (bivalent HPV vaccine, produced by GSK) and received travel funding from Sanofi-Pasteur MSD (manufacturer of a quadrivalent HPV vaccine). MS, JS, TJ, and PS have declared that no competing interests exist.

* E-mail: rutach@uhkt.cz

Introduction

Human papillomaviruses (HPVs) have been established as etiological agents of invasive cervical cancer (CC) [1,2] and they are the most common viral sexually transmitted infection worldwide. Persistent infection with high-risk (HR) HPVs is necessary for the development of premalignant lesions and/or progression of the disease [3]. Furthermore, HPV has carcinogenic effects at several other anatomical sites in women and men [4]. HPV genotype distribution varies across different populations and geographical regions [5]. Recently, meta-analyses and systematic reviews of HPV type distribution in diseases linked to HPV infections worldwide have been published [6–13]. CC is the second most common cancer among women worldwide, with 492,800 incident cases during 2002 [14]. The burden of noncervical anogenital, i.e. anal, vaginal and vulvar, cancers approximates 53,872 cases worldwide annually (i.e. 28,272 anal and 25,600 vaginal and vulvar cancer cases). In the Czech Republic , 990 CC cases, 189 vulvar cancer cases and 121 anal cancer cases occur annually [15].

In spite of the high burden of cervical cancer in Central and Eastern Europe [16], few data are available regarding the

prevalence of HPV [17–20]. Therefore, our study which collects the available data on Czech patients with a wide variety of HPV-associated diseases will contribute to a better understanding of the HPV type distribution in the Czech Republic. Importantly, it will help in estimating the potential local impact of HPV vaccines on the prevention of HPV-associated diseases in women and men.

Materials and Methods

Population studied

Squamous cell cervical carcinoma (SCC) samples as well as precancerous lesion samples from different anatomical locations were selected from the biobank of the National Reference Laboratory for Papillomaviruses in Prague. These samples were collected between 1993 and 2005, stored at −20°C and analyzed in previous studies.

Cervical scrape and biopsy specimens were obtained from women visiting hospital gynecology departments and selected centers of gynecologic-oncology prevention in the Czech Republic [21]. These settings are located in different districts across the Czech Republic and serve wide catchment areas. Therefore, the patients included in our study are representative of the population of the whole of the Czech Republic. Additionally, samples from patients treated for cervical intraepithelial neoplasia garde 1 to 3 (CIN1/2/3) were used. The patients characteristics and sample processing were published before [22]. The classification of all CIN2/3 and SCC specimens and of the majority of CIN1 (86%) specimens was done by histology as specified before. Overall, 86 SCC specimens (patient mean age 49.7 years; age range 28–87 years), 338 CIN1 specimens (mean age 33.8 years; age range 16–76 years), 111 CIN2 specimens (mean age 34.5 years; age range 20–59 years), and 200 CIN3 specimens (mean age 33.9 years; age range 20–66 years) were selected for the purpose of the present study.

Samples from patients surgically treated in the Department of Obstetrics and Gynecology of the 2nd Faculty of Medicine, Charles University, Prague for squamous cell vulvar carcinoma (VC), vulvar intraepithelial neoplasia (VIN) and vulvar condylomata acuminata (VCA) were also included in the study. The patients characteristics and histological data were published before [23]. For HPV typing, 49 VC samples (patient mean age 70.7 years; age range 32–95 years), 46 samples from patients with different grades of usual VIN (u-VIN) (patient mean age 52.5 years; age range 29–85 years) and 54 VCA samples (patient mean age 30.6; age range 15–59 years) were available.

Twenty-two samples from patients with squamous cell carcinoma of the anus (AC) (mean age 64.2 years; age range 47–86 years, 18 women & 4 men) and 10 samples of anal condylomata acuminata (ACA) samples (patient mean age 41.4 years; age range 21–69 years, 1 woman & 9 men), were analyzed. Details on the population, sample preparation and pathological classification were published before [24].

Overall, 157 cancer samples from multiple locations, 695 pre-malignant neoplasia samples, and 64 condylomata acuminata samples were included in this study.

Ethic statement

No informed consent was needed from the patient by the course of law in the Czech Republicbefore 2000. All patients enrolled after the year 2000 signed an informed consent form and the study was approved by the institutional ethics committee [25].

HPV detection and genotyping

PCR and reverse line blot hybridization (RLB) were used for the detection and genotyping of the HPV DNA in samples [26]. RLB is able to identify 37 different HPV types in a single assay. The HPV detection was performed in a PCR thermocycler PTC 200 (MJ Research, Inc, Waltham, MA, USA) by the PCR assay with primers GP5+ and 5′-end biotin labelled GP6+ primer which amplify the 150 bp fragment of the L1 gene. The PCR was performed for 40 cycles and the biotinylated PCR product was hybridized with the oligonucleotide probes labelled with the 5′-terminal amino-group. These probes were covalently linked to an activated negatively charged Biodyne C membrane. After washing, the membrane was incubated for 60 min at 42°C with peroxidase labelled streptavidin conjugate. For chemiluminescent detection of hybridising DNA, the membrane was incubated in ECL detection liquid (Amersham Biosciences, Uppsala, Sweden) and exposed to LumiFilm (Roche, Indianapolis, IN, USA) for 5 min.

Detected HPV types were classified into low-risk (LR) (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89), high-risk (HR) (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68,) and probably high-risk (pHR) (HPV 26, 53, 66, 73, 82) types of the genus Alpha that contains the mucosal types of HPV [27–29]. In our analyses, we defined HPV 31 and 45 as closely related to and HPV 33, 52 and 58 as a distantly related to HPV 16 and/or 18.

To confirm the presence and integrity of the human DNA, beta-globin PCR analysis by PC03/04 primer set [30] was performed for all RLB assay negative specimens. Beta-globin negative specimens were excluded from our study.

The laboratory is accredited according to ČSN EN ISO 15 189 and participates regularly in external control of quality programs organized by INSTAND (Germany) and Mendel Center for Biomedical Sciences (Cyprus). Furthermore, the laboratory participated twice in WHO HPV LabNet Proficiency Study of HPV DNA Typing organized by the WHO HPV Global Reference Laboratory [31].

HPV sequencing

To determine the type of HPV in the specimens positive by RLB only on the agarose gel but not by RLB hybridization, the remaining aliquots of PCR amplicons were used for nucleotide sequencing. The 150 bp products were cut out of the 2% NuSieve GTG agarose gel (BMA, Rockland, ME), purified using the MinElute™Gel Extraction Kit (Qiagen, Hilden, Germany) and directly sequenced using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). The sequence analysis was performed on the ABI PRISM 310 genetic analyzer (Applied Biosystems) and the sequences were analyzed by Chromas software and evaluated by BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistical analyses

Multiply infected samples were those in which two or more HPV types had been detected. Such samples were counted as positive for one type of HPV and also included among positives for the others. Type-specific HPV prevalence rates are expressed as percentages of all cases tested for HPV, and thus represent the HPV prevalence in either single or multiple infections. The differences in the mean age were assessed by a one-way analysis of variance (ANOVA) test. For contingency tables, the standard chi-square test and the Fisher exact test were used. The prevalence ratios in SCC in comparison to CIN2, 3 and CIN1 with 95% confidence intervals (CI) were determined using GraphPad InStat (version 3.00) (GraphPad Software, San Diego, CA). All tests were two sided and the significance level was $p = 0.05$. For assessing the possible impact of HPV vaccines on the prevention of HPV-associated cancer, we estimated the number of cervical cancer

cases attributed to 8 HPV types most prevalent in the Czech Republic. We used the numbers of incident cases of cervical, vulvar and anal cancers in the Czech Republic published in 2010 [15] and type-specific HPV distribution derived from this study. A woman with multiple infections was assigned in proportional fractions to each genotype but counted only once [32].

Results

HPV genotyping of carcinomas

Altogether, 157 carcinoma samples were available for HPV DNA testing. Patients with SCC were significantly younger ($P<0.001$) than those with other types of carcinomas (see materials and methods). One hundred and eighteen (75%) carcinomas samples were HPV DNA positive. The presence of HR HPV was detected in 95% (82/86) of SCC samples, 35% (17/49) of VC samples and 82% (18/22) of AC samples. One vulvar carcinoma sample was only infected with a LR HPV genotype only (HPV 42). No LR types as a single infection were found in carcinoma samples from other anatomical locations (Table 1). Multiple infection (coinfection with two or more HPV types) was only found in 20% of SCC (17/86). Coinfection with HPV 16 and 18 was the most commmon of multiple infections (5/17). HPV 16 coinfection with HR HPV types other than HPV18 was also often observed (11/17).

Table 1. HPV prevalence in carcinomas of different anatomical locations.

	Diagnosis			
	SCC	**VC**	**AC**	**Total**
Sample N	86	49	22	**157**
	Prevalence [%]			
HPV +	95.3	36.7	81.8	**75.2**
Single HPV	75.6	36.7	81.8	**89.2**
Multiple HPV	19.8	0.0	0.0	**10.8**
Any HR type	95.3	34.7	81.8	**74.5**
16	73.3	24.5	81.8	**59.2**
18	8.1	0.0	0.0	**4.5**
31	7.0	0.0	0.0	**3.8**
33	10.5	8.2	0.0	**8.3**
39	1.2	0.0	0.0	**0.6**
45	9.3	2.0	0.0	**5.7**
52	1.2	0.0	0.0	**0.6**
53	1.2	0.0	0.0	**0.6**
56	2.3	0.0	0.0	**1.3**
58	3.5	0.0	0.0	**1.9**
73	1.2	0.0	0.0	**0.6**
16/18[#]	75.6	24.5	81.8	**60.5**
31/45*	8.1	2.0	0.0	**5.1**
33/52/58**	8.1	8.2	0.0	**7.0**
Any LR type	0.0	2.0	0.0	**0.6**
42	0.0	2.0	0.0	**0.6**
6/11***	0.0	0.0	0.0	**0.0**

SCC = squamous cell cervical carcinoma, VC = vulvar carcinoma, AC = squamous cell anal carcinoma.
[#]samples HPV 16 and/or 18 positive.
*samples which do not contain HPV 16 and/or 18.
**samples which do not contain HPV 16 and/or 18 and/or 31 and/or 45.
***samples which do not contain HPV 16 and/or 18.
doi:10.1371/journal.pone.0021913.t001

Overall, we detected 9 HR (HPV 16, 18, 31, 33, 39, 45, 52, 56, 58) 2 pHR (HPV 53, 73) and 1 LR (HPV 42) HPV types in different types of carcinomas, of which 11 different HPV genotypes were found in SCC samples while the spectrum of HR HPV types in other types of carcinomas was much narrower. Only HR HPV types 16, 33 and 45 were found as a single infection. HPV 16 was the most prevalent type in cervical 73% (63/86), vulvar 25% (12/49) and anal 82% (18/22) carcinomas, followed by HPV 33, 45, 18, and 31 in descending order.

HPV vaccinal types (HPV 16 and/or 18) were detected as a single infection in 50% (79/157) of tumors and as a coinfection with other HR HPV types in additional 10% (16/157) of samples. Altogether, 61% (95/157) of analyzed malignant tumors contained one or both vaccinal types. The presence HPV types either closely or distantly related to the vaccinal ones, i.e. HPV 31 and 45 and HPV 33, 52, and 58, respectively, was detected in additional 5% (8/157) and 11% (11/157) of carcinoma specimens, respectively. Sixty-five (76%) of 86 cervical cancer samples contained HPV 16 and/or 18 as a single or multiple infection. The presence of HPV types either closely or distantly related to the vaccinal ones, i.e. HPV 31 and 45 and HPV 33, 52, and 58, was detected in additional 8% (7/86) and 8% (7/86) of SCC samples, respectively.

HPV genotyping of precancerous lesions

A total of 695 precancerous lesion samples were available for our analyses: 338 from CIN1 cases, 111 from CIN2 and 200 from CIN3 cases and 46 from VIN cases. Median age of women with cervical lesions was substantially and statistically significantly lower ($P<0.0001$) compared to that of patients with VIN (see study design). Overall, the prevalence of HPV DNA was 76% (528/695). HPV infection was detected in 62% (209/338) of CIN1 samples, 77% (85/111) of CIN2 and 94% (188/200) of CIN3 samples and 100% (46/46) of VIN samples (Table 2). Among the HPV-positive samples, 4% (19/528) were infected with LR HPV types only.

In comparison to carcinomas, precancerous lesions contained a larger variety of HPV types. As shown in Table 2, altogether 28 different HPV genotypes were detected: 13 were HR (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68), 4 pHR (HPV 26, 53, 66, 82) and 9 LR (HPV 6, 11, 40, 42, 43, 54, 70, , 81, 89) and 2 undetermined (HPV 55, 71). Most of these types were present in cervical lesions. The VIN samples were infected with 6 different HR (HPV 16, 18, 33, 45, 56, 59) and 3 LR (HPV 6, 11, 43) HPV types. Similar to carcinomas, HPV 16 was the most prevalent type in all types of precancerous lesions followed by HPV 33, 31, 18, and 45. HPV 16 was observed in 28% (96/338) of CIN1 samples, 41% (46/111) of CIN2 and 68% (135/200) of CIN3 samples and 72% (33/46) of VIN samples.

More than a half of samples contained a single HPV type (56%). Multiple infection was found most commonly in cervical precancerous lesion samples: in 20% (66/338) of CIN1, 21% (23/111) of CIN2 and 22% (44/200) of CIN3 samples. Most multiple infections were coinfections with two or three HPV types. Coinfection with four HPV genotypes was only found in six cervical lesion samples.

The vaccinal types HPV 16 and/or 18 were present in 34% (239/695) of precancerous lesion samples as a single infection, in 1% (8/695) as a multiple infection (combined HPV16/18 infection), and in 13% (87/695) in combination with other HPV types. The presence of the types either closely or distantly related types to the vaccinal ones: HPV 31 and 45 and HPV 33, 52, and 58, respectively, was detected in 9% (59/695) and 11% (79/695) of samples, respectively.

Table 2. HPV prevalence in precancerous lesions of different anatomical locations.

	Diagnosis				
	CIN1 (%)	CIN2 (%)	CIN3 (%)	VIN (%)	Total (%)
Sample N	338	111	200	46	695
HPV +	61.8	76.6	94.0	100.0	76.0
Single HPV	42.0	55.9	72.0	87.0	56.0
Multiple HPV	19.8	20.7	52.0	13.0	28.8
Any HR type	57.4	73.9	93.5	93.5	72.5
16	28.4	41.4	67.5	71.7	44.6
18	5.3	4.5	5.5	4.3	5.2
26	0.3	0.9	0	0	0.3
31	8.6	8.1	14.5	0	9.4
33	6.8	11.7	14.0	17.4	10.4
35	3.0	2.7	1.0	0	2.2
39	0.3	1.8	0	0	0.4
45	4.7	6.3	3.0	4.3	4.5
51	3.8	3.6	1.5	0	2.9
52	2.1	5.4	2.0	0	2.4
53	0	0.9	0	0	0.1
56	3.6	3.6	3.0	2.2	3.3
58	4.4	2.7	5.5	0	4.2
59	0.9	0	0	2.2	0.6
66	1.8	1.8	1.5	0	1.6
68	0.6	0	0	0	0.3
82	0	0	2.0	0	0.6
Undetermined	0.3	0.9	0	0	0.3
55	0	0.9	0	0	0.1
71	0.3	0	0	0	0.1
16/18#	32.5	43.2	70.5	71.7	43.0
31/45*	9.2	10.8	7.0	4.3	8.3
33/52/58**	8.9	14.4	12.5	17.4	11.4
Any LR type	12.4	4.5	4.0	10.9	8.6
6	4.1	1.8	0.5	6.5	2.9
11	2.4	0	0.5	2.2	1.4
40	0.3	0	0	0	0.1
42	2.1	0	1.5	0	1.4
43	0.6	0.9	0	2.2	0.6
54	0.9	0.9	0.5	0	0.7
70	1.5	0.9	0.5	0	1.0
81	1.2	0.9	0.5	0	0.9
89	0.9	0	0	0	0.4
6/11***	5.3	0.9	0.5	8.7	2.9

CIN1 = cervical intraepithelial neoplasia grade 1, CIN2/3 = cervical intraepithelial neoplasia grade 2 and 3, VIN = vulvar intraepithelial neoplasia.
#samples HPV 16 and/or 18 positive.
*samples which do not contain HPV 16 and/or 18.
**samples which do not contain HPV 16 and/or 18 and/or 31 and/or 45.
***samples which do not contain HPV 16 and/or 18.
doi:10.1371/journal.pone.0021913.t002

Altogether, 33% (110/338), 43% (48/111) and 71% (141/200) of CIN1, CIN2 and CIN3 samples, respectively, were positive for HPV 16 and/or 18. Closely related types HPV 31 and 45 were present in 9% (31/338),11% (12/111) and 7% (14/200) of samples, respectively, and distantly related types HPV 33, 52, and 58 in 9% (30/338), 14% (16/) and 13% (25/100) of samples, respectively. LR HPV types were detected in 9% of precancerous lesion samples. HPV 6 and/or 11 were present as either a single or multiple infection in 3% of all precancerous lesion samples, more precisely in 5% (18/338) of CIN1 samples, 1% (1/111) of CIN2 and 1% (1/200) of CIN 3 samples and 9% (4/46) of VIN samples.

HPV genotyping of condylomata acuminata

HR HPV types as a single infection were only detected in 4% (2/54) of VCA samples and as a multiple infection together with LR types in additional 15% (8/54) of VCA samples, while ACA samples did not contain any HR HPV type, with a single sample being positive for two LR types. In VCA samples, 11 different HPV types were detected: 4 were HR (HPV 16, 33, 45, 51), 2 pHR (HPV 26, 73) and 5 LR (HPV 6, 11, 42, 84, 81) HPV types (Table 3).

Altogether, vaccinal HPV types 6 and/or 11 were present in 84% (54/64) of condyloma acuminatum samples from different anatomical locations, in 87% (47/54) of VCA samples, and 70% (7/10) of ACA samples.

Table 3. HPV prevalence in condyloma acuminata of different anatomical locations.

	Diagnosis		
	VCA	ACA	Total
Sample N	54	10	**64**
	Prevalence [%]		
HPV +	94.4	70.7	**90.6**
Single HPV	75.9	60.0	**73.4**
Multiple HPV	18.5	10.0	**17.2**
Any HR type	18.5	0	**15.6**
16	1.9	0	**1.6**
26	3.7	0	**3.1**
33	3.7	0	**3.1**
45	1.9	0	**1.6**
51	1.9	0	**1.6**
73	5.6	0	**4.7**
16/18#	1.9	0	**1.6**
31/45*	1.9	0	**1.6**
33/52/58**	3.7	0	**3.1**
Any LR type	90.7	100.0	**89.1**
6	75.9	50.0	**71.9**
11	18.5	30.0	**20.3**
42	3.7	0	**3.1**
84	1.9	0	**1.6**
81	1.9	0	**1.6**
6/11***	87.0	70.0	**84.4**

VCA = vulvar condyloma acuminatum, ACA = anal condyloma acuminatum.
#samples HPV 16 and/or 18 positive.
*samples which do not contain HPV 16 and/or 18.
**samples which do not contain HPV 16 and/or 18 and/or 31 and/or 45.
***samples which do not contain HPV 16 and/or 18.
doi:10.1371/journal.pone.0021913.t003

Comparison of HPV prevalence in SCC with CIN1, CIN2 and CIN3 cases

The overall prevalence of HPV was higher in SCC (95%) in comparison to CIN1 (62%) (SCC:CIN1 ratio 1.5, 95% CI 1.4–1.7), CIN2 (77%) (SCC:CIN2 ratio 1.2, 95% CI 1.1–1.4) but not in CIN3 (94%) (SCC:CIN3 ratio 1.0, 95% CI 1.0–1.1) cases. Similar results were obtained for any HR HPV type detected and for seven HR HPV types most prevalent in SCC samples (Table 4). For each of the seven HPV types, the SCC:CIN1, SCC:CIN2 and SCC:CIN3 ratios were also calculated. The respective ratios were 2.6, 1.8 and 1.1 for HPV 16, 1.5, 1.8 and 1.5 for HPV 18 and 2.0, 1.5 and 3.1 for HPV 45. For HPV types closely related (HPV 31, 33, 56), or distantly related (HPV 58) the SCC:CIN3 ratios were 0.5 to 0.8, respectively (Table 4).

Cervical, vulvar and anal cancers associated with specific HPV types

In the Czech Republic 1300 cervical, vulvar and anal incident cancer cases occur, more precisely 990 cervical cancer cases, 189 vulvar cancer cases, and 121 anal cancer cases [15]. We estimated the number of cases that can be attributed to the 8 most prevalent HPV types from this study (HPV 16, 18, 31, 33, 45, 52, 58, and 73). The proportion of cancer cases attributed to the 8 most prevalent HPV types was 74.5% which corresponds to 961 cancer cases. The particular rates were 94.2% for SCC, 34.7% for VC and 81.8% for AC. The eight most prevalent HPV types account for 932 SCC, 66 VC, and 99 AC cases in the Czech republic (Figure 1).

Discussion

In this study we provide the largest summary data on the type-specific HPV type specific prevalence in the population of Czech women and men with diseases of the anogenital tract associated with HPV infection. Importantly, the prevalence rates of the vaccinal HPV types as well as those of HPV types which have shown a partial cross protection in clinical trials [33–36] are reported. The results of this study allow estimating potential benefit that can be achieved by the implementation of routine vaccination in the Czech Republic. Furthermore, these results will be used as inputs for models for estimating the impact of different strategies for the prevention of HPV-associated diseases.

In this study we analyzed 157 squamous cell carcinoma samples, 695 precancerous lesion samples and 64 condylomata acuminata samples from different anatomical locations. A very sensitive method was used, which is based on the amplification of a short DNA fragment of HPV L1 ORF and allows detection of multiple HPV infections [26]. This method is also recommended for HPV detection in primary screening by the European Guidelines for Quality Assurance in Cervical Cancer Screening [37]. Furthermore, the classification of all samples analyzed in this study, except for 14% of CIN1 samples, was confirmed by histology.

The data on HPV prevalence in precancerous cervical lesions and invasive cervical cancer cases in the Czech Republic were evaluated previously on another set of specimens [21]. The HPV prevalence was much lower in the previous study compared to the present one (53% vs. 62% in CIN1 samples, 58% vs. 88% in CIN2/3 samples and 74% vs. 95% SCC samples) as were the numbers of different HPV types (16 HR, 5 LR und 1 undetermined HPV types vs. 17 HR, 9 LR HPV types and 2 undetermined). Even though yielding very important results, our previous study had some limitations. Relatively small numbers of precancerous cervical lesion samples and cervical carcinoma samples were analyzed (87 CIN1, 88 CIN2+, and 49 SCC samples). The PCR method then used for HPV detection, in wide use at that time, has shown limited sensitivity in comparison to other newly introduced ones [38]. Finally, the severity of precancerous lesions was not confirmed for all cases by histology in our previous study. Therefore, the discrepancy in results between our two studies can be most likely attributed to the above-mentioned factors.

The published data on the type-specific HPV prevalence in patients with HPV-associated diseases in the Central and East European countries are scarce and most studies have analyzed only very small numbers of specimens. While more studies from Central and East Europe on HPV prevalence in CC were published and included in the meta-analyses [9], the HPV

Table 4. Comparison of overall and type-specific HPV prevalence between CIN1 and SCC, CIN2 and SCC, and CIN3 and SCC cases.

HPV type	CIN1:SCC			CIN2:SCC			CIN3:SCC		
	PREVALENCE RATIO			PREVALENCE RATIO			PREVALENCE RATIO		
	RR	95%CI	P	RR	95%CI	P	RR	95%CI	P
All**	1.5	1.40–1.70	<0.0001	1.2	1.11–1.39	0.0002	1.0	0.96–1.08	0.784
Any HR***	1.7	1.50–1.84	<0.0001	1.3	1.13–1.43	<0.0001	1.0	0.96–1.08	0.786
7 HR****	1.8	1.61–2.05	<0.0001	1.7	1.44–2.06	<0.0001	1.0	0.95–1.09	0.814
16	2.6	2.09–3.19	<0.0001	1.8	1.37–2.28	<0.0001	1.1	0.92–1.27	0.402
18	1.5	0.66–3.54	0.312	1.8	0.59–5.50	0.372	1.5	0.59–3.69	0.430
31	0.8	0.35–1.90	0.826	0.9	0.32–2.33	1.000	0.5	0.22–1.21	0.157
33	1.5	0.74–3.20	0.256	0.9	0.40–1.99	0.824	0.8	0.37–1.52	0.450
45	2.0	0.87–4.44	0.117	1.5	0.56–3.91	0.589	3.1	1.11–8.67	0.034
56	0.7	0.15–2.87	0.745	0.7	0.12–3.44	0.698	0.8	0.16–3.77	1.000
58	0.8	0.23–2.66	1.000	1.3	0.27–6.24	1.000	0.6	0.18–2.22	0.564

95%CI = 95% confidence interval, RR = relative risk, P = probability.
**all HPV types detected (LR and HR).
***all HR HPV types detected.
****HR HPV types 16, 18, 31, 33, 45, 56, and 58.
doi:10.1371/journal.pone.0021913.t004

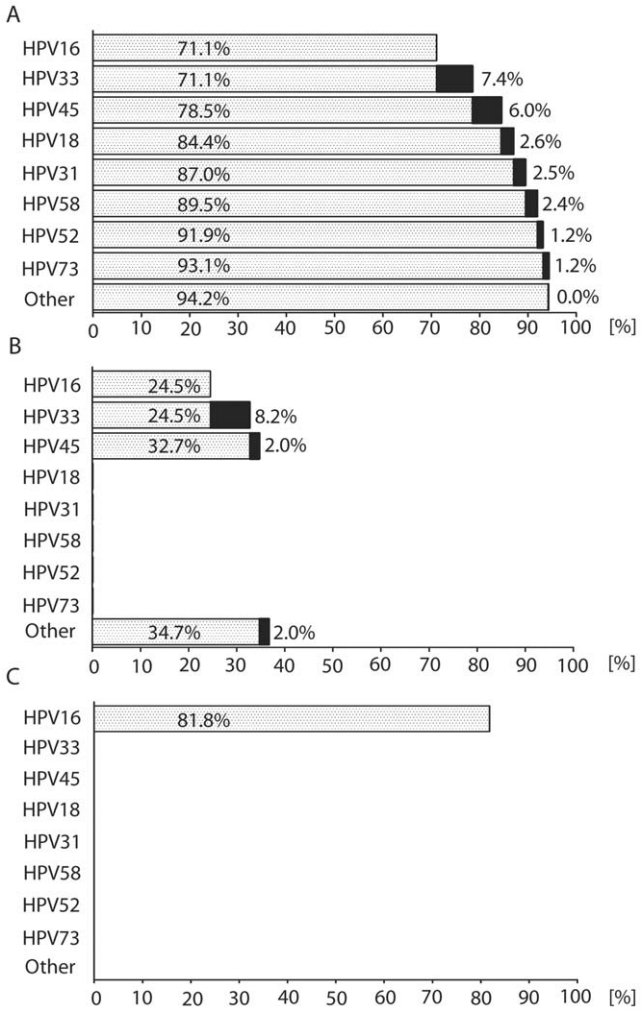


Figure 1. Cumulative percentages of cancer cases of women and men in the Czech Republic. Cumulative percentages of cervical (A), vulvar (B) and anal (C) cancer cases in women and men occurring every year in the Czech Republic that are attributed to eight most prevalent HPV types (990, 189 and 121 incident cancer cases, respectively). (Sdapted from Munoz, 2004)[46].
doi:10.1371/journal.pone.0021913.g001

prevalence in CIN1 samples was only reported for the Czech republic [7] and that in CIN2+ samples for the Czech Republic and Hungary [9,13]. Recently few additional studies from the Central and East European region have been published [17,39,40], but only that of Bardin et al. [17] reported on a larger number of SCC patients. Two meta-analyses [9,13] have concordantly shown an overall rate of 85% of HPV positivity of SCC samples in Europe. The prevalence rates ranged from 53 to 100% and that for the Czech Republic is 95%, as determined in this study. The most prevalent HPV types in SCC in Europe are, in descending order, HPV 16, HPV 18, and HPV 45. In our study, the second most common type was HPV 33, followed by HPV 45 and HPV 18. Since only squamous cell carcinoma samples were included in our study, the reported HPV type prevalence data is in agreement with the results of the meta-analysis of Smith et al. who has shown variation in type-specific HPV prevalence between squamose cell carcinomas and adeno-carcinomas [9]. In accordance with Smiths data for the Europe region, HPV 56 is more common in the Czech Republic than HPV 52 which is more prevalent in SCC in other regions of the world [41]. Despite the fact that HPV 35 was the sixth most prevalent type in SCC in the recently published study by de Sanjose et al. [41], we didnt detect this HPV type in our cohort of SCC patients. In the previous meta-analyses the majority of studies from Europe didnt find HPV 35 in SCC as well, regardless of method used for HPV detection [9,13]. Since in the WHO proficiency study [31] both methods; SPF-10 PCR used in the recent study and GP5+/6+ RLB used in our study, proofed to be very sensitive for the detection of HPV 35, we conclude that discrepant findings can be explained only by the differences in the number of cases studied (86 SCC specimens vs. 2093 SCC specimens from Europe) [41].

The type-specific HPV prevalence in CIN2+ samples found in our study, is the same as the data reported for Europe (88%), with the exception of HPV 16 and/or 18 (61 vs. 52%) [9]. The prevalence rates of other HPV types detected were similar to those observed in Europe, apart from HPV 73 that was not recovered from CIN2+ cases in the Czech Republic, most likely as a result of the use of a less sensitive assay for the detection of HPV 73 [42].

In comparison to the summary data for Europe as published by Clifford et al. [7], the type-specific HPV prevalence in CIN1 cases in our study was quite different. We detected about a one third higher prevalence of HPV 16 (28 vs. 19%), but much lower prevalence rates of HPV 59 (1% vs. 3%), HPV 39 (0.3% vs. 3%), HPV 66 (2% vs. 6%), HPV 52 (2% vs. 5.4%), and HPV 53 (0% vs. 3.7%). The spectrum of HPV types present in CIN1 cases is much wider in comparison to CIN2+ and SCC, with the low prevalent types being more common. Our group has previously reported that the detection of low prevalent types can vary greatly between different assays and that RLB with GP5+/6+ primers has lower sensitivity for HPV 52, 53, and 59 [42]. This could explain some of the discrepant findings.

The present study has shown a significantly higher prevalence of any HPV type, as well as of HR HPV type among SCC cases in comparison to CIN1 ($p<0.0001$ for both)and CIN2 cases ($p = 0.0002$ and $p<0.0001$, respectively) (Table 4). The prevalence was also higher for the seven HPV types most prevalent in SCC. HPV 16 was significantly more prevalent in SCC cases in comparison both to CIN1 ($p<0.0001$) and CIN2 ($p<0.0001$) cases but not to CIN3 cases ($p = 0.402$). Prevalence ratios above one were recorded for HPV 18, 45 and 58, while for other HPV types, the ratios ranged between 0.5 and 0.9, but except for HPV 45 ($p = 0.034$) in CIN3 in comparison to SCC, the differences were not statistically significant. Since our data are comparable to the ratios reported by Clifford et al. [6] for a large number of cases, we conclude that the lack of statistical significance for HPV types other than HPV 16 is due to the small numbers of subjects positive for HPV types other than HPV 16.

A meta-analysis of HPV prevalence studies in precancerous lesions and vulvar and anal carcinomas [11,12] included our previously published data from the Central and Eastern European region and those from Poland and Austria. Recently studies of Kowalewska et al. [43] and Garland et al. [44] have reported on HPV prevalence in vulvar cancer in Poland and Austria. In our study, the HPV prevalence in VIN cases was 100%. Most other studies which have reported comparably high prevalence rates only included patients with VIN 3. We have detected HPV in all VIN samples, including VIN 1 and 2. The most prevalent type was HPV 16, followed by HPV 33, 18, and 45. This finding is in agreement with the summary data reported by de Vuyst et al. [12], except the prevalence rates of HPV 33 and 45 were higher in this study. The lower average rates in the meta-analysis are due much

lower prevalence of HPV 33 in many studies and almost no detection of HPV 45 in VIN.

The overall prevalence of HPV as well as HPV type distribution in VC is in our study comparable to other studies. Nowadays it is widely accepted that about 40% of VC cases can be etiologically linked to HPV.

Importantly, focused on the prevalence of both vaccinal and cross-reactive HPV types, this study revealed that altogether 43% of precancerous lesions of the cervix and vulva (33% of CIN1, 43% of CIN2, 71% of CIN3 and 72% of VIN) are caused by HPV 16 and/or 18 and additional 20% by HPV types related to the vaccinal ones (HPV 31, 45, 33, 52, 58). Therefore, a substantial number of precancerous lesions can be considered preventable by prophylactic vaccination in the Czech Republic. The vaccinal LR HPV types HPV 6 and/or 11 were detected in 5% of CIN1, as few as 1% of CIN2 and 0.5% CIN3 and 9% of VIN cases.

The overall prevalence of HPV 16 and/or 18 among the analyzed cancer cases was 61% and that of the closely or distantly related types was 12%. The lowest prevalence of HPV 16/18 was observed in VC cases. Based on our data, the development of vulvar cancer can be prevented in about half of cases, thus reducing the need for mutilating surgery that dramatically reduces quality of life for patients.

The rate of SCC cases attributable to HPV 16/18 infection in the Czech population is 76%. Even higher is the involvement of HPV 16/18 in AC cases (82%). In view of cross-protective effect of the available vaccines, we can expect the potential benefit from vaccination against HPV in preventing SCC to be as high as 92% for the Czech population.

Finally, specimens with the histologically confirmed presence of condylomata acuminata were analyzed. Even though only a limited number of samples were available, the information is very important for the planning of the preventive strategies. We have shown that 89% of these lesions are infected by LR HPV types, with vaccinal types HPV 6/11 being present in 84% of them. These data should be taken into account when considering population-based prophylactic vaccination against HPV.

In conclusion, our study reports on the type-specific prevalence of HPV in benign, premalignant and malignant lesions of the anogenital tract in women and man. The prevalence and spectrum of HPV types detected in the Czech Republic are comparable to the data reported for European countries. The observed differences can be mostly attributed to the variation in the methods used for HPV detection. The proportion of patients infected with vaccinal and closely or distantly related HPV types is much higher than originally proposed. Approximately 952 of 1300 incident cancer cases (CC, VC and AC) and 921 of 990 CC cases can be attributed to these HPV types in the Czech Republic. Furthermore, it has been shown that the implementation of routine vaccination not only resulted in decrease in incidence of atypical cervical cytology and precancerous cervical lesions but also in the reduced need for colposcopy and invasive treatment procedures [45]. Therefore, we strongly advocate a rapid implementation of routine HPV vaccination in the Czech Republic which can significantly reduce the burden of HPV-associated diseases as well as the national healthcare expenditures.

Acknowledgments

The authors thank Bohumír Prochazka for statistical analyses and Pavlína Jarolímková for expert technical assistance.

Author Contributions

Conceived and designed the experiments: RT EH LR. Performed the experiments: MS JS PS TJ. Analyzed the data: RT EH LR MA. Contributed reagents/materials/analysis tools: RT LR. Wrote the paper: RT EH MA JS.

References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, et al. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189: 12–19.
2. zur Hausen H, Gissmann L, Steiner W, Dippold W, Dreger I (1975) Human papilloma viruses and cancer. Bibl Haematol. pp 569–571.
3. Ho GY, Burk RD, Klein S, Kadish AS, Chang CJ, et al. (1995) Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. J Natl Cancer Inst 87: 1365–1371.
4. Gillison ML, Shah KV (2003) Chapter 9: Role of mucosal human papillomavirus in nongenital cancers. J Natl Cancer Inst Monogr. pp 57–65.
5. de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, et al. (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 7: 453–459.
6. Clifford GM, Smith JS, Aguado T, Franceschi S (2003) Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 89: 101–105.
7. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, et al. (2005) Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 14: 1157–1164.
8. Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005) Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 14: 467–475.
9. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, et al. (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 121: 621–632.
10. Hoots BE, Palefsky JM, Pimenta JM, Smith JS (2009) Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int J Cancer 124: 2375–2383.
11. Smith JS, Backes DM, Hoots BE, Kurman RJ, Pimenta JM (2009) Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. Obstet Gynecol 113: 917–924.
12. De VH, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S (2009) Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer 124: 1626–1636.
13. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S (2003) Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 88: 63–73.
14. Parkin DM, Bray F (2006) Chapter 2: The burden of HPV-related cancers. Vaccine 24 Suppl 3: S3–11-S3/25.
15. Cancer incidence 2007 in the Czech Republic (2010) UZIS CR, NOR CR.
16. Arbyn M, Autier P, Ferlay J. Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. Ann Oncol 2007 18: 1423–5.
17. Bardin A, Vaccarella S, Clifford GM, Lissowska J, Rekosz M, et al. (2008) Human papillomavirus infection in women with and without cervical cancer in Warsaw, Poland. Eur J Cancer 44: 557–564.
18. Kornya L, Cseh I, Deak J, Bak M, Fulop V (2002) The diagnostics and prevalence of genital human papillomavirus (HPV) infection in Hungary. Eur J Obstet Gynecol Reprod Biol 100: 231–236.
19. Kulmala SM, Shabalova IP, Petrovitchev N, Syrjanen KJ, Gyllensten UB, et al. (2007) Prevalence of the most common high-risk HPV genotypes among women in three new independent states of the former Soviet Union. J Med Virol 79: 771–781.
20. Speich N, Schmitt C, Bollmann R, Bollmann M (2004) Human papillomavirus (HPV) study of 2916 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. J Med Microbiol 53: 125–128.
21. Tachezy R, Hamsikova E, Hajek T, Mikyskova I, Smahel M, et al. (1999) Human papillomavirus genotype spectrum in Czech women: correlation of HPV DNA presence with antibodies against HPV-16, 18, and 33 virus-like particles. J Med Virol 58: 378–386.
22. Tachezy R, Mikyskova I, Ludvikova V, Rob L, Kucera T, et al. (2006) Longitudinal study of patients after surgical treatment for cervical lesions: detection of HPV DNA and prevalence of HPV-specific antibodies. Eur J Clin Microbiol Infect Dis 25: 492–500.
23. Skapa P, Zamecnik J, Hamsikova E, Salakova M, Smahelova J, et al. (2007) Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. Am J Surg Pathol 31: 1834–1843.
24. Tachezy R, Jirasek T, Salakova M, Ludvikova V, Kubecova M, et al. (2007) Human papillomavirus infection and tumours of the anal canal: correlation of histology, PCR detection in paraffin sections and serology. APMIS 115: 195–203.

25. Arbyn M, Van Veen EB, Andersson K, Bogers J, Boulet G, et al. (2010) Cervical cytology biobanking in Europe. Int J Biol Markers 25: 117–125.
26. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, et al. (2002) GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 40: 779–787.
27. IARC Monograph Working Group (2007) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 90, Lyon: IARC.
28. Bernard HU, Burk RD, Chen Z, Van DK, Hausen H, et al. (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401: 70–79.
29. Munoz N, Bosch FX, de SS, Herrero R, Castellsague X, et al. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518–527.
30. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, et al. (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230: 1350–1354.
31. Eklund C, Zhou T, Dillner J (2010) Global proficiency study of human papillomavirus genotyping. J Clin Microbiol 48: 4147–4155.
32. Wentzensen N, Schiffman M, Dunn T, Zuna RE, Gold MA, et al. (2009) Multiple human papillomavirus genotype infections in cervical cancer progression in the study to understand cervical cancer early endpoints and determinants. Int J Cancer 125: 2151–2158.
33. Paavonen J, Naud P, Salmeron J, Wheeler CM, Chow SN, et al. (2009) Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 374: 301–314.
34. Jenkins D (2008) A review of cross-protection against oncogenic HPV by an HPV-16/18 AS04-adjuvanted cervical cancer vaccine: importance of virological and clinical endpoints and implications for mass vaccination in cervical cancer prevention. Gynecol Oncol 110: S18–S25.
35. Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, et al. (2009) The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. J Infect Dis 199: 926–935.
36. Wheeler CM, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, et al. (2009) The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16-26 years. J Infect Dis 199: 936–944.
37. Arbyn M, Anttila A, Jordan J, Ronco G, Schenck U, et al. (2010) European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition--summary document. Ann Oncol 21: 448–458.
38. Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kuhndel K, et al. (2004) PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. J Clin Virol 30: 302–308.
39. Szostek S, Klimek M, Zawilinska B, Kosz-Vnenchak M (2008) Genotype-specific human papillomavirus detection in cervical smears. Acta Biochim Pol 55: 687–692.
40. Shipitsyna E, Zolotoverkhaya E, Kuevda D, Nasonova V, Romanyuk T, et al. (2011) Prevalence of high-risk human papillomavirus types and cervical squamous intraepithelial lesions in women over 30 years of age in St. Petersburg, Russia. Cancer Epidemiol 35: 160–164.
41. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, et al. (2010) Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 11: 1048–1056.
42. Sabol I, Salakova M, Smahelova J, Pawlita M, Schmitt M, et al. (2008) Evaluation of different techniques for identification of human papillomavirus types of low prevalence. J Clin Microbiol 46: 1606–1613.
43. Kowalewska M, Szkoda MT, Radziszewski J, Ptaszynski K, Bidzinski M, et al. (2010) The frequency of human papillomavirus infection in polish patients with vulvar squamous cell carcinoma. Int J Gynecol Cancer 20: 434–437.
44. Garland SM, Insinga RP, Sings HL, Haupt RM, Joura EA (2009) Human papillomavirus infections and vulvar disease development. Cancer Epidemiol Biomarkers Prev 18: 1777–1784.
45. Munoz N, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, et al. (2010) Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. J Natl Cancer Inst 102: 325–339.
46. Munoz N, Bosch FX, Castellsague X, Diaz M, de SS, et al. (2004) Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 111: 278–285.

Příloha III.

Škapa P., Robová H., Rob L. a Zámečník J. (2012) Prekancerózní léze vulvy. ***Cesk Patol*** 48:15-21.

Prekancerózní léze vulvy

Škapa P.[1], Robová H.[2], Rob L.[2], Zámečník J.[1]

[1] Ústav patologie a molekulární medicíny UK 2. LF a FN Motol, Praha, Česká republika
[2] Gynekologicko-porodnická klinika UK 2. LF a FN Motol, Praha, Česká republika

SOUHRN
Klasifikace prekancerózních lézí vulvy je založena na konceptu vulvární intraepiteliální neoplázie (VIN) s třístupňovým hodnocením závažnosti dysplastických změn (VIN I, II a III). Na základě histologického vzhledu se dysplázie vulvy dělí na VIN obvyklého typu (u-VIN) a VIN diferencovaného typu (d-VIN), které reprezentují dvě základní cesty patogeneze dlaždicobuněčného karcinomu vulvy. Zatímco je u-VIN etiologicky spojená s infekcí lidským papillomavirem (HPV) a histologickým vzhledem odpovídá cervikální intraepiteliální neoplázii, představuje d-VIN HPV negativní směr karcinogeneze spojený s vulvárními dermatózami typu lichen sclerosus (LS) a lichen simplex chronicus (LSC). u-VIN většinou postihuje relativně mladé ženy s anamnézou dalších prekanceróz děložního hrdla, vagíny nebo vulvy. Typickou pacientkou s d-VIN je naopak postmenopauzální žena bez anamnestických dat ostatních dysplastických lézí dolního ženského pohlavního systému. d-VIN převyšuje u-VIN v tendenci ke stromální invazi a biologickým chováním odpovídá karcinomu in situ (VIN III). Paradoxně se v histologickém vyšetření d-VIN jeví jako nenápadná léze s atypiemi v buňkách bazální vrstvy a s dobře zachovanou diferenciací povrchových partií dlaždicového epitelu, proto bývá při bioptickém vyšetření zaměňována za u-VIN I, LS anebo LSC. Především z důvodu nízké diagnostické reprodukovatelnosti kategorie u-VIN I, pro pochybnosti o jejím maligním potenciálu a také kvůli problematickému rozlišení u-VIN II a III byla v roce 2004 předložena revidovaná klasifikace VIN, ve které bylo od gradingu prekancerózních lézí vulvy upuštěno - jednotka u-VIN I byla zrušena a u-VIN II a III byly spojeny do jediné kategorie. Termín u-VIN tedy v novém názvosloví reprezentuje high grade prekancerózní léze vulvy asociované s HPV (dříve u-VIN II a III) a d-VIN i nadále odpovídá HPV negativním high grade dysplázií.

Klíčová slova: vulvární intraepiteliální neoplázie – VIN obvyklého typu – VIN diferencovaného typu – lichen sclerosus – lichen simplex chronicus – HPV

Review of precancerous vulvar lesions

SUMMARY
Classification of squamous vulvar precancerous lesions is based on the concept of vulvar intraepithelial neoplasia (VIN) and incorporates a three grade evaluation of the intensity of dysplastic changes (VIN I, II and III). On the basis of histological features, VIN has been subdivided into the usual VIN (u-VIN) and differentiated VIN (d-VIN), which represent the two basic pathways of the pathogenesis of vulvar squamous cell carcinoma. Although u-VIN is etiologically associated with the human papillomavirus (HPV) infection and histologically corresponds to cervical intraepithelial neoplasia, d-VIN represents the HPV-negative sequence of vulvar carcinogenesis, which is linked to lichen sclerosus (LS) and lichen simplex chronicus (LSC). u-VIN preferentially occurs in relatively young women with a history of cervical, vaginal or vulvar premalignant lesions. On the other hand, d-VIN usually affects postmenopausal women without anamnestic data of other dysplastic lesions of the lower female genital tract. d-VIN is characterized by a higher tendency of stromal invasion than u-VIN and its malignant potential is analogous to carcinoma in situ (VIN III). The histological appearance of d-VIN is subtle with basal atypia and a well-preserved differentiation of the superficial parts of the squamous epithelium, therefore it is frequently misdiagnosed for u-VIN I, LS or LSC in vulvar biopsies. Primarily because of the low diagnostic reproducibility of the u-VIN I category and the doubts about its precancerous potential as well as due to the questionable differentiation between u-VIN II and III, a revised VIN classification was proposed in 2004. The grading of vulvar precancerous lesions was abandoned, the u-VIN I category was discontinued and u-VIN II and III were merged. In the revised terminology, the term u-VIN represents HPV-associated high grade precancerous vulvar lesions (formerly u-VIN II and III) and d-VIN encompasses HPV-negative high grade dysplasias.

Keywords: vulvar intraepithelial neoplasia – VIN of the usual type – VIN of the differentiated type – lichen sclerosus – lichen simplex chronicus – HPV

Cesk Patol 2012; 48(1): 15–21

SPOLEČNÝ KONCEPT PREKANCERÓZ DOLNÍHO ŽENSKÉHO POHLAVNÍHO SYSTÉMU

Vulva, vagína a děložní hrdlo tvoří komplexní anatomickou jednotku s obdobnou histologickou stavbou a predispozicí ke stejným rizikovým faktorům pro vznik prekancerózních lézí a karcinomů. V anglosaské literatuře existuje pro výše popsanou anatomickou oblast termín *lower female genital tract* (LFGT) odpovídající zřídka užívanému českému ekvivalentu *dolní ženský pohlavní systém*. Základním pojítkem v histologické stavbě orgánů LFGT je přítomnost povrchového dlaždicového epitelu, který je vnímavý k sexuálně pře-

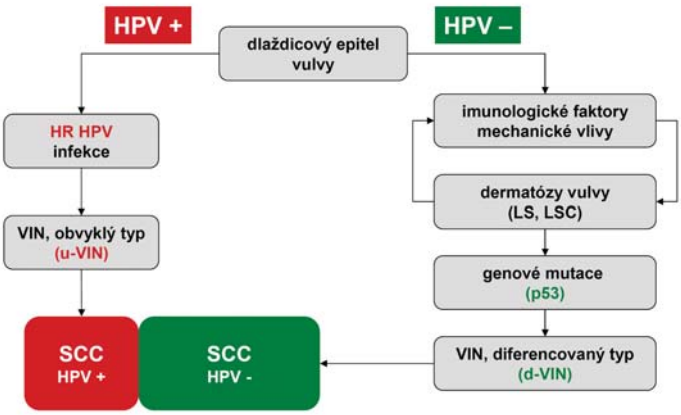
✉ ***Adresa pro korespondenci:***
MUDr. Petr Škapa
Ústav patologie a molekulární medicíny
UK 2. LF a FN Motol
V Úvalu 84, 150 06 Praha 5
tel: +420-224 435 623
fax: +420-224 435 620
e-mail: petr.skapa@lfmotol.cuni.cz

nesené infekci lidským papilomavirem (HPV). Nízce rizikové (LR) HPV typy (např. 6 a 11) jsou příčinou benigních afekcí bez prekancerózního potenciálu charakteru condylomata acuminata nebo plochých kondylomatózních lézí, zatímco vysoce rizikové (HR) typy (např. 16 a 18) mohou za určitých podmínek vyvolat sekvenci dysplastických změn směřujících až k invazivnímu dlaždicobuněčnému karcinomu (SCC) (1). Klinicky důležitou vlastností HPV pozitivních prekanceróz a karcinomů LFGT je jejich multicentrický výskyt nejen v rámci jednotlivého orgánu, ale i v rozsahu celého LFGT (včetně oblasti anu), kde se mohou v libovolných kombinacích rozvíjet simultánně nebo sukcesivně (2,3). Tento jev je popisován termínem *multicentrická neoplázie dolního ženského pohlavního systému* a v širším kontextu zahrnuje nejen dlaždicobuněčné léze LFGT, ale i glandulární dysplastické změny a invazivní adenokarcinomy endocervikální části děložního hrdla. Určitou výjimku v konceptu LFGT představuje pouze vulva, kde se ve významné míře kromě lézí asociovaných s HPV vyskytují i HPV negativní prekancerózy a karcinomy vznikající odlišnou etiopatogenetickou cestou (4–6).

Dysplastické léze dlaždicového epitelu LFGT jsou již tradičně popisovány v konceptu tzv. intraepiteliální neoplázie a dle anatomické lokalizace tedy odpovídají vulvární (VIN), vaginální (VaIN) a cervikální (CIN) intraepiteliální neoplázii. Závažnost dysplastických změn je ve shodě s aktuální WHO klasifikací vyjádřena třístupňovým gradingem (I, II a III) podle vertikálního rozsahu ztráty diferenciace, cytologických atypií a mitotické aktivity (7).

KLASIFIKACE PREKANCERÓZNÍCH LÉZÍ VULVY

Terminologie VIN vytěsnila z histopatologické praxe starší anebo vágně definované diagnostické jednotky charakteru Bowenova choroba, bowenoidní atypie, bowenoidní dysplázie, bowenoidní karcinom in situ, bowenoidní papulóza, erythroplasia de Queyrat a carcinoma in situ simplex. Moderní klasifikace dysplastických lézí vulvy vychází z návrhu *International Society for the Study of Vulvovaginal Disease (ISSVD)* (8), která byla předložena v roce 1986 a následně akceptována WHO jako diagnostický standard (7). Na rozdíl od CIN a VaIN jsou VIN heterogenní skupinou prekanceróz, které lze na základě jejich histologického vzhledu rozdělit do dvou základních jednotek: VIN obvyklého typu (usual VIN; u-VIN) a VIN diferencovaného (resp. simplexního) typu (differentiated VIN; d-VIN) (7,9). Tyto diagnostické jednotky se liší svými klinicko-patologickými vlastnostmi (tab. 1) a reprezentují dvě základní cesty etiopatogeneze SCC vulvy (obr. 1) (4,5).



Obr. 1. Grafické znázornění dvou hlavních cest etiopatogeneze SCC vulvy. u-VIN – vulvární intraepiteliální neoplázie obvyklého typu; d-VIN – vulvární intraepiteliální neoplázie diferencovaného typu; LS – lichen sclerosus; LSC – lichen simplex chronicus; SCC – dlaždicobuněčný karcinom; HPV – lidský papillomavirus; HR HPV – vysoce rizikové typy lidského papilomaviru

VIN obvyklého typu (u-VIN)

Hlavním etiologickým faktorem vzniku u-VIN je sexuálně přenesená infekce HR HPV (3,10,11). Nejpočetnější rizikovou skupinu proto tvoří relativně mladé ženy premenopauzálního věku, které mají i vyšší riziko vzniku ostatních dysplastických a nádorových procesů LFGT a častější výskyt pohlavních chorob (12). SCC asociované s u-VIN jsou též HPV pozitivní, rozvíjejí se v mladším věku a představují asi 30–40 % všech karcinomů vulvy (12). Histologicky odpovídají bazaloidním a warty variantám SCC (obr. 2).

Na základě histologického obrazu lze rozlišit 3 hlavní podtypy u-VIN: bazaloidní, warty (kondylomatózní) a smíšený (warty/bazaloidní) (tab. 2) (obr. 2) (9). Z praktického hlediska však toto členění postrádá většího významu, protože se od sebe jednotlivé subtypy u-VIN neliší klinickým chováním ani spektrem přítomných HPV typů (9). Identifikace u-VIN II a III jako dysplastické léze je většinou bezproblémová (13), protože u-VIN histologicky připomíná ostatní prekancerózy LFGT charakterizované ztrátou zralosti keratinocytů, cytologickými atypiemi, zvýšenou mitotickou aktivitou a případně známkami HPV infekce. Interpersonální a intrapersonální reprodukcibilita diagnózy u-VIN I je naopak velmi nízká (13) a bude blíže diskutována níže v souvislosti se změnami v klasifikaci VIN. Při jakýchkoli pochybnostech o diagnóze u-VIN doporučujeme provést imunohistochemický průkaz **proteinu p16^{INK4a}**, který se jako inhibitor cyklin dependentní kinázy uplatňuje v regu-

Tab. 1. Porovnání dvou základních typů vulvárních prekanceróz (u-VIN a d-VIN).

	VIN, obvyklý typ (u-VIN)	*VIN, diferencovaný typ (d-VIN)*
Celková četnost	více než 80 %	méně než 20 %
Věková predilekce	premenopauzální ženy	postmenopauzální ženy
Asociace s CIN a VaIN (multicentrická neoplázie LFGT)	ano	ne
Asociace s pohlavně přenosnými chorobami a s condylomata acuminata	ano	ne
Etiologický faktor	HR HPV	genové mutace (p53)
Kofaktory	kouření cigaret imunosuprese	dermatózy vulvy (LS, LSC)
Tendence k multifokalitě	silná	slabá
Potenciál ke stromální invazi	slabý	silný
Imunohistochemický marker	p16^{INK4a}	p53
Asociovaný SCC	bazaloidní SCC warty SCC	dobře diferencovaný keratinizující SCC

VIN – vulvární intraepiteliální neoplázie; CIN – cervikální intraepiteliální neoplázie; VaIN – vaginální intraepiteliální neoplázie; LFGT – lower female genital tract; HR HPV – high risk typy lidského papillomviru; LS – lichen sclerosus; LSC – lichen simplex chronicus; SCC – dlaždicobuněčný karcinom

Tab. 2. Základní histologické charakteristiky prekancerózních lézí vulvy a dalších asociovaných afekcí.

Diagnostická jednotka		Histopatologická kritéria
VIN, obvyklý typ (u-VIN)	*warty*	- kondylomatózní vzhled - akantóza, hyperkeratóza a parakeratóza - koilocytóza a koilocytární atypie - vícejaderné keratinocyty
	bazaloidní	- plochá léze bez hyperkeratózy a parakeratózy - dysplastické keratinocyty jsou malé a připomínají bazální buňky epidermis - absence známek HPV infekce
	smíšená	- komplexní léze s warty i bazaloidními rysy
VIN, diferencovaný typ (d-VIN)		- akantóza epidermis s elongací rete ridges a s parakeratózou - ztráta stratum granulosum - atypické buňky lokalizované v bazálních partiích epidermis s mitotickou aktivitou a aberantní keratinizací - suprabazilární partie epidermis tvořené abnormálně diferencovanými keratinocyty s objemnou eozinofilní cytoplazmou a vezikulárními jádry s jadérky - absence známek HPV infekce
Lichen sclerosus (LS)		- atrofie epidermis s hyperkeratózou - edém a hyalinizace papilární dermis - lymfocytární infiltrát v horní dermis
Lichen simplex chronicus (LSC)		- hyperplázie epidermis s elongací rete ridges - akantóza, hypergranulóza a hyperkeratóza epidermis - fibrotizace papilární dermis s lymfocytárním infiltrátem - hyalinizace horní dermis (pokud je LSC superponovaný na LS)

laci buněčného cyklu a bývá imunohistochemicky prokazatelný v dysplastických lézích a karcinomech vulvy asociovaných s HR HPV infekcí (5,14). K interpretaci nálezu ve smyslu $p16^{INK4a}$ pozitivity je nutná silná difuzní jaderná anebo současně jaderná a cytoplazmatická pozitivita, fokální slabé přibarvování cytoplazmy neopravňuje k hodnocení léze jako pozitivní (5,14).

Pagetoidní VIN je vzácná vulvární prekanceróza připomínající Pagetovu chorobu vulvy, která je většinou autorů vnímána jako histologická varianta u-VIN (9). Léze je charakterizovaná přítomností atypických buněk se světlou cytoplazmou, které se intraepiteliálně šíří ve formě jednotlivých buněk anebo buněčných skupin. Diferenciálně diagnostickými rozdíly oproti Pagetově chorobě jsou nepřítomnost intracytoplazmatického mucinu a imunohistochemická negativita GCDFP-15, cytokeratinu 7 a karcinoembryonálního antigenu (CEA) (9).

VIN diferencovaného typu (d-VIN)

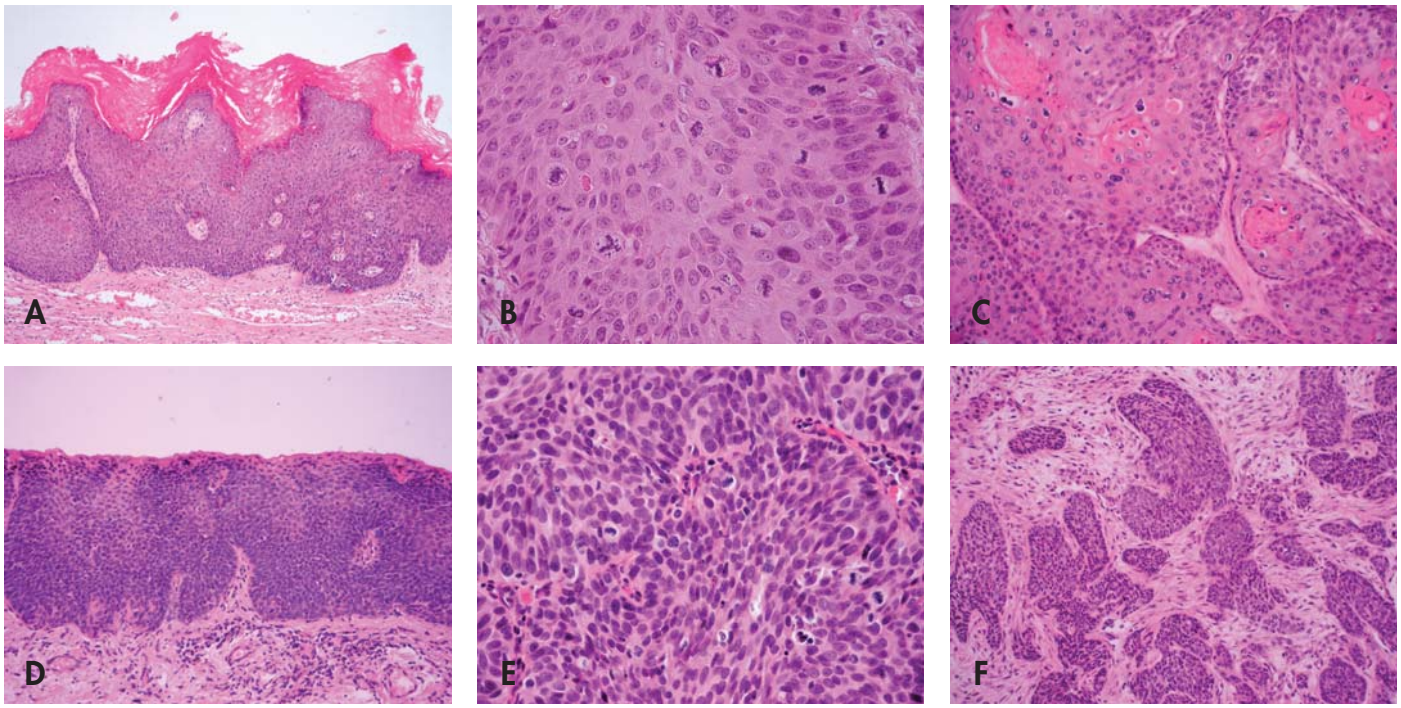
Jako samostatná diagnostická jednotka byla d-VIN poprvé popsána už v 60. letech 20. století jako *carcinoma in situ simplex*, ale do povědomí širší odborné veřejnosti se dostává až v posledních letech. Jde o poměrně vzácnou dysplastickou lézi s frekvencí výskytu kolísající v rozsahu 2–18 % všech prekanceróz vulvy, která postihuje především postmenopauzální ženy a nemá etiopatogenetický vztah k HPV infekci (10,15). Vyvolávající faktory d-VIN nebyly dosud zcela jednoznačně identifikovány, jako nejpravděpodobnější se však jeví genové mutace v terénu vulvárních dermatóz typu lichen sclerosus (LS) a lichen simplex chronicus (LSC), které se rozvíjejí v terénu imunologické predispozice (16). Pruritus způsobený vulvární dermatózou vede k chronické mechanické iritaci vulvy s možností vzniku ulcerací, reaktivní proliferací epitelu a následnému zhoršení vyvolávající vulvární dermatózy. Tento efekt se v literatuře někdy označuje jako *itch-scratch* cyklus (16). V pokročilé fázi onemocnění bývá jeho následkem většinou LSC, který může být superponován na LS (tab. 2). Termín „dlaždicobuněčná hyperplázie" používaný dříve v gynekopatologii pro hyperplastické dermatózní procesy vulvy je pouze popisné vyjádření histologického vzhledu epidermis a v naprosté většině případů odpovídá právě nozologické jednotce LSC (17). Ve vulvární dermatóze je při chronické mechanické iritaci vyšší riziko vzniku genových mutací především v tumor supresorovém genu p53 (18). Následkem je rozvoj d-VIN a progrese v keratinizující typ SCC, který bývá většinou dobře diferencovaný, HPV negativní a reprezentuje asi 60–70% všech karcinomů vulvy (16,19). Literární údaje dokládají, že až 2–6 % pacientek s diagnózou LS rozvine SCC vulvy (12).

d-VIN je makroskopicky i histologicky velmi nenápanou lézí, u které nemusí být na první pohled patrné, že se jedná o dysplázii (tab. 2) (obr. 3, 4) (15). Pokročilý stupeň histologické diferenciace a epiteliální maturace ostatně naznačuje již vlastní název této diagnostické jednotky. S relativně klidným histologickým vzhledem d-VIN však ostře kontrastuje její biologické chování, které odpovídá karcinomu in situ. Léze tedy automaticky spadá do kategorie VIN III a grading v pravém slova smyslu s hodnocením rozsahu ztráty vyzrávání, cytologických atypií a mitotické aktivity se proto neprovádí. Pro d-VIN je typický větší invazivní potenciál spojený s kratší intraepiteliální fází a rychlejší progresí do SCC ve srovnání s u-VIN (15).

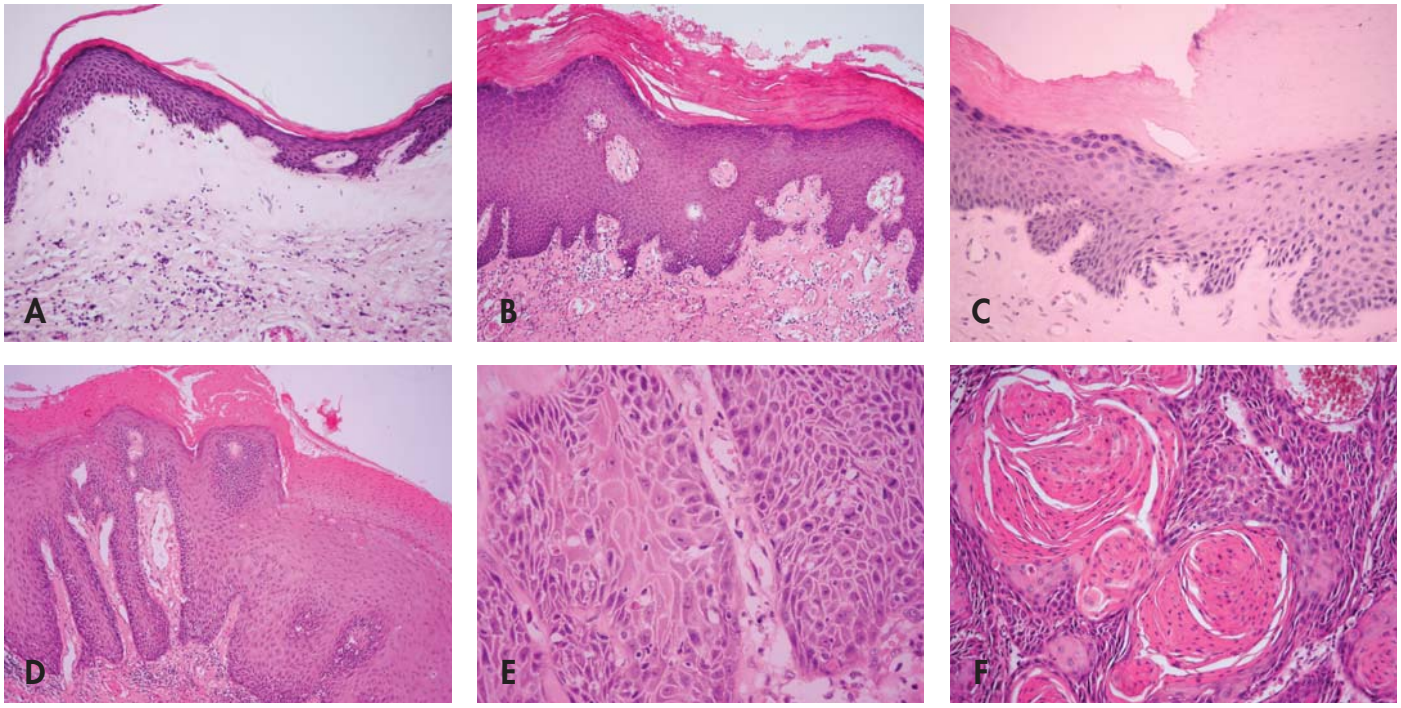
Pro gynekologa je d-VIN v terénu kožních změn při vulvární dermatóze obtížně identifikovatelná, zvláště pokud se jedná o lézi solitární. Podobný problém čeká i patologa během histologického vyšetření, protože dlaždicový epitel d-VIN směrem k povrchu dostatečně vyzrává a celá léze tak připomíná vulvární dermatózu. Podle našich zkušeností bývá přechod mezi d-VIN a dermatózou náhlý a je charakterizován následujícími histologickými znaky (obr. 3 a obr. 4):

1) výskyt cytologických atypií a zvýšená mitotická aktivita v bazální zóně epidermis,
2) dyskeratotické změny v bazálních a suprabazilárních zónách epidermis,
3) výskyt suprabazilárních keratinocytů s objemnou eozinofilní cytoplazmou a vezikulárními jádry,
4) ztráta granulární vrstvy epidermis,
5) přechod superficiální hyperkeratózy do parakeratózy.

K diagnóze d-VIN je nutná kombinace výše uvedených kritérií, protože žádné z nich není samo o sobě specifické pro d-VIN a mohou se vyskytovat při výraznější iritaci a proliferaci i v dermatózách vulvy. To platí i pro atypie v buňkách bazální vrstvy epitelu, které



Obr. 2. Prekancerózní léze vulvy a dlaždicobuněčné karcinomy asociované s HPV infekcí.
A – u-VIN III, warty (HE, 40x); **B** – u-VIN III, warty (HE, 400x); **C** – SCC, warty (HE, 200x); **D** – u-VIN III, bazaloidní (HE, 100x); **E** – u-VIN III, bazaloidní (HE, 400x); **F** – SCC, bazaloidní (HE, 100x)



Obr. 3. Nenádorové, prekancerózní a nádorové léze vulvy sdružené v HPV negativní cestě karcinogeneze.
A – LS (HE, 100x); **B** – LSC (HE, 40x); **C** – přechodová zóna mezi LSC a d-VIN (HE, 200x); **D** – d-VIN (HE, 40x); **E** – d-VIN (HE, 400x); **F** – SCC keratinizující (HE, 200x)

jsou někdy popisovány ve vulvárních dermatózách a je pro ně vyhrazen termín *atypický lichen sclerosus* (19,20). Obtížnost diferenciální diagnózy mezi d-VIN a vulvární dermatózou typu LS anebo LSC je tedy zřejmá. Z praktického hlediska doporučujeme patologům zaměřit se při skenovacím prohlížení preparátu především na náhlou transformaci hyperkeratózy v parakeratózu a s tím související ztrátu granulární vrstvy epidermis a tyto suspektní úseky dále důkladně vyšetřit při větším zvětšení s důrazem na morfologické změny v bazálních vrstvách epitelu. V případě diagnostických pochybností lze provést imunohistochemické vyšetření proteinového produktu tumor supresorového genu p53, které bývá v případě mutace genu pozitivní (15,18). Ke správné interpretaci imunohistochemického nálezu je však nutno mít na vědomí, že v bazálních vrstvách epitelu vulvárních dermatóz dochází nejspíše z důvodu oxi-

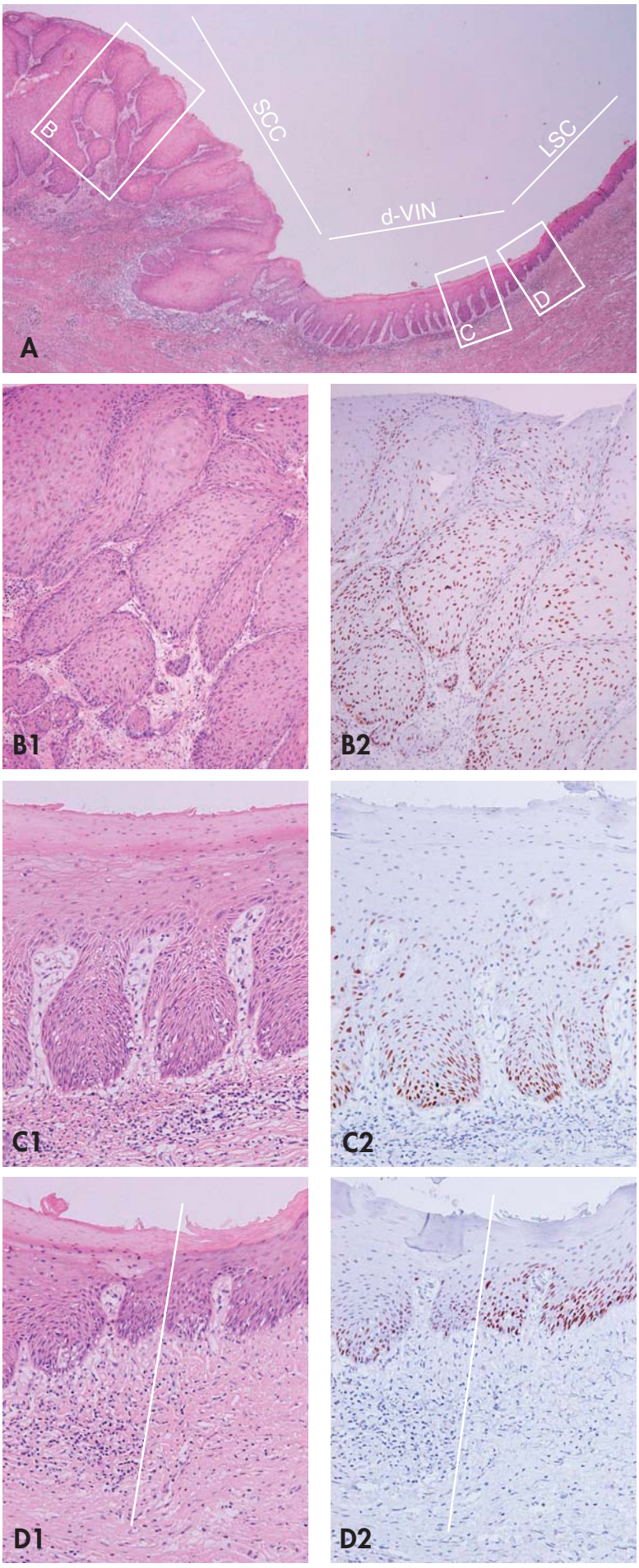
dačního stresu k přibarvování jader keratinocytů (21) a že p53 pozitivita d-VIN je proto definována *suprabazilární extenzí* pozitivity a zvýšeným *labeling indexem* (poměr počtu p53 pozitivních jader k jádrům negativním) často až nad 90 % (15). Specificita a senzitivita imunohistochemického průkazu p53 však není optimální (22), proto je význam tohoto vyšetření některými autory zpochybňován (21). Z naší zkušenosti můžeme potvrdit, že imunohistochemická pozitivita markeru p53 bývá u části d-VIN skutečně variabilní nebo neprůkazná a že p53 pozitivitu lze často pozorovat i v bazálních partiích epitelu vulvárních dermatóz (obr. 4). Při obtížnější diferenciální diagnóze mezi d-VIN a u-VIN lze s úspěchem provést imunohistochemický průkaz proteinu $p16^{INK4a}$, jehož pozitivita svědčí pro u-VIN a negativita pro d-VIN (5). Materiálně náročnější metodou může být stanovení přítomnosti HR HPV metodou polymerázové řetězové reakce (PCR). Pokud přes veškerou snahu není možno prekancerózu vulvy zařadit ani do jedné ze dvou základních kategorií, doporučujeme lézi klasifikovat jako VIN, blíže nezařazenou (VIN, NOS).

Nesoulad mezi procentuálním zastoupením u-VIN a d-VIN a téměř opačným poměrem četnosti HPV pozitivních a HPV negativních SCC vulvy lze vysvětlit výrazným poddiagnostikováním d-VIN (15). Příčinou je makroskopická i histologická podobnost s dermatózami vulvy, proto je d-VIN často přehlédnuta gynekologem a uniká bioptickému vyšetření nebo je nesprávně klasifikována patologem jako vulvární dermatóza. K nižšímu záchytu d-VIN dále vede i vyšší věk pacientek, který je spojen se sporadickou frekvencí návštěv gynekologa. V neposlední řadě má vliv i vysoký sklon d-VIN ke stromální invazi, což vede ke zkrácení intervalu, kdy je možno lézi klinicky zachytit a biopticky ověřit.

I když je existence d-VIN většinou gynekopatologů v současnosti přijímána, objevují se i názory, které ji zpochybňují jako samostatnou diagnostickou jednotku a považují ji pouze za intraepiteliální šíření simultánně se vyskytujícího SCC (21,23). Proti tomuto tvrzení však svědčí dobře zdokumentovaná pozorování rychlé progrese d-VIN do SCC (24,25).

MODIFIKACE KLASIFIKAČNÍHO SYSTÉMU VULVÁRNÍCH INTRAEPITELIÁLNÍCH NEOPLÁZIÍ

V předchozích odstavcích jsme nastínili etiologickou i histologickou podobnost u-VIN s CIN, nicméně v tomto srovnání existují i určité podstatné rozdíly, které v konečném důsledku vedly ke změně klasifikace vulvárních pre-



Obr. 4. Biopsie vulvy zastihující komplexní HPV negativní lézi tvořenou prekancerózou (d-VIN) v přímé kontinuitě s vulvární dermatózou (LSC) a dlaždicobuněčným karcinomem (SCC). Imunohistochemické vyšetření antigenu p53 demonstruje téměř difuzní pozitivitu jader nádorových buněk v SCC a pozitivitu buněk bazálních vrstev epitelu d-VIN se suprabazilární extenzí. Povšimněte si též silné jaderné pozitivity p53 v bazálních a suprabazilárních partiích LSC, která je v přechodové zóně intenzivnější než pozitivita v d-VIN. Nález příkladem z praxe demonstruje problematickou specificitu imunohistochemického vyšetření p53 pro diagnózu d-VIN (blíže diskutováno v textu).
A – kontinuální přechod LSC – d-VIN – SCC s vyznačením jednotlivých zón (20x); **B1** – SCC (HE, 40x), **B2** – SCC (p53, 40x); **C1** – d-VIN (HE, 100x); **C2** – d-VIN (p53, 100x); **D1** – přechodová zóna mezi LSC a d-VIN (HE, 100x); **D2** – přechodová zóna mezi LSC a d-VIN (p53, 100x)

Tab. 3. Srovnávací tabulka klasifikace ISSVD 1986 (aktuální WHO terminologie), modifikovaného schématu ISSVD 2004 a historických termínů dříve užívaných ke klasifikaci vulvárních prekanceróz.

ISSVD 1986		ISSVD 2004		Historická terminologie
Histologický typ	*Grade*	*Histologický typ*	*Grade*	
VIN, obvyklý typ (u-VIN)	I	Reaktivní změny HPV infekce Plochá kondylomatózní léze	–	Bowenova choroba Bowenoidní atypie Bowenoidní dysplázie Bowenoidní karcinom in situ Bowenoidní papulóza Erythroplasia de Queyrat
	II III	VIN, obvyklý typ (u-VIN)	–	
VIN, diferencovaný typ (d-VIN)	III	VIN, diferencovaný typ (d-VIN)	–	Carcinoma in situ simplex

VIN – vulvární intraepiteliální neoplázie; ISSVD – International Society for the Study of Vulvovaginal Disease

kanceróz. Hlavní nesoulad tkví v rozdílu frekvencí low grade dysplázií děložního hrdla a vulvy (CIN I a u-VIN I) (9). CIN I je běžnou skupinou lézí, mezi které se v širším kontextu mohou řadit i HPV indukované změny charakteru plochého kondylomu. Ploché kondylomatózní léze jsou však v oblasti vulvy velmi vzácné a naopak se zde často vyskytuje condyloma acuminatum, které postrádá maligní potenciál a diagnosticky nespadá do kategorie u-VIN I. Naprostou většinu případů u-VIN tedy tvoří high grade dysplastické léze a u-VIN I je v biopsiích vulvy diagnostikována zřídka, často pouze v přímé kontinuitě s u-VIN II a III. Solitárně se vyskytující u-VIN I bez asociace s u-VIN II a III jsou výjimečným nálezem a jejich interpersonální a intrapersonální reprodukcibilita při histopatologickém vyšetření je velmi nízká (13). Při retrospektivní expertní analýze souboru u-VIN I byla podstatná část těchto lézí překlasifikována a striktní diagnostická kritéria jich splnilo pouze 19 % (26). Další poměrně častou chybou v interpretaci atypií buněk bazální vrstvy epitelu ve vulvární biopsii je záměna u-VIN I s d-VIN anebo s vulvární dermatózou charakteru LS anebo LSC (15). Z důvodu nepřesné histopatologické diagnostiky proto může být v rutinní praxi kategorie u-VIN I tvořena směsí lézí s různým prekancerózním potenciálem, což poněkud snižuje její význam jako samostatné diagnostické jednotky. Dalším problémem v histopatologickém gradingu u-VIN je nízká reprodukcibilita odlišení kategorií u-VIN II a III. Jejich rozpoznání je často obtížné až zcela nemožné, neboť část lézí je komplexně stavěná a tvořená oběma komponentami.

Výše uvedené skutečnosti vedly v uplynulé dekádě k pokusům o změnu klasifikačního schématu VIN s hlavním cílem zlepšit diagnostickou reprodukcibilitu jednotlivých kategorií. Výsledkem této snahy je klasifikace *ISSVD 2004* a klasifikace vycházející z cytologické Bethesda terminologie.

Klasifikace ISSVD 2004

V roce 2004 *ISSVD* předložila zjednodušenou verzi původní *ISSVD 1986* klasifikace (tab. 3) (27). Mezi původní a modifikovanou klasifikací VIN jsou dva zásadní rozdíly:

1) bylo zcela upuštěno od gradingu VIN a
2) byla zrušena diagnostická jednotka u-VIN I.

Základní rozdělení VIN na u-VIN a d-VIN zůstává zachováno, termín u-VIN je však vyhrazen pouze pro high grade dysplastické léze asociované s HPV, které dříve odpovídaly kategoriím u-VIN II a u-VIN III. Epiteliální proliferace s histologickým vzhledem u-VIN I již nejsou považovány za dysplázie, ale měly by se popisovat pouze jako reaktivní změny anebo projevy HPV infekce (koilocytóza, plochá kondylomatózní léze).

Modifikovaná klasifikace VIN bohužel nenabízí adekvátní terminologickou alternativu pro vzácné vulvární léze s (atypickou) koilocytózou a ztrátou diferenciace, atypiemi a známkami proliferace v bazálních vrstvách epitelu, které tak splňují všechny morfologické podmínky pro dysplázii. Tyto léze jsou nyní v souladu se schématem *ISSVD 1986* klasifikovány jako u-VIN I a v části z nich byla prokázána přítomnost HR HPV (10,11). Proto byly vysloveny pochybnosti o oprávněnosti zrušení diagnostické kategorie u-VIN I (10,28). Upuštění od rozlišování u-VIN II a III se může v dlouhodobém horizontu také jevit poněkud problematickým. Lze to demonstrovat na modelovém příkladu CIN II, která je v současnosti vnímána jako heterogenní skupina lézí s různým potenciálem k regresi anebo transformaci do CIN III a eventuálně SCC (29). Z pohledu klinika je tedy CIN II nárazníkovou zónou, která je terapeuticky řešena stejným způsobem jako CIN III pouze z „bezpečnostních důvodů" (30). S rostoucími diagnostickými možnostmi a definováním nových imunohistochemických nebo molekulárních markerů se však léčebný přístup k CIN II může v budoucnu změnit. Obdobná situace může nastat i v případě u-VIN II, jejíž heterogenita na základě obsažených typů HPV byla též pozorována (10). Spojením u-VIN II a III do jedné kategorie tedy může dojít k začlenění minimálně progresivních u-VIN II mezi vysoce agresivní u-VIN II a III. Z výše popsaných důvodů by měla být původní *ISSVD 1986* klasifikace dle našeho názoru i nadále používána.

Diagnostické schéma *ISSVD 1986* je v současnosti pro histopatologický popis VIN stále závazné, neboť je součástí aktuálně platné WHO klasifikace chorob ženského genitálu. Některá pracoviště však používají terminologii *ISSVD 2004* v rutinní praxi již nyní a existují silné tendence k jejímu začlenění do WHO klasifikace. Před tímto zásadním krokem by však bylo vhodné ověřit biologické chování u-VIN I a II dalšími studiemi.

Klasifikace VIN vycházející z Bethesda terminologie

Alternativně lze pro popis prekanceróz děložního hrdla použít dvoustupňovou terminologii vycházející z Bethesda cytologické klasifikace – low grade (LSIL) a high grade (HSIL) skvamózní intraepiteliální léze. Snaha o revizi klasifikace VIN vedla k obdobnému návrhu s představením termínů low grade (LGVIL) a high grade (HGVIL) vulvární intraepiteliální léze (31). Do kategorie LGVIL měly být zahrnuty nejen u-VIN I a ploché kondylomatózní léze vulvy, ale i klasická condylomata acuminata. Oba hlavní typy high grade dysplastických lézí vulvy (u-VIN II, III a d-VIN) měly být sdruženy do společné jednotky HGVIL. Návrh Bethesda-like klasifikace však nebyl širší gynekopatologickou obcí akceptován, protože v dostatečné míře nevyčleňoval HPV negativní prekancerózy a řadil léze postrádající maligní potenciál mezi procesy dysplastické (12).

ZÁVĚR

V rutinní bioptické praxi se patolog může setkat nejen s HPV pozitivními prekancerózami vulvy (u-VIN), které mají dobře známý histologický vzhled připomínající dysplázie děložního hrd-

la, ale i s HPV negativními prekancerózními lézemi (d-VIN), které jsou mikroskopicky velmi diskrétní, a proto obtížně identifikovatelné. Přesné učení typu VIN je však nedílnou součástí každé histopatologické diagnózy dysplázie vulvy. Tato informace je pro ošetřujícího lékaře důležitá, neboť klinické chování u-VIN a d-VIN se v některých faktorech liší, a to především v riziku progrese do invazivního karcinomu. Dokud nedojde k začlenění revidované terminologie *ISSVD* 2004 do WHO klasifikace VIN, mělo by být součástí bioptické diagnózy u-VIN i určení stupně diferenciace léze. Doporučujeme však zvážit možnost pokračovat v gradingu u-VIN i v budoucnosti. Obě klasifikace VIN jsou totiž dobře převoditelné a údaj o gradu u-VIN může být potenciálně využit k modifikaci terapie pacientek s lézemi s variabilním maligním potenciálem (u-VIN II).

PODĚKOVÁNÍ
Publikace byla podpořena výzkumným záměrem Ministerstva zdravotnictví ČR. MZ0FNM2005

LITERATURA

1. **Munoz N, Castellsague X, de Gonzales AB, Gissmann L.** Chapter 1: HPV in the etiology of human cancer. *Vaccine* 2006; 24 Suppl 3: S1–10.
2. **van Beurden M, ten Kate FW, Tjong AHSP, et al.** Human papillomavirus DNA in multicentric vulvar intraepithelial neoplasia. *Int J Gynecol Pathol* 1998; 17: 12–16.
3. **Hampl M, Sarajuuri H, Wentzensen N, Bender HG, Kueppers V.** Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. *Obstet Gynecol* 2006; 108: 1361–1368.
4. **van der Avoort IA, Shirango H, Hoevenaars BM, et al.** Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. *Int J Gynecol Pathol* 2006; 25: 22–29.
5. **Hoevenaars BM, van der Avoort IA, de Wilde PC, et al.** A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell carcinoma. *Int J Cancer* 2008; 123: 2767–2773.
6. **Toki T, Kurman RJ, Park JS, et al.** Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol* 1991; 10: 107–125.
7. **Wilkinson EJ, Teixeira MR.** Epithelial tumours of the vulva. *In:* **Tavassoli FA, Devilee P**, eds. Pathology and genetics of tumours of the breast and female genital organs. Lyon: IARC Press; 2003: 316–325.
8. **Wilkinson EJ, Kneale B, Lynch PJ.** Report of the ISSVD terminology committee. *J Reprod Med* 1986; 31: 973.
9. **Hart WR.** Vulvar intraepithelial neoplasia: historical aspects and current status. *Int J Gynecol Pathol* 2001; 20: 16–30.
10. **Škapa P, Zámečník J, Hamšíková E, et al.** Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. *Am J Surg Pathol* 2007; 31: 1834–1843.
11. **Srodon M, Stoler MH, Baber GB, Kurman RJ.** The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 2006; 30: 1513–1518.
12. **van de Nieuwenhof HP, van der Avoort IA, de Hullu JA.** Review of squamous premalignant vulvar lesions. *Crit Rev Oncol Hematol* 2008; 68: 131–156.
13. **Preti M, Mezzetti M, Robertson C, Sideri M.** Inter-observer variation in histopathological diagnosis and grading of vulvar intraepithelial neoplasia: results of an European collaborative study. *Br J Obstet Gynaecol* 2000; 107: 594–599.
14. **Santos M, Montagut C, Mellado B, et al.** Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. *Int J Gynecol Pathol* 2004; 23: 206–214.
15. **Yang B, Hart WR.** Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000; 24: 429–441.
16. **Scurry J.** Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. *Int J Gynecol Cancer* 1999; 9: 89–97.
17. **Ambros RA, Malfetano JH, Carlson JA, Mihm MC, Jr.** Non-neoplastic epithelial alterations of the vulva: recognition assessment and comparisons of terminologies used among the various specialties. *Mod Pathol* 1997; 10: 401–408.
18. **Pinto AP, Miron A, Yassin Y, et al.** Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol* 2010; 23: 404–412.
19. **Chiesa-Vottero A, Dvoretsky PM, Hart WR.** Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosus. *Am J Surg Pathol* 2006; 30: 310–318.
20. **van de Nieuwenhof HP, Bulten J, Hollema H, et al.** Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 24: 297–305.
21. **Liegl B, Regauer S.** p53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). *Histopathology* 2006; 48: 268–274.
22. **Choschzick M, Hantaredja W, Tennstedt P, et al.** Role of TP53 Mutations in Vulvar Carcinomas. *Int J Gynecol Pathol* 2011; 30: 497–504.
23. **Poulsen H, Junge J, Vyberg M, Horn T, Lundvall F.** Small vulvar squamous cell carcinomas and adjacent tissues. A morphologic study. *APMIS* 2003; 111: 835–842.
24. **Roma AA, Hart WR.** Progression of simplex (differentiated) vulvar intraepithelial neoplasia to invasive squamous cell carcinoma: a prospective case study confirming its precursor role in the pathogenesis of vulvar cancer. *Int J Gynecol Pathol* 2007; 26: 248–253.
25. **Mulvany NJ, Allen DG.** Differentiated intraepithelial neoplasia of the vulva. *Int J Gynecol Pathol* 2008; 27: 125–135.
26. **Micheletti L, Barbero M, Preti M, et al.** Vulvar intraepithelial neoplasia of low grade: a challenging diagnosis. *Eur J Gynaecol Oncol* 1994; 15: 70–74.
27. **Sideri M, Jones RW, Wilkinson EJ, et al.** Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. *J Reprod Med* 2005; 50: 807–810.
28. **Wilkinson EJ.** Premalignant and malignant tumors of the vulva. *In:* **Kurman RJ, Ellenson LH, Ronnett BM**, eds. Blaustein's pathology of the female genital tract (6th ed). New York, NY: Springer; 2011: 55–103.
29. **Castle PE, Schiffman M, Wheeler CM, Solomon D.** Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. *Obstet Gynecol* 2009; 113: 18–25.
30. **Wright TC, Massad LS, Dunton CJ, et al.** 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *Am J Obstet Gynecol* 2007; 197: 340–345.
31. **Medeiros F, Nascimento AF, Crum CP.** Early vulvar squamous neoplasia: advances in classification, diagnosis, and differential diagnosis. *Adv Anat Pathol* 2005; 12: 20–26.

Příloha IV.

Škapa P., Pichlík T., Pluta M., Halaška M., Robová H., Rob L., Tachezy R., Zámečník J. (2014) Klasifikace vulvárních prekanceróz pohledem patologa. ***Actual Gyn*** 6:26-32.

Aktuální gynekologie a porodnictví

AGP

www.actualgyn.com

Klasifikace vulvárních prekanceróz pohledem patologa

P. Škapa[1], T. Pichlík[2], M. Pluta[2], M. Halaška[2], H. Robová[2], L. Rob[2], R. Tachezy[3], J. Zámečník[1]

[1]Ústav patologie a molekulární medicíny 2. LF UK a FN Motol, Praha, Česká republika
[2]Gynekologicko-porodnická klinika 2. LF UK a FN Motol, Praha, Česká republika
[3]Oddělení experimentální virologie, Ústav hematologie a krevní transfúze, Praha, Česká republika

Korespondenční adresa: MUDr. Petr Škapa, Ústav patologie a molekulární medicíny 2. LF UK a FN Motol, V Úvalu 84, 150 06 Praha 5, tel.: +420 224 435 623, fax: +420 224 435 620, e-mail: petr.skapa@lfmotol.cuni.cz

Publikováno: 18. 3. 2014 Přijato: 19. 1. 2014 Akceptováno: 3. 3. 2014
Actual Gyn 2014, 6, 26-32 ISSN 1803-9588

Citujte tento článek jako: Škapa P, Pichlík T, Pluta M, Halaška M, Robová H, Rob L, Tachezy R, Zámečník J. Klasifikace vulvárních prekanceróz pohledem patologa. Actual Gyn. 2014;6:26-32

CLASSIFICATION OF VULVAR PRECANCEROUS LESIONS: THE PATHOLOGIST'S VIEW

Review article

Abstract
Classification of vulvar precancerous lesions is based on the concept of vulvar intraepithelial neoplasia (VIN). Two basic types of VIN are recognized histologically: VIN of the usual type (u-VIN) and VIN of the differentiated type (d-VIN). The three grade evaluation system is used to assess the intesity of dysplastic changes in u-VIN (u-VIN I, II and III). The precancerous potential of d-VIN corresponds to carcinoma in situ and therefore grading is not applied. u-VIN is etiologically linked to HPV infection, whereas d-VIN is HPV negative and emerges in the environment of chronic vulvar dermatoses (lichen sclerosus and lichen simplex chronicus). u-VIN has a tendency to multifocality and prolonged recurrent progression to the invasive squamous cell carcinoma. On the other hand, d-VIN represents rather a solitary lesion with a propensity to the rapid stromal invasion. u-VIN usually affects premenopausal women with a higher incidence of other precancerous lesions of the lower female genital tract including the perineum and anal area. Patients diagnosed with d-VIN are of postmenopausal age without any association with aforementioned dysplastic lesions of other anatomic locations. The low diagnostic reproducibility of the u-VIN I category, doubts about the precancerous potential of u-VIN I and the problematic distinction between u-VIN II and u-VIN III resulted in the modification of the current terminology. The grading of u-VIN was abandoned, u-VIN II and u-VIN III categories were merged and u-VIN I was removed from the classification scheme. The revised u-VIN category therefore represents high grade dysplastic lesions associated with HPV infection (former u-VIN II and u-VIN III categories) and the term d-VIN is still reserved for the HPV negative high grade vulvar precancerosis.

Key words: vulvar intraepithelial neoplasia, VIN of the usual type, VIN of the differentiated type, lichen sclerosus, lichen simplex chronicus, HPV

Přehledový článek

Abstrakt
Pro klasifikaci prekanceróz vulvy se používá termín vulvární intraepiteliální neoplázie (VIN). Histologicky lze rozlišit dva základní typy VIN: VIN obvyklého typu (u-VIN) a VIN diferencovaného typu (d-VIN). Rozsah a intenzita dysplastických změn se v případě u-VIN vyjadřuje třístupňovým gradingem (u-VIN I, II a III). Prekancerózní potenciál d-VIN odpovídá

vždy karcinomu in situ, proto se u této diagnostické jednotky grading neprovádí. Etiologicky je u-VIN spojena s infekcí vysoce rizikovými typy lidského papillomaviru (HPV), zatímco d-VIN je HPV negativní a rozvíjí se v terénu chronických vulvárních dermatóz typu lichen sclerosus a lichen simplex chronicus. u-VIN má tendenci k multifokalitě a k dlouhodobému recidivujícímu průběhu před transformací v invazivní dlaždicobuněčný karcinom. d-VIN se naopak vyskytuje spíše solitárně a má sklon k rychlé stromální invazi. u-VIN typicky postihuje ženy premenopauzálního věku s vyšší incidencí ostatních prekancerózních lézí dolního ženského pohlavního systému včetně perinea a anu. Pacientky s d-VIN bývají postmenopauzální ženy a není u nich pozorována asociace s výše zmíněnými dysplaziemi ostatních anatomických lokalizací. Nízká diagnostická reproducibilita kategorie u-VIN I, pochyby o prekancerózním potenciálu u-VIN I a problematické rozlišení u-VIN II a u-VIN III vedly k revizi současného diagnostického schématu. Podstatou změn je zrušení gradingu u-VIN se sloučením kategorií u-VIN II a u-VIN III a dále zrušení u-VIN I jako diagnostické jednotky. V revidované klasifikaci prekanceróz vulvy proto termín u-VIN reprezentuje high grade dysplastické léze asociované s HPV (dříve u-VIN II a u-VIN III) a d-VIN i nadále zůstává označením pro HPV negativní high grade prekancerózu.

Klíčová slova: vulvární intraepiteliální neoplázie, VIN obvyklého typu, VIN diferencovaného typu, lichen sclerosus, lichen simplex chronicus, HPV

Prekancerózy dolního ženského pohlavního systému

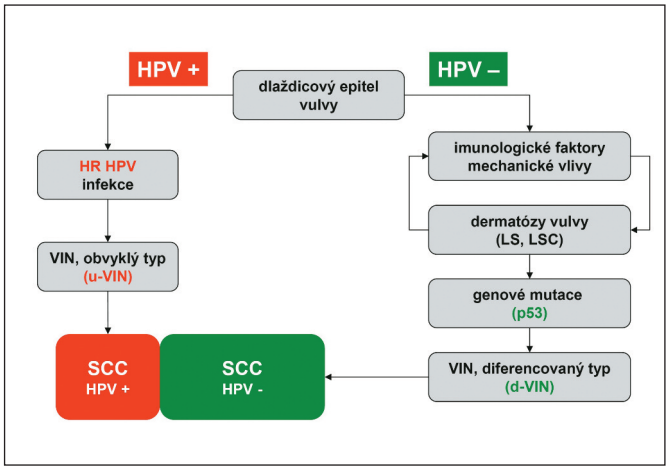
Z hlediska kancerogeneze nevystupují vulva, vagína a děložní hrdlo jako samostatné orgánové jednotky, ale vytvářejí komplexní anatomický systém, pro který se v odborné literatuře vžil termín *lower female genital tract* (LFGT; dolní ženský pohlavní systém). Společnou vlastností všech výše uvedených lokalizací je přítomnost povrchového dlaždicového epitelu, který může být infikován sexuálně přeneseným lidským papillomavirem (HPV). Při infekci vysoce rizikovými typy HPV (HR HPV, např. typu 16 a 18) se mohou v dlaždicovém epitelu za určitých podmínek rozvíjet prekancerózní změny vedoucí až ke vzniku dlaždicobuněčného karcinomu (squamous cell carcinoma, SCC) (1).

Dlaždicobuněčné dysplastické léze LFGT jsou v současnosti klasifikovány jako tzv. *intraepiteliální neoplázie* a dle anatomické lokalizace tedy terminologicky odpovídají vulvární (VIN), vaginální (VaIN) a cervikální (CIN) intraepiteliální neoplázii. Závažnost dysplastických změn vyjadřuje aktuální WHO klasifikace třístupňovým gradingem (I, II a III) podle vertikálního rozsahu ztráty diferenciace, cytologických atypií a mitotické aktivity (2).

Pro HPV asociované prekancerózy a dlaždicobuněčné karcinomy LFGT je typický jejich multicentrický výskyt v libovolných anatomických lokalizacích LFGT, kde mohou v různých kombinacích vznikat simultánně nebo sukcesivně (3,4) a postihovat i perineum a perianální oblast. Tato vlastnost je popisována termínem *multicentrická neoplázie dolního ženského pohlavního systému*. Z klinického pohledu jde o velmi důležitý jev, neboť při diagnóze prekancerózy nebo SCC v jedné anatomické oblasti je nutno vždy počítat s možnou přítomností anebo s rizikem následného rozvoje obdobných patologických změn i v ostatních částech LFGT. Určitou výjimkou jsou pouze prekancerózy vulvy, kde se ve významné míře kromě dysplastických a nádorových lézí asociovaných s HPV vyskytují i HPV negativní prekancerózy a SCC vznikající odlišnou etiopatogenetickou cestou (5-9).

Klasifikace prekancerózních lézí vulvy

Dysplastické léze vulvy jsou v současnosti, na rozdíl od CIN a VaIN, vnímány jako etiologicky heterogenní skupina prekanceróz, které mohou vést nejen k rozvoji HPV asociovaných, ale i HPV negativních SCC (***Obr. 1***) (5,6). Mo-



Obr. 1 *Přehled dvou hlavních cest etiopatogeneze dlaždicobuněčného karcinomu vulvy (se svolením převzato z Škapa et al., 2012 (9)).*
u-VIN – vulvární intraepiteliální neoplázie obvyklého typu; d-VIN – vulvární intraepiteliální neoplázie diferencovaného typu; LS – lichen sclerosus; LSC – lichen simplex chronicus; SCC – dlaždicobuněčný karcinom; HPV – lidský papillomavirus; HR HPV – vysoce rizikové typy lidského papillomaviru

derní klasifikace VIN předložená roku 1986 *International Society for the Study of Vulvovaginal Disease (ISSVD)* (10) proto zohledňuje tyto dvě základní etiopatogenetické cesty vzniku SCC vulvy a rozlišuje HPV asociovanou **VIN obvyklého typu (usual VIN; u-VIN)** a HPV negativní **VIN diferencovaného typu (differentiated VIN; d-VIN)** (2,11). Významným faktem je skutečnost, že u-VIN a d-VIN se liší nejen svými klinicko-patologickými vlastnostmi (***Tab. 1***), ale lze je v naprosté většině případů spolehlivě rozlišit histologicky při běžném bioptickém vyšetření bez použití speciálních technik jako je imunohistochemie nebo detekce přítomnosti HPV molekulárními metodami. Starší nebo nepřesně definované diagnostické jednotky typu Bowenova choroba, bowenoidní atypie, bowenoidní dysplázie, bowenoidní karcinom in situ, bowenoidní papulóza, erythroplasia de Queyrat a carcinoma in situ simplex jsou nyní pro popis prekanceróz vulvy již obsolentní

Tab. 1 *Klinicko-patologické rozdíly dvou základních typů vulvárních prekanceróz (u-VIN a d-VIN).*

	VIN, obvyklý typ (u-VIN)	VIN, diferencovaný typ (d-VIN)
Frekvence výskytu	více než 80 %	méně než 20 %
Věková predispozice	premenopauzální ženy	postmenopauzální ženy
Asociace s CIN a VaIN (multicentrická neoplázie LFGT)	ano	ne
Asociace s condylomata acuminata a s ostatními pohlavně přenosnými chorobami	ano	ne
Hlavní etiologický faktor	HR HPV	genové mutace (p53)
Kofaktory	kouření cigaret imunosuprese	dermatózy vulvy (LS, LSC)
Tendence k multifokalitě	silná	slabá
Potenciál k invazi do stromatu	slabý	silný
Imunohistochemický marker	$p16^{INK4a}$	p53
Asociovaný SCC	bazaloidní SCC warty SCC	keratinizující SCC

VIN – vulvární intraepiteliální neoplázie; CIN – cervikální intraepiteliální neoplázie; VaIN – vaginální intraepiteliální neoplázie; LFGT – lower female genital tract; HR HPV – high risk typy lidského papillomviru; LS – lichen sclerosus; LSC – lichen simplex chronicus; SCC – dlaždicobuněčný karcinom

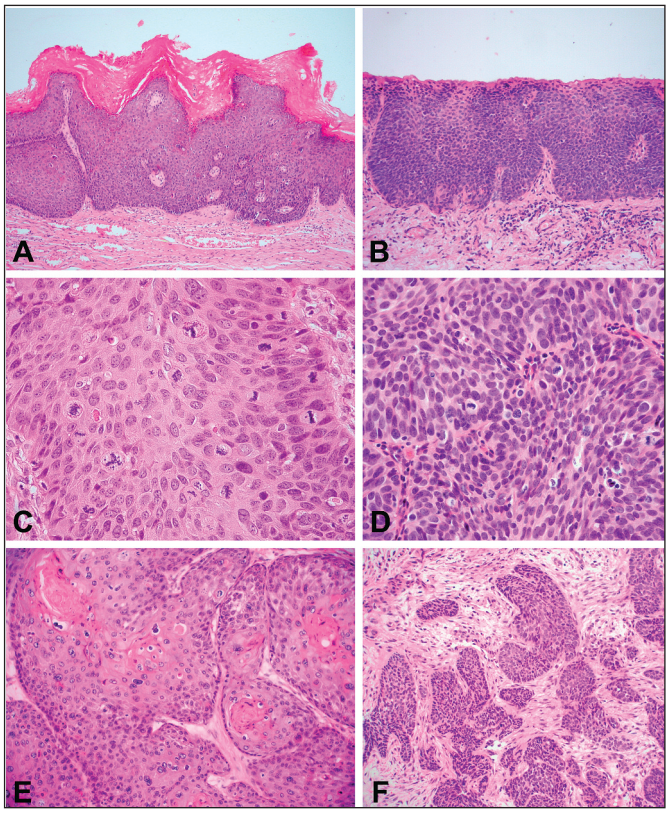
a jejich používání se neslučuje s terminologickým doporučením WHO (2).

VIN obvyklého typu (u-VIN)

V etiopatogenezi u-VIN se jako základní faktor uplatňuje HR HPV infekce (4,8,12), často doprovázená dalšími kofaktory, mezi které spadají především kouření cigaret a imunosupresivní stavy. Sexuální mechanismus přenosu hlavního etiologického agens se odráží i v charakteristických klinicko-patologických vlastnostech tohoto typu dysplastické léze. Typickými pacientkami s u-VIN jsou mladší ženy premenopauzálního věku, které mají zároveň i zvýšenou incidenci ostatních prekanceróz a SCC v oblasti LFGT a je u nich pozorován častější výskyt pohlavních chorob (13). SCC vulvy vzniklé na podkladě u-VIN jsou též HPV pozitivní, rozvíjejí se v mladším věku a představují asi 30–40 % všech karcinomů vulvy (8,13).

Histologický obraz u-VIN se prakticky shoduje s CIN a VaIN a je definován ztrátou zralosti keratinocytů, cytologickými atypiemi, zvýšenou mitotickou aktivitou a případně známkami HPV infekce. Podle vertikálního rozsahu výše popsaných patologických změn se na základě aktuální WHO klasifikace provádí třístupňový grading na u-VIN I, II a III. Zatímco identifikace u-VIN II a III jako high grade prekancerózy je většinou bezproblémová interpersonální a intrapersonální reproducibilita diagnózy u-VIN I je naopak velmi nízká (14). Teoreticky lze na základě morfologického obrazu rozlišit 3 hlavní podtypy u-VIN: bazaloidní, warty (kondylomatózní) a smíšený (warty/bazaloidní) (***Obr. 2***) (11). Z praktického hlediska však toto dělení postrádá význam, neboť se od sebe jednotlivé subtypy u-VIN neliší klinickým chováním ani spektrem HPV typů (11). Obdobně je možno na warty a bazaloidní variantu histologicky subklasifikovat i HPV pozitivní SCC vulvy (***Obr. 2***).

Histologickou diagnózu u-VIN lze v diferenciálně diagnosticky problematických případech podpořit imunohistochemickým vyšetřením markeru **$p16^{INK4a}$** (5,15), který se jako inhibitor cyklin dependentní kinázy uplatňuje v regulaci buněčného cyklu. Patologická overexprese proteinu



Obr. 2 *Histologické typy prekancerózních lézí a dlaždicobuněčných karcinomů vulvy v HPV asociované cestě karcinogeneze (se svolením převzato z Škapa et al., 2012 (9)).*

A – u-VIN III, warty (HE, 40x); B – u-VIN III, bazaloidní (HE, 100x); C – u-VIN III, warty (HE, 400x); D – u-VIN III, bazaloidní (HE, 400x); E – SCC, warty (HE, 200x); F – SCC, bazaloidní (HE, 100x)

$p16^{INK4a}$ doprovázená silnou difuzní jadernou a cytoplazmatickou pozitivitou bývá imunohistochemicky detekovatelná nejen v u-VIN, ale i v HPV pozitivních karcinomech vulvy a v dalších HPV asociovaných prekancerózních a nádorových lézích LFGT (16,17).

VIN diferencovaného typu (d-VIN)

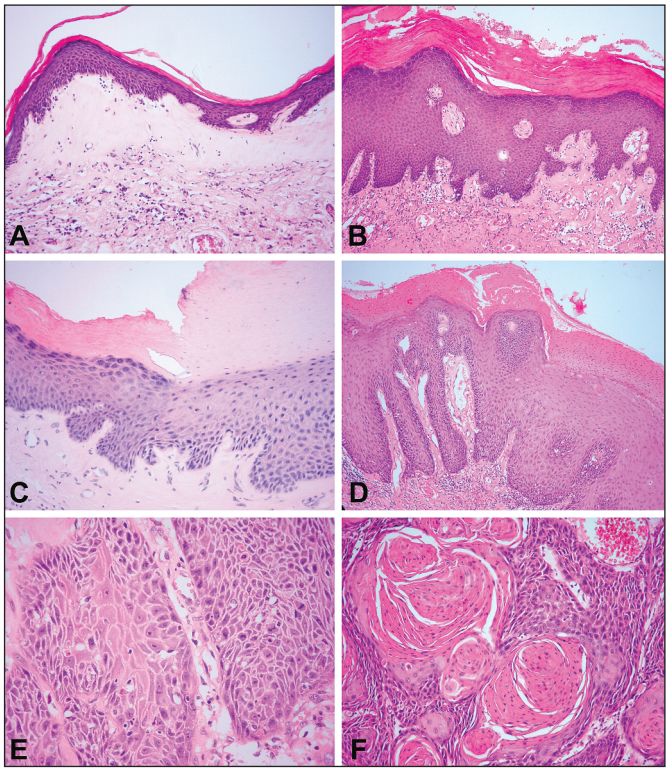
Diagnostická kategorie d-VIN byla popsána již v 60. letech 20. století jako carcinoma in situ simplex, nicméně upadla v zapomnění a svoji renesanci v odborné literatuře a v dia-

gnostické praxi zažívá až v posledních letech, kdy byly identifikovány markantní klinicko-patologické rozdíly mezi oběma základními typy prekanceróz vulvy. Stále však jde o poměrně málo známou terminologickou jednotku jak mezi gynekology, tak i mezi patology. Na rozdíl od u-VIN je d-VIN vzácnější prekancerózou, která nemá etiopatogenetický vztah k HPV infekci a není proto svázána se zvýšeným výskytem pohlavních chorob a ostatních prekancerózních a nádorových lézí LFGT. Nemá též výraznější tendenci k multicentrickému výskytu a typicky se vyskytuje u žen postmenopauzálního věku (8,9,18).

Při současném stavu poznání se za příčinu vzniku d-VIN považují genové mutace vznikající v terénu chronických vulvárních dermatóz typu *lichen sclerosus (LS) a lichen simplex chronicus (LSC)*, které se rozvíjejí v terénu imunologické predispozice (19). Tato kožní onemocnění jsou doprovázena různě intenzivním pruritem vedoucím k volní i mimovolní chronické mechanické iritaci postižené oblasti s možností vzniku ulcerací a reaktivní proliferací dlaždicového epitelu. Následkem může být zhoršení vulvární dermatózy a tím i zintenzivnění pruritu za vzniku *circulus vitiosus*, který se v literatuře někdy označuje jako *itch-scratch cyklus* (19). V pokročilé fázi onemocnění mívá vulvární dermatóza často charakter LSC, který může být superponován na primárně vzniklý LS. Termín „dlaždicobuněčná hyperplázie", používaný dříve pro hyperplastické procesy vulvy, je pouze popisné vyjádření histologického vzhledu epidermis a v naprosté většině případů odpovídá právě nozologické jednotce LSC (20). Hyperplastické a zánětlivé procesy v dlaždicovém epitelu vulvy jsou doprovázeny vyšším rizikem genových mutací především v tumor supresorovém genu p53 (21), které mohou vést k transformaci v d-VIN. Pro d-VIN je typický větší invazivní potenciál spojený s kratší intraepiteliální fází a rychlejší progresí do SCC ve srovnání s u-VIN (18). Cestou d-VIN vznikají HPV negativní SCC keratinizujícího typu, které jsou většinou dobře diferencované (grade 1) a představují přibližně 60–70 % všech karcinomů vulvy (***Obr. 3***) (8,9,19,22). Literární údaje dokládají, že u 2–6 % pacientek s diagnózou LS se rozvine SCC vulvy (13).

Biologickým chováním odpovídá d-VIN karcinomu in situ a spadá tak automaticky do kategorie VIN III. Histologický grading s hodnocením rozsahu ztráty vyzrávání, cytologických atypií a mitotické aktivity se proto u d-VIN neprovádí. Se značným prekancerózním potenciálem d-VIN ostře kontrastuje nenápadný a relativně klidný histologický vzhled léze, který na první pohled nevzbuzuje výraznější podezření z dysplázie a nenaplňuje běžná histologická kritéria pro karcinom in situ (***Obr. 3***) (8,9,18). Při podrobnější histologické analýze by však už měla být patrná následující diagnostická kritéria d-VIN (***Obr. 3***):

1. akantoticky rozšířený dlaždicový epitel s elongovanými rete ridges
2. cytologické atypie, mitotická aktivita a aberantní keratinizace lokalizované v bazálních partiích dlaždicového epitelu
3. suprabazilární oblasti epidermis tvořené abnormálně diferencovanými keratinocyty s objemnou eozinofilní cytoplazmou, s vezikulárními jádry s výraznými jadérky a s jasně patrnými intercelulárními můstky
4. ztráta stratum granulosum
5. superficiální parakeratóza
6. absence známek HPV infekce.



Obr. 3 *Vulvární dermatózy, prekancerózní léze a dlaždicobuněčný karcinom vulvy v HPV negativní cestě karcinogeneze (se svolením převzato z Škapa et al., 2012 (9)). A – LS (HE, 100x); B – LSC (HE, 40x); C – přechodová zóna mezi LSC a d-VIN (HE, 200x); D – d-VIN (HE, 40x); E – d-VIN (HE, 400x); F – SCC, keratinizující (HE, 200x)*

Výše popsané změny vyniknou především na hranici mezi vulvární dermatózou a d-VIN, která je typicky ostrá a přechod mezi lézemi je náhlý (***Obr. 3***) (9). Diferenciální diagnóza mezi vulvárními dermatózami typu LS, LSC a d-VIN je nicméně velmi problematická. K diagnóze d-VIN je nutná kombinace výše uvedených kritérií, neboť žádné z nich není samo o sobě specifické pro d-VIN. Dokonce i atypie v bazálních keratinocytech jsou popisovány ve vulvárních dermatózách a je pro ně vyhrazen termín *atypický lichen sclerosus* (22,23). V případě diagnostických pochybností lze provést imunohistochemické vyšetření proteinového produktu tumor supresorového genu **p53**, které může být v případě mutace genu pozitivní (18,21). Specificita a senzitivita imunohistochemického průkazu p53 však není optimální (24), proto je význam tohoto vyšetření některými autory zpochybňován (25). Zkušenosti z našeho pracoviště tyto rozpaky bohužel jenom potvrzují (9). Reprodukcibilita histologické diagnózy d-VIN proto zůstává i nadále poměrně nízká a ve sporných případech je doporučována konzultace s patologem specializovaným v gynekopatologické problematice (26).

Zajímavým paradoxem je nesoulad mezi frekvencí výskytu u-VIN a d-VIN a téměř inverzním poměrem HPV pozitivních a HPV negativních SCC vulvy. Příčinou je pravděpodobně značné poddiagnostikování d-VIN, které může být způsobeno několika faktory (8,18):

1. Vyšší věk pacientek s d-VIN je spojen se sporadickou frekvencí návštěv u gynekologa a d-VIN tak unikne bioptickému vyšetření.

2. Vysoká tendence d-VIN ke stromální invazi vede ke zkrácení intervalu, kdy je možno lézi klinicky zachytit a biopticky ověřit před vznikem SCC.
3. Makroskopická podobnost s vulvárními dermatózami vede k přehlédnutí d-VIN gynekologem a léze unikne bioptickému vyšetření.
4. Histologická podobnost s vulvárními dermatózami vede k přehlédnutí d-VIN patologem a léze je chybně klasifikována jako LS nebo LSC.
5. Cytologické atypie v bazálních partiích epitelu jsou patologem chybně interpretovány a d-VIN je klasifikována jako u-VIN I.

Kontroverze ale panují i ve věci samotné existence d-VIN. Někteří autoři nepovažují d-VIN za samostatnou diagnostickou jednotku, ale pouze za intraepiteliální propagaci simultánně se vyskytujícího SCC (25,27). Proti tomuto tvrzení však svědčí dobře zdokumentovaná pozorování rychlé progrese d-VIN do SCC (28,29).

Modifikace klasifikačního systému vulvárních intraepiteliálních neoplázií

Ačkoliv třístupňový grading HPV asociovaných prekanceróz vulvy (u-VIN I, II a III) spadá do jednotného konceptu klasifikace dysplastických lézí LFGT a vykazuje paralely s prekancerózami děložního hrdla a vagíny, během rutinní diagnostické praxe vyvstalo několik konfliktních bodů, které v konečném důsledku vedly k modifikaci terminologického systému VIN:

1. *u-VIN I a CIN I se zásadně liší ve frekvenci výskytu* (11). Zatímco CIN I je relativně běžnou skupinou lézí, mezi které se v širším kontextu řadí i HPV indukované epiteliální změny charakteru plochého kondylomu, jsou ploché kondylomatózní léze v oblasti vulvy velmi vzácné. V oblasti vulvy se naopak často vyskytuje *condyloma acuminatum*, které postrádá prekancerózní potenciál a klasifikačně nespadá do kategorie u-VIN I. Drtivou většinu případů u-VIN tedy tvoří high grade dysplastické léze a u-VIN I je v biopsiích vulvy diagnostikována zřídka (8), většinou pouze v přímé kontinuitě s u-VIN II a u-VIN III.
2. *Diagnostická reproducibilita u-VIN I je velmi nízká*. Solitárně se vyskytující u-VIN I bez asociace s u-VIN II a III jsou zcela výjimečným nálezem a jejich interpersonální a intrapersonální reproducibilita při histopatologickém vyšetření je velmi nízká (14). Při retrospektivní expertní analýze souboru u-VIN I splnilo striktní diagnostická kritéria pouze 19 % lézí (30). Z důvodu cytologických nepravidelností v bazálních partiích epitelu jsou jako u-VIN I chybně klasifikovány epiteliální proliferace spojené se zánětlivými a reaktivními změnami, různými dermatózami včetně LS a LSC a v neposlední řadě může dojít i k záměně u-VIN I s d-VIN (18). Vzhledem k obtížné histopatologické diagnostice je proto kategorie u-VIN I tvořena směsí lézí s diametrálně odlišným prekancerózním potenciálem, což značně snižuje její význam jako samostatné diagnostické jednotky.
3. *Diagnostická reproducibilita u-VIN II a u-VIN III je velmi nízká.* Většina high grade dysplastických lézí vulvy je komplexně stavěná a buď obsahuje obě vzájemně přecházející komponenty anebo je nelze striktně zařadit do jednotlivých kategorií u-VIN II a u-VIN III. Z našich zkušeností vyplývá, že u-VIN II je v čisté formě vzácným nálezem (8).

Výše uvedené skutečnosti vedly v uplynulé dekádě k pokusům o změnu klasifikačního schématu VIN s hlavním cílem zlepšit diagnostickou reproducibilitu a prediktivní význam jednotlivých kategorií. Výsledkem těchto snah je především návrh klasifikace ISSVD 2004 (31). Další modifikací je klasifikace vycházející z cytologické Bethesda terminologie (32), která se však mezi gynekopatology neujala a přesahuje proto rozsah této publikace.

Klasifikace ISSVD 2004

V roce 2004 předložila ISSVD z výše uvedených důvodů zjednodušenou verzi původní ISSVD 1986 klasifikace s následujícími změnami (***Tab. 2***) (31):

1. *zrušení kategorie u-VIN I*
   Epiteliální proliferace vulvy dříve klasifikované jako u-VIN I jsou nyní považovány pouze za reaktivní změny anebo projevy HPV infekce (koilocytóza, plochá kondylomatózní léze). Sporným bodem tohoto kroku je existence vzácných lézí se známkami HPV infekce a s atypiemi v bazálních partiích epitelu, u kterých byla prokázána přítomnost HR HPV a pro které modifikovaná klasifikace nenabízí alternativu (8,33).
2. *zrušení gradingu u-VIN*
   High grade prekancerózy vulvy dříve klasifikované jako u-VIN II a u-VIN III jsou nyní sloučeny do společné kategorie u-VIN, která tak vytváří paralelu high grade skvamózním intraepiteliálním lézím děložního hrdla (HSIL). u-VIN II je tedy podobně jako CIN II vnímána jako nárazníková zóna, která je terapeuticky řešena obdobným způsobem jako u-VIN III, resp. CIN III (34). Prekancerózní potenciál u-VIN II a CIN II však nejspíše nedosahuje stupně u-VIN III a CIN III. CIN II je v současnosti považována za nejednotnou skupinu dysplastických lézí s variabilním potenciálem k regresi anebo transformaci do CIN III a případně SCC (35). Podobné vlastnosti mohou být dříve či později identifikovány i u u-VIN II, u které byla již nyní na rozdíl od u-VIN III prokázána heterogenita HPV typů, včetně přítomnosti typů nízce rizikových (8). Terminologickým sloučením u-VIN II a u-VIN III proto může dojít k začlenění minimálně progresivních u-VIN II mezi vysoce agresivní u-VIN III, což může učinit revidovanou ISSVD 2004 klasifikaci nevýhodnou pro přesnější odhad biologického potenciálu léze a pro budoucí výzkumné studie.

Modifikovaná *ISSVD 2004* terminologie prekancerózních lézí vulvy bude v blízké budoucnosti s nejvyšší pravděpodobností začleněna do klasifikačního schématu WHO a stane se tak závaznou pro rutinní diagnostiku.

Závěr

Prekancerózní léze vulvy se svou etiologickou heterogenitou částečně vymykají ze společného konceptu dysplastických lézí LFGT. Kromě relativně častých HPV asociovaných prekanceróz (u-VIN) se gynekolog i patolog mohou ve své běžné praxi setkat i se vzácnější HPV negativní dysplastickou lézí (d-VIN). Přesné určení typu prekancerózy na základě histologického vzhledu je nedílnou součástí výstupu bioptického vyšetření. Jde o klinicky důležitou informaci, neboť prekancerózní potenciál i ostatní vlastnosti u-VIN a d-VIN se vzájemně liší. Bohužel je klinická i histopatologická diagnostika d-VIN stále zatížena vysokou mírou nejistoty a nízkou reproducibilitou, proto nemusejí být dysplastické léze tohoto typu včas zachy-

Tab. 2 *Převodní tabulka klasifikace ISSVD 1986, modifikovaného schématu ISSVD 2004 a historických termínů dříve užívaných ke klasifikaci vulvárních prekanceróz.*

ISSVD 1986		ISSVD 2004		Historická terminologie
Histologický typ	Grade	Histologický typ	Grade	
VIN, obvyklý typ (u-VIN)	I	Reaktivní změny HPV infekce Plochá kondylomatózní léze	-	Bowenova choroba Bowenoidní atypie Bowenoidní dysplázie Bowenoidní karcinom in situ Bowenoidní papulóza Erythroplasia de Queyrat
	II	VIN, obvyklý typ (u-VIN)	-	
	III			
VIN, diferencovaný typ (d-VIN)	III	VIN, diferencovaný typ (d-VIN)	-	Carcinoma in situ simplex

VIN – vulvární intraepiteliální neoplázie; ISSVD – International Society for the Study of Vulvovaginal Disease

ceny. Klasifikační schéma VIN bylo nedávno zrevidováno, třístupňový grading u-VIN byl zrušen a byla vypuštěna kategorie u-VIN I. V modifikované klasifikaci prekanceróz vulvy proto termín u-VIN reprezentuje high grade dysplastické léze asociované s HPV (dříve u-VIN II a u-VIN III) a d-VIN i nadále zůstává označením pro HPV negativní high grade prekancerózu.

Literatura

1. Munoz N, Castellsague X, de Gonzalez AB, et al. Chapter 1: HPV in the etiology of human cancer. Vaccine. 2006;24 Suppl 3:S3/1-10
2. Wilkinson EJ, Teixeira MR. Epithelial tumours of the vulva. In: Tavassoli FA, Devilee P, eds. Pathology and genetics of tumours of the breast and female genital organs. Lyon, PA: IARC Press; 2003:316-325
3. van Beurden M, ten Kate FW, Tjong AHSP, et al. Human papillomavirus DNA in multicentric vulvar intraepithelial neoplasia. Int J Gynecol Pathol. 1998;17:12-16
4. Hampl M, Sarajuuri H, Wentzensen N, et al. Effect of human papillomavirus vaccines on vulvar, vaginal, and anal intraepithelial lesions and vulvar cancer. Obstet Gynecol. 2006;108:1361-1368
5. van der Avoort IA, Shirango H, Hoevenaars BM, et al. Vulvar squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. Int J Gynecol Pathol. 2006;25:22-29
6. Hoevenaars BM, van der Avoort IA, de Wilde PC, et al. A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2 pathways leading to vulvar squamous cell carcinoma. Int J Cancer. 2008;123:2767-2773
7. Toki T, Kurman RJ, Park JS, et al. Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. Int J Gynecol Pathol. 1991;10:107-125
8. Škapa P, Zámečník J, Hamšíková E, et al. Human papillomavirus (HPV) profiles of vulvar lesions: possible implications for the classification of vulvar squamous cell carcinoma precursors and for the efficacy of prophylactic HPV vaccination. Am J Surg Pathol. 2007;31:1834-1843
9. Škapa P, Robová H, Rob L, et al. Prekancerózní léze vulvy. Cesk Patol. 2012;48:15-21
10. Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD terminology committee. J Reprod Med. 1986;31:973
11. Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. Int J Gynecol Pathol. 2001;20:16-30
12. Srodon M, Stoler MH, Baber GB, et al. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). Am J Surg Pathol. 2006;30:1513-1518
13. van de Nieuwenhof HP, van der Avoort IA, de Hullu JA. Review of squamous premalignant vulvar lesions. Crit Rev Oncol Hematol. 2008;68:131-156
14. Preti M, Mezzetti M, Robertson C, et al. Inter-observer variation in histopathological diagnosis and grading of vulvar intraepithelial neoplasia: results of an European collaborative study. Br J Obstet Gynaecol. 2000;107:594-599
15. Santos M, Montagut C, Mellado B, et al. Immunohistochemical staining for p16 and p53 in premalignant and malignant epithelial lesions of the vulva. Int J Gynecol Pathol. 2004;23:206-214
16. O'Neill CJ, McCluggage WG. p16 expression in the female genital tract and its value in diagnosis. Adv Anat Pathol. 2006;13:8-15
17. Škapa P, Robová H, Rob L, et al. p16(INK4a) immunoprofiles of squamous lesions of the uterine cervix-implications for the reclassification of atypical immature squamous metaplasia. Pathol Oncol Res. 2013;19:707-714
18. Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. Am J Surg Pathol. 2000;24:429-441
19. Scurry J. Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. Int J Gynecol Cancer. 1999;9:89-97
20. Ambros RA, Malfetano JH, Carlson JA, et al. Non-neoplastic epithelial alterations of the vulva: recognition assessment and comparisons of terminologies used among the various specialties. Mod Pathol. 1997;10:401-408
21. Pinto AP, Miron A, Yassin Y, et al. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. Mod Pathol. 2010;23:404-412
22. Chiesa-Vottero A, Dvoretsky PM, Hart WR. Histopa-

thologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosus. Am J Surg Pathol. 2006;30:310-318
23. van de Nieuwenhof HP, Bulten J, Hollema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. Mod Pathol. 2011;24:297-305
24. Choschzick M, Hantaredja W, Tennstedt P, et al. Role of TP53 mutations in vulvar carcinomas. Int J Gynecol Pathol. 2011;30:497-504
25. Liegl B, Regauer S. p53 immunostaining in lichen sclerosus is related to ischaemic stress and is not a marker of differentiated vulvar intraepithelial neoplasia (d-VIN). Histopathology. 2006;48:268-274
26. van den Einden LC, de Hullu JA, Massuger LF, et al. Interobserver variability and the effect of education in the histopathological diagnosis of differentiated vulvar intraepithelial neoplasia. Mod Pathol. 2013;26:874-880
27. Poulsen H, Junge J, Vyberg M, et al. Small vulvar squamous cell carcinomas and adjacent tissues. A morphologic study. APMIS. 2003;111:835-842
28. Roma AA, Hart WR. Progression of simplex (differentiated) vulvar intraepithelial neoplasia to invasive squamous cell carcinoma: a prospective case study confirming its precursor role in the pathogenesis of vulvar cancer. Int J Gynecol Pathol. 2007;26:248-253
29. Mulvany NJ, Allen DG. Differentiated intraepithelial neoplasia of the vulva. Int J Gynecol Pathol. 2008;27:125-135
30. Micheletti L, Barbero M, Preti M, et al. Vulvar intraepithelial neoplasia of low grade: a challenging diagnosis. Eur J Gynaecol Oncol. 1994;15:70-74
31. Sideri M, Jones RW, Wilkinson EJ, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. J Reprod Med. 2005;50:807-810
32. Medeiros F, Nascimento AF, Crum CP. Early vulvar squamous neoplasia: advances in classification, diagnosis, and differential diagnosis. Adv Anat Pathol. 2005;12:20-26
33. Wilkinson EJ. Premalignant and malignant tumors of the vulva. In: Kurman RJ, Ellenson LH, Ronnett BM, eds. Blaustein's pathology of the female genital tract (6th ed). New York, NY, PA: Springer; 2011:55-103
34. Wright TC, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. Am J Obstet Gynecol. 2007;197:340-345
35. Castle PE, Schiffman M, Wheeler CM, et al. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. Obstet Gynecol. 2009;113:18-25

Publikace byla podpořena projekty IGA MZ ČR NT 13167-4 a MZ ČR – RVO, FN v Motole 00064203.

Příloha V.

Škapa P., Robová H., Rob L. a Zámečník J. (2013) p16^{INK4a} immunoprofiles of squamous lesions of the uterine cervix – implications for the reclassification of atypical immature squamous metaplasia. ***Pathol Oncol Res*** 19:707-714.

IF 1,555

Pathol. Oncol. Res. (2013) 19:707–714
DOI 10.1007/s12253-013-9633-1

RESEARCH

p16^{INK4a} Immunoprofiles of Squamous Lesions of the Uterine Cervix–Implications for the Reclassification of Atypical Immature Squamous Metaplasia

Petr Skapa · Helena Robova · Lukas Rob · Josef Zamecnik

Received: 15 January 2013 / Accepted: 27 March 2013 / Published online: 18 May 2013

Abstract p16^{INK4a} immunoprofiles of non-precancerous and dysplastic squamous cervical lesions were defined and applied to the reclassification of atypical immature squamous metaplasia (AIM). The immunoexpression of cytokeratin 17 (CK 17) in AIM was also evaluated. Totally, 295 cervical cone biopsies representing squamous metaplasia, reactive changes, koilocytosis, flat condyloma, CIN I, CIN II, CIN III and AIM were subjected to p16^{INK4a} immunohistochemistry. AIM cases were analyzed using CK 17 antibody. Typical p16^{INK4a} immunoprofiles for the metaplastic, LSIL/HPV and HSIL phenotypes were recorded and used for the categorization of AIM into particular phenotype groups. Results were correlated with CK 17 immunoexpression. All CIN II and CIN III lesions, all but one case of CIN I and all flat condylomas overexpressed p16^{INK4a}. Other non-precancerous lesions, including koilocytosis, were predominantly negative. Contrary to the sporadic and focal immunostaining, diffuse positivity was associated with the dysplastic features of the lesion. CIN II and CIN III were characterized by a diffuse, strong/weak, full-thickness staining, whereas CIN I showed a heterogeneous diffuse/focal, weak/strong, lower half positivity. One third of AIM lesions may be reclassified as HSIL, one third as LSIL/HPV and one third shows metaplastic phenotype. All AIM cases with metaplastic and LSIL/HPV phenotypes expressed CK 17 diffusely, whereas focal positivity slightly prevailed in AIM with HSIL phenotype. We conclude that p16^{INK4a} immunohistochemistry is a supporting method for the differential diagnosis of cervical lesions, which may be especially useful for the reclassification of AIM. The efficacy of CK 17 immunohistochemistry seems to be controversial for these purposes.

Keywords Uterine cervix · p16 · Cytokeratin 17 · Cervical intraepithelial neoplasia · Atypical immature squamous metaplasia · Flat condyloma

P. Skapa (✉) · J. Zamecnik
Department of Pathology and Molecular Medicine, Charles University, 2nd Medical School, University Hospital Motol, V Uvalu 84, 150 06 Prague 5, Czech Republic
e-mail: petr.skapa@lfmotol.cuni.cz

H. Robova · L. Rob
Department of Obstetrics and Gynecology, Charles University, 2nd Medical School, University Hospital Motol, V Uvalu 84, 150 06 Prague 5, Czech Republic

Introduction

Cervical intraepithelial neoplasias (CIN) are traditionally classified into three grades: CIN I–III, or an alternative terminology of low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) may be applied. Unfortunately, the histopathological evaluation of cervical biopsies may be influenced by a significant inter- and intraobserver variation [1] that affects especially CIN I [2] and CIN II [3] categories. Further differential diagnostic issues emerge because of a spectrum of benign lesions, which may mimic cervical dysplasias microscopically. One of the most enigmatic entities from this group, initially described by Crum et al. [4], is the atypical immature squamous metaplasia (AIM). It probably represents a heterogeneous group of lesions of various precancerous potential, including LSIL, HSIL and reactive or inflammatory conditions [5–7]. Regrettably, its biologic behavior and clinical significance as a diagnostic category remain unclear.

Cyclin-dependent kinase inhibitor p16^{INK4a}, which is involved in the regulation of cell cycle, may be overexpressed as a consequence of infection with oncogenic high-risk human

papillomavirus (HR-HPV). The immunostaining for $p16^{INK4a}$ is therefore a feature of HPV-associated precancerous lesions and carcinomas of the lower female genital tract [8] and it may be used as an auxiliary method for histopathological evaluation. It was also shown that the level of $p16^{INK4a}$ upregulation correlates with the increasing grade of CIN [9].

Cytokeratin 17 (CK 17) was identified as a marker of cervical stem cells [10], it is consistently expressed in immature squamous metaplasia of the cervical transformation zone [11] and its immunoexpression was also observed in all grades of CIN [11, 12]. However, the prevalent negativity of CK 17 in CIN III was reported and the suggestion to use the reciprocal immunoreactivity of $p16^{INK4a}$ and CK 17 for the distinction between AIM and high-grade CIN was postulated [13].

In this study, we primarily aimed to analyze the expression of $p16^{INK4a}$ in a broad spectrum of squamous lesions of the uterine cervix with various precancerous potential: non-precancerous proliferations (squamous metaplasia, reactive changes), LSIL/HPV group (koilocytosis, flat condylomas, CIN I), HSIL group (CIN II, CIN III) and AIM. Our objective was to estimate typical $p16^{INK4a}$ immunoprofiles of each type of lesion and to define their diagnostic value for the potential reclassification of AIM. Secondarily, the immunoexpression of CK 17 in all cases of AIM was performed to assess its role in the differential diagnosis between AIM and high-grade CIN.

Materials and Methods

Case Selection

In total, 351 cervical cone biopsies were included into the study. Incomplete cone excisions and punch biopsies were discarded because they often fail to demonstrate a representative portion of the transformation zone for the evaluation. Slides were reviewed independently by two consultant pathologists (P.S. and J.Z.): only cases with the concurrent diagnostic interpretation from both observers were enrolled into the study. Finally, 295 cone biopsies were available for the analysis and 56 cases were excluded due to the diagnostic disagreement. All lesions were classified into the following groups: mature and immature squamous metaplasia (SM, $n=33$), metaplastic squamous epithelium with reactive changes (RC, $n=23$), metaplastic squamous epithelium with koilocytosis (Kc, $n=15$), flat condyloma (Co, $n=8$), CIN I ($n=35$), CIN II ($n=82$), CIN III ($n=67$) and AIM ($n=32$). Generally accepted histopathological criteria were used for the classification of SM, RC, Co, CIN I, CIN II and CIN III. Koilocytosis was defined as a non-dysplastic, non-acanthotic and non-papillomatous squamous epithelium containing monucleated or multinucleated cells with perinuclear halos, nuclear enlargement and irregular nuclear contours. The criteria proposed by Crum et al. [4] were applied to the diagnosis of AIM. Provided that more lesions with a different biologic behavior were present in one specimen, only the lesion with the highest precancerous potential was considered for further analysis.

Immunostaining Protocols

Tissue sections intended for $p16^{INK4a}$ immunohistochemistry were subjected to the heat-induced epitope retrieval in water bath at 98 °C for 30 min and incubated overnight at 4 °C with primary monoclonal mouse anti-human antibody $p16^{INK4a}$ (diluted 1:100) (clone G175-405, cat. No. 551154, BD Biosciences, Franklin Lakes, NJ). The immunocomplexes of the antigen and the primary antibody were visualized using N-Histofine Simple Stain MAX PO (MULTI) detection system (cat. No. 414154F, Nichirei Biosciences, Tokyo, Japan). The positive control (squamous cell carcinoma of the uterine cervix) was used in each series of immunohistochemistry.

Nuclear staining or a combination of nuclear and cytoplasmic staining was considered for a positive result of immunoreaction with $p16^{INK4a}$ antibody. Cytoplasmic staining without nuclear staining was interpreted as negativity. All cases were reviewed by two observers and consensually assessed according to the scoring system summarized in Table 1. Three basic parameters were used for evaluation of the immunoreaction: horizontal distribution, vertical distribution and intensity. The horizontal distribution of staining was

Table 1 A standardized scoring system used in this study for the evaluation of $p16^{INK4a}$ immunostaining

Parameter	Value	Histopathological criteria
Horizontal distribution	Negative	Positivity of solitary cells (<1 %)
	Sporadic	Positivity of solitary cells (≥1 % and <5 %)
	Focal	Positivity of solitary cells or clusters of cells (≥5 % and <25 %)
	Diffuse	Band-like confluent positivity (≥25 %)
Vertical distribution	Lower half	Horizontal staining pattern contained to the lower half of the epithelium
	Full-thickness	Horizontal staining pattern extending above the lower half of the epithelium
Intensity	Weak	Light brown staining of substantially lower intensity than a positive control sample, nuclear membranes clearly visible, chromatin pattern distinguishable
	Strong	Dark brown staining comparable with a positive control sample, nuclear membranes and chromatin pattern poorly recognizable

scored according to Klaes et al. [14]. The vertical distribution was interpreted on the basis of maximal vertical alignment of the horizontal staining pattern. The two-grade scoring system (lower half and full-thickness positivity) was used for this purpose instead of the three-grade scheme (lower third, middle third and full-thickness positivity) to ensure the sufficient standardization and reproducibility of the histologic assessment. The comparison with a positive control sample was applied for the evaluation of staining intensity. The three basic parameters of staining were combined into 13 possible immunoprofiles (summarized in Fig. 1).

Tissue sections intended for CK 17 immunohistochemistry were immersed in Target Retrieval Solution (cat. No. S 1700, DakoCytomation, Glostrup, Denmark) for the epitope retrieval at 98 °C for 30 min and subsequently incubated overnight at 4 °C with primary monoclonal mouse anti-human antibody Cytokeratin 17 (diluted 1:100) (clone E3, cat. No. M 7046, DakoCytomation, Glostrup, Denmark). The immunocomplexes of the antigen and the primary antibody were visualized using the streptavidin-biotin detection kit LSAB+, Dako REAL™ Detection Systems, HRP/DAB+, Rabbit/Mouse (cat. No. K 5001, DakoCytomation, Glostrup, Denmark). The positive control (immature squamous metaplasia of the uterine cervix) was used in each series of immunohistochemistry.

All slides immunostained for CK 17 were reviewed by two observers and consensually evaluated. Cytoplasmic staining was considered for a positive result of the immunoreaction. The two-grade scoring system was used to scale the extent of CK 17 staining. Focal positivity was defined as a non confluent staining of single cells or clusters of cells and the diffuse positivity corresponded with a confluent band-like staining.

Results

p16INK4a Immunoprofiles of Lesions

The rates of p16INK4a positive cases in particular groups of patients are calculated in Table 2 and the frequency of all 13 possible immunoprofiles is shown in Fig. 1. Samples of p16INK4a immunostaining are exemplified in Figs. 2 and 3. p16INK4a negativity prevailed in the SM (87.9 %), RC (78.4 %) and Kc (60.0 %) groups. All cases from the Co group were p16INK4a positive and the typical immunostaining profile was a focal, weak, full-thickness positivity (87.5 %). Although the majority of lesions from the CIN I group were p16INK4a positive (97.1 %), their immunoprofiles were diverse and showed mostly diffuse positivity of varying intensity in the lower half of the epithelium (71.4 %) and a focal, weak, lower half staining (17.1 %). The most common p16INK4a immunoprofile in the CIN II and CIN III groups was a diffuse, strong, full-thickness positivity (84.1 % in the CIN II and 94.0 % in the CIN III group). p16INK4a positive lesions from the AIM group (68.8 %) showed four immunoprofiles of approximately similar frequencies (none of these immunoprofiles significantly prevailed).

CK 17 Immunoexpression in AIM

In total, 31 cases of AIM were available for CK 17 immunohistochemistry. One lesion with a diffuse, strong, full-thickness p16INK4a positivity was lost during the previous serial sectioning. The immunoexpression of CK 17 was observed in all AIM lesions. The majority (80.6 %) showed diffuse staining which was usually intense and affected full-thickness of the epithelium. Focal positivity (19.4 %) of single cells or clusters of cells was typically limited to the basal zones of the epithelium and its intensity was more heterogeneous. The diffuse CK 17 staining was constantly observed in all AIM lesions which were p16INK4a negative or showed low level of p16INK4a expression (sporadic, weak, full-thickness and focal, weak, full-thickness staining). In the group of AIM lesions with diffuse, strong, full-thickness p16INK4a positivity, 50.0 % of cases showed diffuse CK 17 immunoexpression and 50.0 % of lesions were focally positive. All AIM cases with focal, strong, full-thickness p16INK4a positivity were focally stained for CK 17. An overview of CK 17 immunostaining in particular groups of lesions is calculated in Table 3 and typical samples are exemplified in Fig. 3.

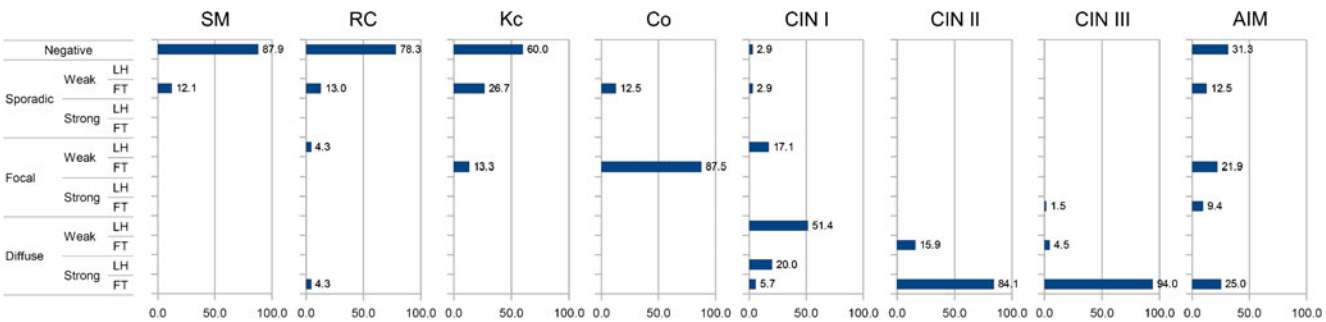


Fig. 1 Percentage frequencies of 13 possible p16INK4a immunoprofiles in particular groups of lesions. *LH* lower half, *FT* full-thickness

Table 2 An overview of $p16^{INK4a}$ positivity and the spectrum of $p16^{INK4a}$ immunoexpression patterns in particular groups of lesions

Lesion	Number of $p16^{INK4a}$ positive cases	$p16^{INK4a}$ positivity (%)	$p16^{INK4a}$ staining parameters in positive cases (%)						
			Horizontal distribution			Vertical distribution		Intensity	
			Sporadic	Focal	Diffuse	Weak	Strong	Lower half	Full-thickness
SM	4	12.1	100.0	–	–	100.0	–	–	100.0
RC	5	21.6	60.0	20.0	20.0	80.0	20.0	20.0	80.0
Kc	6	40.0	66.7	33.3	–	100.0	–	–	100.0
Co	8	100.0	12.5	87.5	–	100.0	–	–	100.0
CIN I	34	97.1	2.9	17.7	79.4	73.5	26.5	91.2	8.8
CIN II	82	100.0	–	–	100.0	15.9	84.1	–	100.0
CIN III	67	100.0	–	1.5	98.5	4.5	95.5	–	100.0
AIM	22	68.8	18.2	45.4	36.4	50.0	50.0	–	100.0

SM mature and immature squamous metaplasia, *RC* metaplastic squamous epithelium with reactive changes, *Kc* metaplastic squamous epithelium with koilocytosis, *Co* flat condyloma, *CIN I* cervical intraepithelial neoplasia I, *CIN II* cervical intraepithelial neoplasia II, *CIN III* cervical intraepithelial neoplasia III, *AIM* atypical immature squamous metaplasia

Discussion

$p16^{INK4a}$ Immunoprofiles of Lesions

Squamous dysplastic lesions of the uterine cervix are generally considered $p16^{INK4a}$ positive, although the results differ between studies according to the grade of lesions and the immunoscoring system used. The highest heterogeneity was seen in the CIN I category, where the rate of $p16^{INK4a}$ expression varied between 35 % [9] and 100 % [15]. Although $p16^{INK4a}$ positivity of the lesions from the CIN II and CIN III categories reached mostly 90–100 % [14, 15], a higher proportion of negative lesions (up to 33 %) has been reported [9]. All but one case of CIN I and all lesions from the CIN II and CIN III groups were $p16^{INK4a}$ positive in our series. Similar to Sano et al. [16], we observed $p16^{INK4a}$ immunoexpression in all cervical flat condylomas. A relatively high rate of $p16^{INK4a}$ negative cases of koilocytosis (60.0 %) in comparison with CIN I and flat condylomas might be explained by different HPV-mediated molecular events in these lesions or by poor reproducibility of koilocytosis.

Our results indicate that the diffuse $p16^{INK4a}$ positivity is strongly associated with the dysplastic behavior of the lesion: it was observed in 98.5 % of CIN III, in 100.0 % of CIN II and in 77.1 % of CIN I, but it was not present in the SM, Kc and Co groups and only one case from the RC group showed this immunostaining pattern. Diffuse staining in the CIN I group was mostly weak (51.4 %), sometimes strong (25.7 %), but predominantly limited to the lower half of the epithelium, whereas it was usually strong and extended to the upper parts of the epithelium in the CIN II and CIN III groups. Similar immunoprofiles of particular grades of CIN were reported in previous studies [14–17]. Focal expression of $p16^{INK4a}$ was observed in our study especially in non-dysplastic lesions associated with HPV infection (87.5 % of Co and 13.3 % of Kc). It occurred in 17.1 % of CIN I as well, but it was only rarely seen in other types of lesions (one case from the RC and CIN III groups). Similar patterns of focal $p16^{INK4a}$ positivity in condylomas and CIN I were detected previously [14, 16], although some papers describe explicitly diffuse positivity in the CIN I category without any focal staining [18]. Our data further showed that sporadic expression of $p16^{INK4a}$ is strongly associated with a non-precancerous behavior of such a lesion. It was observed in the SM (12.1 %), RC (13.0 %), Kc (26.7 %) and Co (12.5 %) groups (only one case of CIN I showed this pattern). Sporadic $p16^{INK4a}$ expression in non-precancerous lesions, including condylomas, was also well documented in previous studies [14–16].

The classification of squamous lesion of the uterine cervix into one of the three diagnostic groups: no dysplasia, LSIL and HSIL, represents the sufficient and clinically relevant information for the appropriate treatment of a patient [19]. These basic phenotypes could be defined in our study as follows: metaplastic (represented by combined SM and RC groups), LSIL/HPV (represented by combined Kc, Co and CIN I groups) and HSIL (represented by combined CIN II and CIN III groups). Our data indicate that the typical $p16^{INK4a}$ immunoprofile of the metaplastic phenotype is negativity. LSIL/HPV phenotype shows a heterogeneous pattern of $p16^{INK4a}$ immunoexpression which may be best defined as a sporadic, weak, full-thickness positivity or a focal, weak, lower half/full-thickness positivity or a diffuse, weak/strong, lower half positivity, where all lesions with

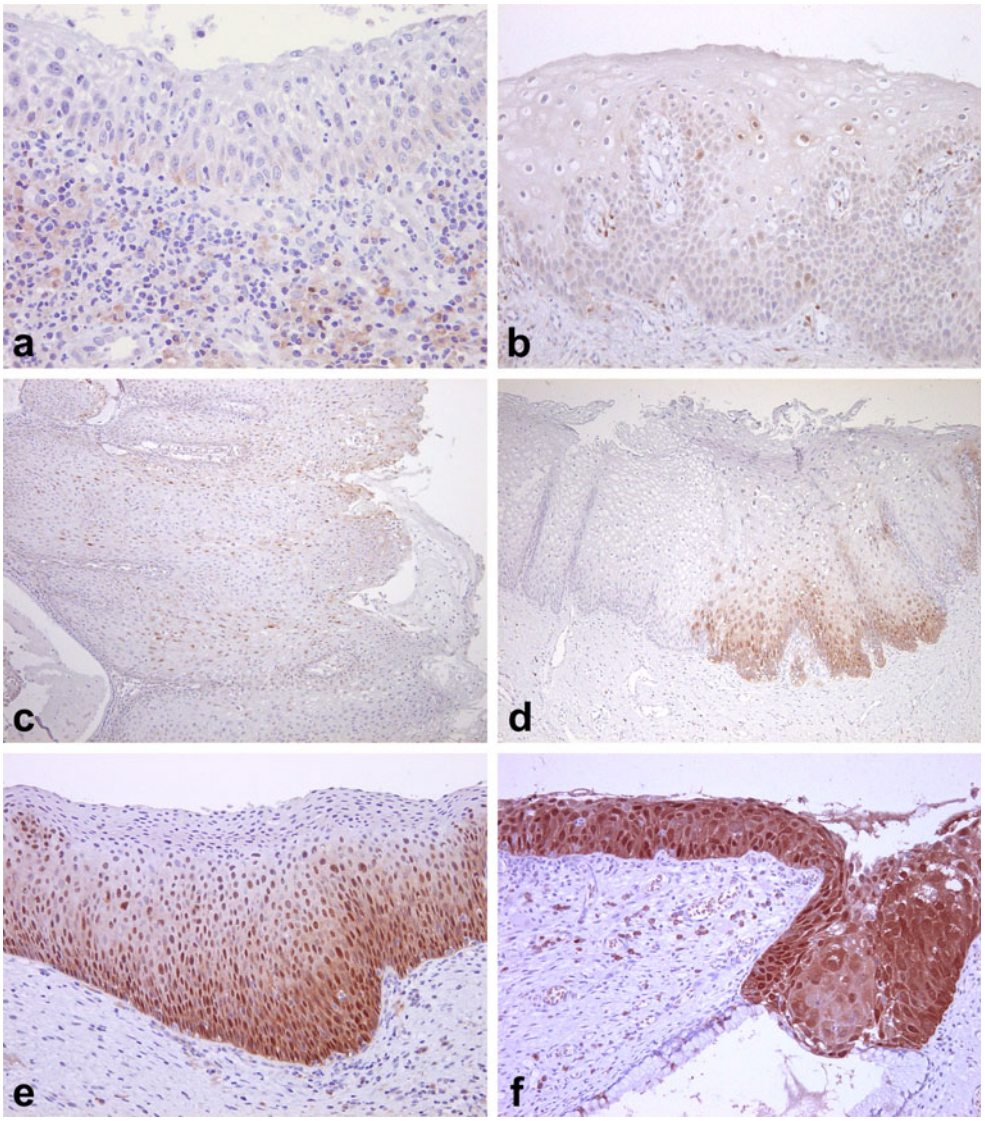


Fig. 2 Examples of p16^{INK4a} immunoprofiles differing between particular groups of lesions. **a** negativity in the metaplastic squamous epithelium with reactive changes (RC) (200×); **b** sporadic, weak, full-thickness positivity in the metaplastic squamous epithelium with koilocytosis (Kc) (200×); **c** focal, weak, full-thickness positivity in flat condyloma (Co) (100×); **d** sharp transition between the non-dysplastic squamous epithelium and CIN I with a diffuse, weak, lower half positivity (100×); **e** immunostaining extending into the upper half of the epithelium in CIN II interpreted as a diffuse, strong, full-thickness positivity (200×); **f** diffuse, strong, full-thickness positivity in CIN III (200×)

diffuse, lower half staining of any intensity correspond to CIN I. HSIL phenotype is clearly defined by a diffuse, strong/weak, full-thickness p16^{INK4a} positivity. Focal, strong, full-thickness p16^{INK4a} staining observed in one case of CIN III probably represent HSIL phenotype as well, as it was not seen in any other type of lesion except AIM.

Predictive Significance of p16^{INK4a} Immunoprofiles

The CIN I group showed the most heterogeneous p16^{INK4a} expression, with a total of six p16^{INK4a} immunoprofiles. Although the majority of CIN I lesions were characterized by the LSIL/HPV phenotype, some of them (5.7 %) with a diffuse, strong, full-thickness positivity displayed the HSIL phenotype. This finding raises the question of whether a heterogeneous spectrum of p16^{INK4a} immunoprofiles in the CIN I group reflects different precancerous potentials of particular lesions. It is well known that p16^{INK4a} expression correlates with the spectrum of HPV types involved in the pathogenesis of the lesion. Diffuse and strong p16^{INK4a} immunostaining was observed mostly in lesions associated with HR-HPV types, whereas sporadic, focal and weak staining or no expression were found in cases infected with low risk HPV [14–17]. In addition, more intense p16^{INK4a} expression was detected in lesions with HPV DNA integrated into the host genome [17].

Given that the level of p16^{INK4a} expression correlates with the grade of CIN as well as with the HPV profile and the HPV integration status, it is not surprising that the diffuse p16^{INK4a} staining was confirmed to be an adverse prognostic factor in CIN I lesions, where it was associated with a higher rate of progression [20] or a shorter interval for progression [21]. Alternatively, p16^{INK4a} negative or non-diffusely stained CIN I did not progress to HSIL [22]. Therefore, we believe that some of the CIN I cases in our series, especially those with the HSIL phenotype, are prone to progress to HSIL.

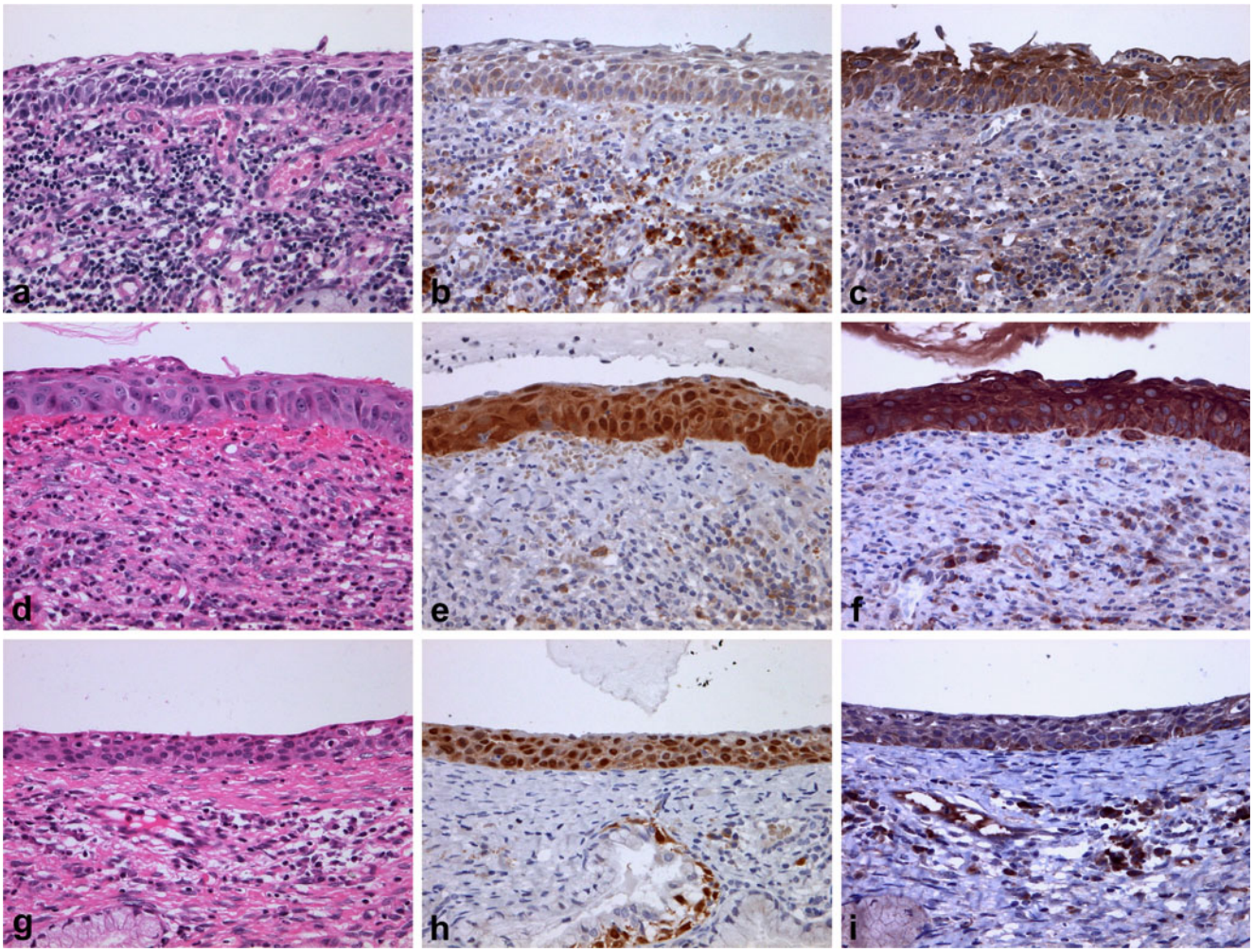


Fig. 3 Examples of AIM with various $p16^{INK4a}$ and CK 17 immunoprofiles (corresponding hematoxylin-eosin (HE) stained sections are shown). **a**, **b**, **c** AIM with the metaplastic phenotype (**a** HE, **b** $p16^{INK4a}$ negativity, **c** diffuse CK 17 positivity, 400×); **d**, **e**, **f** AIM with the HSIL phenotype (**d** HE, **e** diffuse, strong, full-thickness $p16^{INK4a}$ positivity, **f** diffuse CK 17 positivity, 400×); **g**, **h**, **i** AIM with the HSIL phenotype (**g** HE, **h** diffuse, strong, full-thickness $p16^{INK4a}$ positivity, **i** focal CK 17 positivity of single cells and clusters of cells in the basal zone of the epithelium, 400×)

Table 3 An overview of rates of CK 17 immunoexpression patterns in particular groups of AIM diversified according to their $p16^{INK4a}$ immunoprofiles and stratified into three basic clinically relevant phenotype groups (metaplastic, LSIL/HPV and HSIL)

$p16^{INK4a}$ immunoprofile	Phenotype	CK 17 immunoexpression	
		Diffuse	Focal
Negative	Metaplastic	10/10	–
Sporadic, weak, full-thickness	LSIL/HPV	2/2	–
Focal, weak, full-thickness	LSIL/HPV	9/9	–
Focal, strong, full-thickness	HSIL	–	2/2
Difuse, strong, full-thickness	HSIL	4/8	4/8

CK 17 cytokeratin 17, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion, *HPV* human papillomavirus

CIN II is also considered a heterogeneous category of lesions with a various tendency to regression [23]. This could be partially caused by a relatively low reproducibility of CIN II diagnosis when compared with CIN III [3]. However, we did not identify any significant difference between $p16^{INK4a}$ immunoprofiles of patients diagnosed with CIN II and CIN III. The CIN II group therefore seems to be consistent in our series. Importantly, the strong immunoexpression of $p16^{INK4a}$ in CIN II was shown to be associated with a persistence or even progression into CIN III [17].

Reclassification of the AIM group

The AIM group showed five $p16^{INK4a}$ immunoprofiles, making it the second most heterogeneous group in our series. The majority of AIM lesions (68.8 %) were $p16^{INK4a}$ positive and

showed HSIL, LSIL/HPV and metaplastic phenotypes in 34.4 %, 34.4 % and 31.2 % of the cases, respectively. These results suggest that approximately one third of the AIM cases in our study should be considered as a HSIL or a lesion with a potential to progress to HSIL, one third of the AIM group can be reclassified as LSIL or a manifestation of HPV infection and one third represents an immature squamous metaplasia. The immunohistochemical assessment of p16INK4a expression was already shown to be beneficial in the estimation of the biologic behavior of AIM [6, 7, 13]. The proportion of lesions with p16INK4a overexpression fluctuated in the interval 41–65 % [6, 13], with 19 % [6] to 65 % [13] subsequently reclassified as HSIL. These data were supplemented by HPV typing studies, which detected intermediate/HR-HPV types in up to 67 % of the AIM cases [5].

CK 17 represents another immunohistochemical marker which has been evaluated in AIM. Regauer et al. [13] reported the reciprocal immunoreactivity of p16INK4a and CK 17 in immature squamous metaplasia and CIN III and suggested that these two antibodies should be used for the reclassification of AIM and that the term AIM should be withdrawn from the terminology. However, this observation was not confirmed by other studies which describe not only the immunoexpression of CK 17 in all grades of CIN, but also its correlation with the increasing grade of the lesion [11, 12]. In our series, all AIM cases with p16INK4a immunoexpression consistent with metaplastic and LSIL/HPV phenotype displayed a diffuse pattern of CK 17 staining. A heterogeneous CK 17 immunoexpression was observed in AIM lesions with HSIL phenotype where focal CK 17 positivity prevailed (60.0 %) and the diffuse CK 17 staining was detected less frequently (40.0 %). Regauer et al. [13] described similar coexpression of p16INK4a and CK 17 immunomarkers in 15 % of AIM cases and reclassified all these lesions as CIN III. We appreciate this opinion and recommend to prefer p16INK4a as a more reliable marker until the role of CK 17 immunohistochemistry in the differential diagnosis between immature squamous metaplasia and CIN III will be clarified on a larger series of cases.

Conclusions

p16INK4a immunohistochemistry based on the evaluation of the intensity and horizontal and vertical distribution of staining appears as a suitable supporting method for the classification of squamous lesions of the uterine cervix. Furthermore, it may also be used for the reclassification of categories with the heterogeneous p16INK4a expression (especially AIM). We strongly encourage pathologists to use the p16INK4a immunohistochemistry in these specific indications. On the other hand, the efficacy of CK 17 immunohistochemistry seems to be controversial for these purposes.

Acknowledgments This study was supported by the research project of Grant Agency of Charles University in Prague, Czech Republic (GAUK 85608) and by the project of the Ministry of Health of the Czech Republic for conceptual development of research organization 00064203 University Hospital Motol, Prague, Czech Republic.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. McCluggage WG, Walsh MY, Thornton CM, Hamilton PW, Date A, Caughley LM, Bharucha H (1998) Inter- and intra-observer variation in the histopathological reporting of cervical squamous intraepithelial lesions using a modified Bethesda grading system. Br J Obstet Gynaecol 105(2):206–210
2. Stoler MH, Schiffman M (2001) Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 285(11):1500–1505
3. Carreon JD, Sherman ME, Guillén D, Solomon D, Herrero R, Jerónimo J, Wacholder S, Rodríguez AC, Morales J, Hutchinson M, Burk RD, Schiffman M (2007) CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. Int J Gynecol Pathol 26(4):441–446
4. Crum CP, Egawa K, Fu YS, Lancaster WD, Barron B, Levine RU, Fenoglio CM, Richart RM (1983) Atypical immature metaplasia (AIM). A subset of human papilloma virus infection of the cervix. Cancer 51(12):2214–2219
5. Geng L, Connolly DC, Isacson C, Ronnett BM, Cho KR (1999) Atypical immature metaplasia (AIM) of the cervix: is it related to high-grade squamous intraepithelial lesion (HSIL)? Hum Pathol 30(3):345–351
6. Iaconis L, Hyjek E, Ellenson LH, Pirog EC (2007) p16 and Ki-67 immunostaining in atypical immature squamous metaplasia of the uterine cervix: correlation with human papillomavirus detection. Arch Pathol Lab Med 131(9):1343–1349
7. Duggan MA, Akbari M, Magliocco AM (2006) Atypical immature cervical metaplasia: immunoprofiling and longitudinal outcome. Hum Pathol 37(11):1473–1481
8. O'Neill CJ, McCluggage WG (2006) p16 expression in the female genital tract and its value in diagnosis. Adv Anat Pathol 13(1):8–15
9. Branca M, Ciotti M, Santini D, Di Bonito L, Giorgi C, Benedetto A, Paba P, Favalli C, Costa S, Agarossi A, Alderisio M, Syrjanen K (2004) p16(INK4A) expression is related to grade of CIN and high-risk human papillomavirus but does not predict virus clearance after conization or disease outcome. Int J Gynecol Pathol 23(4):354–365
10. Martens JE, Arends J, Van der Linden PJ, De Boer BA, Helmerhorst TJ (2004) Cytokeratin 17 and p63 are markers of the HPV target cell, the cervical stem cell. Anticancer Res 24(2B):771–775
11. Smedts F, Ramaekers F, Troyanovsky S, Pruszczynski M, Robben H, Lane B, Leigh I, Plantema F, Vooijs P (1992) Basal-cell keratins in cervical reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. Am J Pathol 140(3):601–612
12. Ikeda K, Tate G, Suzuki T, Mitsuya T (2008) Coordinate expression of cytokeratin 8 and cytokeratin 17 immunohistochemical staining in cervical intraepithelial neoplasia and cervical squamous cell carcinoma: an immunohistochemical analysis and review of the literature. Gynecol Oncol 108(3):598–602
13. Regauer S, Reich O (2007) CK17 and p16 expression patterns distinguish (atypical) immature squamous metaplasia from high-grade cervical intraepithelial neoplasia (CIN III). Histopathology 50(5):629–635

14. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U, Dallenbach-Hellweg G, Schmidt D, von Knebel Doeberitz M (2001) Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer 92(2):276–284
15. Keating JT, Cviko A, Riethdorf S, Riethdorf L, Quade BJ, Sun D, Duensing S, Sheets EE, Munger K, Crum CP (2001) Ki-67, cyclin E, and p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. Am J Surg Pathol 25(7):884–891
16. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T (1998) Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Am J Pathol 153(6):1741–1748
17. Omori M, Hashi A, Nakazawa K, Yuminamochi T, Yamane T, Hirata S, Katoh R, Hoshi K (2007) Estimation of prognoses for cervical intraepithelial neoplasia 2 by p16INK4a immunoexpression and high-risk HPV in situ hybridization signal types. Am J Clin Pathol 128(2):208–217
18. Focchi GR, Silva ID, Nogueira-de-Souza NC, Dobo C, Oshima CT, Stavale JN (2007) Immunohistochemical expression of p16(INK4A) in normal uterine cervix, nonneoplastic epithelial lesions, and low-grade squamous intraepithelial lesions. J Low Genit Tract Dis 11(2):98–104
19. Wright TC, Massad LS, Dunton CJ, Spitzer M, Wilkinson EJ, Solomon D (2007) 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. Am J Obstet Gynecol 197(4):340–345
20. Negri G, Vittadello F, Romano F, Kasal A, Rivasi F, Girlando S, Mian C, Egarter-Vigl E (2004) p16INK4a expression and progression risk of low-grade intraepithelial neoplasia of the cervix uteri. Virchows Arch 445(6):616–620
21. Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS, Wallin KL (2004) Predictive significance of the alterations of p16INK4A, p14ARF, p53, and proliferating cell nuclear antigen expression in the progression of cervical cancer. Clin Cancer Res 10(7):2407–2414
22. Hariri J, Oster A (2007) The negative predictive value of p16INK4a to assess the outcome of cervical intraepithelial neoplasia 1 in the uterine cervix. Int J Gynecol Pathol 26(3):223–228
23. Castle PE, Schiffman M, Wheeler CM, Solomon D (2009) Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. Obstet Gynecol 113(1):18–25

Příloha VI.

Rob L., Charvát M., Robová H., Pluta M., Strnad P., Hrehorčák M. a Škapa P. (2007) Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. ***Int J Gynecol Cancer*** 17:304-310.

IF 1,427

Int J Gynecol Cancer 2007, **17**, 304–310

Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer

L. ROB*, M. CHARVAT*, H. ROBOVA*, M. PLUTA*, P. STRNAD*,
M. HREHORCAK* & P. SKAPA†
**Division of Gynecologic Oncology, Department of Obstetrics and Gynecology and †Department of Pathological Anatomy and Molecular Medicine, Charles University Prague, 2nd Medical Faculty, Prague, Czech Republic*

Abstract. Rob L, Charvat M, Robova H, Pluta M, Strnad P, Hrehorcak M, Skapa P. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. *Int J Gynecol Cancer* 2007;**17:**304–310.

The purpose of this pilot study was to determine feasibility and safety of a novel and less radical fertility-preserving surgery; laparoscopic lymphadenectomy with sentinel lymph node identification (SLNI) followed by large cone or simple trachelectomy. Obstetrical and oncologic outcomes were evaluated. Twenty-six patients (6-IA2, 20-IB1) selected on basis of favorable cervical tumor characteristics and the desire to maintain fertility underwent laparoscopic SLNI, frozen section (FS), and a complete pelvic lymphadenectomy as first step of treatment. All of nodes were submitted for microscopic evaluation (sentinel nodes for ultramicrostaging). After a 7-day interval, large cone or simple vaginal trachelectomy was performed in patients with negative nodes. The average of sentinel nodes per side was 1.50 and the average of total nodes was 28.0. Four FS were positive (15.4%). In these cases, Wertheim radical hysterectomy type III was immediately performed. We had no false-negative SLN neither on FS nor on final pathology assessment. Median follow-up was 49 months (18–84). One central recurrence (isthmic part of uterus) was observed 14 months after surgery. This patient was treated with radical chemoradiotherapy, and there was no evidence of the disease 36 months after treatment. Fifteen women planned pregnancy, 11 women became pregnant (15 pregnancies), and 7 women delivered eight children (one in 24 weeks, one in 34 weeks, one in 36 weeks, and five between 37 and 39 weeks). We conclude that lymphatic mapping and SLNI improves safety in this fertility sparing surgery. Large cone or simple trachelectomy combined with laparoscopic pelvic lymphadenectomy can be a feasible method with a high successful pregnancy rate.

KEYWORDS: cervical cancer, fertility-sparing surgery, radical trachelectomy, sentinel lymph node, simple trachelectomy.

The mean age of primiparous women has recently been found to increase. Similarly, the number of women planning motherhood after the age of 30. It is therefore evident that the desire to preserve fertility is strong in many of these patients. The development of laparoscopic surgery led to the first fertility-preserving laparoscopic lymphadenectomy with radical trachelectomy performed in 1987 by Dargent et al[1,2]. Since then laparoscopic lymphadenectomy with radical trachelectomy has become the most frequent procedure for conservative, uterus preserving treatments employed in cases of early-stage invasive cervical carcinoma in women planning pregnancy[2–7]. We performed the first lymphadenectomy with radical trachelectomy at our department in cooperation with Dargent in 1997. This procedure gradually became an accepted solution for tumor stages IA2 and IB1 up to a maximum of 20 mm in largest diameter. Oncologic results of more than 300 published cases are acceptable and do not differ significantly from radical procedures[2–7]. Likewise, the reproductive results are satisfactory despite the significantly increased number of premature deliveries[1,4,7–11]. The sentinel lymph node mapping technique improved our knowledge about the lymphatic drainage of cervical carcinoma[12–17]. Our experience with sentinel lymph node mapping enabled us to

Address correspondence and reprint requests to: Lukas Rob, MD, PhD, Department of Obstetrics and Gynaecology, 2nd Medical Faculty, Charles University Prague, V uvalu 84, 150 00 Prague 5, Czech Republic. Email: lukas.rob@lfmotol.cuni.cz

doi:10.1111/j.1525-1438.2007.00758.x

employ this method in the management of conservative surgeries in cases of early cervix carcinoma and to reduce the radicality of the medial part of the lateral parametria[15,16]. We performed large cone (stage IA2) or simple trachelectomy (IB1) in women with negative histologic findings of sentinel nodes (frozen section [FS] and ultramicrostaging) and negative findings of other pelvic nodes. In this article, we are presenting our management of less radical surgery as compared with radical trachelectomy, as well as oncologic results and reproductive outcomes.

Materials and methods

From January 1999 to July 2004, 26 women (mean age 28.0 years) underwent laparoscopic sentinel lymph node identification (SLNI) (blue dye or blue dye + Tc) as a first step of conservative uterus sparing surgery. The desire to preserve fertility was strong in all 26 patients. Inclusion criteria were a tumor size less than 20 mm in the largest diameter representing less than half of the cervical stroma on magnetic resonance imaging (MRI) and ultrasonography (US) volumetry and serum squamous cell carcinoma levels within a normal range. All the diagnostic biopsies performed were subjected to second readings and systematically processed. We routinely performed preoperational tumor volumetry by transvaginal ultrasound in all women exploiting an 8 MHz transvaginal probe on Acuson Sequoia 512 and performed preoperative magnetic resonance volumetry using Gyroscan ACS-15NT Power Track 1000 (Phillips, The Netherlands), with a magnetic field of 1.5 T[16]. The study was approved by the ethical committee of our institution. All patients signed informed consent and all were counseled that the procedure was not standard therapy at the time. The timing of application (short protocol), technique of application (blue dye or Tc and blue dye), laparoscopic SLNI technique, and pathologic evaluation (FS and ultramicrostaging) of the sentinel nodes technique were described in our previous publication[16]. Having sent the sentinel nodes for FS, we performed a complete pelvic laparoscopic lymphatic node dissection and parametrial node dissection as the first step of our management. If the FS was found positive, laparoscopy was terminated and we continued with laparotomic radical hysterectomy (Wertheim type III) and lower para-aortic lymphadenectomy. All nodes were submitted to microscopic assessment: sentinel nodes for serial sectioning and immunohistochemical staging. Other nodes were subjected to standard assessment[16]. In 7-day intervals in patients with negative nodes, large cone in stage IA2 or simple trachelectomy in stage IB1 was performed as a second step in our procedure (Fig. 1). Large cone, which spare more stromal tissue than simple trachelectomy, was performed with needle (0.7 × 40 mm, Biogyn s.n.c., Florence, Italy) that was connect to LETZ (loop excision transformation zone) equipment (RF 300 Electrosurgery System, Engineering Production Equipment Medical, Italy). We cut endocervical edge with scissors. In our modification, simple trachelectomy begins with paracervical and intracervical instillation of 60–80 mL of solution with a vasoconstrictive substance (adrenaline in normal saline solution diluted 1:100,000). Depending on the extent of the tumor on the exocervix, the incision of vaginal mucosa was made circumferentially to create a vaginal cuff. The cervix was then paracervically skeletonized by dissection with scissors to the extent of 25–30 mm, we mobilized bladder and paracervical tissue, then we ligated descendent branches of uterine artery. This step is the same as the first step of simple vaginal hysterectomy. When we do not performed resection of parametrium, we do not have to mobilize ureters upward. The cervix was amputated with an incision approximately 7–10 mm above the tumor. We then employed a high frequency of 10–12 mm small loop (Biogyn s.n.c.) to create an endocervical channel to the depth of approximately 5 mm. The vaginal mucosa was then reapproximated circumferentially by individual sutures in the outer fringe created by the small loop. Cerclage was not performed. All pregnancies were managed as high risk and prophylactic administration of antibiotics was advocated. We elaborated the experimental protocol of the antibiotics administration after the first premature labor in this group of patients. Between weeks 15 and 17, we routinely administered prophylactic antibiotics in 5 days (clindamycin 300 mg every 8 h orally or metronidazole 250 mg every 8 h orally); between weeks 20 and 22, we prescribed vaginal treatment (Clindamycin 2% cream one times daily 5 days); and between weeks 24 and 26, we repeated the antibiotic treatment orally. Prior to the treatment, we examined the vaginal culture, the results of which might lead to the modification of antibiotic administration. We do not use routinely steroid prophylaxis to accelerate lung maturity. All patients in our study completed the minimum 18-month follow-up.

Results

From January 1999 to July 2004, 26 women fulfilled the inclusion criteria and were admitted into the study. Table 1 summarizes patient and tumor characteristics. The mean age of the 26 patients was 28.0 years

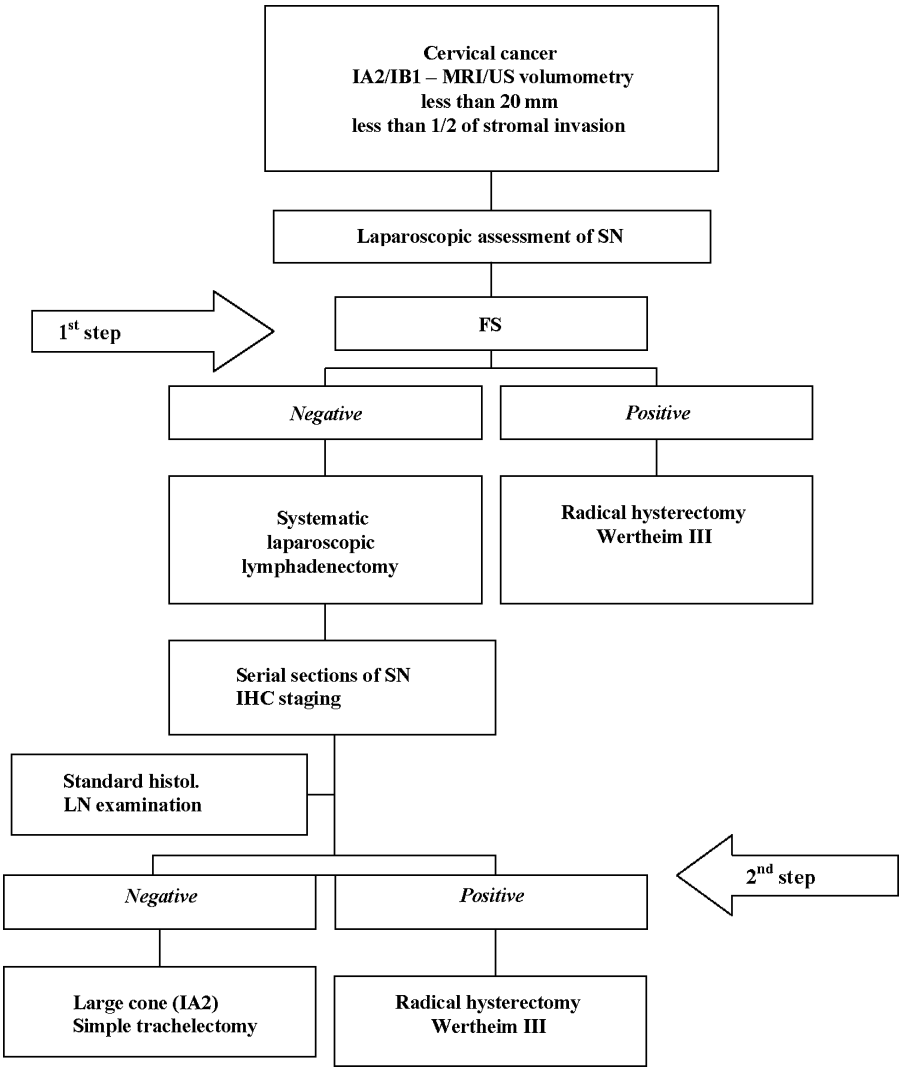


Figure 1. SNLI and conservative surgery.

(24–35). Fifteen of the women were nulliparous, ten had one child, and one woman had three children. Of the cancers, 80.8% were squamous cell (21 cases), 15.4% were adenocarcinomas (4 cases), and 3.8% were adenosquamous (1 case). The majority of patients 76.9% (20 cases) were classified as stage IB1 disease and 23.5% (6 cases) as stage IA2. Of the tumors, 46.2% were histologic grade 1, 34.6% were grade 2, and 19.2% were grade 3. Vascular space invasion (VSI) was found in 10 cases (38.5%) and was not proven in the remaining 16 cases (61.5%). In four cases, VSI was not mentioned in the primary reference record but was described in the second reading. The preoperative diagnosis was established by loop electro excision procedure or conisation in 16 patients (61.5%) and by cervical biopsy in 10 patients (38.5%).

Table 2 describes the operative details and surgical outcomes. The first step of our procedure was laparoscopic detection of sentinel nodes. We detected sentinel nodes in all women (detection rate per patient = 100%). The specific side detection rate of SLN in patients was 83.3% (Patent blau) and 94.1% (Patent blau + Tc). The average sentinel nodes per side was 1.50 (range 1–3). Four women had positive lymph nodes on FS (15.4%); in these cases, Wertheim radical hysterectomy (type III) with low para-aortic lymphadenectomy was performed. In cases of negative FS after SLNI, systematic laparoscopic pelvic lymphadenectomy was continued. The average total gain of lymphatic nodes was 28.0 (range 14–36). After laparoscopic lymphadenectomy, the procedure was completed and deep cone (7 women) or simple trachelectomy (15 women) was performed 7 days after the final histopathologic processing of the dissected nodes. In this study, all women with positive nodes were detected on FS. Serial sectioning and immunohistochemical detection did not confirm any further metastasis. Furthermore, there were no false-negative SLN results on final pathology. Residual tumor was determined in all ten women after biopsy and in six women after conisation.

Table 1. Patient and tumor characteristics

Characteristics	Number of patients = 26
Mean age	28.0 (24–35)
Prior pregnancies	
None	11
Abortions	4
One child	10
Two children	0
Three children	1
Stage IA2	6
Stage IB1	20
Invasion <5 mm	2
Invasion 5–10 mm	12
Invasion >10 mm	6
Grade 1	12
Grade 2	9
Grade 3	5
Histology	
Squamous	21
Adenocarcinoma	4
Adenosquamous	1
VSI positive	10
VSI negative	16
Diagnosis	
Cone or loop electro excision procedure	16
Biopsy alone	10

We found in one case mild dysplasia in posttrachlectomy loop specimen, the lesion did not involve margins.

Intraoperative laparoscopic complications occurred in two patients: one vascular injury of the external iliac vein related to the lymphadenectomy, which was repaired laparoscopically, and one uterine vascular injury during laparoscopic identification of lymphatic channel and sentinel nodes in the medial part of the lateral parametrium. There were no complications related to large cone or simple trachelectomy procedures. No blood loss that required a blood transfusion occurred and there were no urethral injuries.

The median follow-up was 49 months (range 18–84). In our sample, we have so far recorded only one recurrence in the isthmic part of the uterus (patient number 5, stage IB1, LETZ invasion 8 mm, diameter 7 mm with angioinvasion, 27 negative nodes, subsequent trachelectomy, only a small foci cervical intraepithelial neoplasia 1 without evidence of invasive carcinoma, small endocervical loop was negative). The recurrence was diagnosed by colposcopy 14 month after the treatment (Papanicolaou smear was negative 4 month ago). MRI shows tumor 23 × 18 mm in isthmic part of the uterus. We indicated chemoradiotherapy with regard to the volume and the localization of tumor and also with regard to the previous surgery. After the treatment, the woman is in complete remission. During the follow-up, an abnormal cytologic finding (high grade squamous intraepithelial lesion) was detected in one of the women who was determined to be high risk human papillomavirus (HR-HPV) positive. The patient preferred uterus removal and the histopathology examination after hysterectomy confirmed cervical intraepithelial neoplasia 2. All other patients demonstrated no colposcopic or cytologic symptoms of the disease.

Table 2. Treatment details and surgical outcomes

DR	
SLNI—blue dye	DR = 100%, SSDR = 83.3%
SLNI—blue dye + Tc99	DR = 100%, SSDR = 94.1%
Average SLN per side	1.5 (1–3)
False-negative SLN	0
FS outcomes	
FS positive	4 (15.4%)
FS negative	22 (84.6%)
Mean pelvic lymph node yield	28.0 (14–36)
Large cone	7 (26.9%)
Trachelectomy	15 (57.7%)
Radical hysterectomy	4 (15.4%)

DR, detection rate; SSDR, specific side detection rate.

Table 3 presents pregnancy details and results. Fifteen out of 20 women whose reproductive ability had been maintained tried to conceive. Altogether, we recorded 15 pregnancies in 11 women. In three cases, assisted reproduction methods were applied (twice by IUI [intrauterine insemination] and once by IVF [in vitro fertilization]). Seven mothers gave birth to eight children; in only one case, premature delivery occurred in the 24th week of pregnancy. The child, with a birth weight of 650 g, is alive and in good health. One born in the 34th week of gestation weighed 2240 g, one born in the 36th week of gestation weighed 2650 g, and the other five deliveries occurred between 37th and 39th weeks. All children are reported to be in good health. One woman decided on elective abortion for personal reasons and one woman was diagnosed with a very rare intraabdominal pregnancy that was treated surgically. Another woman miscarried twice in

Table 3. Pregnancy outcome

Saving fertility	20
Wishing pregnancy	15
Pregnant women	11
Eight children (1 × IUI, 1 × IVF)	1 × 650 g (24th week) 1 × 2240 g (34th week) 1 × 2650 g (36th week) 5 (37–39th week)
Interruption	1
Abdominal ectopic pregnancy	1
Spontaneous abortion I trimester	2
Spontaneous abortion II trimester (1 × IUI)	2
Ongoing pregnancy (18 weeks)	1

Table 4. Comparison of obstetrical outcome

	Schlaerth *et al.*[7], $n = 12$	Burnett *et al.*[6], $n = 21$	Shepherd *et al.*[4], $n = 30$	Mathevet *et al.*[11], $n = 95$	Bernardini *et al.*[9], $n = 80$	Plante *et al.*[5], $n = 72$	Rob *et al.*, $n = 26$
Pregnancies/women	4/4 women	3/3 women	14/8 women	56/34 women	22/18 women	50/31 women	15/11 women
First trimester losses							
SAB	0	0	4 (29%)	9 (16%)	3 (14%)	8 (16%)	2 (13%)
TAB	0	0	0	3	0	2	1
Ectopic	0	0	0	2	0	0	1
Second trimester losses	2 (50%)	1 (33%)	1 (7%)	8 (14%)	1 (4.5%)	2 (4%)	2 (13%)
Third trimester deliveries	2 (50%)	2 (66%)	9 (64%)	34 (61%)	18 (82%)	36 (72%)	8 (53%)
24–28 weeks	0	1	3		2	2	1
29–32 weeks	1	0	1	5	1	1	0
33–36 weeks	0	0	3		3	5	2
>37 weeks	1 (50%)	1 (50%)	2 (22%)	29 (85%)	12 (67%)	28 (72%)	5 (63%)
Ongoing pregnancy							1 (18 weeks)

SAB, spontaneous abortion; TAB, therapeutic abortion.

the first trimester. In one case, the IUI resulted in the conception of triplets, and at our recommendation, the patient agreed to a reduction to a singleton. In this case, septic miscarriage occurred in the 16th week. One woman miscarried in 22nd week of pregnancy after the premature rupture of membrane. Obstetrician did not respect our recommendation about antibiotic prophylaxis. At the time of evaluation (by March 2006), there was one pregnancy in progress (18th week of gestation).

Discussion

It is agreed that for pre- and intraoperational considerations about the patients eligibility to perform conservative fertility preserving treatment in early stages of cervical carcinoma, it is important to determine precisely the extent of the stromal involvement and to define histologic features as well as to exclude lymphonodal spread. Involvement of the cervical stroma can be determined by the evaluation of the depth of the invasion, the largest tumor diameter and (probably the best method) the tumor "volumetry" that defines tumor size and cervix dimensions. Currently, the best method in the measurement of residual tumor after biopsy or conisation is the combination of magnetic resonance and expert ultrasonographic examination[18,19,20]. Most centers accept the tumor size of less than 20 mm in the largest diameter as a limiting criterion for conservative uterus preserving treatments[3,4,5,7,9]. In our view, the extent of cervix infiltration should not exceed 50% of the stroma in MRI and US volumetric assessment as the most important factor. More extensive infiltration would not allow us to achieve the intact tissue cuff in the amputated section of the cervix. The question of how much of endocervical margin suffices as adequate therapy is currently under debate[7,8,21,22]. We consider 7 mm in the stroma and from the endocervical margin as an acceptable minimum. For the preservation of fertility, it is important at the same time to preserve the functional remaining cervical stroma of more than 10 mm. Therefore, it is clear that infiltration of 50% or more of the stroma is not compatible with the above mentioned criteria.

The preoperation biopsy yields further information about the tumor type and the degree of VSI. There is good agreement concerning the prognostic importance of these factors in conservative operations[3,4,5,7]. We considered some rare and aggressive histopathologic carcinoma types (neuroendocrine, small cell) unsuitable for conservative surgeries. In our sample, VSI, which is probably the most debated factor in early stages of cervical carcinoma, was evidenced in 38.5% (10/26) of the

cases. VSI depends on histopathologic processing, and its presence increases the risk of lymphatic nodes involvement. Although we consider the VSI finding a risk factor, we do not think it is a contraindication of conservative surgery if the pelvic nodes are negative.

The most important prognostic factor in early stages of cervical carcinoma is the positive or negative status of regional pelvic nodes. The sensitivity of all accessible preoperative imaging methods (lymphography, computed tomography, magnetic resonance imaging, positron emission tomography-computed tomography) to be insufficient for the identification of metastases smaller than 10 mm. The studies of sentinel nodes detection with radiocolloid technetium and blue dye further contributed to a better understanding of pelvic lymphatic drain[16,23]. SLNI is a highly sensitive method that enables intraoperative detection of metastatic foci in regional nodes and subsequent treatment of the most risky nodes[12–17,23]. Laparoscopic identification of sentinel nodes includes identification of blue-stained lymphatic channels in the medial part of the lateral parametrium, potential identification of blue-stained nodes in this region and their dissection and extirpation. Narrow lymphatic channels in the medial part of the lateral parametrium mostly run across the obliterated umbilical artery in the direction of supra-obturator or external iliac region to the first sentinel node, and less often in the direction of the presacral or along the common iliac vessels. Sentinel nodes in this medial part of the parametrium are found only very rarely in early stages of cervical carcinoma[16,24]. Positive findings in lymphatic nodes should lead to the withdrawal of the conservative approach. In early stages of cases of negative pelvic nodes, it is very rare to detect positivity in the medial part of the parametrium, which is resected during radical hysterectomy or radical trachelectomy[16,23,24]. The less radical procedure we currently employ in conservative operations follows the hypothesis that the removal of the medial part of the parametrium in such an early stage (IA2, IB1 less than half of stromal invasion) with negative ultramicrostaging of sentinel nodes and negativity in other pelvic nodes might not be important in preventing recurrence.

Recently, Plante et al.[5] analysed the oncologic results of 310 women (6 centers) subjected to radical trachelectomy (4.1% recurrences and 2.5% deaths). One central recurrence (1/22, 4.5%) occurred (in the isthmic part of the uterus) 14 months after surgery. This patient was treated with radical chemoradiotherapy, and 36 months later there was no evidence of the disease. Even though this sample of "reduced radicality" conservative operations is small, the oncologic results are comparable with the results of a radical trachelectomy sample.

In our study, we experienced complications and morbidity only in relation to the laparoscopic part of the operation. Large cone or simple trachelectomy was without complications in our study, and we did not have problems at follow-up with stenosis of the cervix stump related to the technique employed.

The pregnancy outcomes in our study were highly satisfactory. Thus, far, 73% (15/11) of the patients who tried to conceive succeeded. Two women after conisation and nine women after simplex trachelectomy conceived. We managed all pregnancies as high risk, the greatest potential risk being the possibility of a decreased barrier for the infection resulting in chorioamnionitis, and the premature rupture of membranes. First pregnancy in this group terminated prematurely in the 24th week as a result of the premature rupture of membranes and chorioamnionitis with delayed onset of labor. We advocate prophylactic administration of antibiotics. In our current management, we do not recommend sexual intercourse after the 12th week of pregnancy. If more than 10 mm of the cervix is successfully preserved, cervical incompetence (dilatation) was not a problem, even without cerclage. However, the newly created and shortened cervix represents an easier entry point for ascendant infections, and we agree with other centers that it is the greatest problem after simple as well as radical trachelectomies[4,8–11]. The obstetrical outcomes after vaginal radical trachelectomy and a detailed review of the literature were published by Plante et al. (Table 4)[10].

The importance of the medial part of parametrium removal in clinical stage IA2 and small IB1, tumors with negative sentinel nodes and negative other pelvic nodes still remains controversial. In our opinion, the subgroup of patients with negative nodes could be candidates for less excessive parametrial tissue removal with less related morbidity rather than for radical trachelectomy. Two-step management facilitated by ultramicrostaging of sentinel nodes increases the safety of conservative procedures. Pregnancy outcomes in this group of patients are excellent.

Acknowledgments

This work was supported by a grant from NR 8434-3 and MZO-00064203, Ministry of Health, Czech Republic.

References

1 Dargent D, Brun JL, Roy M, Remy I. Pregnancies following radical trachelectomy for invasive cervical cancer [abstract]. *Gynecol Oncol* 1994;**52:**105.

2 Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. *Cancer* 2000;**88:**1877–82.
3 Covens A, Shaw P, De Petrillo J, Lickrish G, Laframboise S. Is radical trachelectomy a safe alternative to radical hysterectomy for patient with stage IA-B carcinoma of the cervix? *Cancer* 1999;**86:**2273–9.
4 Shepherd JH, Mould T, Oram DH. Radical trachelectomy in early stage carcinoma of the cervix: outcome as judged by recurrence and fertility rates. *BJOG* 2001;**108:**882–5.
5 Plante M, Renaud MC, Francois H, Roy M. Vaginal radical trachelectomy: an oncologically safe fertility—preserving surgery. An update series of 72 cases and review on the literature. *Gynecol Oncol* 2004;**94:**614–23.
6 Burnett AF, Roman LD, O'Meara AT, Morrow CP. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. *Gynecol Oncol* 2003;**88:**419–23.
7 Schlaerth JB, Spirtos NM, Schlaerth AC. Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. *Am J Obstet Gynecol* 2003;**188:**29–34.
8 Petignat P, Stan C, Megevand E, Dargent D. Pregnancy after trachelectomy: a high-risk condition of preterm delivery. Report of a case and review of the literature. *Gynecol Oncol* 2004;**94:**575–7.
9 Bernardini M, Barrett J, Seaward G, Covens A. Pregnancy outcomes in patients after radical trachelectomy. *Am J Obstet Gynecol* 2003;**189:** 1378–82.
10 Plante M, Renaud MC, Hoskins IA, Roy M. Vaginal radical trachelectomy: a valuable fertility-preserving option in the management of early—stage cervical cancer. A series of 50 pregnancies and review of the literature. *Gynecol Oncol* 2005;**98:**3–10.
11 Mathevet P, de Kaszon EL, Dargent D. Fertility preservation in early cervical cancer. *Gynecol Obstet Fertil* 2003;**31:**706–12.
12 O'Boyle JD, Coleman RL, Bernstein SG, Lifshitz S, Muller CY, Miller DS. Intraoperative lymphatic mapping in cervix cancer undergoing radical hysterectomy: a pilot study. *Gynecol Oncol* 2000;**79:**238–43.
13 Malur S, Krause N, Kohler C, Schneider A. Sentinel lymph node detection in patients with cervical cancer. *Gynecol Oncol* 2001;**80:**254–7.
14 Levenback C, Coleman RL, Burke TW *et al*. Lymphatic mapping and sentinel node identification in patients with cervix cancer undergoing radical hysterectomy and pelvic lymphadenectomy. *J Clin Oncol* 2002;**20:**688–93.
15 Rob L, Pluta M, Strnad P, Robova H, Charvat M, Schlegerova D. Sentinel node identification in uterine cervix cancer stage I [abstract]. A pilot study. *Gynecol Oncol* 2002;**84:**521.
16 Rob L, Strnad P, Robova H *et al*. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. *Gynecol Oncol* 2005;**98:**281–8.
17 van Dam PA, Hauspy J, Vanderheyden T *et al*. Intraoperative sentinel node identification with Technetium-99m-labeled nanocolloid in patients with cancer of the uterine cervix: a feasibility study. *Int J Gynecol Cancer* 2003;**13:**182–6.
18 Sahdev A, Jones J, Shepherd JH, Reznek RH. MR imaging appearance of the female pelvis after trachelectomy. *Radiographics* 2005;**25:** 41–52.
19 Wagenaar HC, Trimbos JB, Postema S *et al*. Tumor diameter and volume assessed by magnetic resonance imaging in the prediction of the outcome for invasive cervical cancer. *Gynecol Oncol* 2003; **82:**474–82.
20 Chou CY, Hsu KF, Wang ST, Huang SC, Tzeng CC, Huang KE. Accuracy of three-dimensional ultrasonography in volume estimation of cervical carcinoma. *Gynecol Oncol* 1997;**66:**89–93.
21 Tanguay C, Plante M, Renaud MC, Roy M, Tetu B. Vaginal radical trachelectomy in the treatment of cervical cancer: the role of frozen section. *Int J Gynecol Pathol*. 2004;**23:**170–5.
22 Morice P, Dargent D, Haie-Meder C, Duvillard P, Castaigne D. First case of a centropelvic recurrence after radical trachelectomy: literature review and implications for the preoperative selection of patients. *Gynecol Oncol* 2004;**92:**1002–5.
23 Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. *Gynecol Oncol* 2003;**91:**494–503.
24 Covens A, Rosen B, Murphy J *et al*. How important is removal of the parametrium at surgery for carcinoma of the cervix? *Gynecol Oncol* 2002;**84:**145–9.

Accepted for publication July 11, 2006

Příloha VII.

Rob L., Pluta M., Strand P., Hrehorčák M., Chmel R., Škapa P. a Robová H. (2008) A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. ***Gynecol Oncol*** 111:S116-120.

IF 2,614

Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 111 (2008) S116–S120
Gynecologic Oncology
www.elsevier.com/locate/ygyno

A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer☆

Lukas Rob [a,*], Marek Pluta [a], Pavel Strnad [a], Martin Hrehorcak [a], Roman Chmel [a], Petr Skapa [b], Helena Robova [a]

[a] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Charles University Prague, 2nd Medical Faculty, Prague, Czech Republic
[b] Department of Pathological Anatomy and Molecular Medicine, Charles University Prague, 2nd Medical Faculty, Prague, Czech Republic

Received 9 July 2008
Available online 23 August 2008

Abstract

The purpose of the two pilot studies was to determine the feasibility and safety of using less-radical fertility-preserving surgery: laparoscopic lymphadenectomy with sentinel lymph node identification (SLNI) followed by a large cone or simple trachelectomy (LAP-I protocol) and the LAP-III protocol, which includes neoadjuvant chemotherapy (NAC).

LAP-I: Forty women underwent laparoscopic SLNI, frozen-section analysis, and a complete pelvic lymphadenectomy as the first step of treatment. Seven days after final histopathological processing of dissected nodes, a large cone or simple vaginal trachelectomy was performed in patients with negative nodes. Nine women had a tumor larger than 20 mm, prompting the administration of three cycles of NAC before surgery.

LAP-I: Six frozen sections were positive (15%). In these cases, a type III Wertheim was immediately performed. There were no false-negative SLNs. There was one central recurrence, but after chemoradiation therapy, there was no evidence of the disease 62 months post-treatment. Twenty-four of 32 women whose reproductive ability had been maintained tried to conceive. Of these 24 women, 17 became pregnant (71% pregnancy rate). Eleven mothers gave birth to 12 children (1 at 24 weeks, 1 at 34 weeks, 1 at 36 weeks, and 9 between 37 and 39 weeks). LAP-III: Nine patients were included. In 7 of these 9 women, reproductive ability was maintained, with 3 women becoming pregnant (1 full term and 2 ongoing).

SLNI improves safety in fertility-sparing surgery. Large cone or simple trachelectomy combined with laparoscopic pelvic lymphadenectomy can be a feasible method that yields a high, successful pregnancy rate. NAC followed by fertility-sparing surgery is an experimental alternative treatment for larger tumors.

Keywords: Simple trachelectomy; Sentinel lymph node; Cervical cancer; Fertility-sparing surgery; Neoadjuvant chemotherapy

Introduction

Due to the effective use of screening, an increasing number of women are being diagnosed with cervical cancer at a younger age. Many of these women are of childbearing age and wish to preserve their fertility. Meanwhile, the mean age of primiparous women and women planning pregnancy has increased. This postponement of childbearing coupled with the comparatively young age at which many women are diagnosed with cervical cancer has led to the innovation of various fertility-sparing procedures. The most common of these procedures is the radical vaginal trachelectomy. Alternative procedures include a less radical simple vaginal trachelectomy or a more radical procedure, such as the radical abdominal trachelectomy or total radical laparoscopic trachelectomy [1–12].

Oncology outcomes are usually very good if the tumor does not exceed 2 cm in diameter at its largest point or if it does not infiltrate more than half of the stroma. The 5-year survival rate is greater than 95% in these cases [1,3,4,7,9–11]. Pregnancy outcome is excellent in women treated with a radical vaginal trachelectomy or simple trachelectomy despite the significantly

☆ This work is supported by grant MZ NS-9914, Ministry of Health, Czech Republic.
* Corresponding author. Fax: +420 224 434 218.
E-mail address: lukas.rob@lfmotol.cuni.cz (L. Rob).

0090-8258/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2008.07.021

increased number of premature deliveries; however, pregnancy outcome is not as good in women treated with a radical abdominal or total radical laparoscopic trachelectomy [3,7,9–11,13–15].

Our experience with sentinel lymph node mapping (SLNM) enabled us to employ this method in the management of conservative surgeries in cases of early cervical carcinoma and to reduce the radicality of the medial part of the lateral parametria [16,17]. We performed a large cone (stage IA2) or simple trachelectomy (IB1) in women with negative sentinel nodes (based on frozen-section analysis and ultramicrostaging) and other negative pelvic nodes [7]. In this article, we present our two-step, less-radical treatment option to the radical trachelectomy, as well as oncological results and reproductive outcomes. We also present a second, new experimental protocol, which highlights the use of high-dose density neoadjuvant chemotherapy (NAC) followed by less-radical surgery.

Materials and methods

From January 1999 to December 2006, 40 women (mean age, 28.3 years) underwent laparoscopic sentinel lymph node identification (SLNI) as the first step for conservative fertility-sparing surgery (LAP-I protocol) at our institution. All 40 patients strongly desired fertility preservation. The inclusion criteria included a tumor size less than 20 mm in largest diameter *or infiltration of less than half of the cervical stroma* based on magnetic resonance imaging (MRI) and ultrasonograph (US) volumetry [7]. The ethical committee of our institution approved the study.

Having sent the sentinel nodes for frozen-section analysis, we perform a complete laparoscopic pelvic lymph node dissection and parametrial node dissection as the first step of our management. If the frozen section is positive, laparoscopy is abandoned and we continue with a laparotomic radical hysterectomy (Wertheim type III) and lower para-aortic lymphadenectomy. During the second step of our LAP-I protocol, patients with negative pelvic nodes and stage IA2 disease are treated with a large cone trachelectomy, and patients with negative pelvic nodes and stage IB1 disease are treated with a simple trachelectomy 7 days after the final histopathological processing of the dissected nodes (Fig. 1). In January 2005, our institutional ethical committee approved a new study, which features the use of high-dose density NAC (cisplatin [75 mg/m^2]+ifosfamide [2 g/m^2] for squamous disease or cisplatin [75 mg/m^2]+adriamycin [35 mg/m^2] for adenocarcinoma) followed by fertility-sparing surgery for early-stage cervical cancer patients (LAP-III–NAC protocol). Nine women under the age of 40 who desired fertility preservation and who had cervical cancer involving more than half of the stroma but not more than two thirds or a tumor larger than 2 cm were included in the study.

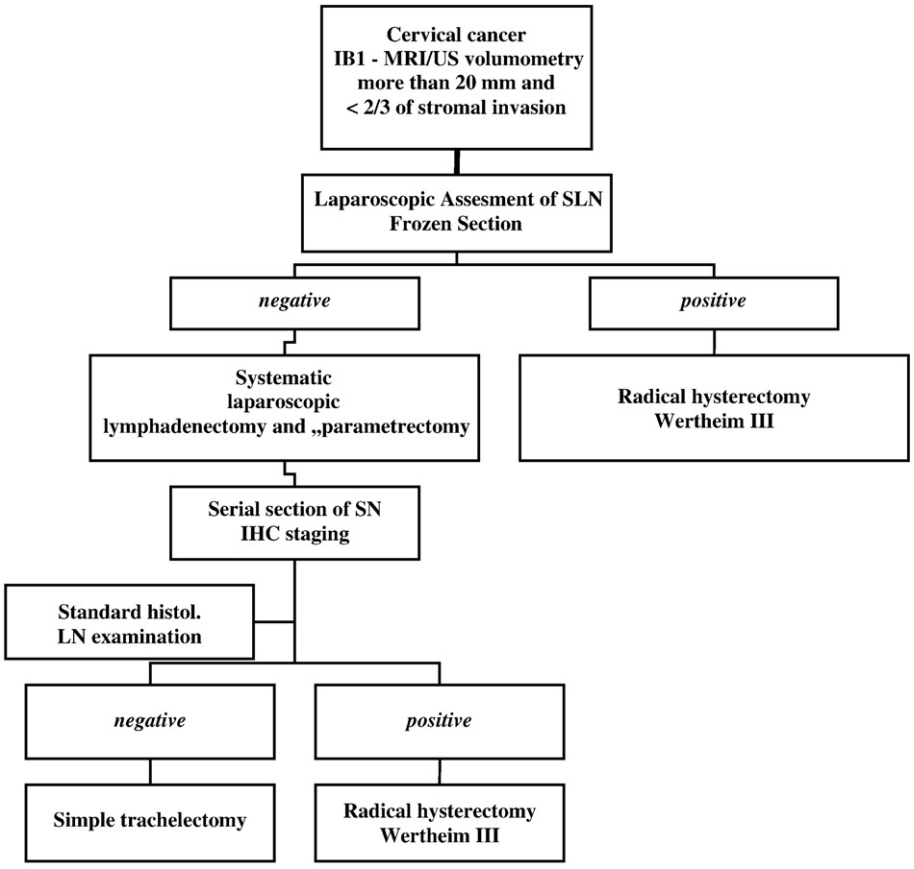


Fig. 1. NAC-SLNM and conservative surgery (NAC, neoadjuvant chemotherapy; SLNM, sentinel lymph node mapping; SLN, sentinel lymph node; LN, lymph node; MRI, magnetic resonance imagings; US, ultrasonography; IHC, imunohistochemistry).

Results

In the LAP-I protocol, there were 3 stage IA1 patients (100% with lymph-vascular space invasion [LVSI]), 10 IA2 patients (40% with LVSI), and 27 IB1 patients (38.5% with LVSI). Of the total 40 cases, 80% (32 cases) were of squamous cell histology, 17.5% (7 cases) were adenocarcinomas, and 2.5% (1 case) were adenosquamous. The preoperative diagnosis was established by a loop electrosurgical excision procedure (LEEP) or conization in 26 patients (65%) and by cervical biopsy in 14 patients (35%).

Operative details and surgical outcomes

We detected sentinel lymph nodes in all of the women (detection rate per patient [DR], 100%; specific side detection rate [SSDR], 95%). The average number of sentinel nodes per side was 1.50 (range, 1–4). Six (15%) frozen sections were positive; in these cases, a Wertheim radical hysterectomy (type III) with low para-aortic lymphadenectomy was performed. In cases of negative frozen section after SLNI, laparoscopic pelvic lymphadenectomy was continued. The average total gain of lymphatic nodes was 24.8 (range, 12–48). As for the second step of our LAP-I protocol, patients with negative pelvic nodes and stage IA2 disease were treated with a large cone trachelectomy 10 women), and patients with negative pelvic nodes and stage IB1 disease were treated with a simple trachelectomy (24 women) 7 days after the final histopathological processing of the dissected nodes. In this study, all of the positive nodes were detected by frozen-section analysis.

The median follow-up was 47 months (range, 12–102 months). One central recurrence occurred (in the isthmic part of the uterus) 14 months after surgery. This patient was treated with radical chemoradiation therapy, and there was no evidence of disease 60 months later. During the follow-up, an abnormal cytological finding showing a high-grade lesion and positivity of high-risk papillomavirus were detected in 1 patient; in this patient, a total abdominal hysterectomy was performed.

Pregnancy details and results

Twenty-four of 32 women whose reproductive ability had been maintained tried to conceive (by January 2008). Altogether, we recorded 23 pregnancies in 17 women. In 4 of these 17 women, assisted reproduction methods were applied (twice by intrauterine insemination [IUI] and twice by in vitro fertilization [IVF]). Eleven women gave birth to 12 children; in only one of these cases premature delivery occurred in the 24th week of pregnancy. The child, with a birth weight of 650 g, is

Table 1
Neoadjuvant chemotherapy and fertility-sparing surgery — patient characteristics

Patient age	Diagnosis	Histopathology	MRI	Chemotherapy	Toxicity	Surgery	Histopathology	Parity Pregnancy
#1, 25 years	Punch biopsy	Squamous cell Grade 2	20×20×10 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	Grade 2 neutropenia	SLNI, PLN Simple trachelectomy	16 LN negative No residual disease	Nulliparous
#2, 26 years	Conization	Adeno Grade 3	44×36×19 mm	DDP 75 mg/m^2 ADM 35 mg/m^2	0	Hyst WIII (patient decision)	29 LN negative No residual disease	Nulliparous
#3, 30 years	Biopsy 20×15 mm	Adeno Grade 1	17×11×5 mm	DDP 75 mg/m^2 ADM 35 mg/m	0	SLNI, PLN, Simple trachelectomy	22 LN negative Residual disease 0.5 mm	Nulliparous Spontaneous delivery at term
#4, 24 years	Punch biopsy	Squamous cell Grade 3	23×10×30 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	Grade 2 neutropenia	SLNI, PLN Simple trachelectomy	15 LN negative Residual disease 2×0.1 mm	Nulliparous Pregnancy 20th week
#5, 32 years	Biopsy 8×8 mm	Squamous cell Grade 3	15×17×24 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	0	SLNI, PLN Simple trachelectomy	22 LN negative Residual disease 13×6 mm	Nulliparous Pregnancy 34th week
#6, 31 years	Punch biopsy	Squamous cell Grade 1	27×17×26 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	0	SLNI, PLN Simple trachelectomy	17 LN negative Residual disease 1×1 mm	Nulliparous
#7, 33 years	Biopsy 18×8 mm	Squamous cell Grade 3	17×13×28 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	Grade 1 neutropenia	SLNI, PLN Simple trachelectomy	21 LN negative Residual disease 2×2 mm	Nulliparous
#8, 31 years	Conization 20×15 mm	Squamous cell Grade 3	17×22×17 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	0	SLNI, PLN Simple trachelectomy	31 LN negative No residual disease	Nulliparous
#9, 32 years	Conization 10×4 mm	Squamous cell Grade 3	18×27×29 mm	DDP 75 mg/m^2 Ifo 2 g/m^2	0	SLNI, PLN Simple trachelectomy Hyst WIII	16 LN negative Residual disease in cranial margins of trachelectomy Residual disease in isthmus	Tertiparous (2 children died)

SLNI, sentinel lymph node identification; PLN, pelvic lymphadenectomy; DDP, cisplatin, ADM, doxorubicin; Ifo, Ifosfamide; LN, lymph node; MRI, magnetic resonance imaging; Hyst WIII, radical hysterectomy type III.

alive and in good health at the time of this writing. Two infants were born in the 34th and 35th weeks (2240 g and 2650 g), while the other 9 deliveries occurred between the 37th and 39th weeks of pregnancy. All children are reported to be in good health. For personal reasons, 2 women decided on elective abortion; one of these women was diagnosed with extrauterine pregnancy. One woman miscarried twice in the first trimester. In addition, there were 3 spontaneous abortions in the second trimester. At the time of the evaluation (January 2008), there were 3 pregnancies in progress.

Neoadjuvant chemotherapy and fertility-sparing surgery

Table 1 presents treatment details of the LAP-III–NAC protocol. From January 2005 to December 2007, 9 women fulfilled the inclusion criteria. In 7 of these 9 women, reproductive ability had been maintained, with 1 full-term pregnancy and 2 pregnancies in progress. During the very short follow-up, there has been no recurrence.

Discussion

Colposcopy, with MRI and US volumetry, can determine the exact amount of tumor infiltration of the cervix. This is the most important step in the decision process. Oncology outcomes are very good in all kinds of fertility-sparing procedures, assuming the tumor does not exceeded 2 cm in diameter at its largest point or if it does not infiltrate beyond half of the stroma. The 5-year survival is greater than 95% in these cases. The extension of the parametrectomy is different in various fertility-sparing procedures. The fundamental question is: How many women after radical trachelectomy with negative sentinel lymph nodes and pelvic nodes had positive findings in the parametrium? Only abdominal radical trachelectomy or total radical laparoscopic trachelectomy fulfill "classical" criteria regarding oncological radicality. Preservation of the uterine artery in a radical vaginal trachelectomy is a withdrawal from radicality. The lymphatic channels are often close to the uterine artery in the parametrium and thus cannot be removed by a radical vaginal trachelectomy. When performing the laparoscopic sentinel lymph node procedure, we remove blue afferent lymphatic channels or nodes from the parametrium. These specimens are then sent separately to the histology laboratory to be investigated [7]. The importance of the radical medial part of parametrium removal in clinical stage IA2 and small IB1 tumors with negative sentinel nodes and negative other pelvic nodes still remains controversial [16–19]. In our opinion, the subgroup of patients with negative nodes could be candidates for less excessive parametrial tissue removal, with less related morbidity, than for a radical abdominal, laparoscopic surgery or radical vaginal trachelectomy. Two-step management facilitated by ultramicrostaging of sentinel nodes increases the safety of conservative procedures.

The obstetrical outcomes after vaginal simple or radical trachelectomy are very good. The crucial factor in second-trimester abortion or premature labor is the amount of remaining stromal tissue. However, the safe margins have to be the same in abdominal as in vaginal procedures [2,4,7–11]. Vaginal surgery provides for a more precise and friendly removal of the essential part of the cervical stroma. The newly created and shortened cervix represents an easier entry point for ascendant infections, and we agree with other centers that this exemplifies the problem of greatest concern following all types of trachelectomy [2–4,7–11]. The abdominal radical trachelectomy or full laparoscopic radical trachelectomy does not have good pregnancy outcome [9–11]. Poor pregnancy results are probably due to the complete discontinuation of the important nerves from the pelvic plexus for tubal motility during an abdominal or full laparoscopic radical trachelectomy.

NAC followed by SLNM and laparoscopic lymphadenectomy and trachelectomy in node-negative patients is a new, experimental modality for women who do not fulfill the criteria for primary surgical treatment (tumor more than 2 cm in largest diameter or infiltration of more than half but less than two thirds of the cervical stroma). The tumor volume reduction after NAC permits the less-radical removal of the cervical stroma, which, in turn, improves the chances for successful pregnancy [20,21].

At the present time, all kinds of fertility-sparing surgery procedures are experimental; we therefore need more data regarding oncological and obstetrical outcomes. The best options for minimizing recurrences are careful patient selection and treatment by skilled surgeons.

Conflict of interest statement

The authors have no conflicts of interest to declare.

References

[1] Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. Cancer 2000;88:1877–82.
[2] Covens A, Shaw P, De Petrillo J, Lickrish G, Laframboise S. Is radical trachelectomy a safe alternative to radical hysterectomy for patient with stage IA–B carcinoma of the cervix? Cancer 1999;86:2273–9.
[3] Shepherd JH, Mould T, Oram DH. Radical trachelectomy in early stage carcinoma of the cervix: outcome as judged by recurrence and fertility rates. Br J Obstet Gynecol 2001;108:882–5.
[4] Plante M, Renaud MC, Francois H, Roy M. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An update series of 72 cases and review on the literature. Gynecol Oncol 2004;94:614–23.
[5] Burnett AF, Roman LD, O'Meara AT, Morrow CP. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. Gynecol Oncol 2003;88:419–23.
[6] Schlaerth JB, Spirtos NM, Schlaerth AC. Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. Am J Obstet Gynecol 2003;188:29–34.
[7] Rob L, Charvat M, Robova H, Pluta M, Strnad P, Hrehorcak M, et al. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. Int J Gynecol Cancer 2007;17:304–10.
[8] Abu-Rustum NR, Sonoda Y, Black D, Levine DA, Chi DS, Barakat RR. Fertility-sparing radical abdominal trachelectomy for cervical carcinoma: technique and review of the literature. Gynecol Oncol 2006;103:807–13.
[9] Fukuchi T. Abdominal radical trachelectomy. International Symposium on Radical Hysterectomy Dedicated to Hidekazu Okabayashi, 2007, Abstract book, 119–120.
[10] Ungar L, Palfalvi L, Hogg R, Siklos P, Boyle DC, Del Priore G, et al. Abdominal radical trachelectomy: fertility-preserving options for women with early cervical cancer. BJOG 2005;112:366–9.
[11] Andou M. Function-preserving surgery for early invasive cancer of the cervix: total laparoscopic radical trachelectomy and nerve-sparing

technique. International Symposium on Radical Hysterectomy Dedicated to Hidekazu Okabayashi, 2007, Abstract book, 121–122.
[12] Cibula D, Ungár L, Palfalvi L, Binó B, Kuzel D. Laparoscopic abdominal radical trachelectomy. Gynecol Oncol 2005;97:707–9.
[13] Plante M, Renaud MC, Hoskins IA, Roy M. Vaginal radical trachelectomy: a valuable fertility-preserving option in the management of early-stage cervical cancer. A series of 50 pregnancies and review of the literature. Gynecol Oncol 2005;98:3–10.
[14] Petignat P, Stan C, Megevand E, Dargent D. Pregnancy after trachelectomy: a high-risk condition of preterm delivery. Report of a case and review of the literature. Gynecol Oncol 2004;94:575–7.
[15] Bernardini M, Barrett J, Seaward G, Covens A. Pregnancy outcomes in patients after radical trachelectomy. Am J Obstet Gynecol 2003;189: 1378–82.
[16] Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, Hrehorcak M. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol 2005;98:281–8.
[17] Rob L, Charvata M, Robova H, Strnad P, Pluta M, Halaska M, et al. Sentinel lymph node identification (SLNI) in the management of conservative surgery in early cervical cancer: is it acceptable? Gynecol Oncol 2005;99(3 Suppl 1):147–8.
[18] Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol 2003;91: 494–503.
[19] Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol 2002;84:145–9.
[20] Plante M, Lau S, Brydon L, Swenerton K, LeBlanc R, Roy M. Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage IB1 cervical cancer: case report. Gynecol Oncol 2006;101: 367–70.
[21] Kobayashi Y, Akiyama F, Hasumi K. A case of successful pregnancy after treatment of invasive cervical cancer with systemic chemotherapy and conisation. Gynecol Oncol 2006;100:213–5.

Příloha VIII.

Strnad P., Robová H., Škapa P., Pluta M., Hrehorčák M., Halaška M. a Rob L. (2008) A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. ***Gynecol Oncol*** 109:280-284.

IF 2,319

Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 109 (2008) 280–284
Gynecologic Oncology
www.elsevier.com/locate/ygyno

A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer

Pavel Strnad [a], Helena Robova [a], Petr Skapa [b], Marek Pluta [a], Martin Hrehorcak [a], Michael Halaska [a], Lukas Rob [a,*]

[a] Department of Gynaecology and Obstetrics, Division of Oncogynecology, Charles University Prague, 2nd Medical Faculty, Czech Republic
[b] Department of Pathology and Molecular Medicine, Charles University Prague, 2nd Medical Faculty, Czech Republic

Received 29 November 2007
Available online 2 April 2008

Abstract

Objective. The purpose of prospective study is to determine incidence and distribution of pelvic lymph node(LN) involvement, sentinel lymph node(SLN) involvement and pathologic parametrial involvement(PI) in stage Ia2 and small Ib1 cervical cancer. PI is defined as positive parametrial LN or discontinuous malignant cells in parametrium.

Methods. After radical abdominal hysterectomy, 158 women patients were stratified into two groups based on tumour size: In Group 1 (91 women) tumours were less than 20 mm and less than half of stromal invasion. In Group 2 (67 women) tumours were between 20 and 30 mm and infiltration was not more than 2/3 of cervical stroma.

Results. In Group 1 positive SLN was detected in 11(12.1%) patients; of these, 3 (27.3%) had positive PI. In 80 women with negative SLN PI was not detected. In Group 2 positive SLN was detected in 14 (20.9%) patients: PI was found in four (28.6%) of these 14 patients. No PI was detected in 53 women with negative SLN.

Conclusion. No PI was observed in early cervical cancer if SLNs were negative. However, we found PI in 28.0% of women with positive SLN. Statistical analysis revealed that the results were highly significant. Based on our results, radical removal of parametrium in SLN negative patients is questionable.

Keywords: Prametrial involvement; Sentinel lymph node; Cervical cancer

Introduction

Radical hysterectomy and pelvic lymphadenectomy are the standard surgical treatments for patients with stage Ia2 and Ib1 cervical cancer [1–3]. Parametrectomy is the most technically difficult aspect of radical hysterectomy and is the main cause of postoperative complications [4–6]. This problem is the primary reason for the numerous modifications of radical hysterectomy and the extent of parametrial resection, including the implementation of nerve sparing surgery. The reduction of radicality did not impact prognosis, but the number and seriousness of late complications were decreased [4,6,7]. A large number of papers have evaluated parametrial involvement (PI) in early-stage cervical cancer with quite high variability regarding the involvement of the resected parametria (1.5–35.0%). One of the most important prognostic factors for the involvement of the parametrium is the depth of stromal invasion, tumour volume, positive sites of pelvic lymph nodes and lymphovascular space involvement (LVSI) [8–14]. Research over the past five years on sentinel lymph nodes (SLNs) has greatly expanded our knowledge on lymphatic propagation of cervical cancer [15–21]. The purpose of the present prospective study is to determine the incidence and distribution of pelvic lymph node involvement, SLN involvement and pathologic PI in clinical stage Ia2 and different tumour volume in small Ib cervical cancer that do not exceed more than two thirds of the cervical stroma. PI was defined either as positive parametrial lymph node (PLN) or discontinuous malignant cells in the parametrium.

* Corresponding author. V Uvalu 84 150 00 Prague 5 Czech Republic. Fax: +420 224434220.
E-mail address: lukas.rob@lfmotol.cuni.cz (L. Rob).

0090-8258/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2008.02.004

Patients and methods

A prospective study design of SLN mapping was used for evaluation of tumour involvement of the medial part of the paracervical tissue (medial part of lateral parametrium, anterior parametrium and posterior parametrium). The technique and timing of the application of blue dye or the combination of blue dye and radiocoloid of technetium were described in a previous study [18]. Sentinel lymph nodes were extirpated separately and were sent for frozen section. Identification of SLN in the parametrium is possible in vivo only by blue dye because radioactivity is very high near the cervix. SLN from the parametrium was extirpated separately and radioactivity was controlled in vitro. SLN from the parametria were also sent for frozen section. Characteristics of the patients are given in Table 1. Only women in which Okabayashi radical hysterectomy (resection of parametrial tissue: note the same as in radical hysterectomy type III of Rutledge and Piver) was performed were included in the study, which was conducted from February 2000 to September 2006. Radical hysterectomy was performed with or without a nerve sparing technique. Women with evident extrauterine dissemination, with tumour-infiltrated bulky lymph nodes more than two thirds of the cervical stroma on definitive histopathological results were excluded from the study group. Women aged between 18 and 70 years signed informed consent forms with "SLNM II study" (protocol detection of SLNs). Protocol "SLNM II study" was approved by local institutional ethical committee in our hospital. Totally, 158 women with cervical cancer Ia2 and Ib1 were included in the study. The women patients were categorised into two groups according to tumour size and tumour volume, because we needed one group which would have the same criteria as the patients indicated for fertility sparing surgery (less than 20 mm and less than half of stromal invasion). Group 1 (91 women) included patients with tumours no bigger than 20 mm or not more than half of stromal invasion. Group 2 (67 women) consisted of patients with tumours between 20 and 30 mm and infiltration not more than two thirds of the cervical stroma. Histopathological elaboration was done by uniform technique. The uterus is oriented and fixed to the polystyrene table immediately after surgical removal and the centre of the anterior cervical labium is marked with the suture by the surgeon. After a 24-hour fixation period in10% buffered formalin, the specimen is described and cut according to standardised protocol. The cervix is amputated from the corpus and cut clockwise in radial sections of 3 mm thickness starting from the suture on the anterior labium, which indicates 12 o'clock. Each section should include exocervical and endocervical segments, the squamocolumnar junction and the corresponding part of the vaginal cuff. The rest of the cervix is cut longitudinally through the endocervical canal and the full thickness of the cervical endometrium, cervical stroma and surrounding soft tissue is processed in one level. Parametria are separated and sectioned in serial 3 mm thick sections. This is the way how we can detect quite well lymphangioinvasis in the parametria. The imunohistochemistry of the parametria was not done. The uterine body and adnexa are processed routinely. Elaboration of the sentinel node and other lymph nodes that were removed was done by standard protocol [18]. Dependence between risk of tumour involvement of the sentinel lymph nodes (SLNs) and risk of tumour involvement of the medial part of the lateral or anterior or posterior parametria in early cervical cancer was evaluated.

Chi-square test with Yates correction and Fisher's exact test (in case of small sample size) were used. The data were analysed as dichotomous variables. Odds ratios (ORs) with 95% confidence intervals (CIs) and *p*-values were computed in particular 2×2 contingency tables using GraphPad Instat 3.05 statistical software (GraphPadSoftware, San Diego, CA, USA). *P*-values < 0.05 were considered statistically significant.

Table 1
Histopathological characteristics and demographics

	Group I *N*=91	Group II *N*=67
Age (years) - median	46.2	47.1
- range	26–69	28–68
Histopathological type - squamous	69 (75.8%)	49 (73.1%)
- adenocarcinoma	19 (20.9%)	16 (23.9%)
- adenosquamous	3 (3.3%)	2 (3.0%)
Depth of invasion (mm) - median	8.1	13.2
- range	3–15	6–22
Number of removed pelvic nodes - mean	28.5	29.6
- range	14–58	15–64

Table 2
Groups of patients — SLN and parametrial involvement

	Number of women (%)	Parametrial involvement	Other pelvic nodes
Group I (91 women)			
SLN negative	80 (87.9%)	80 negative (100%)	80 negative (100%)
SLN positive	11 (12.1%)	3 positive (Table 2)	3 positive (Table 2)
Group II (67 women)			
SLN negative	53 (79.1%)	53 negative (100%)	53 negative (100%)
SLN positive	14 (20.9%)	4 positive (Table 3)	5 positive (Table 3)

Results

Group 1

Positive SLN was detected in 11 patients (12.1%). No false-negatives were noted in any of the 91 women in this group. No positive findings in the parametrium were detected in women with negative SLN and all pelvic lymph nodes were negative (Table 2). Table 3 summarises the analysis of women with positive SLN. In 9 women positive was just only SLN. One woman (patient number 42) had positive SLN in the right medial part of the lateral parametrium. All other women with positive findings in the medial part of the lateral parametrium had positive pelvic SLN. Risk of involvement of the medial part of the lateral parametrium was 27.3% (3 of 11 patients) in women with positive SLN.

Group 2

Positive SLN was detected in 14 patients (20.9%). No false positive SLN was found in this group of patients and no positive

Table 3
Patients in Group I with positive SLN

Patient number	SLN positive	Other pelvic lymph node	Paraaortic LN	Parametrial involvement (PI)
15	1× SUP	34 negative	4 negative	Negative
21	1× EXT+ 1× EXT	28 negative	5 negative	Negative
25	1× SUP	39 negative	0	Negative
39	1× EXT	45 negative	7 negative	Negative
45	1× PRS	40 negative	2 negative	Negative
54	1× SUP+ 1× EXT	29 negative	4 negative	Negative
83	1× EXT	33 negative	0	Negative
12	1× SUP	1 positive 25 negative	0	Negative
42	1× parametrial LN	28 negative	5 negative	SLN +
63	1× SUP	2 positive 39 negative	3 negative	1× LN positive
76	1× EXT+ 1× SUP	2 positive 22 negative	1 positive	LVSI positive

LN — lymph node, SLN — sentinel lymph node, LVSI — lympho vascular space involvement, SUP — supraobturator LN, EXT — external LN, PRS — presacral LN, COM — common iliac LN.

findings in the medial part of lateral parametrium was noted in women with negative SLN and pelvic lymph nodes (Table 2). Table 4 shows the women with positive SLNs. One woman (patient number 66) had positive SLN in the right parametrium; however, final histopathology of this patient did not reveal any other positive lymph nodes. Four of 14 women (28.6%) with positive SLN had involvement of the medial part of the lateral parametria. Only one woman (patient number 58) with positive pelvic nodes also presented positive suprapelvic lymph node (precaval lymph node).

No positive findings in the parametrium were detected in 133 women with negative SLN (women from both groups). Positive findings in the parametrium were observed in 7 of 25 (28%) women with positive SLNs. This difference was statistically significant, $p<0.0001$ (OR 71.63, 95% CI 3.81–1345.4).

Table 5 gives the distribution of SLNs for 156 women (SLN was not detected in two women). In the external iliac area 205 SLNs (42.9%) were detected, 201 SLNs (42.1%) in the supraobturator area, 20 SLNs (4.2%) in medial part of the lateral parametria, 26 SLNs (5.4%) in the common iliac area and bifurcation and 26 SLNs (5.4%) in the presacral area. No SLN was found in the suprapelvic (paraaortal) area. SLN was not detected in only 2 of 158 patients (detection rate, DR=98.8%). SLN was detected on only one side in 14 women (specific side detection rate, SSDR=94.3%). These results are consistent with our earlier paper in smaller cervical cancer Ib1 [18].

Table 5 shows the distribution of 33 positive SLNs in 25 women. In these data no false-negative SLN was recorded. The distribution of positive SLNs was similar to the distribution of all SLNs. In the external iliac area 14 positive SLNs (42.4%) were detected, 13 positive SLNs (39.4%) in the supraobturator area, 2 positive SLNs (6.1%) in the common iliac area and bifurcation, 2 positive SLNs (6.1%) in the medial part of the lateral parametria and 2 SLNs (6.1%) in the presacral area. No positive SLN was found in the paraaortal area.

Table 4
Patients in Group II with positive SLN

Patient number	SLN positive	Other pelvic lymph node	Paraaortic LN	Parametrial involvement (PI)
3	1× EXT	48 negative	2 negative	Negative
9	1× SUP+ 1× EXT	52 negative	3 negative	Negative
15	1× EXT	19 negative	0	Negative
21	1× SUP+ 1× COM	33 negative	5 negative	Negative
24	1× EXT	28 negative	2 negative	Negative
29	1× EXT	23 negative	3 negative	Negative
35	1× SUP	28 negative	0	Negative
48	1× SUP+ 1× PRS	45 negative	4 negative	Negative
66	1× parametrial LN	28 negative	5 negative	SLN +
11	1× SUP	2 positive 29 negative	3 negative	1× LN positive
38	1× EXT+ 1× SUP	1 positive 30 negative	0	LVSI positive
47	1× SUP	1 positive 36 negative	0	Negative
49	1× COM	1 positive 38 negative	7 negative	Negative
58	1× EXT+ 1× EXT	5 positive 21 negative	1 positive	LVSI positive

LN — lymph node, SLN — sentinel lymph node, LVSI — lympho vascular space involvement, SUP — supraobturator LN, EXT — external LN, PRS — presacral LN, COM — common iliac LN.

Table 5
Distribution of the sentinel lymph nodes and positive sentinel lymph nodes

	SLN number — 478	Positive SLN number — 33
External iliac area	205 (42.9%)	14 (42.4%)
Supraobturator area	201 (42.1%)	13 (39.4%)
Common iliac area	26 (5.4%)	2 (6.1%)
Presacral area	26 (5.4%)	2 (6.1%)
Medial part of the lateral parametrium	20(4.2%)	2 (6.1%)

Discussion

Extension of the resection of the anterior, posterior and lateral parametria (paracervical tissue) in radical hysterectomies and fertility sparing surgery have been discussed intensively over the past 15 years [4–7, 12–14, 27–31]. The Rutledge and Piver classification system of radical surgery in cervical cancer was widely used in the 1970s. [1]. Complete resection of the parametria by Rutledge and Piver type III was considered a standard operation in invasive stage Ia2 and Ib1 cervical cancer during the 1970s and 1980s. Burghardt later confirmed the significance of the performed radical resection of the parametria [8]. Burghardt documented dissemination of the tumour to the medial and lateral parts of the lateral parametria. The giant section technique was used for histopathological processing in this study. Other papers dealt with risk of involvement of the parametria which is described in 1.5–31% [5–12, 22–26]. Detailed analysis of these papers showed that most of the women included in these studies had bulky tumours and very few had cervical cancer Ia2 and small Ib1. A large number of studies have confirmed a correlation between size of the tumour or infiltration of the cervical stroma and positive lymph nodes and involvement of the parametria [6,7,10,22,24,25]. The largest retrospective study of women ($n=536$) was published by Covens in 2002. In this study, involvement of the parametria was found in 0.6% of the patients having stage Ia2 and Ib1 tumours with less than 10 mm of stromal invasion [22]. In another retrospective study of 125 women no involvement was noted in the medial part of the lateral parametria in patients with negative pelvic lymph nodes [24].

We try to find in our study save way how to decrease morbidity which is caused by radical parametrial resection. We utilized our experiences with sentinel lymph node mapping in cervical cancer [18]. In the 1990s many authors advocated the use of "modified" radical hysterectomy. The primary aim of this procedure was to reduce long-term morbidity, which is associated with the radical resection of the parametria [4–7]. These studies could not demonstrate impaired prognosis in patients with less radical resection of the parametria in early-stage cervical cancer. None of these studies were randomised studies, however. Only one prospective study from Italy (Landoni) compared standard radical hysterectomy type III with limited resection of the parametria in radical hysterectomy type II. As in the other studies, this study found no difference in overall survival and percentage of

recurrence in stage Ib cervical cancer between type II and type III radical hysterectomy. There was a statistically significant difference in long-term morbidity, especially in urological morbidity [4].

Our study is the first prospective study that has evaluated risk of PI in dependence of involvement of the SLNs in early cervical cancer. In the first group of "small" cancer less than 20 mm at the largest diameter and infiltrating not more than half of the cervical stroma 11 of 91 women (12.1%) had positive SLNs. One positive SLN was detected in the medial part of the lateral parametria, where the other pelvic lymph nodes were negative. In two other cases of PI positive pelvic SLN was detected. A positive finding in the parametrium was detected in 3.3% of the patients in this extremely prognostically favourable group. In cases of positive SLNs the parametrial involvement is 27.3%. Very good timing in the application of the blue dye is necessary for detection of lymphatic channels and eventually SLN in the paracervical tissue (medial part of the lateral parametria) [18]. We did not detect any cases of a positive finding in the parametrium and negative SLN was noted. No false-negative SLN was diagnosed in our group of patients when we combined technetium and blue dye.

In the second group of patients with a tumour between 20 and 30 mm and infiltration not more than two thirds of the cervical stromal 14 of 67 women (20.9%) had positive SLNs. In this group one positive SLN (1.9%) was detected in the medial part of the parametrium while other pelvic nodes were negative. The other three women with positive findings in the parametrium had positive pelvic SLN. Risk of involvement of the parametria was 6% in this group of women, but in cases involving the SLN the risk of a positive parametrial finding increased markedly to 28.6%. No false-negative SLN was found.

We observed only one recurrence between the negative SLN patients from the second group. The recurrence was on the pelvic wall and grew into the gluteus muscles. We expect more recurrences during the next period because now the follow up is too short.

Our study demonstrates that the risk of tumour involvement of the parametria is minimal in cervical cancer infiltrating less than two thirds of the cervical stoma if the SLNs are negative. In contrast, if the SLNs are positive, there is a high risk of tumour involvement of the parametrium (27.3% in Group 1 and 28.6% in Group 2). Our study confirms the clinical significance of detection and final histopathological evaluation of SLNs. Sentinel lymph node mapping is a procedure that makes it possible to perform less radical surgeries of parametria with greater safety in SLN negative patients thus enabling us to involve less radical approaches like modified radical hysterectomy, nerve sparing surgery and possibly simple trachelectomy as a fertility sparing procedure. Simple hysterectomy and simple trachelectomy in SLN negative patients are still experimental. We need a prospective randomized study or multiinstitutional observation study to confirm our study before we will include them in oncogynecological standards.

Acknowledgment

This work was supported by grant MZO-00064203, Ministry of Health, Czech Republic.

References

[1] Piver S, Rutledge F, Smith JP. Five classes of extended hysterectomy. Am J Obstet Gynecol 1974;44:265–72.
[2] Rob L, Svoboda B, Robova H, Stankusova H, Cwiertka K, Neumannova R, et al. Guideline for gynecological malignant tumors — primary complex therapy in operable stages of malignant tumors of uterus cervix. Ceska Gynekol 2004;69(5):376–383.
[3] Plante M, Roy M. New approaches in the surgical management of early stage cervical cancer. Cur Opin Obstet Gynecol 2001;13(1):41–6.
[4] Landoni F, Maneo A, Cormio G, Pereno P, Milani R, Caruso O, et al. Class II versus class III radical hysterectomy in stage Ib–IIa cervical cancer: a prospective randomised study. Gynecol Oncol 2001;80:3–12.
[5] Margina JF, Goodrich MA, Weaver AL, Podratz KC. Modified radical hysterectomy: morbidity and mortality. Gynecol Oncol 1995;59 (2):277–82.
[6] Kinney WK, Hodge DO, Egorshin EV, Ballard DJ, Podratz KC. Identification of low risk subset of patients with stages Ib invasive squamous cancer of the cervix possibly suited to less radical surgical treatment. Gynecol Oncol 1995;57(1):3–6.
[7] Benedetti-Panici P, Angioli R, Palaia I, Muzzi L, Zullo MA, Manci N, et al. Tailoring the parametrectomy in stages IA2-IB1 cervical carcinoma: is it feasible and safe? Gynecol Oncol 2005;96(3):792–8.
[8] Burghardt E, Pickel H. Local spread and lymph node involvement in cervical cancer. Obstet Gynecol 1978;52(2):138–45.
[9] Landoni F, Bocciolone L, Perego P, Maneo A, Bratina G, Mangioni C. Cancer of cervix, FIGO stages Ib and IIa: patterns of local growth and paracervical extension. Int J Gynecol Oncol 1995;5(5):329–34.
[10] Inoue T. Prognostic significance of the depth of invasion relating to nodal metastase, parametrial extension, and cell types. A study of 628 cases with stage Ib, IIa and IIb cervical carcinoma. Cancer 1984;54(2): 3035–42.
[11] Delgado G, Bundy GN, Fowler WC, Stehman FB, Sevin B, Creasman WT, et al. A prospective surgical pathological study of stage I squamous carcinoma of the cervix: a Gynecology Oncology Group study. Gynecol Oncol 1989;35(3):314–20.
[12] Benedetti-Panici P, Maneschi F, Gutillo G, Rabitti C, Congiu M, et al. Early cervical carcinoma: the natural history of lymph node involvement redefined on the basis of thorough parametomy and giant section study. Cancer 2000;88:2267–74.
[13] Girardi G, Lichtenegger W, Tamussino K, Haas J. The importance of parametrial lymph nodes in the treatment o cervical cancer. Gynecol Oncol 1989;34(2):206–11.
[14] Burghardt E, Pickel H, Haas J, Lahousen M. Prognostic factors and operative treatment of stages Ib–IIb cervical cancer. Am J Obstet Gynecol 1987;156(4):988–96.
[15] O'Boyle JD, Coleman RL, Bernstein SG, Lifshitz S, Muller CY, Miller DS. Intraoperative lymphatic mapping in cervix cancer undergoing radical hysterectomy: a pilot study. Gynecol Oncol 2000;79:238–43.
[16] Malur S, Krause N, Kohler C, Schneider A. Sentinel lymph node detection in patients with cervical cancer. Gynecol Oncol 2001;80:254–7.
[17] Levenback C, Coleman RL, Burke TW, Lin WM, Erdman W, Deavers M, et al. Lymphatic mapping and sentinel node identification in patients with cervix cancer undergoing radical hysterectomy and pelvic lymphadenectomy. J Clin Oncol 2002;20:688–93.
[18] Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol 2005;98(3):281–8.
[19] van Dam PA, Hauspy J, Vanderheyden T, Sonnenemans H, Spaepen A, Eggenstein G. Intraoperative sentinel node identification with Technetium-99m-labeled nanocolloid in patients with cancer of the uterine cervix: a feasibility study. Int J Gynecol Cancer 2003;13:182–6.
[20] Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol 2003;91(3): 494–503.
[21] Marnitz S, Kohler Ch, Bongardt S, Braig U, Hertel H, Schneider A. Topographic distribution of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol 2006;103(1):35–44.

[22] Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol 2002;84(1):145–9.
[23] Winter R, Haas J, Reich O, Koemetter R, Tamussino K, Lahousen M, et al. Parametrial spread of cervical cancer in patients with negative pelvic lymph nodes. Gynecol Oncol 2002;84(2):252–7.
[24] Steed H, Capstick V, Schepansky A, Honore L, Hiltz M, Faught W. Early cervical cancer and parametrial involvement: is it significant? Gynecol Oncol 2006;103(1):53–57.
[25] Puente R, Guzman S, Israel E, Poblete MT. Do the pelvic lymph nodes predict the parametrial status in cervical cancer stages IB–IIA? Int J Gynecol Cancer 2004;14,:832–40.
[26] Rob L, Charvat M, Robova H, Strnad P, Pluta M, Halaska M, et al. Sentinel lymph node identification (SLNI) in the management of conservative surgery in early cervical cancer: is it acceptable? Gynecol Oncol 2005;99:114–47 S.
[27] Covens A, Shaw P, De Petrillo J, Lickrish G, Laframboise S. Is radical trachelectomy a safe alternative to radical hysterectomy for patient with stage IA–B carcinoma of the cervix? Cancer 1999;86:2273–9.
[28] Shepherd JH, Mould T, Oram DH. Radical trachelectomy in early stage carcinoma of the cervix: outcome as judged by recurrence and fertility rates. Br J Obstet Gynecol 2001;108:882–5.
[29] Plante M, Renaud MC, Francois H, Roy M. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An update series of 72 cases and review on the literature. Gynecol Oncol 2004;94:614–23.
[30] Burnett AF, Roman LD, O'Meara AT, Morrow CP. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. Gynecol Oncol 2003;88:419–23.
[31] Rob L, Charvat M, Robova H, Pluta M, Strnad P, Hrehorcak M, et al. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. Int J Gynecol Cancer 2007;17(1):304–10.

Příloha IX.

Robová H., Pluta M., Hrehorčák M., Škapa P. a Rob L. (2008) High-dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. ***Int J Gynecol Cancer*** 18:1367-1371.
IF 1,425

High–dose density chemotherapy followed by simple trachelectomy: full-term pregnancy

H. ROBOVA*, M. PLUTA*, M. HREHORCAK*, P. SKAPA† & L. ROB*
*Departments of *Obstetrics and Gynecology and †Pathology and Molecular Biology, University Hospital Motol, 2nd Medical Faculty, Charles University, Prague, Czech Republic*

Abstract. Robova H, Pluta M, Hrehorcak M, Skapa P, Rob L. High–dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. *Int J Gynecol Cancer* 2008;**18:**1367–1371.

We report five patients with early-stage cervical cancer who do not fulfill criteria of fertility-sparing surgery (tumor more than 2 cm in the biggest diameter or infiltrating more than half of stroma). Five patients received three cycles of dose density neoadjuvant chemotherapy (NAC) at a 10-day interval: cisplatin plus ifosfamide in squamous cell cancer or plus doxorubicin in adenocarcinoma with good tolerance. After NAC, they underwent laparoscopic pelvic lymphadenectomy and vaginal simple trachelectomy. Two patients had no residual tumor, two had only microscopic residual disease, and one had macroscopic residual disease. Two women became pregnant 5 and 8 months after surgery, one delivered in term healthy baby and one is now in the second trimester of pregnancy without any complications. NAC followed by fertility-sparing surgery seems to be feasible treatment for women with tumor bigger than 2 cm or infiltrated more than half of the stroma.

KEYWORDS: neoadjuvant chemotherapy, pregnancy, sentinel lymph node biopsy, simple trachelectomy.

Address correspondence and reprint requests to: Lukas Rob, MD, PhD, Department of Obstetrics and Gynecology, University Hospital Motol, 2nd Medical Faculty, Charles University, V uvalu 84, 150 00 Prague, Czech Republic. Email: lukas.rob@lfmotol.cuni.cz

doi:10.1111/j.1525-1438.2007.01178.x

Increasing age at time of the first delivery brings with it certain problems. There are more women with cervical cancer who wish to be pregnant and an increasing number of oncogynecologic departments perform

fertility-sparing surgery. The most common procedure is radical vaginal trachelectomy; an alternative is simple trachelectomy or radical abdominal trachelectomy[1–4]. Oncologic outcomes are normally very good if the tumor does not exceed 2 cm in diameter at its largest point or if it does not infiltrate more than half of the stroma. Five-year survival is more than 95% in these cases with good prognosis. Pregnancy outcome is very good in women after radical vaginal trachelectomy or simple trachelectomy, but not so good after radical abdominal trachelectomy[2,5–7]. Adequate free margins are essential for the safety of trachelectomy (7 mm is a minimum). The preservation of an adequate amount of stromal tissue is fundamental for good pregnancy outcome; at our department, 10 mm is considered a minimum requirement. Fertility-sparing surgery is usually performed in nulliparous women who quite often have a very small cervix, and thus, trachelectomy can be a serious problem when performed on tumors that are about 20 mm. In such cases, it is not possible to save a sufficient amount of stroma. It makes little sense to perform fertility-sparing surgery if we do not believe in successful pregnancy results. The literature confirms that neoadjuvant chemotherapy (NAC) works well in stage IB2 cervical cancer before performing Wertheim radical hysterectomy[8]. NAC can decrease the volume of a tumor before surgery to achieve adequate margins without too much loss of cervical stroma. Based on the results of our cervical cancer studies ("Simple trachelectomy in cervical cancer IA1 and small IB1" and "Neoadjuvant chemotherapy in cervical cancer IB2"), the ethical committee approved the study protocol regarding NAC and fertility-sparing surgery in January 2005. All women under the age of 40 years with a desire to be pregnant and who have cervical cancer involving more than half of the stroma but no more than two thirds or a tumor larger than 2 cm were included in the study. The surgical procedure has been described earlier[6]. All women signed an informed consent form that radical hysterectomy should be performed in the event of positivity of lymph nodes or inadequate margins. The treatment protocol is depicted in Figure 1.

Case reports

Case 1

Invasive squamous cell cancer grade 2 with lymphvascular invasion (LVSI) was diagnosed in a 25-year-old nulliparous woman by punch biopsy. Colposcopic findings showed an exophytic tumor 20 × 20 mm. Magnetic resonance imagining (MRI) diagnosed tumor 20 × 20 × 10 mm which involved more than half of the cervical stroma, vaginal ultrasonography (US) confirmed MRI. The patient received three cycles of dose density chemotherapy in a 10-day period (cisplatin 75 mg/m^2 and ifosfamide 2 g/m^2). She presented thrombocytopenia grade 3 and leukopenia grade 2 after a third course of chemotherapy. No other toxicity was observed. Sentinel lymph node (SLN) biopsy and laparoscopic lymphadenectomy were performed 44 days after initiation of the chemotherapy. A frozen section of two SLNs was negative and final histopathology of 16 pelvic nodes also proved negative. Simple trachelectomy was undertaken 1 week after the lymphadenectomy. Histopathology showed only degenerative and regenerative changes: no tumor or precancer lesion was found. The patient is without evidence of disease 27 months later. As of this writing, the patient does not plan pregnancy.

Case 2

Large loop excision of transformation zone was performed on a 26-year-old nulliparous woman. This procedure revealed endometrioid adenocarcinoma grade 3 with massive LVSI. MRI showed a residual tumor 44 × 36 × 19 mm which infiltrated two thirds of the cervical stroma and US showed tumor 31 × 28 × 24 mm.

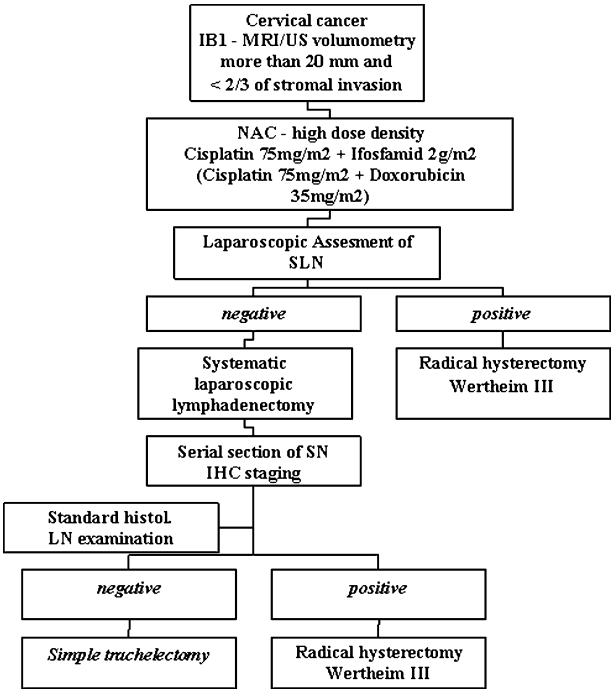


Figure 1. NAC–sentinel lymph node mapping and conservative surgery. LN, lymph node; IHC, immunohistochemistry.

The patient received three cycles of platinum 75 mg/m^2 and doxorubicin 35 mg/m^2 in a 10-day interval. She had no hematologic or other toxicity. After the third course of chemotherapy, the patient decided to undergo radical hysterectomy as opposed to conservative surgery. We therefore performed radical hysterectomy. Histopathology did not reveal any cancer in the specimen, but it did show some necrotic changes. Totally, 29 lymph nodes were negative.

Case 3

Adenocarcinoma mucinous endocervical type grade 1 with LVSI was diagnosed in a 30-year-old nulliparous woman by excisional biopsy (20 × 15 mm). Colposcopy detected a 15-mm exophytic tumor on a very small cervix. MRI showed a residual tumor (17 × 11 × 5 mm), which involved two thirds of the cervical stroma (US confirmed MRI findings). Three cycles of the dose density chemotherapy were applied (cisplatin + doxorubicin). We did not observe any serious toxicity. SLN biopsy and laparoscopic lymphadenectomy were performed after 37 days from the beginning of chemotherapy. A frozen section of two SLNs was negative as were all 22 pelvic lymph nodes. Simple trachelectomy was indicated: a residual tumor (0.5 mm) and necrotic tissue were found. The woman was treated with antibiotics 3 weeks after surgery for inflammatory lymphocyst. She became pregnant 8 months after surgery. No complications occurred during her pregnancy and she delivered in the 39th week of gestation a spontaneously healthy boy weighing 3620 g. She is without evidence of disease 23 months after the surgery.

Case 4

Punch biopsy diagnosed squamous cell cancer grade 3 with LVSI in a 24-year-old nulliparous woman. Colposcopy detected a 30 × 25-mm tumor and MRI confirmed the diagnosis from the colposcopy (tumor 30 × 23 × 10 mm); US showed a 20 × 10 × 10 mm tumor. Three cycles of platinum plus ifosfamide were applied. The patient had grade 2 gastrointestinal toxicity. After the third course, grade 2 thrombocytopenia and neutropenia were noted. Surgery was done 40 days from the first course of chemotherapy. Two SLNs were perioperatively negative as was the final histopathology of the pelvic lymph nodes. The residual tumor measured 2 mm. Stenosis of the endocervical canal occurred 9 months after surgery, which led us to perform dilatation of the cervix. A Papanicolaou smear proved negative, but high-risk human papillomavirus are still positive.

Case 5

A 31-year-old nulliparous woman showed evidence of squamous cell cancer grade 3 with LVSI as diagnosed by excisional biopsy. Colposcopy indicated that the exophytic cancer was 25 mm in diameter. MRI showed that a residual tumor (24 × 17 × 15 mm) infiltrated more than half but not more than two thirds of the cervical stroma (US confirm MRI results). Three cycles of platinum plus ifosfamide chemotherapy were administered. The patient showed no toxicity after chemotherapy. The interval between the surgery and the first course of chemotherapy was 40 days. SLNs were negative as were all 22 pelvic lymph nodes. The residual tumor was 13 × 6 mm. This patient is without evidence of disease 9 months postsurgery. She became pregnant 5 months after completion of therapy with no indications of pathology.

Discussion

The oncologic outcomes of fertility-sparing surgery in cervical cancers that infiltrate more than half of stroma or are bigger than 2 cm are classified as unsatisfactory[9–11]. The inclusion criterion in most centers is that the largest diameter of the tumor must be less than 2 cm. In the present study with young patients, the tumor volume and the amount of infiltrated stroma are more important determinants than the biggest diameter because they usually have a very small uterine cervix not bigger than 25 mm in longitudinal axis. We performed tumor volumometry by MRI and vaginal US in all these patients in order to evaluate how much of the stroma is involved. Before starting with the NAC protocol, all women with tumor infiltrated more than half of the cervical stroma or there existed a tumor bigger than 2 cm underwent radical hysterectomy Wertheim, type III.

The lymphvascular space involvement is also a very important prognostic factor in early-staged cervical cancer[12]. Some authors exclude patients with LVSI from conservative protocols. In our department, LVSI is not considered an exclusion criterion[6,7]. Massive LVSI was diagnosed in all women from our group with NAC.

There is a question regarding safety of the simple trachelectomy in patients with SLN and other negative pelvic nodes. We consider SLN mapping as a safe method, even after NAC, because the SLN specific side detection rate in cervical cancer stage IB2 after NAC is high (90.4%) when we use a combination of blue dye and ^{99}Tc[13]. The lymphatic channels are often close to the uterine artery in the parametrium and thus cannot be removed by radical vaginal trachelectomy; rather,

laparoscopy or abdominal trachelectomy has to be employed. In these cases, some authors recommend abdominal radical trachelectomy. The parametria, including the uterine artery, are resected during the abdominal procedure, as this approach seems to increase safety. When we perform the SLN procedure, we remove blue afferent lymphatic channels or nodes from the parametrium; these specimens are then sent separately to the histopathology. Thus, the surgical procedure has the same safety as radical abdominal trachelectomy. Abdominal trachelectomies do not have good pregnancy outcome though the oncologic results are very good[2]. For good pregnancy outcome, the crucial factor is the amount of remaining stromal tissue. However, the safe margins have to be the same in abdominal as in vaginal procedures and the same amount of cervical stroma has to be removed. Another factor for poor pregnancy results is that the important nerves for tubal motility are completely severed during abdominal radical trachelectomy.

NAC followed by radical hysterectomy is used in Europe in stage IB2 bulky cervical cancer as an alternative treatment to chemoradiotherapy, but no data exist about NAC followed by radical hysterectomy in stage IB1 cervical tumor. NAC reduces tumor volume and number of positive lymph nodes. Our results are very good if we use NAC in dose density regiments with short intervals between courses[8]. Our experience with high–dose density NAC is adapted from bulky stage IB2 cervical cancers that we used from 1998 without any serious adverse events[14]. NAC can be an opportunity for patients who wish to preserve fertility without increasing the risk of recurrence. The tumor volume reduction after NAC permits less radical removal of the cervical stroma, which therefore improves the chance for successful pregnancy[15,16]. The incision is performed approximately in the level of proximal border of the original tumor (before NAC). Without NAC, we are not able to obtain safe margins with preserving of sufficient amount of cervical stromal tissue. Two of our patients had no residual tumor (after NAC one patient decided to have radical hysterectomy), two had only microscopic residual disease, and one had macroscopic residual tumor, but the volume in that tumor was reduced by 50%. No inadequate tumor margins were seen in our group of patients. No radical hysterectomy was performed till this time from indication of positive lymph nodes or positive margins. In our patient group, we had two spontaneous pregnancies (one delivery in term and one ongoing pregnancy) and no recurrence.

The use of an alkylating agent in women in reproductive age can result in impairment of the ovarian function. In the past, we have had good experience with cyclophosphamide in high-risk malignant gestational trophoblastic disease with no impact on ovarian function. In our patients, we use 2 g/m^2 of ifosfamide and no more than a cumulative dose of 3 g per one cycle, which means that the cumulative dose is no more than 9 g. All our patients had regular menstrual cycles within 3 months.

Because of toxicity and mutagenicity of chemotherapy, we typically recommend pregnancy 1 year after the end of chemotherapy. One woman from our group became pregnant 8 months after the conclusion of chemotherapy. The first trimester screening was normal and the second trimester screening and US in the 20th week of pregnancy were within the normal range. She delivered in term a healthy baby. Another woman became pregnant 5 months after chemotherapy treatment. This patient has shown normal pregnancy in the second trimester.

Acknowledgment

This work was supported by Grant IGA MZ CR NR 8434-3.

References

1 Plante M, Renaud MC, Harel F, Roy M. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An update series of 72 cases and review of literature. *Gynecol Oncol* 2004; **94:**614–23.
2 Ungar L, Palfalvi L, Hogg R *et al*. Abdominal radical trachelectomy: a fertility-preserving options for women with early cervical cancer. *BJOG* 2005;**112:**366–9.
3 Marchiole P, Benchaib M, Buenerd A, Lazslo E, Dargent D, Mathevet P. Oncological safety of laparoscopic-assisted vaginal radical trachelectomy (LARVT or Dragnet's operation): a comparative study with laparoscopic-assisted vaginal radical hysterectomy (LARVH). *Gynecol Oncol* 2007;**106:**132–41.
4 Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. *Cancer* 2000;**88:**1877–82.
5 Plante M, Renaud MC, Hoskins IA, Roy M. Vaginal radical trachelectomy: a valuable fertility preserving option in the management of early-stage cervical cancer. A series of 50 pregnancies and review of literature. *Gynecol Oncol* 2005;**98:**3–10.
6 Rob L, Charvat M, Robova H *et al*. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. *Int J Gynecol Cancer* 2007;**17:**304–10.
7 Rob L, Charvata M, Robova H *et al*. Sentinel lymph node identification (SLNI) in the management of conservative surgery in early cervical cancer: is it acceptable? *Gynecol Oncol* 2005;**99**(Suppl. 1):147–8.
8 Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer: a systematic review and meta-analysis of individual patient data from 21 randomised trials. *Eur J Cancer* 2003;**39**:2470–86. Review.
9 Dargent D, Frazosi F, Ansquer Y, Martin X, Mathevet P, Adeline P. Extended trachelectomy relapse: plea for patient involvement in the medical decision. *Bull Cancer* 2002;**89:**1027–30.
10 Del Priore G, Ungar L, Smith J, Heller PB. Regarding first case of centropelvic recurrence after radical trachelectomy: literature review and implication for the preoperative selection of patients. *Gynecol Oncol* 2004;**95:**414–6.
11 Morice P, Dargent D, Haie-Meder C, Duvillard P, Castaigne D. First case of a centropelvic recurrence after radical trachelectomy:

literature review and implications for the preoperative selection of patients. *Gynecol Oncol* 2004;**92**:1002–5. Review.
12 Milam MR, Frumovitz M, dos Reis R, Broaddus RR, Bassett RL Jr, Ramirez PT. Preoperative lymph-vascular space invasion is associated with nodal metastases in women with early-stage cervical cancer. *Gynecol Oncol* 2007;**106:**12–5.
13 Rob L, Strnad P, Robova H *et al*. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. *Gynecol Oncol* 2005;**98:**281–8.
14 Kacirek J, Robova H, Rob L, Pluta M, Schlegerova D, Strnad P. High dose density neoadjuvant chemotherapy before radical hysterectomy in patients with IB2 squamous cell cervical cancer—results in group 1998–2004 [abstract]. *Int J Gynecol Oncol* 2005;**15**:135. Abstract 305.
15 Plante M, Lau S, Brydon L, Swenerton K, LeBlanc R, Roy M. Neoadjuvant chemotherapy follows by vaginal radical trachelectomy in bulky stage IB1 cervical cancer: case report. *Gynecol Oncol* 2006;**101:** 367–70.
16 Kobayashi Y, Akiyama F, Hasumi K. A case of successful pregnancy after treatment of invasive cervical cancer with systemic chemotherapy and conisation. *Gynecol Oncol* 2006;**100:**213–5.

Accepted for publication November 4, 2007

Příloha X.

Pluta M., Rob L., Charvát M., Chmel R., Halaška M., Škapa P. a Robová H. (2009) Less radical surgery than radical hysterectomy in early stage cervical cancer: A pilot study. ***Gynecol Oncol*** 113:181-184.
IF 2,614

Gynecologic Oncology 113 (2009) 181–184

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Less radical surgery than radical hysterectomy in early stage cervical cancer – A pilot study

Marek Pluta [a], Lukas Rob [a,*], Martin Charvat [a], Roman Chmel [a], Michael Halaska Jr. [a], Petr Skapa [b], Helena Robova [a]

[a] Department of Gynecology and Obstet., Division of Oncogynecology, Charles University Prague, 2nd Medical Faculty, V Uvalu 84, Prague 5, 15006, Czech Republic
[b] Department of Pathology and Molecular Medicine, Charles University Prague, 2nd Medical Faculty, V Uvalu 84, Prague 5, 15006, Czech Republic

ARTICLE INFO

Article history:
Received 29 December 2008
Available online 4 March 2009

Keywords:
Sentinel lymph node
Early cervical cancer IB1
Laparoscopical lymphadenectomy
Conservative surgery
Parametrectomy
Morbidity

ABSTRACT

Objective. The purpose of this pilot study was to evaluate the feasibility and safety of a less radical surgery; laparoscopic lymphadenectomy followed by a simple vaginal hysterectomy in sentinel lymph node (SLN) negative early cervical cancer patients. Treatment-associated morbidity and oncological outcome were evaluated.

Patients and methods. From December 2000 to September 2007, 60 patients (50 squamous and 10 adenocarcinoma patients) in stages 3-IA1, 11-IA2 and 46-IB1 with median age of 44.6 years (range 33–64 years) were enrolled. Patients were selected based on favorable cervical tumors (IA1 with lymph-vascular space invasion [LVSI], IA2 and IB1 with tumor size less than 20 mm and less than half of stromal invasion). All patients underwent laparoscopic SLN identification using frozen section (FS). Negative SLN patients underwent complete pelvic laparoscopic lymphadenectomy and vaginal hysterectomy. FS positive patients underwent radical hysterectomy with low paraaortic lymphadenectomy.

Results. The average number of sentinel nodes per side was 1.4 with detection rate per side of 95%. The average number of removed nodes was 23.2. Five patients (8.3%) were SLN positive. There were two false negative FS results (both were micrometastases in SLN). Median follow-up was 47 months (range 12–92). There were no recurrences in 55 SLN negative patients and in 5 SLN positive patients.

Conclusion. Lymphatic mapping and SLN identification improved safety in less radical surgery in early stage cervical cancer. This preliminary study showed that it is both feasible and safe to reduce the radicality of parametrial resection for small tumor volume in SLN negative patients. Results also indicated that treatment-associated morbidity is low.

Introduction

Because of the effective use of screening, an increasing number of women are being diagnosed with cervical cancer in an early stage of the disease. Individualization of treatment to reduce therapy-associated early and late morbidity is the most current trend in cervical cancer surgery [1,2]. Radical hysterectomy and pelvic lymphadenectomy in different modifications are the standard surgical treatment for patients with stage IA2 and IB1 cervical cancer [3–6]. Extensive lymphadenectomy and parametrectomy are the main cause of postoperative complications [6–11]. However, the majority of patients with early stage disease do not present lymph node metastases and parametrial involvement is rare. Quite a large number of retrospective studies show that the incidence of parametrial involvement is very low in the subgroup of patients with a tumor less than 2 cm in diameter, less than 10 mm of invasion and negative lymph nodes [12–16]. Stegeman et al., who performed a review of the literature, found that only 0.6% (5 of 799) of the patients with low-risk pathologic characteristics had parametrial involvement [16]. Research over the past 8 years on sentinel lymph nodes (SLNs) has greatly expanded our knowledge on lymphatic propagation of early stage cervical cancer [17–24]. Our prospective study demonstrates that, if the SLNs are negative, the risk of tumor involvement of the parametria is minimal in cervical cancer less than 2 cm in diameter and infiltrating less than one half of the cervical stroma [25].

The purpose of this pilot study was to evaluate the feasibility and safety of a less radical surgery; namely laparoscopic lymphadenectomy followed by a simple vaginal hysterectomy in SLN negative early cervical cancer patients. Treatment-associated morbidity and oncological outcome were also evaluated.

* Corresponding author. Obstetrics and Gynecology Department, 2nd Medical Faculty, Faculty Hospital Motol, V uvalu 84, 150 00 Prague 5, Czech Republic.
E-mail address: lukas.rob@lfmotol.cuni.cz (L. Rob).

0090-8258/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2009.02.005

Patients and methods

From December 2000 to September 2007, 60 female patients (3 patients — IA1, 11 patients — IA2 and 46 patients — IB1) were enrolled in the prospective study. The patients were selected based on favorable cervical tumors (I A1 with lymph–vascular space invasion {LVSI} and IA2 and IB1 with tumor size less than 20 mm in diameter and infiltration of less than half of the cervical stroma). Mean age was 44.6 years (range 33–64 years). MRI and ultrasound volumometry were performed after biopsy (wide excision, large loop excision of the transformation zone or needle cone) to identify residual disease. On bases of these examinations when the tumor was more than 20 mm in the biggest diameter or stromal invasion was more than half of stroma, all women were excluded from the study and underwent radical hysterectomy. A second reading of the histopathology was done in all cases if the biopsy was performed out of our hospital. Protocol of the study was approved by the local institutional ethical committee in our hospital (Fig. 1) and informed consent was signed by all patients.

All 60 patients underwent laparoscopic SLN identification. The most important aspect of SLN mapping is timing, the isotope injection technique and the blue dye into the cervix. We used radiocolloid 20 MBq ^{99m}Tc-labeled Sentiscint colloidal albumin measuring 100–600 nm in diameter (MEDI-RADIOPHARMA LTD, Hungary), diluted in 2 ml of saline solution and 2 ml of Patent blue (BLEU PATENTÉ V 2.5% — Guerbet, France) diluted in 2 ml of saline solution. In the operating room, after the introduction to general anesthesia, ^{99m}Tc was very slowly injected peritumorally into the tumor bed (5–8 s to each quadrant); 10 to 15 min later laparoscopic visualization of the small pelvis was performed. As in the case of ^{99m}Tc, Patent blue is then applied using the vaginal approach under direct visualization. Direct visualization of the pelvis allows the surgeon to observe the individual blue-colored lymphatic channels. The retroperitoneum was open for 5 min after the injection of Patent blue, which means 15 to 20 min after the injection of the radioisotope. We used a short protocol of ^{99m}Tc administration and did not employ preoperative lymphoscintigraphy. Upon identification of the individual blue-dyed lymphatic channels and blue-colored nodes, we performed radioactivity detection with a laparoscopic gamma probe (Neoprobe, Johnson and Johnson, USA). SLNs were extirpated separately. Identification of SLNs in the medial part of the lateral parametrium (between the cervical fascia and obliterated umbilical artery) is possible in vivo only by blue dye because radioactivity is very high near the cervix [20]. In case of detection of blue node in the parametrium of the patients radioactivity was measured out of the body. The blue lymphatic channel from the medial part of lateral parametrium was removed separately and sent for histopathological evaluation. Having sent the sentinel nodes from frozen section analysis, we performed a complete laparoscopic pelvic lymph node dissection. If the frozen section was

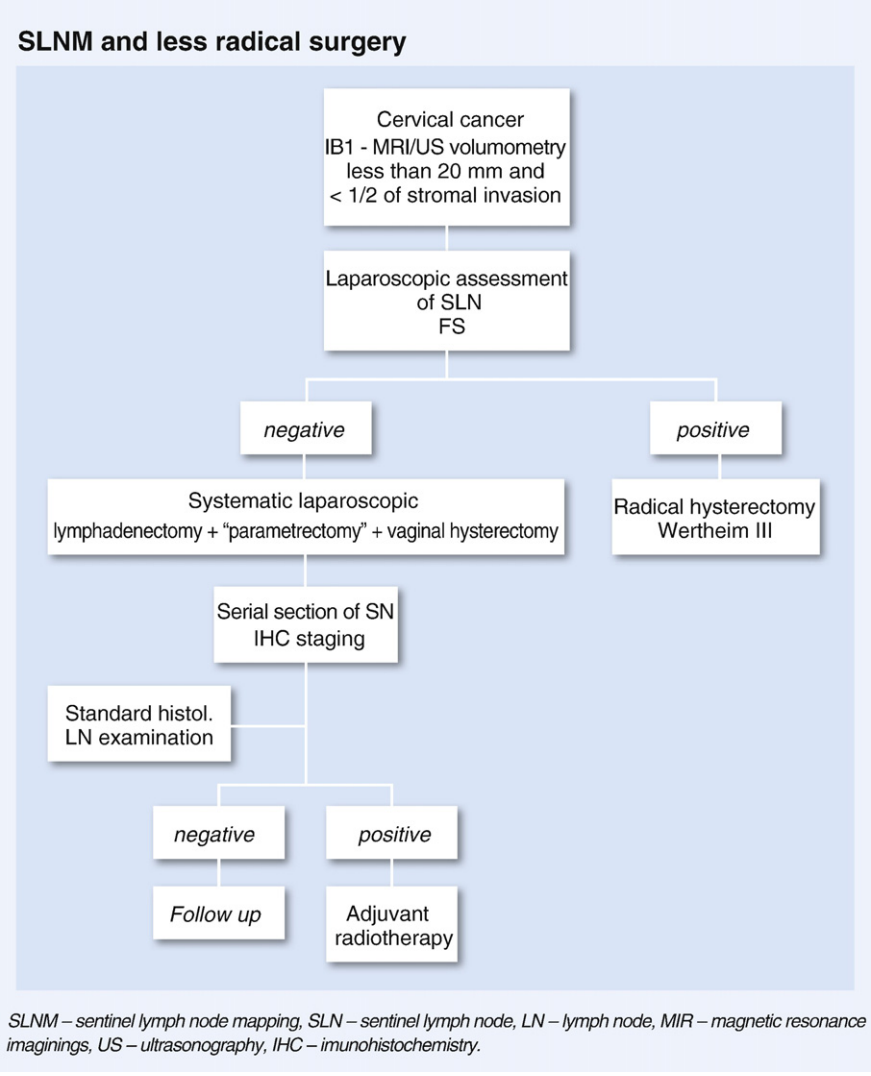


Fig. 1. SLNM and less radical surgery.

positive, laparoscopy was abandoned and we continued with laparotomy and radical hysterectomy as well as lower paraaortic lymphadenectomy. If the frozen section was negative, patients were treated with a simple vaginal hysterectomy. Laparoscopically assisted vaginal hysterectomy or total laparoscopic hysterectomy would be other option to vaginal hysterectomy. All patients underwent US of abdomen and kidneys before they were discharged from the hospital and 6 weeks after surgery.

Elaboration of the sentinel node (ultramicrostaging) and other lymph nodes that were removed was done by standard protocol [20]. Nodes larger than 8 mm were divided into two halves, parallel to the long axis of the node. Both halves were frozen in one block. Nodes less than 8 mm were frozen in one piece. Nodes were cut at intervals 40 μm by 4 μm section cut and were stained with Harris hematoxylin. After the frozen section the lymph node slices were fixed in a buffered 4% formaldehyde, pH 7.2. The tissue was embedded in a paraffin wax by standard serial sectioning techniques. Each level was stained for hematoxylin and eosin (HE). Every third slide was immunostained with an anti-cytokeratin antibody — large spectrum, cytokeratines mixture CK 5, 6, 8, 17, 19 (Immunotech, France). All other surgically removed lymph nodes were examined histopathologically using routine hematoxylin and eosin (HE) staining.

Results

In the protocol there were 3 patients with stage IA1 (100% with LVSI), 11 IA2 patients (36.4% with LVSI) and 46 IB1 patients (26.1% with LVSI). Of the 60 cases, 83.3% (50 cases) were of squamous cell histology and 16.7% (10 cases) adenocarcinoma. The preoperative diagnosis was established by a loop electrosurgical excision procedure (LEEP) or cervical conisation in 45 patients (75%) and cervical biopsy in 15 patients (25%).

We detected SLNs in all of the patients. The detection rate (DR) per patient was 100% and the specific side detection rate (SSDR) 94.2%. The average number of sentinel nodes per side was 1.4 (range 1–4). Positive SLNs were detected in 5 patients (8.3%) Table 1. All node-positive patients were only with SLN positive. No false negative SLNs were noted in any of the 60 patients. Three frozen sections were positive, and in these cases a Wertheim radical hysterectomy with low paraaortic lymphadenectomy was performed. In cases of negative frozen sections after SLN identification laparoscopic pelvic lymphadenectomy was continued. Two false negative frozen section results were observed. In both cases it was micrometastasis less than 2 mm that was detected by serial sectioning of the SLNs. The average total gain of lymphatic nodes was 26.8 (15–56) in the first 25 SLN FS negative patients. In the second period radicality of lymphadenectomy in patients with a negative frozen section was reduced (patient numbers 26 to 60) and the average number of lymphatic nodes was 16.5 (range 5–28). Median follow-up was 47 months (range 12–92). No serious intraoperative complication was registered (injury of big vessels, bladder, ureter or bowels). The bladder catheter was removed in 56 women 24 h after surgery. In one patient it was necessary to retain the bladder catheter 5 days after laparoscopic lymphadenectomy and vaginal hysterectomy because a large residual of urine was found. No injury of the ureter was noted and no blood transfusion was administered after less radical surgery. Average length of stay in hospital was 3 days in patients after less radical surgery. Standard length of stay after the radical hysterectomy is in our hospital 7 days. Lymphocysts were diagnosed in 8 patients, but only three of these patients were symptomatic. From the first 25 patients after conservative surgery, 6 (24%) had clinically detectable lymphocysts and 3 (12%) had more than 24 lymph nodes removed. In three (12%) patients clinical lymphedema was diagnosed in the first year after treatment. From the next 32 patients only 2 (6.3%) had lymphocysts (one lymphocyst (3.1%) was symptomatic).

No recurrence was noted in 55 LN-negative patients. Median follow-up was 47 months (range12–92). From the first part of the study, persisting symptomatic lymphedema of the legs was limited to two patients (8%) while the remaining 58 patients were without significant long-term morbidity. All five patients with positive nodes were without evidence of disease after the 24-, 42-, 44-, 60- and 74-month follow-ups; four of these positive-node patients received adjuvant teleradiotherapy without chemotherapy.

Table 1
Characteristics of patients with positive lymph nodes

Patient	Age (years)	Stage	Tumor diameter	LVSI	FS-SLN	Size of meta in SLN	Non SLN meta
No. 1	48	IA2	6×3.5 mm	Yes	3 negative	1×1.5 mm 1×ITC (IHC)	No
No. 2	52	IB1	9×3 mm	No	1 positive 2 negative	1×5 mm	No
No. 3	50	IB1	10×2.5 mm	Yes	2 negative	1×0.5 mm	No
No. 4	42	IB1	8×5 mm	Yes	1 positive 2 negative	1×8 mm	No
No. 5	56	IB1	12×4 mm	no	1 positive 1 negative	1×10 mm	No

LVSI — lymphovascular space involvement, ITC — isolated tumor cells, IHC — immunohistochemical staging, SLN — sentinel lymph node, and FS — frozen section.

Discussion

Complete resection of the parametria by radical hysterectomy was considered a standard operative procedure in invasive stage Ia2 and Ib1 cervical cancer during the 1970s and 1980s [28–30]. In the 1990s, the concept of modified radical hysterectomy was accepted [3–5], which reduced the radicality of resection of the parametria. The primary aim of this procedure was to reduce long-term morbidity, which is associated with the radical resection of the parametria, where potential injury to the innervation of the rectum and bladder can occur. Extension and utility of the resection of the paracervical tissue (parametrium) in radical hysterectomies in women with early stage cervical cancer have been discussed extensively in the literature over the past 15 years [2–5,9,12–16]. Numerous studies have confirmed a correlation between size of the tumor or infiltration of the cervical stroma and positive lymph nodes with involvement of the parametria [3,4,9,12–16]. When we performed a detailed analysis of the data, risk of *parametrial involvement* is lower than 1% in cervical cancer Ia2 and Ib1 when the tumor size is less than 20 mm in diameter and invasion is less than 10 mm and the pelvic lymph nodes are negative [4,12,15]. When we excluded from this prognostically favorable group patients with LVSI, the parametrium involvement is very rare. In this pilot study we do not consider LVSI as an exclusion criterion for either less radical surgery or fertility sparing surgery, because intraoperative sentinel lymph nodes evaluation is in our institution accurate except micrometastases [26–27].

The most important prognostic factor in our opinion is patients with positive SLNs. We did not register any recurrences in our group of patients after less radical surgery with negative SLNs, including patients with LVSI. Our prospective study, which was the first of its kind that had evaluated risk of parametrial involvement independent of the involvement of the SLNs, confirmed the minimal risk of parametrial involvement in SLN negative patients. When the SLNs are positive, risk of parametrial involvement is about 28% [25]. Involvement of isolated parametrial SLNs without the incidence of other pelvic lymph nodes is very rare (about 1%) in early cervical cancer [4,12,20,25]. In our view, identification of SLNs and blue lymphatic channels and eventually blue lymph nodes in paracervical tissue should be a part of less radical surgery in early cervical cancer. Measuring of radioactivity of blue parametrial lymph nodes has to be done out of the operation field after extirpation. Identification of SLNs is an important component of our management and reduces the risk of less radical surgery. The pivotal element of successful SLN detection

in early cervical cancer is good timing in the administration of radiocolloid and blue dye, the right technique with respect to peritumoral injection of radioactive colloid, experience with the method and good cooperation with a histopathologist [20,27,28]. When all these conditions are met, we obtain a 95% detection rate of SLN, with more than 99% sensitivity [20,22].

Morbidity of radical surgery is not only dependent on the reduction of resecting the parametria but also on good pelvic lymphadenectomy (more than 25 lymph nodes), even laparoscopic-related morbidity. Another benefit of SLN detection is a reduction in radicality of lymphadenectomy in patients with peroperative negative SLNs [28,30]. In patients who underwent surgery at the end of the study radicality of lymphadenectomy was reduced in all cases with a negative frozen section of the SLNs. In these patients there were fewer lymphocysts and almost no clinical lymphedema of the legs.

Using frozen sections of SLNs is still controversial issue. We routinely perform FS of SLNs in cervical cancers, where a metastasis larger than 2 mm was always diagnosed with FS in our group of more than 300 cervical cancers. Only micrometastases less than 2 mm can be diagnosed as late as during the serial sectioning of SLNs. There is a question regarding what is considered clinical significance of micrometastases from removed SLNs. Metastatic disease was never diagnosed in other pelvic nodes in cases of false negative FS of SLNs. Furthermore, in this group of patients who underwent less radical surgery only micrometastases less than 2 mm were not identified by FS.

Radical hysterectomy type III when the frozen section of SLN was positive is controversial. If we diagnose positive SLN by frozen section, it is not micrometastatic disease. There is a risk for involvement of other nodes and risk for parametrial involvement is about 28% [25]. Debulking in these cases has sense by our opinion. In cases when only SLN is positive and other upper nodes are negative, we used for adjuvant treatment only small radiation field on the pelvis.

In select early cervical cancer the combination of lymphatic mapping and SLNI is a procedure that makes it possible to perform less radical surgeries of the parametria and to reduce the radicality of pelvic lymphadenectomy in SLN negative patients. This preliminary study showed that it is feasible and safe to reduce the radicality of parametrial resection when tumor volume is small, when the patients are sentinel node negative and when treatment associated with morbidity is low. Lymphatic mapping and SLNI improved the safety of less radical surgery in early cervical cancer. However, simple hysterectomy and simple trachelectomy in SLN negative patients are still experimental. We need a prospective multiinstitutional observation study to confirm our pilot work before we can include simple hysterectomy and simple trachelectomy in oncogynecological standards. Now MD Anderson Cancer Center Houston, Texas, USA prepares multicentric study about less radical surgery in early cervical cancer.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

Acknowledgment

This work is supported by grant IGA MZCR NS9914-4.

References

[1] Rob L, Svoboda B, Robova H, Stankusova H, Cwiertka K, Neumannova R, et al. Guideline for gynecological malignant tumors — primary complex therapy in operable stages of malignant tumors of uterus cervix. Ceska Gynekol 2004;69 (5):376–83.
[2] Plante M, Roy M. New approaches in the surgical management of early stage cervical cancer. Cur Opin Obstet Gynecol 2001;13(1):41–6.
[3] Margina JF, Goodrich MA, Weaver AL, Podratz KC. Modified radical hysterectomy: morbidity and mortality. Gynecol Oncol 1995;59(2):277–82.
[4] Kinney WK, Hodge DO, Egorshin EV, Ballard DJ Podratz KC. Identification of low risk subset of patients with stages Ib invasive squamous cancer of the cervix possibly suited to less radical surgical treatment. Gynecol Oncol 1995;57(1):3–6.
[5] Landoni F, Maneo A, Cormio G, Pereno P, Milani R, Caruso O, et al. Class II versus class III radical *hysterectomy* in stage Ib–IIa cervical cancer: a prospective randomised study. Gynecol Oncol 2001;80:3–12.
[6] Hoffman MS. Extent of radical hysterectomy: evolving emphasis. Gynecol Oncol 2004;94:1–9.
[7] Kadar N, Saliba N, Nelson JH. The frequency, causes and prevention of severe urinary dysfunction after radical hysterectomy. Br J Obstet Gynecol 1983;90 (9):858–63.
[8] Low JA, Mauger GM, Carmichael JA. The effect of Wertheim hysterectomy upon bladder and urethral function. Am J Obstet Gynecol 1981;139(7):826–34.
[9] Benedetti-Panici P, Angioli R, Palaia I, Muzzi L, Zullo MA, Manci N, et al. Tailoring the parametrectomy in stages IA2–IB1 cervical carcinoma: is it feasible and safe? Gynecol Oncol 2005;96(3):792–8.
[10] Sood AK, Nygaard I, Shahin MS, Sorosky JI, Lutgendorf SK, Rao SS. Anorectal dysfunction after surgical treatment for cervical cancer. J Am Coll Surg 2002;195 (4):513–9.
[11] Frumovitz M, Sun CC, Schover LR, Munsell MF, Jhingran A, Wharton JT, et al. Quality of life and sexual functioning in cervical cancer survivors. J Clin Oncol 2005;23:7428–36.
[12] Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol 2002;84(1):145–9.
[13] Puente R, Guzman S, Israel E, Poblete MT. Do the pelvic lymph nodes predict the parametrial status in cervical cancer stages IB–IIA? Int J Gynecol Cancer 2004;14:832–40.
[14] Wright JD, Grigsby PW, Brooks R, Powell MA, Gibb RK, Gao F, et al. Utility of parametrectomy for early stage cervical cancer treated with radical hysterectomy. Cancer 2007;110(6):1281–6.
[15] Steed H, Capstick V, Schepansky A, Honore L, Hiltz M, Faught W. Early cervical cancer and parametrial involvement: is it significant? Gynecol Oncol 2006;103 (1):53–7.
[16] Stegeman M, Louwen M, van der Velden J, den Kate FJ, den Bakker MA, Burger CW, et al. The incidence of parametrial tumor involvement in select patients with early cervical cancer is too low to justify parametrectomy. Gynecol Oncol 2007;105 (2):475–80.
[17] O'Boyle JD, Coleman RL, Bernstein SG, Lifshitz S, Muller CY, Miller DS. Intraoperative lymphatic mapping in cervix cancer undergoing radical hysterectomy: a pilot study. Gynecol Oncol 2000;79:238–43.
[18] Malur S, Krause N, Kohler C, Schneider A. Sentinel lymph node detection in patients with cervical cancer. Gynecol Oncol 2001;80:254–7.
[19] Levenback C, Coleman RL, Burke TW, Lin WM, Erdman W, Deavers M, et al. Lymphatic mapping and sentinel node identification in patients with cervix cancer undergoing radical hysterectomy and pelvic lymphadenectomy. J Clin Oncol 2002;20:688–93.
[20] Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol 2005;98(3):281–8.
[21] van Dam PA, Hauspy J, Vanderheyden T, Sonnenemans H, Spaepen A, Eggenstein G. Intraoperative sentinel node identification with Technetium-99m-labeled nano-colloid in patients with cancer of the uterine cervix: a feasibility study. Int J Gynecol Cancer 2003;13:182–6.
[22] Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol 2003;91(3):494–503.
[23] Marnitz S, Kohler Ch, Bongardt S, Braig U, Hertel H, Schneider A. Topographic distribution of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol 2006;103(1):35–44.
[24] Frumovitz M, Ramirez PT, Levenback C. Lymphatic mapping and sentinel nod detection in gynaecologic malignancies of the lower genital tract. Curr Oncol Rep 2005;7(6):435–43.
[25] Strnad P, Robova H, Skapa P, Pluta M, Hrehorcak M, Halaska M, et al. A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. Gynecol Oncol 2008;109(2):280–4.
[26] Rob L, Charvat M, Robova H, Pluta M, Strnad P, Hrehorcak M, et al. Less radical fertility — sparing surgery than radical trachelectomy in early cervical cancer. Int J Gynecol Cancer 2007;17(1):304–10.
[27] Rob L, Pluta M, Strnad P, Hrehorcak M, Chmel R, Skapa P, et al. A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. Gynecol Oncol 2008;111(2):S116–20.
[28] Gien LT, Covens A. Quality control in sentinel lymph node biopsy in cervical cancer. J Clin Oncol 2008;26(18):2930–1.
[29] Abu-Rustum NR, Barakat RR. Observations on the role of circumflex iliac node resection and the etiology of lower extremity lymphedema following pelvic lymphadenectomy for gynecologic malignancy. 2007;106:4–5
[30] Rob L, Charvat M, Robova H, Strnad P, Pluta M, Halaska M, Hrehorcak M. Sentinel lymph node identification (SLNI) in the management of conservative surgery in early cervical cancer: is it acceptable? Gynecol Oncol 2005;99:S147–8.

Příloha XI.

Rob L., Pluta M., Škapa P. a Robová H. (2010) Advances in fertility-sparing surgery for cervical cancer. ***Expert Rev Anticancer Ther*** 10:1101-1114.
IF 2,493

EXPERT REVIEWS

Advances in fertility-sparing surgery for cervical cancer

Expert Rev. Anticancer Ther. 10(7), 1101–1114 (2010)

Lukas Rob†[1], Marek Pluta[1], Petr Skapa[2] and Helena Robova[1]

[1]*Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Charles University Prague, 2nd Medical Faculty, Prague, Czech Republic*
[2]*Department of Pathological Anatomy and Molecular Medicine, Charles University Prague, 2nd Medical Faculty, Prague, Czech Republic*
†*Author for correspondence:*
Tel.: +420 224 434 203
Fax: +420 224 434 218
lukas.rob@lfmotol.cuni.cz

This article reviews the literature on fertility-sparing surgery in early cervical cancer. The article evaluates selection criteria, preoperative management and the most frequent surgical procedures used for preservation of fertility in cervical cancer. The article also analyzes oncological, fertility and pregnancy results. Oncological outcomes are not statistically different among single groups (vaginal radical trachelectomy, abdominal radical trachelectomy, simple trachelectomy or cone with or without neoadjuvant chemotherapy). Oncological results after fertility-sparing procedures in women with tumors smaller than 2 cm are comparable with women with the same risk factors after radical hysterectomy. Pregnancy following fertility-sparing surgery is associated with a variety of adverse pregnancy outcomes, especially second-trimester loss and preterm delivery. Less radical procedures (simple trachelectomy or cone with or without neoadjuvant chemotherapy) show statistically significant better pregnancy results. The pregnancy rate after abdominal radical trachelectomy was dramatically lower than in women treated with other types of fertility-sparing surgery. In the future, it will be necessary to optimize the technique and management of fertility-sparing surgery in order to attain good oncological results. Pregnancy outcomes should be given high priority. Fertility-sparing surgery is valuable for women who want to preserve their reproductive capability.

Keywords: cervical cancer • fertility-sparing surgery • radical trachelectomy • sentinel lymph node • simple trachelectomy

It is estimated that 25–40% of cervical cancer cases diagnosed in developed countries occur in women of reproductive age [1,2]. For various reasons, many of these women may have postponed conception because delayed childbearing is a trend in developed countries. For decades, the only fertility-sparing surgical option for women who wished to retain reproductive function was cervical conization in patients with FIGO stage IA1 cervical cancer. Radical hysterectomy with pelvic lymphadenectomy or radiation therapy were the only options for women with cervical cancer of more than 3-mm invasion (FIGO stage IA2 and more). Pregnancy was not possible using either of these therapeutic approaches. In developed countries, cervical screening has led to an impressive reduction of incidence and mortality, resulting in a relative increase of early-stage cancer occurring in young women [1,3]. In the future, fertility preservation may be of concern to more and more women with newly diagnosed cervical cancer.

At the Society of Gynecological Oncologists (SGO) Annual Meeting in 1994, Daniel Dargent and his group presented 8 years of experience with laparoscopic pelvic lymphadenectomy and vaginal radical trachelectomy (VRT) as a fertility-sparing therapy for early cervical cancer [4]. This presentation led to the question of whether it is possible to perform less radical procedures than radical hysterectomy in order to preserve the uterus without increasing the risk of recurrence (oncological outcome), and to afford the opportunity for successful fertility (fertility outcome) and successful pregnancy leading to delivery of a healthy infant (pregnancy outcome). In a short time, a number of groups presented studies with laparoscopic lymphadenectomy and slightly modified VRT [5–8]. The number of reported cases increased over the next 15 years, which resulted in the answering of many questions, but also the raising of new questions. Currently, VRT is referred to as Dargent's operation, despite a number of small technical modifications being introduced. We estimate that approximately 1000 VRTs have been performed, but only papers that have more than ten cases and that analyzed oncologic and pregnancy outcome were included in this article. These criteria were fulfilled by 683 women.

 10.1586/ERA.10.61 ISSN 1473-7140

Box 1. Criteria for performing fertility-sparing surgery.

Criteria for woman
- Strong fertility desire
- Age that supposes a real chance for pregnancy – reproductive potential (40–45 years)
- Women are fully informed in order to allow a choice to be made

Criteria for tumor
- Tumor is limited to the cervix (less than 20 mm in the biggest diameter and less than half of stroma invaded)†
- Neuroendocrine small-cell cancer of the cervix is an exclusion criterion
- Negative pelvic lymph nodes

Criterion for center
- Well elaborated management of fertility-sparing surgery with quality control

†Women with a tumor larger than 2 cm are potential candidates for neoadjuvant chemotherapy.

An alternative fertility-sparing procedure is abdominal radical trachelectomy (ART). This approach was first described in 1932 by the Romanian surgeon Eugen Aburel. However, none of the women he treated with this procedure had a successful pregnancy. The international group of Ungar, Del Priore and Smith restored the use of this surgical approach, and they published their initial experiences in 1997 [9]. The extent of radicality of ART is the nearest to our oncogynecological representation of a radical surgical procedure in invasive cervical cancer that supposes extirpation of paracervical tissues to the extent of Piver type III (new classification Querle–Morrow C1,2). The same radicality of paracervical resection was described using the total laparoscopic approach [10–12] or robotically [13,14]. To date, approximately 250 ARTs have been performed, but only papers that have more than ten cases and analyzed oncological and pregnancy outcome were included in this article. These criteria were met by 207 women for oncological outcome and 221 women for pregnancy outcome.

New trends in fertility-sparing surgery have led to less radical approaches. 'Simple trachelectomy', as described by Rob in 2007 [15], or 'chemo-conization' by Landoni in 2007 [16], are two less radical approaches. Less radical surgery is divided into two steps. The first step involves laparoscopic pelvic lymphadenectomy with or without sentinel lymph node mapping (SLNM). The second step concerns conization or simple trachelectomy, which is performed when the sentinel lymph nodes (SLNs), and other pelvic nodes are negative. These procedures (conization and simple trachelectomy) significantly reduce the radicality of paracervical resection in node-negative patients [15,16].

Another fertility-sparing approach that attempts to reduce radicality and retain the childbearing potential in women with bulky cervical cancer is neoadjuvant chemotherapy (NAC). All NAC protocols use chemotherapy with cisplatin [7–19].

This article is a review of our knowledge of fertility-sparing procedures in cervical cancer patients. The review discusses selection criteria, presents an analysis of oncological and pregnancy results, examines current surgical approaches and evaluates present perspectives.

Patient selection & preoperative (work-up) assessment

Careful patient selection is important for the desired oncological and pregnancy outcome. Box 1 summarizes criteria for potential candidates for fertility-sparing surgery.

Precise management of fertility-sparing surgery must include not only a good selection of women with a strong desire to preserve fertility, but also complete information about preoperative examinations, surgery, specific late complications and in particular the risk of premature delivery. The patients must know that these procedures are not the standard of care, but are available to a few select motivated individuals. The women must be informed that, in 10–15% of the cases, more extensive endocervical disease or positive pelvic lymph nodes are found. In such cases, the fertility-sparing surgery must be abandoned and replaced by chemoradiation or radical hysterectomy. Informed consent must include information about the risk of infertility and second-trimester abortion and premature delivery [15,20–22].

Tumor characteristics

Appropriate candidates for fertility-sparing surgery are patients with tumors smaller than 2 cm in diameter (FIGO stage IA1 with lymphovascular space invasion [LVSI], IA2 and IB1). In IB1 tumors larger than 2 cm, there is a higher risk of extrauterine spread and, statistically, the risk of recurrence is significantly higher [23]. Tables 1 & 2 show that this criterion (i.e., tumors <2 cm in size) is the same for VRT (recurrences in 2.9% oversus recurrences in 20.8% in tumors >2 cm) and ART (1.6% of recurrences vs 18.2%). In most centers, 30–70% of patients underwent conization, which allows the exact measurement of tumor size histopathologically. A second careful pathological review of the previous biopsies by an expert gynecopathologist is strongly recommended.

A second histopathological examination is important for determination of histopathological type, depth of invasion, pattern of invasion and LVSI. Many centers examine LVSI separately as two components: lymphatic vessel invasion (LVI) and blood vessel invasion (BVI). LVSI is detected more often in early-stage cervical cancers, and it increases the risk of positive lymph nodes. BVI, which is more frequent in adenosquamous and neuroendocrine cancers increases the risk for hematogenous metastases. Small-cell neuroendocrine carcinoma is not suitable for fertility-sparing surgery because the prognosis is poorest in comparison with other types [23–25]. When these risk factors (i.e., adenosquamous type, LVSI, LVI, BVI, pattern of invasion and depth of invasion) are reviewed separately, they do not provide sufficient sensitivity in predicting tumor behavior *in vivo* and, therefore, their use is debatable [25]. Although most oncologists consider these findings possible risk factors for recurrence, the majority do not use them as exclusion criteria for fertility-preserving surgery. LVSI was present in 30% of the ART and 30.1% of the VRT (Tables 1 & 2). Women must be informed of these risk factors, as well as of the risk of malignant extrauterine spread and the increased risk of recurrence.

Table 1. Vaginal radical trachelectomy: characteristics and oncological outcome.

Parameter	Pahisa [39]	Sonoda [38]	Chen [40]	Hertel [42]	Dargent [41]	Shepherd [22,64]	Plante [20,63]	Covens [65,51]	Burnett [37,66]	Schlaerth [67]	Total[§]
Year	2008	2008	2008	2006	2007	2006[‡]/2008	2004/2008[‡]	2007/2008[‡]	2003/2006[‡]	2003	
Location	Barcelona, Spain	New York, USA	Chongqing, China	AGO, Germany	Lyon, France	London, UK	Quebec, ON, Canada	Toronto, ON, Canada	Los Angeles, CA, USA	Pasadena	
Period	2000–2007	2001–2006	2000–2004	1995–2005	1986–2003	1994–2005[‡] 1994–2007	1991–2003 1991–2008[‡]	1999–2003 1999–2007[‡]	1995–2001	1995–1999	
Planned VRT (n)	15	43	16	108	135	158	82	93	21	12	683
Fertility spared (%)	13 (87)	36 (84)	16 (100)	106 (98.1)	118 (87.40)	138 (87)	72 (87.8)	91 (97.8) 137[‡]	18 (85.7)	10 (83.3)	618 (90.5%)
N1 (%)	0/15 (0)	2/43 (4.70)	0/16 (0)	4/108 (4)	9/135 (6.7)	7/123 (5.7)[‡]	4/82 (4.9)	2/93 (2.1)	1/21 (4.8)	0/12 (0)	29/648 (4.5%)
LVSI (%)	1/15 (6.70)	NA	1/16 (6.3)	38/108 (35.2)	43/118 (36.4)	49/158 (31)	14/72 (20)	31/93 (33)	6/21 (28.6)	1/10 (10)	184/611 (-30.1%)
Average age (years)	NA	31	27.6	32	NA	30.6	31	30	30	30.9	31
Range (years)	NA	20–40	24–31	21–41	NA	21–45	21–42	NA	23–41	22–44	20–44
Nulliparous women (%)	NA	35/43 (81.4)	14/16 (87.5)	92/108 (85.2)	NA	97/123 (78.9)[‡]	54/72 (75)	NA	16/21 (76.8)	12/12 (100)	320/395 (81)
Histology											
• SCC (%)	9[†] (60)	24 (56)	14 (87.5) 2 (12.5)	74 (68.5) 33 (30.6)	90[†] (76.3) 25[†] (21.2)	103 (65)	42 (58)	40 (43)	12 (57.1)	4[†] (40)	412 (63)
• AC (%)	6[†] (40)	16 (37)	0	1 (0.9)	3[†] (2.5)	51 (33)	27 (38)	50 (54)	9 (42.9)	5[†] (50)	224 (34.3)
• Other (%)	0	3 (7)				4 (2)	3 (4)	3 (3)	0	1[†] (10)	18 (2.7)
Size											
• <2cm/ recurrence	1/11	1/36	0/9	3/105	1/91	NA	1/64	5/83	NA	0/10	12/409 (2.9)
• >2cm/ recurrence	1/2	0	0/7	1/1	1/27	NA	8/2	1/ 8	NA	0	11/53 (20.8)
Recurrences	2/13	1/36	0/16	4/106	7/118	4/138	3/72 4/115[‡]	6/91	2/18[‡]	0/10	29/618 (4.7)
Death	1	1	0	2	5	4	2	4	1	0	20 (3.2)

[†]Only patients after VRT.
[‡]Author published data from extended group of patients, the marked numbers are from these marked publications.
[§]Only numbers that are available.
AC: Adenocarcinoma; LVSI: Lymphovascular space involvement; N1: Positive lymph node; SCC: Squamous cell cancer; VRT: Vaginal radical trachelectomy.

Table 2. Abdominal radical trachelectomy: characteristics and oncological outcome.

Parameters	Abu Rustum (2008) [52]	Pajera (2008) [68]	Duska (2009) [69]	Nishio (2008) [73]	Cibula (2009) [70]	Ungar (2005) [59]	Kim (2010) [10]	Total§
Location	NY, USA	Medelin	MA, USA	Tokyo, Japan	Prague, Czech Republic	Budapest, Hungary/ London, UK/ NY, USA	Seoul, Korea	
Period	2005–2008	2002–2008	1999–2007	2002–2008	2001–2008	1997–2002	2004–2009	
Planned ART	22	15	10	71	24	33	32	207
Fertility spared	15	14	10	61	17†	30	27	174 (84%)
N1 (%)	6/22 (27)	1/15 (6.7)	0/10 (0)	15/71 (21.1)	4/24 (16.7)	2/33 (6.1)	4/32 (12.5)	32/207 (15.5)
LVSI (%)	9/22 (41)	5/15 (33)	NA	31/71 (43.7)	2/24 (7)	8/33 (24.2)	4/32 (12.5)	59/197 (29.9)§
Average age (range/years)	33 (23–43)	30 (25–38)	31.7 (25–38)	33 (26–44)	32.4 (23–37)	30.5 (23–37)	29 (22–37)	32 (22–44)
Nulliparous women (%)	20/22 (91)	NA	9/10 (90)	NA	21/24 (87.5)	NA	20/27 (74.1)	70/83 (84.3)§
Histology								
• SCC (%)	9 (41)	11 (73.3)	3 (30)	58 (95.1)‡	14 (58)	26 (86.7)‡	20 (74.1)	141 (74.6)
• AC (%)	13 (59)	4 (26.7)	7 (70)	2 (3.3)‡	10 (42)	1 (3.3)‡	6 (22.2)	43 (22.8)
• Other (%)	0	0	0	1 (1.6)‡	0	3 (10)‡	1 (3.7)	5 (2.6)
Size								
• <2cm/ recurrence	NA	0/14	0/10	1/48	1/14	0/21	0/19	2/126 (1.6%)
• >2cm/ recurrence	NA	0	0	5/13	0/3	0/9	1/8	6/33 (18.2%)
Recurrences	0/15	0/14	0/10	6/61	1/17	0/30	1/27	8/174 (4.6%)
Death	0	0	0	NA	NA	0	1	NA

†Three of these were performed completely laparoscopically.
‡Only patients after ART.
§Only numbers that are available.
AC: Adenocarcinoma; ART: Abdominal radical trachelectomy; LVSI: Lymphovascular space involvement; N1: Positive lymph nodes; NA: Not applicable; SCC: Squamous cell cancer.

Imagining modalities to determine tumor burden

Colposcopy is the standard examination in clinically detected tumors before fertility-sparing surgery and is important in assessing the exocervical diameter and in excluding spread to the vagina [6,15,20]. Tumor volumetry by MRI is an important preoperative diagnostic method for determining exact tumor size, amount of cervical stroma infiltration and amount of healthy stroma (determination of tumor growth in anteroposterior, craniocaudal and transverse directions) or for accurately determining residual disease after conization [20,26–28]. Since it is necessary to have a 1-cm free margin, several authors have suggested that infiltration of less than half of the cervical stroma is the limit for safe trachelectomy [8,15,29]. Regardless of type of trachelectomy, at least 1 cm of healthy stroma should be saved, because this increases the chance of successful pregnancy. Imaging techniques (e.g., computed tomography [CT], MRI and PET-CT) are insufficient to detect pelvic and parametrial lymph node metastases [27,30]. In patients with early cervical cancer, small lymph node metastases are more common and are not detectable by current imaging techniques. A new generation of PET-CT and MRI techniques that use ultra-small iron particles seem to be feasible for preoperative evaluation of lymph nodes [31,32].

Intraoperative assessment: sentinel lymph node mapping & endocervical involvement

The majority of centers try to perform intraoperative detection of extrauterine spread (involvement of pelvic and paraaortal lymph nodes) because it is very important for safe and feasible fertility-sparing procedures.

Sentinel lymph node mapping

Since squamous cell cancer (e.g., adenocarcinoma) metastasizes primarily to lymph nodes, hematogenous spread is late. Currently, the SLNM procedure is being incorporated in fertility-sparing surgery management in many centers. The SLN is the first lymph node that receives direct drainage from the primary tumor and is useful in detecting lymph nodes with the highest metastatic risk. A number of studies have confirmed that SLNM is feasible and highly accurate in predicting the status of regional lymph nodes in early cervical

Table 3. Vaginal radical trachelectomy: pregnancy outcome.

Author	Patients	Pregnant women	Conceptions	First-trimester loss	Second-trimester loss	Delivery			Ongoing pregnancy	Ref.
						Before 32nd week	*32–36th week*	*Term*		
Pahisha and Alonso	13	3 (23%)	3	0	0	0	0	1	2	[39]
Chen *et al.*	16	5 (31%)	5	0	2	0	1	1§	1	[40]
Hertel *et al.*	100	18 (18%)	18	3	0	12			3	[42]
Schlaerth and Spirtos	10	4 (40%)	4	0	2	0	1	1	NA	[67]
Burnett	18	3 (17%)	3	0	1	0	0	2	NA	[66]
Sonoda *et al.*	36	11 (31%)	11	3	0	0	0	4	4	[38]
Plante	115	51 (44%)	90	23	3	4	9§	51	NA	[20]
Dargent and Milliken	95	33 (35%)	56	14	8	5		29	NA	[41]
Shepherd	138	NA	88	22	12	10	35 + 2†		7	[64]
Covens *et al.*	80	18 (23%)	22	3	1	3§	3	12	NA	[71]
Total	621	146‡	300	68 (22.7%)	29 (9.7%)	186+2† (62%)			17 (5.7%)	

†Surrogate live birth.
‡Missed numbers from Shepherd group of patients.
§Twins.
NA: Not applicable.

cancer tumors with a diameter smaller than 2 cm [33–35]. SLNs are currently detected by the application of two techniques: blue dye and radioactive tracer ^{99}mTc. Good timing of blue dye injection allows the identification of SLNs, and also identifies and removes blue afferent lymphatic channels or nodes from the parametrium. These specimens are then sent separately to the histology laboratory for analysis. Detection of SLNs increases the procedure's safety since it allows us to perform frozen-section (FS) analysis of SLNs and precise histopathological elaboration of the most high-risk lymph nodes.

Intraoperative endocervical assessment

Perioperative evaluation of endocervical involvement is routinely performed in many centers. The indication and methods of FS diagnosis of SLNs were discussed in detail in a paper from the Quebec Center (Canada) [36]. FS examination of a cross section of the superior portion of the separated cervix is conducted to evaluate the tumor-free status of the endocervical resection margin. There is no consensus as to how many millimeters of free endocervical margin is safe, although the majority of centers recommend more than 5 mm [15,20,37–41]. Perioperative FS assessment is not without its difficulties, especially in adenocarcinoma [36]. Thus, some centers do not routinely perform FS and instead take exact measurement of free endocervical margins wait for definitive histopathological evaluation [15,42].

Sentinel lymph nodes: histopathological procedures

The TNM staging book (International Union Against Cancer [UICC], 6th edition), divides tumor deposits in SLNs into three categories: macrometastasis (diameter >2 mm), micrometastasis (diameter >0.2 mm but not >2 mm) and isolated tumor cells (ITCs; diameter <0.2 mm). The presence of macrometastatic or micrometastatic disease in SLNs is described as positivity in the N category of the TNM staging system. On the other hand, the clinical significance of ITCs is still unknown, and SLN containing ITCs is recommended to be interpreted as tumor free (N0). SLNs are traditionally evaluated postoperatively in hematoxylin–eosin (H&E) sections. The pathologic assessment of SLN should be primarily aimed at identification of macrometastatic and micrometastatic tumor deposits because the detection of ITCs is still a question of experimental studies. Macrometastases are usually easily detected by routine histopathological evaluation, and serial sectioning is used to increase the sensitivity for the eventual detection of micrometastases and ITCs. However, there is no standard protocol today on pathologic assessment of SLN and the interval of sections varies widely. The identification of most micrometastases requires serial sectioning of the whole SLN at thicknesses of 200–250 µm [43]. Minimal requirements defined by cutting levels between 0.5 [44] and 1.0 mm [45] were established in axillary SLN of breast cancer patients to avoid excessive laboratory workload. Immunohistochemistry (pan-cytokeratin staining) increases the sensitivity of identification of tumor deposits in SLN, but this sensitivity mostly falls into the category of ITCs because macrometastases and micrometastases are usually found in H&E sections [46,47]. Therefore, the use of immunohistochemistry is questionable and, if it is applied, it is often restricted to cases that are difficult to interpret from H&E staining or to experimental studies.

The intraoperative assessment of SLN potentially modifies the surgical procedure and the subsequent treatment scheme. FSs are used in this indication. Despite the obvious benefits of

FS for the patient (especially reduction of extent and number of operations), this technique has some serious limitations. The intraoperative serial cutting of the entire SLN is not applicable because of the prolongation of operating time, technical limitations of processing of frozen material and loss of tissue for postoperative evaluation. Usually, small SLNs are processed in one piece, whereas larger ones are bisected. The number of levels taken from each tissue block is not standardized, and varies between departments. This algorithm of tissue sectioning in FS, therefore, enables one to primarily reveal macrometastatic tumor deposits. However, it leads to reduced sensitivity in detecting micrometastases and results in false negatives [48,49].

Postoperative care: follow-up

Early postoperative care differs among centers and it is dependent on the surgical procedure, especially trachelectomy. Perioperative morbidity and early postoperative morbidity of fertility-sparing surgery are less than, or similar to, abdominal radical hysterectomy or laparoscopic radical hysterectomy, with an incidence of 5–14% [50–52]. In contrast to perioperative and early postoperative morbidity, late morbidity and quality of life (QoL) after fertility-sparing surgery in cervical cancer have only been evaluated in a few papers. Some late complications (deep dyspareunia, excessive vaginal discharge and upper thigh paresthesia) are more often reported in the trachelectomy group than in the control group after radical hysterectomy [53]. Specific problems, such as dysmenorrhea, cervical stenosis (external os or isthmic stenosis), irregular menstruation, recurrent candidiasis and cervical cerclage suture problems cohere with retained uterus. These complications are mentioned in 10–20% of patients [20,53], and correspond with subsequent infertility. These complications are related to surgical technique [28,54–56].

Table 4. Comparison of abdominal and vaginal radical trachelectomies.

Parameter	VRT	ART	p-value
Planned (%)	683	207	
Fertilities spared (%)	618/683 (90.5)	174/207 (84.1)	NS
N1 (%)	29/648 (4.5)	32/207 (15.5)	0.001
LVSI (%)	184/611 (30.1)	59/197 (29.9)	NS
Age (range) (years)	31 (20–44)	31 (22–44)	NS
Nuliparous women	320/395 (81%)	70/83 (84.3%)	NS
Histology			
• SCC (%)	412 (63)	141 (74.6)	0.0041
• AC (%)	224 (34.3)	43 (22.8)	0.0037
• Other (%)	18 (2.7)	5 (2.6)	NS
Recurrences			
• <2 cm (%)	12/409 (2.9)	2/126 (1.6)	NS
• >2 cm (%)	11/53 (20.8)	6/33 (18.2)	NS
Recurrences (%)	29/618 (4.7%)	8/174 (4.6%)	NS

AC: Adenocarcinoma; ART: Abdominal radical trachelectomy; LVSI: Lymphovascular space involvement; N1: Positive lymph node; NS: Not significant; SCC: Squamous cell cancer; VRT: Vaginal radical trachelectomy.

Protocols of follow-up vary only slightly among centers. Some centers prefer the use of contraceptive pills for the first 6 months of follow-up [22]. During the first 2 years, the follow-up interval is usually between 3 and 4 months, which then increases to 6 months. Basic follow-up examination includes colposcopy and Pap smear. Cervical stenosis can limit the quality of the Pap smear from the endocervical canal and it is necessary to consider the option of dilatation. Following trachelectomy, the Pap smear should be performed by an expert because clumps of endocervical and endometrial cells are present [57,58]. Many centers use human papillomavirus high-risk (HPVHR) testing to increase the safety of cytology [28,42,58]. In some centers, gynecological clinical examination is completed by ultrasonography and MRI every 6–12 months [22,28]. Nowadays, PET-CT is not used in follow-up.

The patients must be informed of follow-up and timing of pregnancy. Some centers do not consider it necessary to have any interval between surgery and pregnancy [8,28], whereas others recommend an interval of 6–12 months [6,7,23,52]. A 2-year interval between surgery and pregnancy has been recommended, but this extreme has no rationale [59]. The infertility rate in women who have had fertility-sparing surgery is higher than in the same age cohort in the normal population. The cause of infertility is attributed to cervical factors (stenosis of cervical canal and reduced mucus production). Noyes *et al.* summarize questions of infertility in a recent clinical commentary [56]. Differences in infertility exist among different procedures, with the highest proportion of infertility coming from techniques with more radical resection of parametrial tissue. The subsequent pregnancy is considered to be a high-risk pregnancy with numerous precautions which differ among centers [20–23,28]. The rate of first-trimester loss is similar to the normal population, whereas the risk of second-trimester abortion doubles compared with the normal population [28,29,60–63]. Preterm delivery is an important issue following fertility-sparing surgery. Shortness of the cervix causes a reduction of cervical mucus. This reduction of mucus can result in ascending infection, which may cause premature rupture of the membrane followed by premature delivery. Ascending infection is the determining factor in premature delivery and plays a more important role than lack of mechanical support. Some authors recommend prophylactic antibiotic therapy (vaginally and systematically) in the 16th, 20th and 24th week of pregnancy to prevent ascending infection, and recommend to abandon intercourse between the 16th and 30th week of pregnancy [15].

Vaginal radical trachelectomy

'Dargent operation'

Vaginal radical trachelectomy was developed by Daniel Dargent in the late 1980s as a modification of the Schauta–Stockel method of radical vaginal hysterectomy. Growing progress in laparoscopic surgery

Table 5. Abdominal radical trachelectomy: pregnancy outcome.

Study	Patients (n)	Pregnant women (n)(%)	Conceptions	First-trimester loss	Second-trimester loss	Delivery			Ongoing pregnancy	Ref.
						Before 32nd week	*32–36th week*	*Term*		
Abu Rustum *et al.*	15	2 (13.3)	2	1	0	0	0	0	1	[52]
Pajera *et al.*	14	3 (21.4)	3	0	0	1	0	2	0	[68]
Duska *et al.*	10	2 (20)	4	1	0	0	1	1	1	[69]
Nishio *et al.*	57	4 (7)	4	0	0	2	0	2	0	[73]
Cibula *et al.*	17†	6 (35.3)	6	1	0	2	0	3	0	[70]
Ungar *et al.*	81	13 (16)	13	4	0	0	1	5	3	[72]
Kim *et al.*	27‡	3 (11.1)	3	2	0	0	1	0	0	[10]
Total	221	33 (14.9)	35	7 (20.0%)	0	5 (14.3%)	3 (8.6%)	13 (37.1%)	5 (14.3%)	

†Three of these were performed completely laparoscopically.
‡All performed laparoscopically.

enables us to perform laparoscopic pelvic lymphadenectomy and eventually paracervical lymph node dissection, with or without SLN identification. The extent of laparoscopic surgery varies from school to school. The second phase of the procedure (vaginal phase) requires experience in vaginal surgery. The vaginal phase starts with resection of the vaginal cuff and opening of the paravesical and pararectal spaces. Vaginally identifying and mobilizing the ureter is difficult, but is necessary for the safe resection of the parametria [5–7]. Some centers use ureteral catheterization before surgery in order to more easily identify and palpate the ureter [37]. Radicality of the resection of the parametria is limited by the goal of preserving the uterine artery and only ligating the vaginal branch of this artery. The cervix should be transacted approximately 1 cm above the endocervical tumor margin and a maximum of 1 cm caudally from the internal os. Most centers perform prophylactic cerclage with a nonresorbable stitch, and suture the vaginal mucosa to the residual exocervical stroma. Finally, the vaginal mucosa is reapproximated to the new exocervix using interrupted sutures.

Characteristics & oncological outcome

Published oncologic results on VRT are summarized in Table 1. In total, 683 women were included in the analysis and 90.5% of those women for whom VRT was planned had their fertility preserved. Positivity of lymph nodes was near to 4.5% of these cases. These findings provide evidence regarding good selection of suitable patients for VRT. When positive pelvic or parametrial nodes are diagnosed perioperatively, either the VRT should be aborted and the patient should undergo chemoradiotherapy instead [41,63,64] or surgery should be radicalized [15]. In clinical practice the number of micrometastases (<2 mm) or ITCs, that were not diagnosed by FS increased because of better histopathological elaboration of SLNs. Today, the management of these patients is extensively discussed. Lyon's group published a report of three women with micrometastases in SLN who refused the recommended adjuvant radiotherapy and no recurrence was diagnosed [41]. Another four women with micrometastasis in lymph nodes underwent chemotherapy, which allows the possibility of future pregnancy. All four of these women were without evidence of disease [42]. The second cause of fertility loss was the clearance of normal cervical tissue beyond the tumor. If perioperative FS or permanent section confirms tumor involvement, resection margin usually leads to some form of immediate radical surgery. The question of how much endocervical margin suffices as adequate therapy is still under debate; however, 5 mm is considered the minimum margin [15,20,36,65]. The recommendation of some centers is that 1 cm of healthy tissue remains [22,66,67].

In 11.5% of cases, VRT was performed in tumors larger than 2 cm. A comparison of recurrences in tumors less than 2 cm (409 women; 12 recurrences = 2.9%) with recurrences in tumors larger than 2 cm (53 women; 11 recurrences = 20.8%) shows that VRT is a risky procedure for tumors larger than 2 cm. In women who received VRT, the recurrence percentage was only 4.7% and the mortality was 3.0%. These results are comparable with women with the same risk factors after radical hysterectomy. It was not possible to analyze data in patients in whom fertility was not spared (9.5%), and VRT was aborted because the majority of centers did not mention the oncological outcome of these patients.

Pregnancy outcome

Table 2 summarizes the pregnancy outcomes following VRT in 621 women. A strong desire to preserve fertility is a strict precondition for fertility-preserving surgery in all centers and, therefore, objective criteria of pregnancy outcome are the number of pregnant women and the number of deliveries. Pregnancy in 30% of the women after VRT can be regarded as very good, especially considering that the average age of the women was 31 years [20,61,65]. It is feasible that actualization of the results would improve the pregnancy results. From 300 pregnancies, there were 186 deliveries (62%) and 190 babies. There were 68 (22.7%) first-trimester losses (spontaneous abortions, therapeutic abortions and extrauterine pregnancies), which is similar to that

Table 6. Less radical simple trachelectomy/cone: characteristics and oncological outcome.

Parameter	Rob (2007/2008) [15,28]
Period	1999–2006
Planned ST	40
Fertility spared (%)	32 (80%)
N1 (%)	6/40 (15%)
Average age (range/years)	28.3 (24–35)
Nulliparous women (%)	25/40 (62.5%)
Histology	
• SCC (%)	32 (80)
• AC (%)	7 (17.5)
• Other (%)	1 (2.5)
Stage	
• IA1 + LVSI (%)	3 (7.5)
• IA2 (%)	10 (25)
• IB1 (%)	27 (67.5)
LVSI (%)	17/40 (42.5)
Recurrences (%)	1/32 (3.1)
Death	0

AC: Adenocarcinoma; LVSI: Lymphovascular space invasion; SCC: Squamous cell cancer; ST: Simple trachelectomy/cone.

of the normal population of the same age group. Second-trimester loss was twice as high compared with the normal population (9.7% or 29 cases). The cause of second-trimester loss is the same as in premature delivery (i.e., ascending infection and premature rupture of membranes [PROMs] as a result of shortening of the cervix and lack of mucus [20,22,37,61,64,65].

Abdominal radical trachelectomy

Abdominal radical trachelectomy is a modification of laparotomic abdominal radical hysterectomy and does not need any special surgical training or instruments and, for this reason, this approach is preferred in some centers. Pelvic lymphadenectomy, with or without SLN identification, is the first step in this procedure [52,54,59,68–73]. Radicality of cervical and parametrial extirpation should be determined on an individual basis as in radical hysterectomy type B, or type C with or without nerve-sparing surgery. 'Classical' ART provides standard radical resection of the parametria with complete resection of the uterine vessels at their origin. Modifications of ART with preservation of the uterine artery have been described elsewhere. Briefly, modified ART involves skeletization of the uterine artery and ligation of the descending branch, followed by tubal reanastomosis of the uterine artery [74]. Suture of the uterus and vagina together, and formation of the neocervix, including catheterization of the neocervix, vary in different clinics. Some authors prefer permanent cervical cerclage [52,69,73], whereas others perform cervical cerclage during pregnancy [68]. However, several authors question the importance of prophylactic cerclage and catheterization of the neocervix and, consequently, do not perform these procedures [54,59].

Published oncologic results on ART are summarized in Table 3. ART was planned in 207 women and fertility was spared in 174 (84%). Positive lymph nodes were detected in 15.5% of the women and LVSI in 30%. Average age was 31.6 years and 84% of the women were nulliparous. In 20.8% of the women, ART was performed in tumors larger than 2 cm. The oncological outcome was very good, with only eight recurrences (4.6%) published. Some papers subdivided the tumors into two groups: tumors less than 2 cm and those larger than 2 cm. Recurrences differed in these groups (126 women: two recurrences = 1.6% in the tumor <2-cm group vs 33 women: six recurrences = 18.2% in the tumor >2-cm group). Thus, it seems that ART, similar to VRT, is a hazardous procedure in node-negative women with tumors larger than 2 cm in the sense that the oncological results are no better.

Table 4 presents a comparison between ART and VRT. There were more women with positive lymph nodes and more squamous cell cancers in the ART group. Differences in oncological results between ART and VRT (recurrence of tumors <2 cm or >2 cm) were not statistically significant.

Table 5 summarizes the pregnancy outcomes following ART in 221 women. In total, 30 laparoscopic ART and 191 ART were included in the analysis. In addition, updated results from Ungar, Smith and Del Priore were included [72]. Of 221 women in whom fertility was preserved there were 35 pregnancies in 33 women (14.9%). However, all centers included only those women with a strong desire to preserve fertility. Percentage of nulliparous women (84%) and average age (31.6 years) in the ART group are comparable with other fertility-sparing groups. From this point of view, the number of pregnant women was dramatically lower than in other types of fertility-sparing surgery.

Simple trachelectomy or large cone biopsy (less radical)

The extension of the parametrectomy differs in various fertility-sparing procedures. The fundamental question is: how many women after radical trachelectomy with a tumor less than or equal to 2 cm in largest dimension and with negative SLNs and pelvic nodes had positive findings in the parametrium? Retrospective studies of parametrial involvement in small tumors with infiltration of less than 10 mm or less than half of the stroma, and which had not spread to the pelvic lymph nodes, support the use of less radical surgery without resection of paracervical tissue [75–79]. The minimal risk of parametrial involvement in cases of negative SLNs was confirmed by the first prospective study [80]. Good timing of injection of Tc radiocolloid and blue dye allow the monitoring of blue lymphatic channels, which usually go through the parametrium without any interruption into the regional SLN. SLNs are identified very rarely (3–5%) in the medial part of the lateral parametrium. A less radical fertility-sparing surgery protocol was first published by Rob [15]. Only women with tumors smaller than 2 cm, infiltration less than half of cervical stroma and with negative SLNs on FSs were included into the protocol. The first step of this procedure is laparoscopic SLN identification and FSs of SLNs, extirpation of parametrial blue channels and, eventually,

extirpation of blue lymph nodes in the medial part of the lateral parametrium. Pelvic lymphadenectomy is applied in FS-negative sentinel nodes. If the FSs were found to be positive, laparoscopy was terminated and laparotomic radical hysterectomy with low para-aortic lymphadenectomy was performed. If nodes are negative, the remaining stages of the procedure are performed in 7-day intervals: reconization (stage IA1 with LVSI and stage IA2 tumors) or simple trachelectomy (stage IB1 tumors <2 cm). The pioneers of this operation chose not to place cerclage or intracervical catheters. Two-step management facilitated by ultramicrostaging of SLNs increases the safety of conservative 'simple trachelectomy' procedures [15,28].

Published oncologic results of the less radical treatment simple trachelectomy are summarized in Table 6. Simple trachelectomy was performed on 40 women and fertility was spared in 32 (80%). Positive lymph nodes were detected in six patients (15.0%), LVSI was detected in 42.5%, and all tumors were less than 2 cm. The average age of these women was 28.3 years and 62.5% of the women were nulliparous. These risks are similar to those with VRT. The oncological outcome was very good. One recurrence was diagnosed in the isthmic part, but the women are still alive without evidence of disease more than 5 years after treatment. Of the 32 woman with spared fertility, 17 (53.1%) became pregnant. A total of 23 pregnancies were noted: five pregnancies had first-trimester loss (three women), three had second-trimester loss and 12 had deliveries (52.2%). In addition, there were three ongoing pregnancies. Prophylactic cerclage was not performed in any of the women. The oncological and pregnancy results of large cone or simple vaginal trachelectomy combined with laparoscopic pelvic lymphadenectomy will be verified in an international multicentric study.

Another less radical approach for preserving fertility is 'chemoconization', which was first described by Landoni [16]. The first step in this approach was laparoscopic pelvic lymphadenectomy and, in cases of negative nodes and tumors less than 2 cm, 'deep' laser conization was performed. When negative prognostic factors were present (LVSI, free margin <3 mm or deep stromal infiltration >10 mm), adjuvant chemotherapy (paclitaxel 175 mg/m^2, ifosfamide 5 g/m^2 plus cisplatin 75 mg/m^2 [TIP] or paclitaxel 175 mg/m^2, epirubicine 80 mg/m^2 plus cisplatin 75 mg/m^2 [TEP] regimen) was performed (Table 7). Three cycles of NAC were administered to patients with node-negative tumors between 2 and 3 cm. Deep-laser conization was performed after

Table 7. Neoadjuvant chemotherapy and fertility sparing surgery: characteristic and oncological outcome.

Parameter	Robova/Rob [18,28]	Plante [17]	Maneo [19]	Total[§]
Year	2007/2008	2006	2008	
Period	2005–2007	2004–2005	1995–2007	
Chemotherapy	IP: cisplatin 75 mg/m^2[‡] ifosfamid 2 g/m^2 every 10 days or AP: cisplatin 75 mg/m^2[‡] doxorubicin 35 mg/m^2 every 10 days	TIP: paclitaxel 175 mg/m^2[‡] cisplatin 75 mg/m^2[‡] ifosfamide 5 g/m^2 or TEP: paclitaxel 175 mg/m^2[‡] cisplatin 75 mg/m^2[‡] epirubicin 80 mg/m^2 every 21 days	TIP: paclitaxel 175 mg/m^2[‡] cisplatin 75 mg/m^2[‡] ifosfamide 5g/m^2 or TEP: paclitaxel 175 mg/m^2[‡] cisplatin 75 mg/m^2[‡] epirubicin 80 mg/m^2 every 21 days	
Planned surgery	9	3	21	33
Fertility spared	7	3	16	26 (78.8%)
N1 (%)	0/9 (0)	0/3 (0)	2/21 (9.5)	2/33 (3)
Average age (years) (range)	29.3 (24–33)	32.3 (26–36)	30 (17–39)	30.5 (17–39)
Nulliparous women (%)	8/9 (89)	2/3 (67)	NA	10/12[‡] (83.3)
Histology				
• SCC (%)	7 (78)	3 (100)	9 (43)	19 (57.6)
• AC (%)	2 (22)	0	12 (57)	14 (42.4)
• Others	0	0	0	0
Size				
• <2 cm (%)	0	0	9 (43)	9 (27.3)
• >2 cm (%)	9 (100)	3 (100)	12 (57)	24 (72.7)
LVSI	9/9 (100)	NA	1/21 (5%)	NA
Recurrences	1/7[†]	0/3	0/16	26/1 (3.8%)

[†]Unpublished case.
[‡]Only numbers that are available.
AC: Adenocarcinoma; LVSI: Lymphovascular space invasion; N1: Positive lymph node; SCC: Squamous cell cancer.

Table 8. Neoadjuvant chemotherapy and fertility-sparing surgery: pregnancy outcome.

Study	Patients (n)	Pregnant women (n) (%)	Conceptions	First-trimester loss	Second-trimester loss	Delivery			Ongoing pregnancy	Ref.
						Before 32nd week	*32–36th week*	*Term*		
Robova/Rob *et al.*	7	5 (71.4)	5	0	0	1	0	3	1	[18,28]
Plante *et al.*	3	2 (66.7)	3	0	0	0	1	2	NA	[17]
Maneo *et al.*	16	6 (37.5)	10	1	0	2	0	7	NA	[19]
Total	26	13 (50)	18	1	0	3	1	12	NA	

chemotherapy. Of 11 women (eight stage IB1 and three stage IA2), the mean age was 32 years and ten were nulliparous. Of these 11 women, three patients received NAC and one patient received adjuvant chemotherapy. No recurrence (7–29 months) was observed in this group, and three babies were delivered.

Neoadjuvant chemotherapy & fertility-sparing surgery

Some centers use NAC in bulky cervical cancer, with the aim of downstaging the disease before radical hysterectomy [81,82]. One of the limitations of fertility-preserving surgery is deep stromal invasion and tumors larger than 2 cm. The idea underlying NAC is to reduce the size of the cervical tumor in order to preserve fertility. Currently, three papers with three approaches have been published on NAC before fertility-sparing surgery. The characteristics and oncological outcome are summarized in Table 7. The pioneering work on NAC and fertility-sparing surgery was presented by the Maneo group at the International Gynecologic Cancer Society (IGCS) meeting in 2004. In 2008, the group published updated results [19]. A total of 21 women with tumors 10–30 mm were included. Fertility was preserved in 16 of these women,. The chemotherapy consisted of three courses of TIP or TEP every third week (Table 7). After three courses of chemotherapy, all 16 patients underwent cold-knife cervical conization and complete pelvic lymphadenectomy. Cervical specimens were evaluated by FS. In the case of massive neoplastic persistence a radical hysterectomy was performed. No patients progressed on chemotherapy and no recurrence was observed; however, three women had a precancerous lesion at follow-up. Fertility results are given in Table 8.

In a case report, Plante reported on three women with NAC [17]. Only tumors between 3 and 4 cm were included, and chemotherapy was the same as in the Maneo study. Surgery consisted of laparoscopic SLNM, pelvic lymphadenectomy and VRT. Complete histopathological remission was achieved in all cases. In August 2009, all three patients were without evidence of disease. Furthermore, one patient delivered one baby and one patient delivered two (Table 8).

The third approach is the Prague LAP-III protocol with high-dose-density chemotherapy [18,28]. Only tumors that were larger than 2 cm, but that had not infiltrated more than two-thirds of the stroma in the MRI volumetry, were included. A total of three cycles of high-dose-density NAC were used (Table 7). Fertility was preserved in seven out of nine women. Complete remission or minimal residuum of less than 2 mm was diagnosed in seven women. One recurrence in the ovary was diagnosed 6 weeks after spontaneous delivery. This woman died of hematogenous dissemination of the tumor [Rob, unpublished data]. It is the first known recurrence and death after NAC and fertility-preserving surgery. Fertility results are given in Table 8.

Table 9. Statistics: pregnancy outcome.

	Method	Women	Pregnancies (%)	Odds ratio	95% CI	p-value	χ^2	p-value
Pregnant women	VRT	483	146 (30.2)	Ref	Ref	Ref	32.6	$p < 0.0001$
	ART	221	33 (14.9)	0.4	0.3–0.7	<0.0001		
	ST	32	17 (53.1)	2.6	1.3–5.4	0.010		
	NAC	26	13 (50.0)	2.3	1.0–5.1	0.049		
Pregnancies	VRT	621	300 (48.3)	Ref	Ref	Ref	81.0	$p < 0.0001$
	ART	221	35 (15.8)	0.2	0.1–0.3	<0.0001		
	ST	32	23 (71.9)	2.5	1.3–5.0	0.011		
	NAC	26	18 (69.2)	2.5	1.0–5.0	0.045		
Deliveries	VRT	621	186 (30.0)	Ref	Ref	Ref	47.9	$p < 0.0001$
	ART	221	21 (9.5)	0.2	0.2–0.4	<0.0001		
	ST	32	12 (37.5)	1.4	0.7–3.3	0.430		
	NAC	26	16 (61.5)	3.3	1.6–10	0.002		

ART: Abdominal radical trachelectomy; NAC: Neoadjuvant chemotherapy; Ref: Referral; ST: Simple trachelectomy or cone; VRT: Vaginal radical trachelectomy.

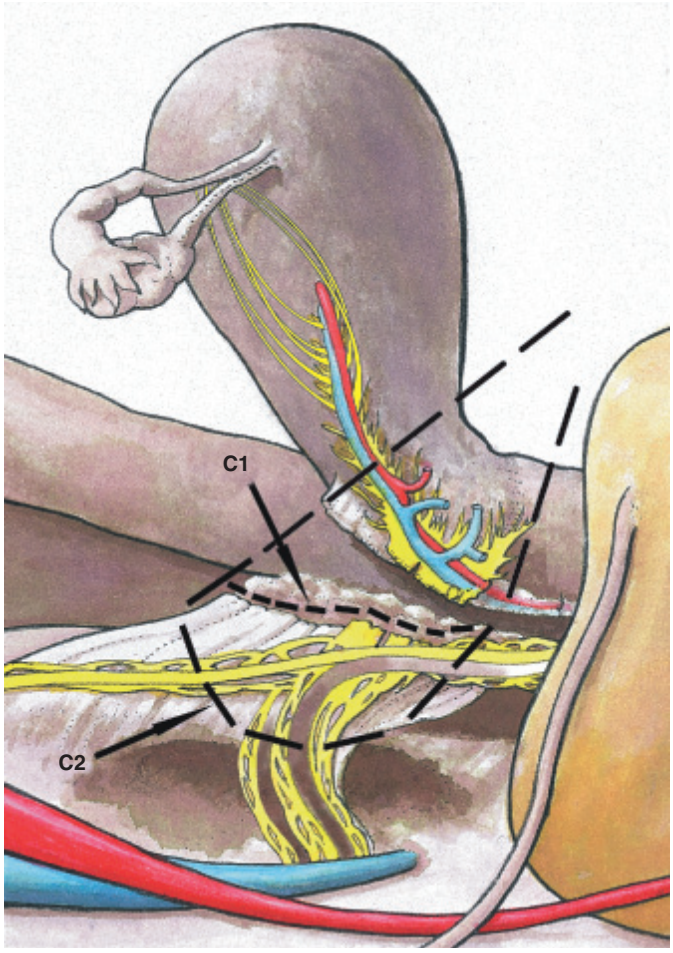


Figure 1. Disruption of innervation of the uterus and paracervix during radical trachelectomy C1 and C2.

Comparison between fertility & delivery in fertility-sparing procedures

Table 9 summarizes pregnancy results after VRT, ART, simple trachelectomy and NAC. VRT with laparoscopic pelvic lymphadenectomy was used as a standard in fertility-preserving procedures. We evaluated the number of pregnant women, number of pregnancies and number of deliveries in women in whom fertility was spared. When all three methods are compared, the pregnancy results are statistically significant in all parameters studied. Both less radical procedures (simple trachelectomy or cone biopsy with or without NAC) produced significantly better results. The different pregnancy results must be discussed in the context of the surgical procedure because average age and duration of follow-up are not statistically different between groups (in the NAC group the follow-up period is even shorter). One factor that can further influence fertility is a higher risk of adhesions after an open abdominal procedure compared with laparoscopic procedures. Another factor that increases the risk for adverse pregnancy is shortening of the cervix, which leads to reduction of cervical mucus. Finally, chronic irritation that is caused by a permanent cervical catheter can lead to cervical stenosis. Insertion of an intracervical catheter for 3 weeks is highly controversial because it can damage the cylindrical epithelium of the rest of the cervix. Risk of stenosis can be minimized by technical modification of the trachelectomy procedure [15]. Abandoning cerclage and insertion of an intracervical catheter seems to be a solution [28,54]. The above-mentioned factors alone do not explain such big differences. The basic differences between the various techniques are the extent of resection of the parametria and the extent of disruption of the pelvic autonomic innervation, inferior hypogastric plexus [83]. That the larger resection of the paracervix implies greater disruption of the uterus end tube innervation is depicted schematically in Figure 1. Radicality in the paracervix decreases the chance for spontaneous pregnancy and potentially increases the need for medically assisted reproduction methods.

Expert commentary

The incorporation of fertility-sparing surgery for cervical cancer in young women is one of the most important breakthroughs in oncogynecology in the past 20 years. Over the years, this procedure has become increasingly accepted. However, the question of oncologic safety has not been definitively resolved. Fertility-sparing procedures (e.g., VRT and ART) in tumors less than 2 cm in diameter and with invasion of less than half of the stromal are now considered to be safe surgical procedures. We still need to develop approaches for tumors bigger than 2 cm in diameter, but NAC now seems to be the most promising approach. Oncological safety of less radical surgeries (deep cone or simple trachelectomy) still needs to be confirmed in multicenter studies. Preliminary results in patients with negative SLNs and other pelvic nodes in tumors less than 2 cm are promising and comparable with the results of VRT and ART. SLNM is a procedure that increases the safety of fertility-sparing surgery. The second aspect of fertility-preserving surgery is pregnancy outcome. To minimize the rate of premature delivery the preservation of at least 1 cm of residual cervical tissue is mandatory. Pregnancy results (number of pregnant women, number of pregnancies and number of deliveries) can differ statistically significantly with different methods. ART proved worse than all the other procedures in all parameters. Significantly better results were observed with less radical procedures. Research groups are still looking for an optimal radicality and precautions to minimize risk of premature labor.

Five-year view

Within the next 5 years, data regarding complete laparoscopic or robotic trachelectomy will be analyzed and long-term data (pregnancy and oncological outcome) in ART and VRT from large centers will be actualized. The authors hope that, within the next few years, it might be possible to use less aggressive surgical treatment than radical trachelectomy for women with low-risk early cervical cancer (squamous or adenocarcinoma <2 cm in diameter and <10 mm invasion). Less radical surgery will involve the reduction of paracervical resection in order that damage to the autonomous nerves, including branches of the inferior hypogastric plexus, will be minimized. Identification and extirpation of

SLNs from the paracervix and pelvis will become an important component of laparoscopic or robotic fertility-sparing surgery. Lymphadenectomy will be restricted to extirpation of SLNs in tumors without angioinvasion. The use of prophylactic cerclage will be re-evaluated. It will not be necessary to use neocervical catheters because the new technique of trachelectomy suture will be used. The group of women that will profit most from NAC can be exactly defined.

Financial & competing interests disclosure

This work was supported by grant NS 9914-4. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Vaginal radical trachelectomy (VRT) and abdominal radical trachelectomy (ART) are safe and feasible procedures in women with early cervical carcinoma (less than 2 cm in diameter) that wish to preserve fertility.
- VRT and ART are risky procedures for tumors larger than 2 cm.
- Precise management of fertility-sparing surgery must include a good selection of women with a strong desire to preserve fertility.
- Sentinel lymph node mapping increases safety in fertility-sparing surgery. In some cases it is appropriate to perform frozen-section to evaluate the tumor-free status of the resection margin.
- After ART, the pregnancy rate was dramatically lower than that following other types of fertility-sparing surgery.
- Less radical procedures such as simple trachelectomy or cone with or without chemotherapy, showed significantly better pregnancy results than ART and VRT.
- Larger resection of the paracervix implies greater disruption of the uterus end-tube innervation. With bigger resection of the paracervical tissue, the probability of spontaneous pregnancy decreases and the necessity to use methods of assisted reproduction increases.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Arbyn M, Autier P, Ferlay J *et al*. Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann. Oncol.* 18(8), 1423–1425 (2007).

2 Sonoda Y, Abu-Rustum NR, Gemignani ML *et al*. A fertility sparing alternative to radical hysterectomy: how many patients may be eligible? *Gynecol. Oncol.* 95(3), 534–538 (2004).

3 Watson M, Saraiya M, Benard V *et al*. Burden of cervical cancer in the United States, 1998–2003. *Cancer* 113(Suppl. 10), 2855–2864 (2008).

4 Dargent D, Burn JL, Roy M, Remi I. Pregnancies following radical trachelectomy for invasive cervical cancer. *Gynecol. Oncol*. 52, 105 (1994) (Abstract 14).

5 Schneider A, Krause N, Kuhne-Heid R, Noschel H. Preserving fertility in early cervix carcinoma: trachelectomy with laparoscopic lymphadenectomy. *Zentralbl Gynaekol.* 118(1), 6–8 (1996).

6 Roy M, Plante M. Pregnancies after radical vaginal trachelectomy for early-stage cervical cancer. *Am. J. Obstet. Gynecol.* 179(6), 1491–1496 (1998).

7 Shepherd JH, Crawford RA, Oram DH. Radical trachelectomy: a way to preserve fertility in the treatment of early cervical cancer. *Br. J. Obstet. Gynaecol.* 105(8), 912–916 (1998).

8 Covens A, Shaw P, Murphy J *et al*. Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA–B carcinoma of the cervix? *Cancer* 86(11), 2273–2279 (1999).

9 Smith JR, Boyle DC, Corless DJ *et al*. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. *Br. J. Obstet. Gynaecol.* 104(10), 1196–1200 (1997).

10 Kim JH, Park JY, Kim DY *et al*. Fertility-sparing laparoscopic radical trachelectomy for young women with early stage cervical cancer. *Br. J. Obstet. Gynaecol.* 117(3), 340–347 (2010).

11 Martin A, Torrent A. Laparoscopic nerve-sparing radical trachelectomy: surgical technique and outcome. *J. Minim. Invasive Gynecol.* 17(1), 37–41 (2010).

12 Cibula D, Ungar L, Palfalvi L *et al*. Laparoscopic abdominal radical trachelectomy. *Gynecol. Oncol*. 97(2), 707–709 (2005).

13 Ramirez PT, Schmeler KM, Malpiga A, Soliman PT. Safety and feasibility of robotic radical trachelectomy in patients with early-stage cervical cancer. *Gynecol. Oncol*. 116(3), 512–515 (2010).

14 Persson J, Kannisto P, Bossmar T. Robot-assisted abdominal laparoscopic radical trachelectomy. *Gynecol. Oncol.* 111(3), 564–567 (2008).

15 Rob L, Charvát M, Robová H *et al*. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. *Int. J. Gynecol. Cancer* 17(1), 304–310 (2007).

16 Landoni F, Parma G, Peiretti M *et al*. Chemo-conization in early cervical cancer. *Gynecol. Oncol.* 107, S125–S126 (2007).

17 Plante M, Lau S, Brydon L *et al*. Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage 1B1 cervical cancer: case report. *Gynecol. Oncol.* 101(2), 367–370 (2006).

18 Robova H, Pluta M, Hrehorcak M *et al*. High dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. *Int. J. Gynecol. Cancer.* 18(6), 1367–1371 (2008).

19 Maneo A, Chiari S, Bonazzi C, Mangioni C. Neoadjuvant chemotherapy and conservative surgery for stage 1B1 cervical cancer. *Gynecol. Oncol.* 111(3), 438–443 (2008).

20 Plante M. Vaginal radical trachelectomy: an update. *Gynecol. Oncol.* 111, S105–S110 (2008).

21 Carter J, Sonoda Y, Chi DS *et al*. Radical trachelectomy for cervical cancer: Postoperative physical and emotional adjustment concerns. *Gynecol. Oncol.* 111(1), 151–157 (2008).

22 Shepherd JH, Spencer C, Herod J *et al*. A radical vaginal trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer cumulative pregnancy rate in a series of 123 women. *Br. J. Obstet. Gynaecol.* 113(6), 719–724 (2006).

23 Mathevet P, Laszlo de Kaszon E, Dargent D. Fertility preservation in early cervical cancer. *Gynecol. Obstet. Fertil.* 31(9), 706–712 (2003).

24 Zivanovic O, Leitao MM, Park JK *et al*. Small cell neuroendocrine carcinoma of the cervix: analysis of outcome, recurrence pattern and the impact of platinum-based combination chemotherapy. *Gynecol. Oncol.* 112(3), 590–593 (2009).

25 Singh N, Arif S. Histopathologic parameters of prognosis in cervical cancer – a review. *Int. J. Gynecol. Cancer* 14(5), 741–750 (2004).

26 Peppercorn PD, Jeyrajah AR, Woolas R *et al*. Role of MR imaging in the selection of patients with early cervical carcinoma for fertility-preserving surgery: initial experience. *Radiology* 212(2), 395–399 (1999).

27 Sahdev A, Sohaib SA, Wenaden AE *et al*. The performance of magnetic resonance imaging in early cervical carcinoma: a long-term experience. *Int. J. Gynecol. Cancer* 17(3), 629–636 (2007).

28 Rob L, Pluta M, Strnad P *et al*. A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. *Gynecol. Oncol.* 111, S116–S120 (2008).

- **Largest series of less radical treatment (simple cone or simple trachelectomy) with sentinel lymph nodes (SLNs) and laparoscopic lymphadenectomy.**

29 Milliken D, Shepherd JH. Fertility preserving surgery for carcinoma of the cervix. *Curr. Opin. Oncol.* 20(5), 575–580 (2008).

30 De Souza NM, Dina R, McIndoe A *et al*. Cervical cancer: value of an endovaginal coil magnetic resonance imaging technique in detecting small volume disease and assessing parametrial extension. *Gynecol. Oncol.* 102(1), 80–85 (2006).

31 Wright JD, Dehdashti F, Herzog TJ *et al*. Preoperative lymph node staging of early-stage cervical carcinoma by /^{18}F/-fluoro-2-deoxy-D-glucose-positron emission tomography. *Cancer* 104(11), 2484–2491 (2005).

32 Rockall AG, Sohaib SA, Harisinghani NG *et al*. Diagnostic performance of nano particle-enhanced MRI in the diagnosis of lymph node metastasis in patients with endometrial and cervical cancer. *J. Clin. Oncol.* 23(12), 2813–2821 (2005).

33 Rob L, Strnad P, Robova H *et al*. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. *Gynecol. Oncol.* 98(2), 281–288 (2005).

34 Plante M, Renaud MC, Tetu B *et al*. Laparoscopic sentinel node mapping in early-stage cervical cancer. *Gynecol. Oncol*. 91(3), 494–503 (2003).

35 Marnitz S, Kohler CH, Bongardt S *et al*. Topographic distribution of sentinel lymph nodes in patients with cervical cancer. *Gynecol. Oncol*. 103(1), 35–44 (2006).

36 Chenevert J, Tetu B, Plante M *et al*. Indication and method of frozen section in vaginal radical trachelectomy. *Int. J. Gynecol. Pathol*. 28(5), 480–488 (2009).

37 Burnett AF, Roman LD, O'Meara AT *et al*. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. *Gynecol. Oncol.* 88(3), 419–423 (2003).

38 Sonoda Y, Chi DS, Carter J *et al*. Initial experience with Dargent's operation: the radical vaginal trachelectomy. *Gynecol. Oncol.* 108(2), 214–219 (2008).

39 Pahisa J, Alonso I, Torné A. Vaginal approaches to fertility-sparing surgery in invasive cervical cancer. *Gynecol. Oncol.* 110, S29–S32 (2008).

40 Chen Y, Xu H, Zhang Q *et al*. A fertility-preserving option in early cervical carcinoma: laparoscopy-assisted vaginal radical trachelectomy and pelvic lymphadenectomy. *Eur. J. Obstet. Gynecol. Rep. Biol.* 136(1), 90–93 (2008).

41 Marchiole P, Benchaib M, Buenerd A *et al*. Oncological safety of laparoscopic-assisted vaginal radical trachelectomy (LARVT or Dargent's operation): a comparative study with laparoscopic-assisted vaginal radical hysterectomy (LARVH). *Gynecol. Oncol.* 106(1), 132–141 (2007).

42 Hertel H, Köhler C, Grund D *et al*. Radical vaginal trachelectomy (RVT) combined with laparoscopic pelvic lymphadenectomy: prospective multicentre study of 100 patients with early cervical cancer. *Gynecol. Oncol.* 103(3), 506–511 (2006).

- **Largest multicenter study series of vaginal radical trachelectomy with 100 women.**

43 Cserni G. A model for determining the optimum histology of sentinel lymph nodes in breast cancer. *J. Clin. Pathol*. 57(5), 467–471 (2004).

44 Kuehn T, Bembenek A, Decker T *et al*. A concept for the clinical implementation of sentinel lymph node biopsy in patients with breast carcinoma with special regard to quality assurance. *Cancer* 103(3), 451–461 (2005).

45 Cserni G, Amendoeira I, Apostolikas N *et al*. Discrepancies in current practice of pathological evaluation of sentinel lymph nodes in breast cancer. Results of a questionnaire based survey by the European Working Group for Breast Screening Pathology. *J. Clin. Pathol.* 57(7), 695–701 (2004).

46 Moore RG, Granai CO, Gajewski W *et al*. Pathologic evaluation of inguinal sentinel lymph nodes in vulvar cancer patients: a comparison of immunohistochemical staining versus ultrastaging with hematoxylin and eosin staining. *Gynecol. Oncol.* 91(2), 378–382 (2003).

47 Euscher ED, Malpica A, Atkinson EN *et al*. Ultrastaging improves detection of metastases in sentinel lymph nodes of uterine cervix squamous cell carcinoma. *Am. J. Surg. Pathol.* 32(9), 1336–1343 (2008).

48 Pluta M, Rob L, Charvat M *et al*. Less radical surgery than radical hysterectomy in early stage cervical cancer: a pilot study. *Gynecol. Oncol.* 113(2), 181–184 (2009).

49 Fader AN, Edwards RP, Cost M *et al*. Sentinel lymph node biopsy in early-stage cervical cancer: utility of intraoperative versus postoperative assessment. *Gynecol. Oncol.* 111(1), 13–17 (2008).

50 Diaz JP, Sonoda Y, Leitao MM *et al*. Oncology outcome of fertility-sparing radical trachelectomy versus radical hysterectomy for stage IB1 cervical carcinoma. *Gynecol. Oncol.* 111(2), 255–260 (2008).

51 Beiner ME, Hauspy J, Rosen B *et al*. Radical vaginal trachelectomy vs radical hysterectomy for small early stage cervical cancer: a matched case–control study. *Gynecol. Oncol.* 110(2), 168–171 (2008).

52 Abu-Rustum NR, Neubauer N, Sonoda Y *et al*. Surgical and pathological outcomes of fertility-sparing radical abdominal trachelectomy for FIGO stage 1B1 cervical cancer. *Gynecol. Oncol.* 111(2), 261–264 (2008).

53 Alexander-Sefre F, Chee N, Spencer C *et al*. Surgical morbidity associated with radical trachelectomy and radical hysterectomy. *Gynecol. Oncol.* 101(3), 450–454 (2006).

54 Cibula D, Slama J, Fischerova D *et al*. Update on abdominal radical trachelectomy. *Gynecol. Oncol.* 111, S111–S115 (2008).

55 Selo-Ojeme DO, Ind T, Shepherd JH. Isthmic stenosis following radical trachelectomy. *J. Obstet. Gynaecol.* 22(3), 327–328 (2002).

56 Noyes N, Abu-Rustum NR, Ramirez PT *et al*. Options in the management of fertility-related issues after radical trachelectomy in patients with early cervical cancer. *Gynecol. Oncol.* 114(1), 117–120 (2009).

57 Feratovic R, Lewin SN, Sonoda Y *et al*. Cytologic findings after fertility-sparing radical trachelectomy. *Cancer* 114(1), 1–6 (2008).

58 Ghorab Z, Ismiil N, Covens A *et al*. Postradical vaginal trachelectomy follow-up by isthmic–vaginal smear cytology: a 13-year audit. *Diagn. Cytopathol.* 37(9), 641–646 (2009).

• **Summarized experience, emphasizing the role of isthmic–vaginal smear for early detection of central recurrence and highlighting the role of lower uterine segment endometrial cells and reactive changes as potential pitfalls.**

59 Ungar I, Palfalvi I, Hogg R *et al*. Abdominal radical trachelectomy: a fertility-preserving option for women with early cervical cancer. *Br. J. Obstet. Gynecol.* 112(3), 366–369 (2005).

60 Jolley JA, Battista L, Wing DA. Management of pregnancy after radical trachelectomy: case reports and systematic review of the literature. *Am. J. Perinatol.* 24(9), 531–539 (2007).

61 Plante M, Renaud MC, Hoskins IA *et al*. Vaginal radical trachelectomy: valuable fertility-preserving option in the management of early-stage cervical cancer. A series of 50 pregnancies and review of the literature. *Gynecol. Oncol.* 98(1), 3–10 (2005).

62 Petignat P, Stan C, Megevand E *et al*. Pregnancy after trachelectomy: a high-risk condition of preterm delivery. Report of a case and review of the literature. *Gynecol. Oncol.* 94(3), 575–577 (2004).

63 Plante M, Renaud MC, Harel F *et al*. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An update series of 72 cases and review of the literature. *Gynecol. Oncol.* 94(3), 614–623 (2004).

64 Shepherd JH, Milliken DA. Conservative surgery for carcinoma of the cervix. *Clin. Oncol.* 20(6), 395–400 (2008).

• **Largest series of vaginal radical trachelectomy, with 158 women.**

65 Beiner ME, Covens A. Surgery insight: radical vaginal trachelectomy as a method of fertility preservation for cervical cancer. *Nat. Clin. Pract. Oncol.* 4(6), 353–361 (2007).

66 Burnett AF. Radical trachelectomy with laparoscopic lymphadenectomy: review of oncologic and obstetrical outcomes. *Curr. Opin. Obstet. Gynecol.* 18(1), 8–13 (2006).

67 Schlaerth JB, Spirtos NM, Schlaerth AC. Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. *Am. J. Obstet. Gynecol.* 188(1), 29–34 (2003).

68 Pareja FR, Ramirez PT, Borrero FM *et al*. Abdominal radical trachelectomy for invasive cervical cancer. A case series and literature review. *Gynecol. Oncol.* 111(3), 555–560 (2008).

69 Olawaiye A, Del Carmen M, Tambouret R *et al*. Abdominal radical trachelectomy: success and pitfalls in a general gynecologic oncology practice. *Gynecol. Oncol.* 112(3), 506–510 (2009).

70 Cibula D, Slama J, Svarovsky J *et al*. Abdominal radical trachelectomy in fertility sparing treatment of early stages cervical cancer. *Int. J. Gynecol. Cancer* 19(8), 1407–1411 (2009).

71 Bernardini M, Barrett J, Seaword G *et al*. Pregnancy outcome in patients post radical trachelectomy. *Am. J. Obstet. Gynecol.* 189(5), 1378–1382 (2003).

72 Ungar L, Palfalvi L, Smith JR *et al*. Update on and long term follow-up of 91 abdominal radical trachelectomies. *Gynecol. Oncol.* 101, 20 (2006) (Abstract 42).

73 Nishio H, Fujii T, Kameyama K *et al*. Abdominal radical trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer in a series of 61 women. *Gynecol. Oncol.* 115(1), 51–55 (2009).

• **Largest series of abdominal radical trachelectomy, with 61 women.**

74 Wan XP, Yan Q, Xi XW *et al*. Abdominal radical trachelectomy: two new surgical techniques for the conservation of uterine arteries. *Int. J. Gynecol. Cancer* 16(4), 1698–1704 (2006).

75 Covens A, Rosen B, Murphy J *et al*. How important is removal of the parametrium at surgery for carcinoma of the cervix? *Gynecol. Oncol.* 84(1), 145–149 (2002).

76 Kinney WK, Hodge DO, Egorshin EV *et al*. Identification of a low-risk subset of patients with stage 1B invasive squamous cancer of the cervix possibly suited to less radical surgery treatment. *Gynecol. Oncol.* 57(1), 3–6 (1995).

77 Steed H, Capstick V, Schepansky A *et al*. Early cervical cancer and parametrial involvement: is it significant? *Gynecol. Oncol.* 103(1), 53–57 (2006).

78 Stegeman M, Louwen M, van der Velden J *et al*. The incidence of parametrial tumor involvement in select patients with early cervix cancer is too low to justify parametrectomy. *Gynecol. Oncol.* 105(2), 475–480 (2007).

79 Frumovitz M, Sun CH, Schmeler KM *et al*. Parametrial involvement in radical hysterectomy specimens for women with early-stage cervical cancer. *Obstet. Gynecol.* 114(1), 93–99 (2009).

• **Study identified a cohort of women with early-stage cervical cancer at very low risk of parametrial involvement.**

80 Strnad P, Robova H, Skapa P *et al*. A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. *Gynecol. Oncol.* 109(2), 280–284 (2008).

•• **Minimal risk of parametrial involvement in the case of negative SLNs was confirmed by the first prospective study.**

81 Benedetti Panici PL, Bellati F, Pastore M *et al*. An update in neoadjuvant chemotherapy in cervical cancer. *Gynecol. Oncol.* 107, S20–S22 (2007).

82 Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer: a systematic review and meta-analysis of individual patient data from 21 randomised trials. *Eur. J. Cancer* 39(17), 2470–2486 (2003).

83 Rob L, Halaska M, Robova H. Nerve-sparing and individually tailored surgery for cervical cancer. *Lancet Oncol.* 11(3), 292–301 (2010).

•• **Focuses on the neuroanatomy of the pelvis and suggests options for the sparing of these nerves during surgery for cervical cancer.**

Příloha XII.

Rob L., Škapa P. a Robová H. (2011) Fertility-sparing surgery in patients with cervical cancer. ***Lancet Oncol*** 12:192-200.
IF 14,470

Fertility-sparing surgery in patients with cervical cancer

Lukas Rob, Petr Skapa, Helena Robova

Lancet Oncol 2011; 12: 192–200

Published Online
July 8, 2010
DOI:10.1016/S1470-2045(10)70084-X

This Review has been corrected. The corrected version first appeared at thelancet.com/oncology on December 30, 2010

Department of Obstetrics and Gynaecology, 2nd Medical Faculty, Charles University, Prague, Czech Republic (Prof L Rob MD, P Skapa MD, H Robova MD)

Correspondence to:
Prof Lukas Rob, Department of Obstetrics and Gynaecology, 2nd Medical Faculty, Charles University, Uvalu 84, 150 00 Prague 5, Czech Republic
lukas.rob@lfmotol.cuni.cz

There are several types of fertility saving procedures that can be done in patients with cervical cancer, which differ in terms of surgical approach and extent of paracervical resection. This review assesses oncological and pregnancy results after different procedures. The oncological results of vaginal radical trachelectomies (VRT) and abdominal radical trachelectomies (ART) are similar for tumours less than 2 cm in size, and are now considered safe surgical procedures. Oncological outcomes of VRT and ART in tumours larger than 2 cm are also identical, but the results cannot be considered satisfactory. Preliminary findings of less radical procedures (ie, deep cone and simple trachelectomy) in patients with tumours less than 2 cm, and negative sentinel and other pelvic lymph nodes, are comparable with the results of VRT and ART. Downstaging tumours larger than 2 cm by neoadjuvant chemotherapy is still an experimental procedure and will need multicentre cooperation to verify its oncological safety. Pregnancy results vary statistically with the different methods.

Introduction

Cervical cancer is the second most common cancer in women in developing countries and the seventh most common cancer in developed countries.[1] More than 500 000 new invasive cervical cancer cases are estimated to be diagnosed worldwide every year. The precise number of diagnosed cancer cases and the age distribution are unknown, because not all countries have complete cancer registration. In developed countries with a good public health infrastructure, screening of cervical cancer has led to an impressive reduction in incidence and mortality. Despite this fact, in 2004, 30 570 new invasive cervical cancer cases were diagnosed in 25 states of the European Union and there were an estimated 10 520 new cases of cervical cancer in the USA.[1,2] More than 25% of women with cervical cancer are under 40 years of age and the age of nulliparous women has increased in developed countries.[3,4]

Radical surgery and radiotherapy (less often) have been the only treatment options for women with cervical cancer of more than 3 mm invasion (International Federation of Gynecology and Obstetrics [FIGO] stage IA2 and more). Most young patients with cervical cancer are diagnosed in the early stages of the disease and the number of these patients that are completely cured is high. However, radical surgery and radiotherapy do not spare fertility and both methods can lead to psychosexual dysfunction and decreased quality of life. Furthermore, infertility increases the frequency of depression, stress, and sexual dysfunction.[5,6] This leads to the question of whether it is possible to preserve the uterus without increasing the risk of recurrence and to afford the opportunity for pregnancy.

The concept of less radical surgery was first introduced in breast and vulvar cancer. In 1986, Professor Daniel Dargent was the first to undertake fertility sparing surgery in patients with cervical cancer. This procedure included laparoscopic pelvic lymphadenectomy and vaginal radical trachelectomy (VRT) also referred to as the "Dargent operation". Dargent presented the first oncological results and successful pregnancies at the Society of Gynecologic Oncologists (SGO) Annual Meeting, Orlando, FL, 1994, and his findings were published in the same year.[7,8] In a short period of time, several centres presented studies regarding slightly modified VRT. These procedures consisted of laparoscopic pelvic lymphadenectomy, and extirpation of the cervix and paracervical tissue vaginally.[9–12] An abdominal fertility sparing surgical procedure called a abdominal radical trachelectomy (ART) was brought into clinical practice in 1997 by an international group.[13] Over the past 10 years, many studies have been published on neoadjuvant chemotherapy and fertility sparing surgery in women with larger cancers,[14–17] and studies have described procedures that reduce the radicality of paracervical resection.[17,18] With these procedures, women have an opportunity to retain their childbearing potential. This paper reviews our present knowledge of fertility sparing procedures in patients with cervical cancer, and presents an analysis of oncological and pregnancy results.

Selection criteria and preoperative management

Patient characteristics

The management of fertility sparing surgery must include a good selection of patients and complete information about them. The patients need to be informed about preoperative examinations, surgery, late complications, and especially about the risk of premature delivery. The patients must know that future pregnancies will be risky and that they will have to reduce their normal lifestyle activities.[19–23] Detailed informed consent is essential. The criterion, which is accepted in all centres, is a strong desire to preserve fertility. In all centres, reported patients have planned pregnancy in the near future. Preservation of the uterus for personal reasons in women who do not plan pregnancy is controversial.[24,25] In some centres, no clinical evidence of previously impaired fertility has also been a selection criterion.[9,26,27] This is currently highly problematic, because methods of assisted reproduction are widely used and most women are nulliparous. Hence, we are not able to estimate reproductive potential before surgery accurately. The reported number of nulliparous women who have undergone fertility sparing surgery ranges between 75% and 100%; 81% of women who have undergone VRT; and 89% of women who have had ART (tables 1 and 2). Most

	Shepherd[22,23]	Sonoda[24]	Pahisa[28]	Chen[29]	Hertel[30]	Dargent[27,31]	Plante[19,32,33]	Covens[34–36]	Burnett[26,37]	Schlaerth[38]
Period	1994–05 1994–07	2001–06	2000–07	2000–04	1995–2005	1986–2003	1991–2003 1991–2008	1999–2003 1999–2007	1995–2001	1995–99
Planned VRT, n	158	43	15	16	108	135	82	93	21	12
Fertilities spared, n	138	36	13	16	106	118	72	91	18	10
N1, n	7/123	2	0	0	4	9	4	2	1	0
LVSI, n	49/158	NA	1/15	1/16	38/108	43/118	14/72	31/93	6/21	1/10
Mean age (range), years	30·6 (21–45)	31 (20–40)	NA	27·6 (24–31)	32 (21–41)	NA	31 (21–42)	30 (NA)	30 (23–41)	30·9 (22–44)
Nulliparous women, n	97/123	35/43	NA	14/16	92/108	NA	54/72	NA	16/21	12/12
Histology, n										
SCC	103	24	9*	14	74	90*	42	40	12	4*
AC	51	16	6*	2	33	25*	27	50	9	5*
Other	4	3	0	0	1	3*	3	3	0	1*
Recurrence, n										
Size <2 cm	NA	1/36	1/11	0/9	3/105	1/91	1/64	5/83	NA	0/10
Size >2 cm	NA	0	1/2	0/7	1/1	6/27	2/8	1/8	NA	0
Deaths, n	4	1	1	0	2	5	2	4	1	0
Pregnant women, n	NA	11	3	5	18/106	33/118	51	18	3	4
Conceptions, n	88	11	3	5	17	56	90	22	3	4
First trimester loss, n	22	3	0	0	3†	14	23	3	0	0
Second trimester loss, n	12	0	0	2	0	8	3	3	1	2
Delivery, n										
Before week 32	10	0	0	0	3		4	3‡	0	0
Weeks 32–36		0	0	1	5§	5¶	9‡	3	0	1
Term	35+2§||**	4	1	1	4	29	51	12	2	1
Ongoing pregnancy	7	4	2	1	3	NA	NA	NA	NA	NA

VRT=vaginal radical trachelectomy. N1=positive lymph nodes. LVSI=lymphovascular space involvement. NA=not available. SCC=squamous-cell carcinoma. AC=adenocarcinoma. *Only patients after VRT. †Two abortions induced at patients' request. ‡Two twin pregnancies. §Twins. ¶These data are for before week 32 and weeks 32–36. ||Surrogate livebirth. **These data are for 32–36 weeks and term.

***Table 1*: Characteristics, oncological outcome, and pregnancy outcome of radical vaginal trachelectomy**

centres do not specify an upper age limit for fertility sparing surgery.[11,19,22,30] In the few centres that do, age varies from 40 years[18,28,29] to 45 years.[17,27] Mean reported ages and age ranges are shown in tables 1 and 2. The mean age of the youngest group of patients was 27·6 years (range 24–31)[29] and the oldest group had a mean age of 33 years (range 26–44).[41] The mean age in the VRT group was 31 years and, in the ART group, it was 32 years.

Imaging modalities

Tumour volume

Tumour size is an important criterion in most centres. Appropriate candidates for fertility sparing surgery are patients with tumours of FIGO stage IA1 with lymphovascular space involvement (LVSI), IA2, and IB1. Most centres include stage IB1 tumours of less than 2 cm only. In IB1 tumours larger than 2 cm in size, there is a higher risk of extrauterine spread[43,47] and, statistically, the risk of recurrence is significantly higher.[25,32,43] Expert colposcopy is the standard examination before fertility sparing surgery, and is important in assessing the exocervical diameter and spread to the vagina.[10,18,19] Magnetic resonance imaging (MRI) volumetry is the second preoperative diagnostic method, important for determination of exact tumour size, amount of cervical stroma infiltration, and amount of healthy stroma (determination of tumour growth in anterioposterior, craniocaudal, and transverse directions). MRI can help identify the proportions of healthy and infiltrated stroma, and can help in choosing appropriate candidates for fertility sparing surgery.[19,48–50] Many clinicians have suggested that infiltration of less than half of the cervical stroma is the limit for a safe trachelectomy, because it is necessary to have a 1-cm-free margin.[12,18,51] All forms of trachelectomy should save at least 1 cm of healthy stroma, because the chance of pregnancy is higher. Preservation of cervical stroma lowers the risk for cervical incompetence, ascending infection, and premature delivery. MRI can also assess tumour involvement of paracervical tissues. However, MRI and CT scans are insufficient for evaluation of microscopic pelvic lymph-node infiltration.[49,52] A new generation of PET–CT and MRI, which use ultra-small iron particles, seem to be feasible for preoperative assessment of lymph nodes.[53,54] Vaginal or rectal ultrasonography is used for tumour volumometry in some centres, with good results.[55]

	Abu Rustum[39,40]	Pajera[41]	Duska[42]	Nishio[43]	Cibula[44]	Ungar[45,46]
Period	2005–08	2002–08	1999–2007	2002–08	2001–08	1997–2002
Planned ART, n	22	15	10	71	24	33‡
Fertilities spared, n	15	14	10	61	17*	30§
N1, n	6	1	0	15	4	2
LVSI, n	9	5	NA	31	2	8
Mean age (range), years	33 (23–43)	30 (25–38)	31·7 (25–38)	33 (26–44)	32·4 (23–37)	30·5 (23–37)
Nulliparous women, n	20	NA	9	NA	21	NA
Histology, n						
SCC	9	11	3	58†	14	26†
AC	13	4	7	2†	10	1†
Other	0	0	0	1†	0	3†
Recurrence, n						
Size <2 cm	NA	0	0	1/48	1/14	0/21
Size >2 cm	NA	0	0	5/13	0/3	0/9
Deaths, n	0	0	0	NA	NA	0
Pregnant women, n	2	3	2	4	6	13
Conceptions, n	2	3	4	4	6	13
First trimester loss, n	1	0	1	0	1	4
Second trimester loss, n	0	0	0	0	0	0
Delivery, n						
Before week 32	0	1	0	2	2	0
Weeks 32–36	0	0	1	0	0	1
Term	0	2	1	2	3	5
Ongoing pregnancy	1	0	1	0	0	3

81 fertilities spared. ART=abdominal radical trachelectomy. N1=positive lymph nodes. LVSI=lymphovascular space involvement. NA=not available. SCC=squamous-cell carcinoma. AC=adenocarcinoma. *Three were done completely laparoscopically. †Only patients after ART. ‡Updated data; 91 Planned ART. §Updated data.

***Table 2*: Characteristics, oncological outcome, and pregnancy outcome of abdominal radical trachelectomy**

Ultrasonography could have an important role in diagnostic management, especially in developed countries, where it is widely available.

Tumour biology

Most centres demand careful review of available histology obtained from previous biopsies taken outside the centre.[18,23,32] A second histopathological examination is important for determination of type, depth of invasion, and LVSI. Small-cell neuroendocrine carcinoma is not suitable for fertility sparing surgery, because the prognosis for this tumour is worse than for other types.[25,32,56,57] Other important prognostic factors are adenosquamous type, vascular invasion, and pattern of invasion. When these risk factors are reviewed separately, they do not provide sufficient sensitivity in predicting tumour behaviour in vivo and, thus, their use is debatable.[58] LVSI is the most commonly discussed risk factor.[8,10,11,24,25] LVSI was present in around a third of women who underwent fertility sparing surgery (tables 1 and 2). Most centres consider these risk factors seriously, but do not regard them as exclusion criteria. Women must be informed of these risk factors and of the risk of malignant extrauterine spread, as well as the increased risk of recurrence.

Intraoperative assessment

During surgery, extrauterine spread to the lymph nodes should be assessed and an adequate margin of healthy stroma assured. These goals can only be met if perioperative frozen section is used. When extrauterine spread or infiltration of the cranial part of the specimen is diagnosed, it becomes necessary to radicalise surgery or initiate chemoradiotherapy.

Perioperative assessment of regional lymph nodes can be done by repeated frozen sections;[24,29] however, this assessment has been replaced in many centres by detection of sentinel lymph nodes (SLNs).[18,25,30,32,59] Although a perioperative frozen section allows detection of metastatic disease, it has limitations in detecting micrometastases. Patients should be told of all viable alternatives before surgery, with informed consent being obtained. In clinical practice, micrometastases (lesions less than 2 mm in size) or isolated tumour cells, which were not diagnosed perioperatively, can be detected on final histopathology. These situations are a therapeutic problem. The Lyon group[31] published a paper on three patients with micrometastases who refused adjuvant radiotherapy and in whom recurrences were not diagnosed. In another centre, four women with micrometastases received adjuvant chemotherapy and recurrences were not diagnosed.[30] Serial sections of SLNs increase the safety of fertility sparing surgery, despite the ongoing debate on management of patients with postoperative detection of micrometastasis or isolated tumour cells. The modality of treatment is dependent on the patient's decision—ie, whether the patient prefers adjuvant chemotherapy, which provides a chance for pregnancy, or radiotherapy, which leaves no chance for pregnancy. There is no published randomised study that compares different methods of adjuvant treatment. Some centres do not undertake perioperative assessment of lymph nodes if they are not suspicious, and, thus, rely on histopathology.[43]

Perioperative assessment of endocervical involvement is done in many centres. A cross section of the superior portion of the separated cervix is sent for frozen-section examination to assess the tumour-free status of the endocervical resection margin. At least 8–10 mm of free endocervical margin should be obtained; otherwise, more of the endocervix needs to be removed or the fertility preserving surgery needs to aborted.[19,26,28,29,31,32] Perioperative assessment is not without its difficulties, especially in adenocarcinoma. Thus, some centres do not routinely undertake frozen sectioning.[18,30]

Vaginal radical trachelectomy

Progress in laparoscopic surgery has led to an improvement in pelvic lymphadenectomy and, therefore, vaginal radical hysterectomies have been reinstated into

the management of cervical cancer (Schauta–Stoeckel procedure). VRT, is a modification of the Schauta–Stockel procedure. The surgeon needs to be experienced in laparoscopy, first learning laparoscopic lymphadenectomy and eventually paracervical lymph-node dissection with or without SLN identification. The extent of laparoscopic surgery varies from school to school. The second phase of the procedure requires experience in vaginal surgery, because vaginally identifying and mobilising the ureter is difficult; this is necessary for the safe resection of the parametria.[10,11,29,34,47] Radicality of the resection of the parametria is limited by the goal of preserving the uterine artery and only ligating the vaginal branch of this artery. The cervix should be transected about 1 cm above the endocervical tumour margin and a maximum of 1 cm caudally from the internal cervical orificium. There is a greater chance of successful pregnancy with a greater volume of cervical stroma—ie, a greater volume decreases the risk of ascending infection and premature rupture of the membranes (PROM). Most centres do prophylactic cerclage with a non-resorbable stitch and suture the vaginal mucosa to the residual exocervical stroma. Only 9·5% of 683 women for whom VRT was planned could not have their fertility preserved (table 1). Positivity of lymph nodes was the cause in half of these cases. When positive nodes are diagnosed perioperatively, either the surgery should be aborted and the patient should undergo chemoradiotherapy instead,[23,31,32,48] or the surgery should be radicalised.[18]

The second cause of fertility loss was the clearance of normal cervical tissue beyond the tumour. The normal recommendation is the remainder of 1 cm of healthy tissue,[23,47] although some clinicians suggest only 5–8 mm are necessary.[18,60] Perioperative frozen-section examination showing a close margin usually leads to some form of immediate radical surgery. In view of the fact that frozen sectioning has its limitations, this form of assessment is rarely considered adequate. If the margin on final pathology is shown to be too close, the implication is that there is less than 5 mm.[31,32] The question of how much endocervical margin suffices as adequate therapy is still under debate; however, 5 mm is considered the minimum margin. Involvement of the paracervical tissue is an exception and is an indication for chemoradiotherapy.[24,47] In more than 10% of reported cases, VRT was done in tumours larger than 2 cm. A comparison of recurrences in tumours less than 2 cm in size (12 recurrences in 409 women [2·9%]) with recurrences in tumours larger than 2 cm (11 recurrences in 53 women [20·8%]) shows that VRT is a risky procedure for tumours larger than 2 cm. In women who underwent VRT, the percentage of recurrence was only 4·7% and mortality was 3·0%. It was not possible to analyse data about patients in whom fertility was not spared (the VRT procedure was terminated in 9·5% of women), because most centres did not report the oncological outcome of these patients. A randomised study comparing fertility sparing surgery with radical hysterectomy is not feasible because of the ethical issue involved in recruiting women who wish to remain fertile and because it is impossible to have enough patients to do meaningful statistical analyses. One possibility is to do a case-control study that compares women with the same risk factors. The biggest case-control study published up to now is the Toronto study, which compares VRT with radical hysterectomy in 90 patients. The oncological outcome was shown to be similar in both groups.[36]

Table 1 summarises the pregnancy outcomes after VRT in 618 women in ten centres. It is feasible that actualisation of the results would improve the pregnancy results. Pregnancy in 30% of women after VRT can be regarded as very good, especially considering that the average age of the women in most groups was over 30 years. A strong desire to preserve fertility is a strict condition for fertility preserving surgery in all centres and, therefore, a more objective criterion of pregnancy outcome is the number of pregnant women than number of women who wish to preserve their fertility. From 300 conceptions, there were 186 deliveries (62%) and 190 babies. There were 68 first trimester losses (22·7%): spontaneous abortions, therapeutic abortions, and extrauterine pregnancies. This is similar to that of the general population at the same age. Second trimester loss was higher than in the general population (9·7% [29 cases]). The cause of second trimester loss is the same as in premature delivery—ie, ascending infection and PROM. A high frequency of preterm deliveries was caused by PROM, after ascending infection as a result of shortening of the cervix and lack of mucus.

Abdominal radical trachelectomy

ART is the second most frequent type of fertility sparing surgery. ART was first described in 1932 by the Romanian surgeon and obstetrician Eugen Aburel; however, Aburel did not have any successful pregnancies after this procedure. Later, J R Smith restored the use of this surgical approach, publishing his first experiences in 1997.[13] ART is a modification of abdominal radical hysterectomy and does not need any special surgical training or instruments. Pelvic lymphadenectomy, with or without SLN identification, is the first step in this procedure.[39–43,45,59] Radicality of cervical extirpation can vary in different schools. "Classical" ART provides standard radical resection of the parametria with complete resection of the uterine artery. If the flow through ovarian vessels goes unchanged, sufficient formation of new collateral circulation in the neocervix can be obtained.[13,59]

Modifications of ART with preservation of the uterine artery have been described: first, by involving skeletisation of the uterine artery and ligation of the descending branch, and, second, by re-anastomosis of the uterine artery.[45,61] Furthermore, resection of the parametria should be on an individual basis, as in radical hysterectomy (type B, or type C with or without nerve-sparing surgery).

Suture of the uterus and cervix together, and formation of the neocervix, including catheterisation of the neocervix, vary in different schools. Some surgeons prefer permanent cervical cerclage,[39,43,51] whereas others undertake cervical cerclage during pregnancy.[41] However, several clinicians have expressed their concerns about this procedure.[45,59]

Table 2 lists six studies on ART in which the characterisation of patients is detailed. ART was planned in 175 women and fertility was spared in 147 (84%). Positive lymph nodes were detected in 16% of the women, which is higher in comparison with VRT. The number of IA1 tumours having ART was lower compared with those having VRT (19·1% *vs* 29·5%, respectively) and there was a higher number of IB1 tumours in patients undergoing ART, including tumours larger than 2 cm. The percentage of LVSI in patients having ART and those having VRT was comparable (33·3% *vs* 30·1%, respectively). The oncological outcome of ART was good, with only seven recurrences (4·8%) reported. Some studies subdivided the tumours into two groups: tumours less than 2 cm and those larger than 2 cm. Recurrences differed in these groups, with 1·9% in tumours less than 2 cm versus 20% in those larger than 2 cm. These proportions follow those in VRT. Thus, it seems clear that ART, like VRT, is a hazardous procedure in tumours larger than 2 cm in size, because the oncological results are no better.

Table 2 shows the pregnancy results of 194 women after ART. Updated results from Ungar and colleagues[44] were included. In total, there were 32 pregnancies in 30 women (15·5%). Premature labour was mentioned in seven cases (35%) from a possible 20 deliveries, which is the same number as in VRT. However, all centres also included only those women with a strong desire to preserve fertility. From this point of view, the number of pregnant women was dramatically lower than in other types of fertility sparing surgery.

	Characteristics
Period	1999–2006
Planned simple trachelectomy/cone, n	40
Fertilities spared, n	32
N1, n	6
Mean age (range)	28·3 (24–35)
Nulliparous women, n	25
Histology, n	
SCC	32
AC	7
Other	1
Stage, n	
IA1+LVSI	3
IA2	10
IB1	27
LVSI, n	17
Recurrences, n	1/32
Deaths, n	0

N1=positive lymph nodes. SCC=squamous-cell carcinoma. AC=adenocarcinoma. LVSI=lymphovascular space.

***Table 3*: Characteristics and oncological outcome of patients who underwent simple trachelectomy, large cone[18,50]**

Less radical fertility sparing surgery

Simple trachelectomy, large cone

There are only a few case reports about conisation or trachelectomy with or without lymphadenectomy in cervical cancers. A less radical surgical protocol was first published by Rob and colleagues.[18] This procedure is divided into two steps: first, laparoscopy, SLN identification and frozen section, and pelvic lymphadenectomy are done; second, conisation (stage IA1 with LVSI and stage IA2 tumours) or simple trachelectomy (stage IB1 tumours, less than 2 cm) are undertaken when the SLNs are negative. Updated findings were published in 2008.[50] Retrospective studies of parametrial involvement in small tumours with infiltration of less than 10 mm or less than half of the stroma, with negative pelvic lymph nodes, support less radical surgery without resection of paracervical tissue.[62–66] The minimum risk of parametrial involvement in cases of negative SLNs was confirmed by the first prospective study.[67] Well-timed injection of blue dye enables the removal of blue afferent lymphatic channels or nodes from the parametrium. Conisation is done using an electrosurgical needle. Simple trachelectomy involves amputation of the cervix with an incision 7–10 mm above the tumour, then removal of the endocervical channel by use of the loop electrosurgical excision procedure with a small loop electrode. Individual sutures to the outer edge created by the small loop reapproximate the vaginal edge circumferentially. Cerclage is not done. This technique keeps the risk of stenosis to a mimimum.[50] In six of 40 women, radical hysterectomy was done due to positive SLNs. Fertility was spared in 32 of these women (table 3). The mean age of the women was 28·3 years (range 24–35); 25 women were nulliparous; LVSI was detected in 17 and IB1 tumours represented 27 tumours. These characteristics are similar to those in women having VRT. One recurrence was diagnosed, but the woman is still alive without evidence of disease more than 5 years after treatment. Of 32 women, 17 became pregnant. Of 23 pregnancies: five were lost in the first trimester (three women) and three in the second trimester. There were 12 deliveries and three ongoing pregnancies. Prophylactic cerclage was not done in any of the women. To verify the findings of less radical fertility sparing surgery, two multicentric studies are being prepared (NTC01048853).

Another less radical approach was published by Landoni and colleagues.[17] 11 patients (ten of whom were nulliparous) with tumours less than 3 cm were included in the study (eight with stage IB1 and three with stage IA2). The first step was laparoscopic pelvic lymphadenectomy and, in cases of negative nodes and tumours less than 2 cm, laser

conisation with deep penetration was done. When negative prognostic factors were present, adjuvant chemotherapy was undertaken (the same as the TIP [paclitaxel 175 mg/m² plus cisplatin 75 mg/m² plus ifosfamide 5 g/m²] or TEP [paclitaxel 175 mg/m² plus cisplatin 75 mg/m² plus epirubicin 80 mg/m²] regimen in the study by Maneo and colleagues;[16] table 4). Three cycles of neoadjuvant chemotherapy were administered to patients with tumours between 2 cm and 3 cm (TIP or TEP). Deep laser conisation was done after the chemotherapy. Of 11 women, three received neoadjuvant chemotherapy and one received adjuvant chemotherapy. No recurrence was noted in this group and there were three full-term babies.

Neoadjuvant chemotherapy and fertility sparing surgery

One of the limitations of fertility preserving surgery is deep stromal invasion and tumours larger than 2 cm. Some centres use neoadjuvant chemotherapy in "bulky" cervical cancers with the aim of downstaging before radical hysterectomy.[68,69] Currently, three approaches have been published on neoadjuvant chemotherapy and fertility sparing surgery (table 4). First, Maneo and colleagues[16] used neoadjuvant chemotherapy before fertility sparing surgery. 21 women with tumours 10–30 mm in size were included in this protocol. In 16 of these women, fertility was preserved. Minimum (less than 3 mm) or no residuum was found in 17 women, and none of the patients' tumours progressed on chemotherapy. Initially, lymphadenectomies were done by laparotomy, and then laparoscopically. Two women had positive lymph nodes. No recurrences were noted, but three women had precancerous lesions during follow-up.

Plante and colleagues[14] reported on three women who had neoadjuvant chemotherapy. The protocol of chemotherapy was the same as in the study by Maneo and colleagues[16] and only tumours between 3 and 4 cm were included. Surgery consisted of laparoscopic pelvic lymphadenectomy, with identification of SLNs, and VRT. Complete histopathological remission was achieved in all cases. In August, 2009, all the patients were without evidence of disease; one patient delivered one baby and one patient delivered two (table 4).

The third approach is known as the Prague protocol.[15,50] Women with tumours larger than 2 cm, but which had not infiltrated more than two thirds of the stroma, were included. High dose density neoadjuvant chemotherapy was used: three cycles of chemotherapy at an interval of ten days. Fertility was preserved in seven of nine women. One woman opted for a radical hysterectomy after chemotherapy. Positive endocervical margins were present in another patient. Complete remission or minimal residuum of less than 2 mm was diagnosed in seven patients (table 4). One recurrence in the ovary was diagnosed 6 weeks after spontaneous delivery. This woman died of haematogenous dissemination of the tumour (unpublished data). To our knowledge, this is the first recurrence and death after neoadjuvant chemotherapy and

	Robova[15] and Rob[50]	Plante[14]	Maneo[16]
Period	2005–07	2004–05	1995–2007
Chemotherapy	Cisplatin 75 mg/m² plus ifosfamide 2 g/m² every 10 days; or cisplatin 75 mg/m² plus doxorubicin 35 mg/m² every 10 days	TIP; or TEP every 21 days	TIP or TEP
Planned surgery, n	9	3	21
Fertility spared, n	7	3	16
N1, n	0	0	2
Mean age (range), years	29·3 (24–33)	32·3 (26–36)	30 (17–39)
Nulliparous women, n	8	2	NA
Histology, n			
SCC	7	3	9
AC	2	0	12
Others	0	0	0
Tumour size, n			
<2 cm	0	0	9
>2 cm	9	3	12
LVSI, n	9	NA	1
Recurrences, n	1/7*	0	0/16
Pregnant women, n	5/7	2	6/16
Conceptions, n	5	3	10
1st trimester loss, n	0	0	1
2nd trimester loss, n	0	0	0
Delivery, n			
Before week 32	1	0	2
Weeks 32–36	0	1	0
Term	3	2	7
Ongoing pregnancy, n	1	NA	NA

N1=positive lymph nodes. NA=not applicable. SCC=squamous-cell carcinoma. AC=adenocarcinoma. LVSI=lymphovascular space invasion. TIP=paclitaxel 175 mg/m² plus cisplatin 75 mg/m² plus ifosfamide 5g/m². TEP=paclitaxel 175 mg/m² plus cisplatin 75 mg/m² plus epirubicin 80 mg/m². *Unpublished case.

Table 4: **Characteristics, oncological outcome, and pregnancy outcome after neoadjuvant chemotherapy and fertility sparing surgery**

	N	n	OR	95% CI	p value	χ^2	p value
Pregnant women							
VRT	483	146 (30%)	Ref	Ref	Ref	32·6	<0·0001
ART	194	30 (15%)	0·4	0·3–0·7	<0·0001		
ST	32	17	2·6	1·3–5·4	0·010		
NAC	26	13	2·3	1·0–5·1	0·049		
Deliveries							
VRT	621	186 (30%)	Ref	Ref	Ref	47·9	<0·0001
ART	194	20 (10%)	0·3	0·2–0·4	<0·0001		
ST	32	12	1·4	0·7–3·3	0·430		
NAC	26	16	3·3	1·6–10·0	0·002		
Pregnancies							
VRT	621	300 (48%)	Ref	Ref	Ref	81·0	<0·0001
ART	194	32 (16%)	0·2	0·1–0·3	<0·0001		
ST	32	23	2·5	1·3–5·0	0·011		
NAC	26	18	2·5	1·0–5·0	0·045		

OR=odds ratio. VRT=vaginal radical trachelectomy. Ref=reference. ART=abdominal radical trachelectomy. ST=simple trachelectomy or cone. NAC=neoadjuvant chemotherapy.

Table 5: **Analysis of pregnancy outcomes**

Search strategy and selection criteria

Data for this review were identified by a search of Medline and PubMed, using the search terms: "trachelectomy", "fertility sparing", "fertility saving", "radical vaginal trachelectomy", and "radical abdominal trachelectomy". Dates of the search on Medline were from January, 1994, to July, 2009. References from the identified articles were investigated for relevance. Abstracts from meetings were included only if they were presented at meetings of the Society of Gynecologic Oncologists and in supplements, and if they actualised already published results from centres undertaking fertility sparing surgery.

fertility sparing surgery. Five women became pregnant: four delivered and one was pregnant as of August, 2009.

Neoadjuvant chemotherapy in fertility sparing surgery is an experimental concept, which requires verification in the future, especially concerning oncological findings. Pregnancy results are very good and neoadjuvant chemotherapy has no effect on fertility. In total, there were 18 pregnancies in 13 women. There was one first-trimester loss, and four premature deliveries from 16 deliveries (one ongoing pregnancy remaining, see table 4).

Conclusion

VRT with laparoscopic pelvic lymphadenectomy is currently the standard fertility preserving procedure. Oncological results are similar in VRT and ART for tumours less than 2 cm in size. Fertility sparing procedures in tumours less than 2 cm in size are now considered to be safe surgical procedures.

Oncological safety of less radical surgeries (deep cone and simple trachelectomy) must be confirmed in prospective multicentre studies. Preliminary findings in patients with negative SLNs and other pelvic lymph nodes in tumours less than 2 cm are promising and comparable with the results of VRT and ART. Downstaging by neoadjuvant chemotherapy is still an experimental procedure and will need multicentre cooperation to verify oncological safety. However, chemotherapy neither affects fertility nor decreases the chance for pregnancy.

Pregnancy results can differ statistically with different methods. Table 5 summarises pregnancy results after VRT, ART, simple trachelectomy, and neoadjuvant chemotherapy, and also compares the number of pregnant women, the number of deliveries, and the number of pregnancies in VRT, which is taken as a standard when compared with other methods. From this point of view, ART proved worse than all the others in all parameters. Less radical procedures showed significantly better results.

Factors of great interest are the extent of the removed cervix, technique of re-anastomosis, and formation of a neocervix, including permanent cerclage. Fertility will be decreased by progressive shortening of the cervix, together with decreased cervical mucus and stenosis of the residual part of the cervix, including the isthmic part. A progressively short cervix provides an easy route for ascending infection during pregnancy, which increases the risk of late miscarriage in the second trimester and of premature delivery.[70–75] Nowadays, all techniques aim to save at least 1 cm of cervical stroma. A factor that can further affect fertility is a higher risk of adhesions after abdominal lymphadenectomy.

The basic difference between techniques is the extent of resection of the parametria and the extent of disruption of pelvic autonomic innervation (inferior hypogastric plexus). Larger resection of the paracervix implies greater disruption of the uterus and tube innervation. A combination of the different factors (extent of extirpation of the cervix, technique of formation of the neocervix, and extent of resection of the paracervix) explains fertility and pregnancy results.

Contributors

LR wrote the paper. HR contributed to the literature search, tables, and editing. PS contribute to the literature search on histopathology and analysed risk factors in cervical cancer.

Conflicts of interest

The authors declared no conflicts of interest.

Acknowledgments

This work was supported by grant MZ0FNM2005.

References

1 Arbyn M, Autier P, Ferlay J, et al. Burden of cervical cancer in the 27 member states of the European Union: estimates for 2004. *Ann Oncol* 2007; **18:** 1423–25.

2 Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58:** 71–96.

3 Watson M, Saraiya M, Benard V, et al. Burden of cervical cancer in the United States, 1998–2003. *Cancer* 2008; **113:** 2855–64.

4 Sonoda Y, Abu-Rustum NR, Gemignani ML, et al. A fertility sparing alternative to radical hysterectomy: how many patients may be eligible? *Gynecol Oncol* 2004; **95:** 534–38.

5 Corney RH, Crowther ME, Everett H, et al. Psychosexual dysfunction in women with gynaecological cancer following radical pelvic surgery. *Br J Obstet Gynaecol* 1993; **100:** 73–78.

6 Alexander-Sefre F, Chee N, Spencer C, et al. Surgical morbidity associated with radical trachelectomy and radical hysterectomy. *Gynecol Oncol* 2006; **101:** 450–54.

7 Dargent D, Burn JL, Roy M, Remi I. Pregnancies following radical trachelectomy for invasive cervical cancer. *Gynecol Oncol* 1994; **52:** 105 (abstr 14).

8 Dargent D, Burn JL, Roy M. La trachélectomie élargie (T.E.). Une alternative á l´hystérectomie radicale dans le traitement des cancers infiltrants développés sur la face externe du col utérin. *J Obstet Gynecol* 1994; **2:** 292–95.

9 Schneider A, Krause N, Kuhne-Heid R, Noschel H. Preserving fertility in early cervix carcinoma: trachelectomy with laparoscopic lymphadenectomy. *Zentralbl Gynaekol* 1996; **118:** 6–8.

10 Roy M, Plante M. Pregnancies after radical vaginal trachelectomy for early-stage cervical cancer. *Am J Obstet Gynecol* 1998; **179:** 1491–96.

11 Shepherd JH, Crawford RAF, Oram DH. Radical trachelectomy: a way to preserve fertility in the treatment of early cervical cancer. *Br J Obstet Gynaecol* 1998; **105:** 912–16.

12 Covens A, Shaw P, Murphy J, et al. Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA-B carcinoma of the cervix? *Cancer* 1999; **86:** 2273–79.

13 Smith JR, Boyle DC, Corless DJ, et al. Abdominal radical trachelectomy: a new surgical technique for the conservative management of cervical carcinoma. *Br J Obstet Gynaecol* 1997; **104:** 1196–200.

14 Plante M, Lau S, Brydon L, et al. Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage 1B1 cervical cancer: case report. *Gynecol Oncol* 2006; **101:** 367–70.

15 Robova H, Pluta M, Hrehorcak M, et al. High dose density chemotherapy followed by simple trachelectomy: full-term pregnancy. *Int J Gynecol Cancer* 2008; **18:** 1367–71.

16 Maneo A, Chiari S, Bonazzi C, Mangioni C. Neoadjuvant chemotherapy and conservative surgery for stage 1B1 cervical cancer. *Gynecol Oncol* 2008; **111:** 438–43.

17 Landoni F, Parma G, Peiretti M, et al. Chemo-conization in early cervical cancer. *Gynecol Oncol* 2007; **107:** 125–26.

18 Rob L, Charvát M, Robová H, et al. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. *Int J Gynecol Cancer* 2007; **17:** 304–10.

19 Plante M. Vaginal radical trachelectomy: an update. *Gynecol Oncol* 2008; **111:** 105–10.

20 Carter J, Sonoda Y, Chi DS, et al. Radical trachelectomy for cervical cancer: postoperative physical and emotional adjustment concerns. *Gynecol Oncol* 2008; **111:** 151–57.

21 Carter J, Sonoda Y, Abu Rustum NR, et al. Reproductive concerns of women treated with radical trachelectomy for cervical cancer. *Gynecol Oncol* 2007; **105:** 13–16.

22 Shepherd JH, Spencer C, Herod J, et al. A radical vaginal trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer—cumulative pregnancy rate in a series of 123 women. *Br J Obstet Gynaecol* 2006; **113:** 719–24.

23 Shepherd JH, Milliken DA. Conservative surgery for carcinoma of the cervix. *Clin Oncol* 2008; **20:** 395–400.

24 Burnett AF, Roman LD, O'Meara AT, et al. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. *Gynecol Oncol* 2003; **88:** 419–23.

25 Mathevet P, Laszlo de Kaszon E, Dargent D. Fertility preservation in early cervical cancer. *Gynecol Obstet Fertil* 2003; **31:** 706–12.

26 Sonoda Y, Chi DS, Carter J, et al. Initial experience with Dargent's operation: the radical vaginal trachelectomy. *Gynecol Oncol* 2008; **108:** 214–19.

27 Diaz JP, Sonoda Y, Leitao MM, et al. Oncology outcome of fertility-sparing radical trachelectomy versus radical hysterectomy for stage IB1 cervical carcinoma. *Gynecol Oncol* 2008; **111:** 255–60.

28 Pahisa J, Alonso I, Torné A. Vaginal approaches to fertility-sparing surgery in invasive cervical cancer. *Gynecol Oncol* 2008; **110:** 29–32.

29 Chen Y, Xu H, Zhang Q, et al. A fertility-preserving option in early cervical carcinoma: laparoscopy-assisted vaginal radical trachelectomy and pelvic lymphadenectomy. *Eur J Obstet Gynecol Rep Biol* 2008; **136:** 90–93.

30 Hertel H, Köhler C, Grund D, et al. Radical vaginal trachelectomy (RVT) combined with laparoscopic pelvic lymphadenectomy: prospective multicentre study of 100 patients with early cervical cancer. *Gynecol Oncol* 2006; **103:** 506–11.

31 Marchiole P, Benchaib M, Buenerd A, et al. Oncological safety of laparoscopic-assisted vaginal radical trachelectomy (LARVT or Dargent's operation): a comparative study with laparoscopic-assisted vaginal radical hysterectomy (LARVH). *Gynecol Oncol* 2007; **106:** 132–41.

32 Plante M, Renaud MC, Harel F, et al. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An update series of 72 cases and review of the literature. *Gynecol Oncol* 2004; **94:** 614–23.

33 Plante M, Renaud MC, Grégorie J, et al. Outcome of 87 pregnancies following radical trachelectomy for the treatment of early-stage cervical cancer. *Gynecol Oncol* 2008; **108:** 92 (abstract 208).

34 Beiner ME, Covens A. Surgery insight: radical vaginal trachelectomy as a method of fertility preservation for cervical cancer. *Nat Clin Pract Oncol* 2007; **4:** 353–61.

35 Bernardini M, Barrett J, Seaword G, et al. Pregnancy outcome in patients post radical trachelectomy. *Am J Obstet Gynecol* 2003; **189:** 1378–82.

36 Beiner ME, Hauspy J, Rosen B, et al. Radical vaginal trachelectomy vs. radical hysterectomy for small early stage cervical cancer: a matched case-control study. *Gynecol Oncol* 2008; **110:** 168–71.

37 Burnett AF. Radical trachelectomy with laparoscopic lymphadenectomy: review of oncologic and obstetrical outcomes. *Curr Opin Obstet Gynecol* 2006; **18:** 8–13.

38 Schlaerth JB, Spirtos NM, Schlaerth AC. Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. *Am J Obstet Gynecol* 2003; **188:** 29–34.

39 Abu-Rustum NR, Neubauer N, Sonoda Y, et al. Surgical and pathological outcomes of fertility-sparing radical abdominal trachelectomy for FIGO stage 1B1 cervical cancer. *Gynecol Oncol* 2008; **111:** 261–64.

40 Abu-Rustum NR, Sonoda Y, Black D, et al. Fertility-sparing radical abdominal trachelectomy for cervical carcinoma: technique and review of the literature. *Gynecol Oncol* 2006; **103:** 807–13.

41 Pareja FR, Ramirez PT, Borrero FM, et al. Abdominal radical trachelectomy for invasive cervical cancer. A case series and literature review. *Gynecol Oncol* 2008; **111:** 555–60.

42 Olawaiye A, Del Carmen M, Tambouret R, et al. Abdominal radical trachelectomy: success and pitfalls in a general gynecologic oncology practice. *Gynecol Oncol* 2009; **112:** 506–10.

43 Nishio H, Fujii T, Kameyama K, et al. Abdominal radical trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer in a series of 61 women. *Gynecol Oncol* 2009; **115:** 51–55.

44 Cibula D, Slama J, Svarovsky J, et al. Abdominal radical trachelectomy in fertility sparing treatment of early stages cervical cancer. *Int J Gynecol Cancer* 2009; **19:** 1407–11.

45 Ungar I, Palfalvi I, Hogg R, et al. Abdominal radical trachelectomy: a fertility-preserving option for women with early cervical cancer. *Br J Obstet Gynecol* 2005; **112:** 366–69.

46 Ungar L, Palfalvi L, Smith JR, et al. Update on and long term follow-up of 91 abdominal radical trachelectomies. *Gynecol Oncol* 2006; **101:** 20 (abstr 42).

47 Dargent D, Martin X, Sacchetoni A, et al. Laparoscopic vaginal radical trachelectomy a treatment to preserve the fertility of cervical carcinoma patients. *Cancer* 2000; **88:** 1877–82.

48 Peppercorn PD, Jeyrajah AR, Woolas R, et al. Role of MR imaging in the selection of patients with early cervical carcinoma for fertility-preserving surgery: initial experience. *Radiology* 1999; **212:** 395–99.

49 Sahdev A, Sohaib SA, Wenaden AE, et al. The performance of magnetic resonance imaging in early cervical carcinoma: a long-term experience. *Int J Gynecol Cancer* 2007; **17:** 629–36.

50 Rob L, Pluta M, Strnad P, et al. A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. *Gynecol Oncol* 2008; **111:** 116–20.

51 Milliken D, Shepherd JH. Fertility preserving surgery for carcinoma of the cervix. *Curr Opin Oncol* 2008; **20:** 575–80.

52 De Souza NM, Dina R, McIndoe A, et al. Cervical cancer: value of an endovaginal coil magnetic resonance imaging technique in detecting small volume disease and assessing parametrial extension. *Gynecol Oncol* 2006; **102:** 80–85.

53 Wright JD, Dehdashti F, Herzog TJ, et al. Preoperative lymph node staging of early-stage cervical carcinoma by [18F]–fluoro-2-deoxy-D-glucose-positron emission tomography. *Cancer* 2005; **104:** 2484–91.

54 Rockall AG, Sohaib SA, Harisinghani NG, et al. Diagnostic performance of nano particle-enhanced MRI in the diagnosis of lymph node metastasis in patients with endometrial and cervical cancer. *J Clin Oncol* 2005; **23:** 2813–21.

55 Fischerova D, Cibula D, Stehnova H, et al. Transrectal ultrasound and magnetic resonance imaging in staging of early cervical cancer. *Int J Gynecol Cancer* 2008; **18:** 766–72.

56 Vinh-Hung V, Bourgain C, Vlastos G, et al. Prognostic value of histopathology and trends in cervical cancer: a SEER population study. *BMC* 2007; **7:** 164.

57 Zivanovic O, Leitao MM, Park JK, et al. Small cell neuroendocrine carcinoma of the cervix: analysis of outcome, recurrence pattern and the impact of platinum-based combination chemotherapy. *Gynecol Oncol* 2009; **112:** 590–93.

58 Singh N, Arif S. Histopathologic parameters of prognosis in cervical cancer—a review. *Int J Gynecol Cancer* 2004; **14:** 741–50.

59 Cibula D, Slama J, Fischerova D, et al. Update on abdominal radical trachelectomy. *Gynecol Oncol* 2008; **111:** 111–15.

60 Covens A. Preserving fertility in early cervical cancer with radical trachelectomy. *Contemp Obstet Gynecol* 2003; **2:** 46–66.

61 Wan XP, Yan Q, Xi XW, et al. Abdominal radical trachelectomy: two new surgical techniques for the conservation of uterine arteries. *Int J Gynecol Cancer* 2006; **16:** 1698–704.

62 Covens A, Rosen B, Murphy J, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? *Gynecol Oncol* 2002; **84:** 145–49.

63 Kinney WK, Hodge DO, Egorshin EV, et al. Identification of a low-risk subset of patients with stage 1B invasive squamous cancer of the cervix possibly suited to less radical surgery treatment. *Gynecol Oncol* 1995; **57:** 3–6.

64 Steed H, Capstick V, Schepansky A, et al. Early cervical cancer and parametrial involvement: is it significant? *Gynecol Oncol* 2006; **103:** 53–57.

65 Stegeman M, Louwen M, van der Velden J, et al. The incidence of parametrial tumor involvement in select patients with early cervix cancer is two low to justify parametrectomy. *Gynecol Oncol* 2007; **105:** 475–80.

66 Rob L, Strnad P, Robova H, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. *Gynecol Oncol* 2005; **98:** 281–88.

67 Strnad P, Robova H, Skapa P, et al. A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. *Gynecol Oncol* 2008; **109:** 280–84.

68 Benedetti Panici PL, Bellati F, Pastore M, et al. An update in neoadjuvant chemotherapy in cervical cancer. *Gynecol Oncol* 2007; **107:** 20–22.

69 Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer: a systematic review and meta-analysis of individual patient data from 21 randomised trials. *Eur J Cancer* 2003; **39:** 2470–86.

70 Jolley JA, Battista L, Wing DA. Management of pregnancy after radical trachelectomy: case reports and systematic review of the literature. *Am J Perinatol* 2007; **24:** 531–39.

71 Plante M, Renaud MC, Hoskins IA, et al. Vaginal radical trachelectomy: valuable fertility-preserving option in the management of early-stage cervical cancer. A series of 50 pregnancies and review of the literature. *Gynecol Oncol* 2005; **98:** 3–10.

72 Petignat P, Stan C, Megevand E, et al. Pregnancy after trachelectomy: a high-risk condition of preterm delivery. Report of a case and review of the literature. *Gynecol Oncol* 2004; **94:** 575–77.

73 Roman LD. Pregnancy after radical vaginal trachelectomy: maybe not such a risky undertaking after all. *Gynecol Oncol* 2005; **98:** 1–2.

74 Selo-Ojeme DO, Ind T, Shepherd JH. Isthmic stenosis following radical trachelectomy. *J Obstet Gynaecol* 2002; **22:** 327–28.

75 Noyes N, Abu-Rustum NR, Ramirez PT, et al. Options in the management of fertility-related issues after radical trachelectomy in patients with early cervical cancer. *Gynecol Oncol* 2009; **114:** 117–20.

Příloha XIII.

Rob L., Robová H., Chmel R., Komár M., Halaška M. a Škapa P. (2012) Surgical options in early cervical cancer. ***Int J Hyperthermia*** 28:489-500.

IF 1,923

Int. J. Hyperthermia, September 2012; 28(6): 489–500

informa
healthcare

REVIEW ARTICLE

Surgical options in early cervical cancer

LUKAS ROB[1], HELENA ROBOVA[1], ROMAN CHMEL[1], MATEJ KOMAR[1], MICHAEL HALASKA[1], & PETR SKAPA[2]

[1]*Division of Gynaecologic Oncology, Department of Obstetrics and Gynaecology, Second Medical Faculty, Charles University Prague, Czech Republic, and* [2]*Department of Pathological Anatomy and Molecular Medicine, Second Medical Faculty, Charles University Prague, Czech Republic*

(Received 3 February 2012; Revised 5 March 2012; Accepted 7 March 2012)

Abstract
Cancer of the cervix is the second most common cancer in women worldwide and the fourth leading cause of cancer mortality in women. Early cervical cancer stage IB1 includes a broad range of disease from clinically undetectable microinvasive cancer to bulky tumours that infiltrated the entire cervix. This article reviews the literature about risk factors and surgical radicality and fertility-sparing surgery in early cervical cancer. The review evaluates selection criteria, preoperative management and the most frequent surgical procedures used for individually tailored surgery for cervical cancer.

Keywords: *Cervical cancer, fertility-sparing surgery, individually tailored surgery, sentinel lymph node, surgical treatment*

Introduction

Cancer of the cervix is the second most common cancer in women worldwide and the fourth leading cause of cancer mortality in women. The latest epidemiologic data underline the heavy burden of cervical cancer: over half a million incident cases and over 300 000 cases of attributable deaths are predicted. Remarkable contrasts were observed on cervical cancer incidence and mortality between different continents, but also within them [1, 2]. These variations are possible to explain by different healthcare and different screening strategies. Because of the effective use of screening in the developing countries, an increasing number of women are being diagnosed with cervical cancer in an early stage of the disease. Individualisation of treatment to reduce therapy associated with early and late morbidity is the current trend in cervical cancer surgery [3–5]. The importance of this type of cancer becomes more apparent when considering that more than 54% of women diagnosed are younger than 50 years of age [6]. Focus is on chronic morbidity that influences quality of life (QoL) because curability of early stages is not a current problem. Despite that surgery and radiotherapy produce similar therapeutic outcomes for treatment of early cervical cancer, surgery remains the preferred primary treatment option, especially in young women. Surgery is probably chosen over radiotherapy because of the negative effects of radiation on ovarian function and vaginal integrity, better lubrication and vaginal elasticity and better quality of sexual life. It is well known that standard radical hysterectomy with pelvic lymphadenectomy can be accompanied by early and late postoperative morbidity [7–9]. Some onco-gynaecological groups performed different modifications (less radical) of radical hysterectomy in the past 30 years [7, 10]. Development of laparoscopy led to a renaissance of vaginal surgery (laparoscope assisted radical vaginal hysterectomy (LARVH) and vagina assisted radical laparoscopic

Correspondence: Lukas Rob, MD, PhD, Department of Obstetrics and Gynaecology, Second Medical Faculty, Charles University Prague, Vuvalu 84, 150 00 Prague 5, Czech Republic. Tel: +420 224 434 203. Fax: +420 224 434 218. E-mail: lukas.rob@lfmotol.cuni.cz

ISSN 0265–6736 print/ISSN 1464–5157 online
DOI: 10.3109/02656736.2012.675116

hysterectomy (VARLH)), as well as radical robotic surgery in the past 10 years [11–15]. Optimal surgical management of early stage cervical cancer should reduce early and late morbidity without compromising oncological outcome. The key question today is how to select patients. Type of surgical radicality in early cervical cancer should be a consequence of exact preoperative and intraoperative assessment of risk factors. The concept of preservation of autonomic nerves during radical hysterectomy and individually tailored less radical surgery, in comparison with standard radical hysterectomy, have led to a much improved QoL [16–25]. The authors have previously covered this topic in detail [3].

Another specific problem related to cervical cancer is that 25–40% of cases that are diagnosed in developed countries occur in women of reproductive age and in women that plan for pregnancy [1, 26]. For various reasons, many of these women may have postponed conception because of delayed childbearing. For decades, the only fertility-sparing surgical option for women who wished to retain reproductive function was cervical conisation in patients with stage IA1 cervical cancer. Different types of radical hysterectomy with pelvic lymphadenectomy or radiation therapy were the only options for women with cervical cancer of more than 3 mm invasion (FIGO stage IA2 and more). Pregnancy was not possible using either of these therapeutic approaches. In 1986, Professor Daniel Dargent was the first to undertake fertility-sparing surgery in patients with invasive cervical cancer [27]. Several fertility-saving procedures can be done in patients with cervical cancer, which in terms of surgical approach and extent of paracervical resection, were introduced during the next few years [28–42]. In the future, fertility preservation can be considered for more and more women with newly diagnosed cervical cancer.

Preoperative (work-up) assessment, patient selection

The extent of cervical cancer surgery should be individualised in accordance with tumour-related and patient-related factors. Preoperative identification of women with low risk of extra-cervical involvement of the pelvic lymph nodes and paracervix has been debated for years [41–51].

Cone biopsy with exact diameter of the tumour is essential for exact diagnosis of clinically undetectable early cervical cancer. Colposcopy is the standard examination in clinically detected tumours and is important in assessing the exocervical diameter as well as in excluding spread to the vagina. Clinical tumour size is an important prognostic factor (less than 2 cm, more than 4 cm). In our opinion, preoperative tumour volumometry is the most important preoperative prognostic factor [3]. Development of imaging methods, particularly magnetic resonance imagining (MRI) and ultrasonography (US), makes possible not only exact tumour measurement but also determines tumour volume (determination of the amount of cervical stroma infiltration and the amount of healthy stroma, determination of tumour growth in *anteroposterior*, craniocaudal and transverse directions or accurately determining residual disease after conisation) as an important preoperative prognostic factor [34, 50, 52]. Tumour volumometry is very important because it is impossible to compare tumour diameters exactly from MRI and histopathology. Increasing evidence suggests that it is also possible to identify women with a higher risk of metastatic involvement of lymph nodes and paracervical tissue in cervical cancer stage IB1. Women with tumours less than 2 cm in the largest diameter, or stroma infiltration of less than half or invasion less than 10 mm have a significantly lower risk of involvement of the paracervix and pelvic lymph nodes [43, 44, 46–50]. On the other hand, tumours with stromal invasion of more than two thirds or reaching the pericervical fascia or infiltrating the uterine corpus have a high risk of involvement of the paracervix and lymph nodes [44, 45].

Preoperative biopsy gives us information about some prognostics factors. Diagnosis of microcarcinoma is done by conisation and principal histopathological prognostic factors are tumour size, depth of invasion, lymphovascular or vascular invasion, perineural involvement and histopathological type [53]. It is important to exclude neuroendocrine tumours that have different management. Malignant neuroendocrine carcinomas (small cells, large cells) are aggressive neoplasms that often occur in younger patients. Treatment in these cases would start with chemotherapy [54]. When tumour is clinically evident, smaller biopsy would be performed, but information about some risk factors is limited (tumour diameter, LVSI and VSI or perineural involvement).

Pre- and intraoperative assessment of lymph nodes

The status of regional lymph nodes is the most important prognostic factor in early cervical cancer patients. Pelvic lymph node dissections are routinely performed as a part of the standard surgical treatment, except stage IA1 (carcinoma confined to the cervix, showing stromal invasion less than 3.0 mm and with a horizontal spread of 7.0 mm or less). Although lymphangiography, computed tomography and magnetic resonance imaging are commonly used

for lymph node assessment as a part of the treatment plan, systematic review and meta-analysis of the literature on the diagnostic accuracy show only moderate sensitivity and specificity to detect lymph node metastasis [55]. Similarly, 18F-fluorodeoxyglucose positron emission tomography is most accurate but had low sensitivity and positive predictive value for pretreatment lymph node staging [51, 56, 57]. In patients with early cervical cancer, imaging techniques such as computed tomography (CT), magnetic resonance imagining (MRI) and positron emission tomography (PET-CT) are insufficient to detect pelvic and parametrial lymph node metastases [51, 52, 55–59]. In patients with early cervical cancer small lymph node metastases (less than 7 mm) are more common and are not detectable by current imaging techniques. A new generation of PET-CT and MRI techniques that use ultra-small iron particles seems to be feasible for preoperative evaluation of lymph nodes [60]. The number of publications about sentinel lymph node biopsy (SLNB) has increased in the past 10 years. Early stages of the most common histological types (squamous and adenocarcinomas) metastasise primarily to lymph nodes (haematogenous spread is late). Currently, the sentinel lymph node mapping (SLNM) procedure has been incorporated in cervical cancer individually tailored surgery and fertility-sparing surgery management in many centres. The sentinel lymph node (SLN) is the first lymph node that receives direct drainage from the primary tumour and is useful in detecting lymph nodes with the highest metastatic risk. A number of studies have confirmed that SLNM is feasible and highly accurate in predicting the status of regional lymph nodes in early cervical cancer [49, 50, 61–63]. SLNs are currently detected by the application of two techniques: blue dye and radioactive tracer 99mTc or combination of both techniques. Radiocolloid is usually injected 2–4 h preoperatively (one-day protocol) or in the operating theatre in the beginning of general anaesthesia 15–30 min before detection (ultra-short protocol). The organisation of preoperative radiocolloid application and subsequent lymphoscintigraphy or single photon emission tomography (SPET)/CT is difficult and more expensive. Intraoperative ultra-short protocol without preoperative lymphoscintigraphy has a high detection rate, easier management of application and is cost effective [49, 50, 61]. Exclusion of preoperative lymphoscintigraphy and use of only intraoperative detection of SLNs by hand-held or laparoscopic gamma probe do not decrease the detection rate of SLNs. The techniques of application are very important for high detection rate. Injection of radiocolloid and blue dye is necessary to perform using a very thin needle peritumourally into the healthy surrounding tissue into all four quadrants. The particle size of the labelled colloid and time interval between application and detection are important. Similarly, no sentinel nodes have been detected in the paraaortal region for particles over 200 nm and for one-day protocol. A good application technique and good timing of blue dye injection allow the identification of SLNs and can identify and remove blue afferent lymphatic channels or nodes from the parametrium. These specimens are then sent separately to the histology laboratory for analysis. Identification of lymphatic channels and lymph nodes in paracervical tissue is possible only by visualisation using blue dye because measurement of activity is difficult because of the high activity in the cervix. A combination of both methods (radiocolloid and blue dye) is superior for these reasons. Detection of SLNs is decreased with increasing tumour volume [61, 63]. The role of SLN mapping in bulky cervical cancer is less clear and there is no clinical role for its use in the management of advanced disease. No difference in SLN detection has been observed between laparoscopy and laparotomy [61].

Sentinel lymph node – histopathological procedures

The TNM staging book (UICC, 6th edition) divides tumour deposits in SLNs into three categories: macrometastasis (diameter greater than 2 mm), micrometastasis (diameter greater than 0.2 mm but not greater than 2 mm) and isolated tumour cells (ITCs) (diameter 0.2 mm or less) [64]. The presence of macrometastatic or micrometastatic disease in SLNs is described as positivity in the N category of the TNM staging system. On the other hand, the clinical significance of ITCs is still unknown and SLNs containing ITCs are recommended to be interpreted as tumour free (N0). Sentinel nodes are sent for pathological evaluation as separate specimens. SLNs are traditionally evaluated postoperatively in haematoxylin-eosin (H&E) sections. The pathological assessment of SLN should primarily be aimed at identification of macrometastatic and micrometastatic tumour deposits because the detection of ITCs is still a question of experimental studies. Usually, macrometastases are easily detected by routine histopathological evaluation and serial sectioning is used to increase the sensitivity for the eventual detection of micrometastases and ITCs. However, there is no standard protocol today on pathologic assessment of SLN and the interval of sections varies widely. The identification of most micrometastases requires serial sectioning of the whole SLN at sections of 200–250 μm thickness [65]. Minimal requirements defined by cutting

sections between 0.5 mm and 1.0 mm [66] were established in axillary SLNs of breast cancer patients to avoid excessive laboratory workload. Immunohistochemistry (pan-cytokeratin staining) increases sensitivity of identification of tumour deposits in SLNs, but this sensitivity mostly falls into the category of ITCs because macrometastases and micrometastases are typically found in H&E sections [67, 68]. Therefore, the use of immunohistochemistry is questionable, and if it is applied, it is often restricted to cases difficult to interpret from H&E staining or to experimental studies.

The intraoperative assessment of SLN potentially modifies the surgical procedure and subsequent treatment management. Frozen sections (FSs) are used in this indication. Despite the obvious benefits of FS examination for the patient (especially reduction of extent and number of operations), this technique has some serious limitations. The intraoperative serial cutting of the entire SLN is not applicable because of the prolongation of operating time, technical limitations of the processing of frozen material, and loss of tissue for postoperative evaluation. Usually, small SLNs are processed in one piece, whereas larger ones are bisected. The number of levels taken from each tissue block is not standardised and varies between the departments. This algorithm of tissue sectioning in FS therefore enables one to reveal macrometastatic tumour deposits. However, it leads to reduced sensitivity in detecting micrometastases and results in false negatives [69, 70]. FS allows reliable detection of clinically important metastases in lymph nodes (metastases bigger than 2 mm). Furthermore, it fails only if the micrometastases are less than 2 mm and ITCs are diagnosed. Selection of SLN-positive patients allows preoperative modifications of the treatment, stops surgery and refers patients for either chemoradiotherapy or surgery that is more radical. In contrast, negative FS in early cervical cancer is an important part of individually tailored, less radical surgery. Sentinel node biopsy allows more precise histopathology evaluation of the 'high risk' nodes because serial sections and ultra-staging of whole material are impossible. In addition, Sentinel node biopsy allows more precise negativity determination of the evaluated 'high risk' lymph nodes.

Microinvasive carcinoma – management

Prognostically, the most favourable subgroup of patients with early stage cervical cancers is patients with microinvasive cancers. The International Federation of Gynaecology and Obstetrics (FIGO) classification of carcinoma of the cervix from 1994 was based on the histological definition of stage IA cervical cancer, which measures invasion depth and horizontal extension. In 2009, the new FIGO staging system was published. In this new version, the definition of stage IA was not changed [71]. Stage IA1 was defined as a tumour that invades to a depth of 3 mm or less with 7 mm or less horizontal spread. Stage IA2 was defined as stromal invasion of more than 3 mm and less than 5 mm with a horizontal spread of 7 mm or less. According to FIGO classification, an IA1 and IA2 can only be diagnosed microscopically, a cervical lesion should not be clinically visible and the involvement of vascular spaces (lymphatic, venous) does not change the stage, even though it has been accepted as a strong prognostic factor. In 1973, the SGO accepted a definition for microinvasive cervical cancer, where the criteria were depth of invasion less than 3 mm and the absence of LVSI without any limits of the tumour horizontal dimensions. Since the end of past century, numerous publications appeared about microcarcinoma that did not specify the horizontal diameter. In addition, some of these studies included tumours with a horizontal tumour spread of more than 7 mm or clinically detectable lesions, although these are FIGO stage IB1 and have a higher incidence of positive lymph nodes (these publications are cited in monographs). The true incidence of lymph node metastases from microinvasive cervical carcinomas was difficult to determine from these retrospective studies because they used different definitions of microcarcinomas. For exact evaluation, type of histopathological elaboration of lymph nodes – levelling and evaluation of angioinvasion are necessary. SLN mapping is a new phenomenon that allows serial section of the most risky lymph nodes and increases detection of micrometastases [67, 68]. Detection and utilisation of SLN mapping are not yet accepted in the FIGO classification of cervical cancer. Risk of positive lymph nodes in stage IA1 cervical cancer is less than 0.5% in cases without angioinvasion and increases to 1-2% in cases when angioinvasion is present. Recurrence risk in IA1 cervical cancer is less than 2% [72]. The majority of studies presented positive lymph nodes of from 3% to 7% in stage IA2 cervical cancer. If strict FIGO criteria for IA2 cancer are observed, Lushley et al. found a risk of positivity of lymph nodes of 0.5% with a recurrence rate of 2.9% [73]. When angioinvasion is not present in stage IA2, risk for lymph node involvement is very low [74]. Reviews about adenocarcinoma stage IA1 and IA2 (strict FIGO criteria) confirm a low incidence of positivity of lymph nodes, although it is still possible to find in the literature more radical management in adenocarcinoma than in squamous cell carcinoma. No difference was found in recurrence after conservative or radical

treatment (less than 2%) [73, 75]. Current data about fertility-sparing surgery confirm that adenocarcinoma is not necessarily managed more aggressively than squamous cancer. The question remains whether LVSI is a prognostic factor in microinvasive stage IA1 or IA2 for squamous cell carcinoma or adenocarcinoma. Controversy still remains whether LVSI affects the management of fertility-sparing surgery [26, 28–34, 69].

Stage IA1 (Figure 1A)

Conisation for the conservative treatment of stage IA1 cervical cancer is a standard treatment option in women wishing to retain their reproductive potential. However, we prefer needle electroexcision cone biopsy (NEEC) or large loop electroexcision of the transformation zone (LLETZ) or cold knife biopsy. Laser conisation has been replaced by high frequency techniques (e.g. needle cone biopsy or LLETZ). From a surgical and pathological point of view (no burnt margins), cold knife biopsy is the best procedure available, but it has the highest risk of preterm delivery. Therapeutic conisation would have a tumour-free surgical margin (exocervical and endocervical). If women do not plan pregnancy, simple hysterectomy is preferred. Hysterectomy in postmenopausal women would be the treatment of choice because stenosis of the endocervical canal is common after conisation and follow-up is limited. If LVSI is present, management is controversial. In our management, SLN mapping is recommended with extirpation only SLN. Complications associated with systematic pelvic lymphadenectomy predominate the risk of pelvic recurrence.

Stage IA2

Convincing data show that the risk of positive lymph nodes is low and positive findings of parametrial involvement are extremely rare in FIGO stage IA2. Risk of involvement of regional lymph nodes and risk of recurrence are the same as in squamous cell cancer as in adenocarcinoma [72–74]. Despite these data, many authorities recommend modified radical hysterectomy (type B) and if LVSI is not present, they suggest extrafascial hysterectomy (type A) with pelvic lymphadenectomy. Radical vaginal trachelectomy (RVT) with laparoscopic pelvic lymphadenectomy is usually indicated as fertility-preserving surgery [28–33, 72–75]. Less radical procedures (conisation and simple trachelectomy) with SLN mapping and laparoscopic pelvic lymphadenectomy are currently alternatives to more radical procedures. These less radical procedures have less morbidity and good pregnancy outcome [34, 49, 69, 76, 80]. Numbers of publications about minimal impact of parametrectomy and systemic lymphadenectomy in stage IA2 regardless of long-term morbidity of these procedures increase. Pilot studies of less radical procedures are already published [34, 49, 69, 76, 80]. SLN mapping with use of minimal invasive techniques and simple hysterectomy or conisation in women seeking pregnancy are logically a next step in individualised and less radical therapy in early stages cervical cancer. Prospective randomised studies that would compare conservative and radical procedures could not be realised because of the low number of patients with stage IA2. Clinical data that allow us to change the management of the treatment of stage IA2 disease include fertility-sparing surgery when the exact FIGO criteria can be retained.

Stage IB1

Stage IB1 includes a broad range of prognostic tumours. Further subgrouping of this stage is necessary for treatment management. Stage IB includes IB1 tumours with invasion less than 10 mm, less than half of stromal invasion and smaller than 20 mm at the largest diameter that have risk of involvement of lymph nodes 7–10% and where risk of parametrial involvement is less than 1% [3–5, 43, 50, 53, 61, 63]. In contrast, IB1 tumours larger than 30 mm that infiltrated more than two thirds of the cervical stroma or with infiltration of the lower uterine segment have a 30–40% risk of positive pelvic nodes and a risk of parametrial involvement of 10–30% [3, 44, 45, 53, 64]. These tumours are similar to stage IB2. Individualisation of treatment to reduce therapy (associated early and late morbidity) is the current trend in cervical cancer surgery. Extensive lymphadenectomy and paracervical resection (parametrectomy) are the main causes of postoperative complications [3, 64, 77]. The basic surgical approach for treatment of stage IB cervical cancer is radical hysterectomy and pelvic lymphadenectomy. The Piver–Rutledge–Smith classification published in 1974 has long been accepted by the medical community [78]. The anatomical landmarks, especially for radical hysterectomy type II–III, were not well defined within this classification system. The term radical hysterectomy was used for different surgical procedures with various levels of radicality. These anatomical landmarks were suggested by Querleu and Morrow in 2008 [79]. This classification, based on international discussions and the consensus of leading experts, divides radical hysterectomy into types A–D (Table I). Despite some objections to this classification, it is regarded as the most suitable classification of radical hysterectomies

and capable of accommodating new trends in cervical cancer surgery [3].

Stage IB1 – 'small volume tumours' (Figure 1B)

The first group includes IB1 tumours that do not fulfil criteria of stage IA2 (i.e. tumours less than 20 mm at the largest diameter, stromal invasion less than 10 mm and stromal infiltration less than half of the stroma) (Figure 1). This group is possible to identify through conisation, excisional biopsy and imaging methods, particularly MRI and ultrasonography in clinically clear cancers. These methods enable exact tumour volumometry (relation tumour and healthy tissue and the distance of the tumour from the pericervical fascia). In this subgroup the majority of patients do not present lymph node metastases and parametrial involvement and node negative patients are extremely rare. A large number of retrospective studies have shown that the incidence of lymph node positivity in this group is 7–10% and parametrial involvement in pelvic lymph node negative patients is less than 1% [5, 43, 46–49]. In their review, Stegeman et al. found only 0.6% of their patients with parametrial involvement [4]. Research over the past 10 years on SLN nodes has greatly expanded our knowledge on lymphatic propagation of early cervical cancer [49, 50, 61–63]. SLN mapping increases the reliability of lymph node negativity because of serial section of the most risky lymph nodes. Good timing of blue dye injection during SLN mapping makes possible the identification and removal of blue lymphatic channel and parametrial lymph nodes. In a prospective study Strnad et al. demonstrated that, if the SLNs are negative, the risk of tumour involvement of the paracervix is minimal in cervical cancer less than 20 mm in diameter and infiltrating less than one half of the cervical stroma [50]. In select early 'small' volume cervical cancer the combination of lymphatic mapping and SLNI makes it possible to perform less radical surgeries of the parametrial in SLN negative patients. A preliminary study showed that it is feasible and safe to reduce the radicality of parametrial resection when tumour volume is small, when the patients are sentinel node negative and when treatment associated with morbidity is low [69, 80,81]. However, extrafascial 'simple' hysterectomy (type A) in situations where future childbearing was not an issue or large cone or simple trachelectomy in SLN negative patients with laparoscopic or robotic lymphadenectomy is still an experimental alternative to modify radical hysterectomy (type B) or radical vaginal trachelectomy. In conclusion, we feel that there is growing evidence to reduce radicality in 'small' volume tumours. The incidence of parametrial tumour involvement in this subgroup of SLN negative patients is too low to justify parametrectomy.

Stage IB1 – 'more than small tumour volume and less than two thirds stromal invasion' (Figure 1C)

The majority of these IB1 tumours are diagnosed clinically (Figure 1). Preoperative MRI or ultrasonographic tumour volumometry can estimate quite exactly tumour extent in the uterine cervix. The involvement of pelvic nodes is from 12–24%. The extent of radical surgery should be individualised with the aim of minimising injury to the pelvic autonomic innervations. The first choice is to preserve the nerves with minimum damage and perform nerve-sparing radical hysterectomy (NSRH) type C1 (type III radical hysterectomy with NS in the old terminology). Studies of type III NSRH (C1) have increased over the years. Precise visualisation is important for exact identification of nerve fibres. NSRHs are performed by the abdominal approach. Laparoscopy and robotic surgery offers many advantages, with the greatest being improvement in visualisation. The schools of surgery that undertake NSRH differ, but all track the same idea: perioperative location and protection of the autonomic pelvic nerves [3].

A second approach to preserving nerves is to use less radical surgery with less radical resection of the paracervix: radical hysterectomy type B1 or B2 (type II radical hysterectomy or modified RH in the old terminology). Over the past three decades, several papers have described a large reduction in morbidity after modified RH compared with standard radical hysterectomy. Our onco-gynaecological school prefers nerve-sparing radical hysterectomy type C1 with SLN detection and frozen section in this group of patients. Fertility-sparing surgery is limited and controversial in women with tumours larger than 20 mm. Recurrence risk in these tumours is about 20% after radical abdominal trachelectomy or radical vaginal trachelectomy. A limitation of fertility-preserving surgery, which has been accepted by the majority of onco-gynaecological centres, is deep stromal invasion and tumours larger than 2 cm. The aim of neoadjuvant chemotherapy (NAC) is to reduce the size of the cervical tumour in order to preserve fertility. Few papers have been published on NAC before fertility-sparing surgery. Three cycles of high-dose density neoadjuvant chemotherapy are used in our centre. All protocols with NAC followed by fertility-sparing surgery are still experimental and hence it will be necessary to have a larger group of

patients to confirm oncological safety and pregnancy outcome.

Stage IB1 – 'more than two thirds stromal invasion' (Figure 1D)

Tumours infiltrating more than two thirds of cervical stroma and bigger than 30 mm are prognostically the most unfavourable and comparable to stage IB2. Tumours with stromal invasion of more than two thirds or reaching the pericervical fascia or infiltrating the low segment of uterine corpus (Figure 1) have a high risk of involvement of the paracervix (10–20%) and pelvic lymph nodes (30–40%).

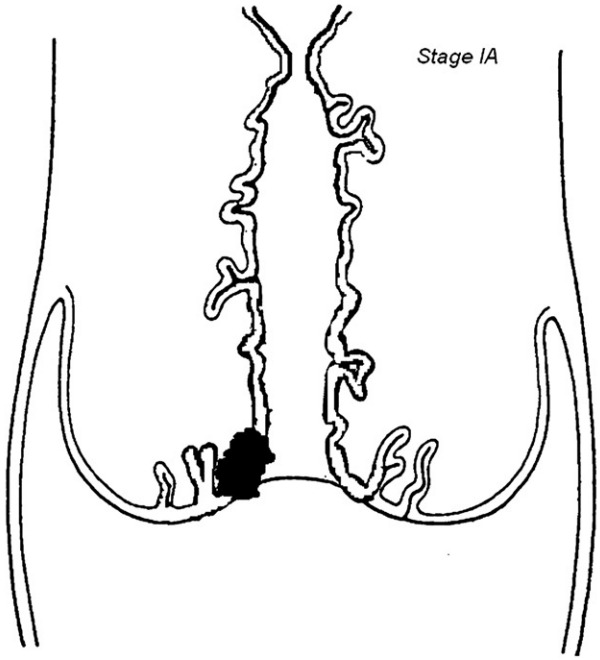


Figure 1. Stage IA.

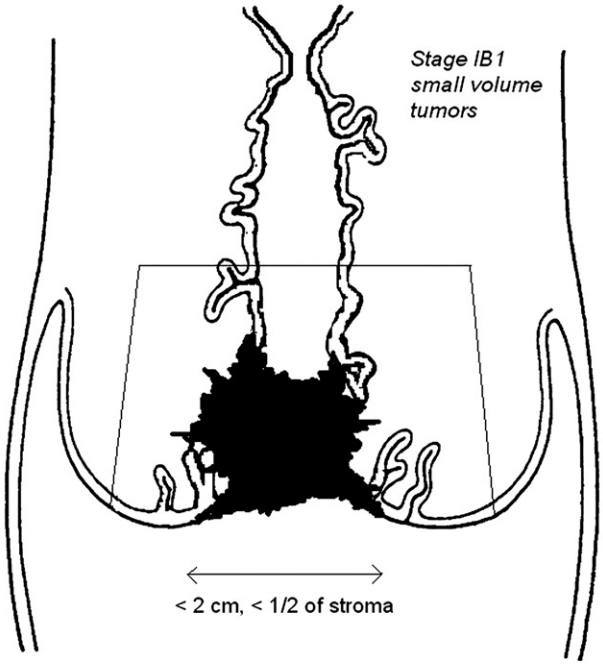


Figure 2. Stage IB1, small volume tumours.

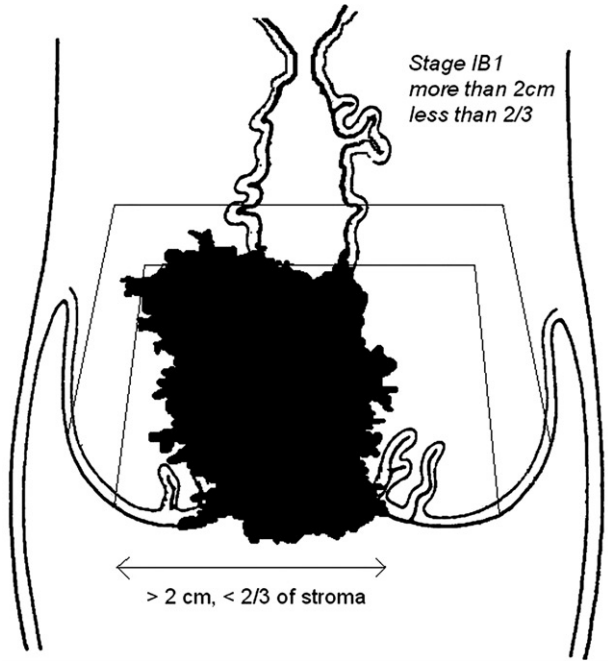


Figure 3. Stage IB1, less than 2/3 of stroma are more than 2 cm.

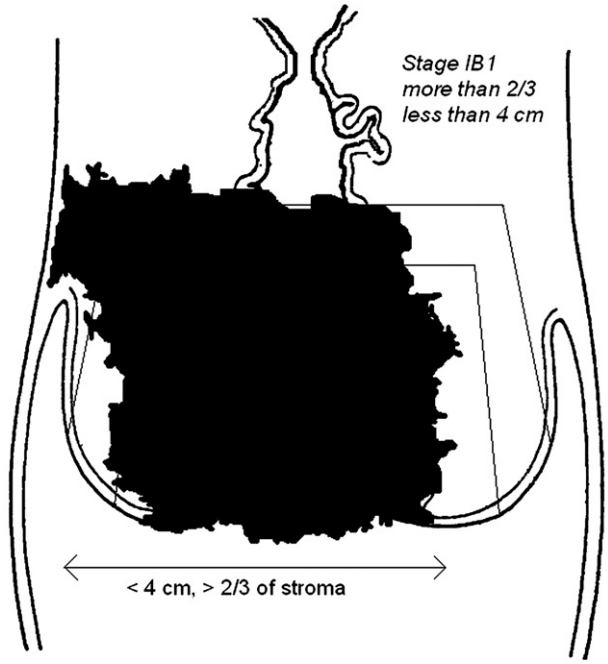


Figure 4. Stage IB1, more than 2/3 of stroma are less than 4 cm.

Table I. Classification of radical hysterectomy [79]

Type of hysterectomy	Resection extend	Ureter	Comment
A Minimum resection of paracervix	Paracervix is transected medial to ureter but lateral to the cervix Uterosacral and vesicouterine ligaments are not transected at a distance from the uterus Vaginal resection – generally at a minimum, without removal of the paracolpos	Palpation or direct visualisation without freeing from bed	
B Transection of paracervix at the ureter	Paracervix is transected at the level of the ureteral tunel Partial resection of uterosacral and vesicouterine ligaments No resection of caudal (deep) neural component of the paracervix (caudal to the deep uterine vein) Vaginal resection – at least 10 mm of the vagina from the cervix or tumour	Unroofed and rolled laterally	The border between paracervical and iliac (parietal) lymph-node dissection is obturator nerve (combination of paracervical and parietal LN dissection is comprehensive pelvic node dissection and may be equivalent to that of type C1 resection)
B1	B1 as described		
B2	B2 as described and with additional removal of the lateral paracervical lymph nodes		
C Transection of paracervix at junction with internal iliac vascular system	Transection of the uterosacral ligaments at the rectum Transection of the vesicouterine ligaments at the bladder Resection 15–20 mm of the vagina from the tumour or cervix and corresponding paracolpos	Completely mobilised	
C1	C1 with autonomic nerve sparing/ preservation		
C2	C2 without autonomic nerve sparing/ preservation		
D Laterally extended resection		Completely mobilised	
D1	D1 resection of the paracervix at the pelvic side wall with the vessels arise from the internal iliac system, exposing the roots of the sciatic nerve		
D2	D2 resection of the paracervix at the pelvic side wall with the hypogastric		

All types of radical hysterectomy are combined with lymph node dissection: Level 1, external and internal iliac level; Level 2, Level 1 + common iliac + presacral; Level 3, Level 2 + aortic infra-mesenteric; Level 4, Level 3 + aortic infra-renal.

The majority of tumours have the largest diameter of over 30 mm but less than 40 mm and thus do not fulfil the criteria for stage IB2. When primary radical hysterectomy is performed in cancers defined in this way, the majority of women are indicated for adjuvant radiotherapy or chemoradiotherapy based on the Gynecologic Oncology Group (GOG) criteria (intermediate or high-risk group). In this group decisions about treatment management are important from the perspective of oncological outcome and QoL. For these women, many onco-gynaecological centres prefer primary chemoradiotherapy to primary surgery with adjuvant radiotherapy, which is indicated in most patients. Chemoradiotherapy, especially in young women, has significant long-term morbidity (especially sexual), which decreases QoL [3, 8, 77]. A third alternative of treatment is neoadjuvant chemotherapy with consecutive radical hysterectomy that is used in some onco-gynaecological centres in 'bulky' cervical cancer with the aim of downstaging the disease before radical hysterectomy [83–86]. The main reason for neoadjuvant chemotherapy is to exclude any radiotherapy from treatment in young sexually active women. The key factor today is to select patients that benefit from surgery and that mostly benefit from chemoradiotherapy.

Patients with tumours infiltrating more than two thirds of the cervical stroma are not suitable for any current fertility-sparing procedures [85].

Conclusion

Optimal surgical management of early cervical cancer should reduce early and late morbidity without compromising oncological disease control. Individualisation of radicality in early cervical cancer should be based on preoperative assessment of risk factors (histopathology, tumour volumometry (US or MRI assessment of stromal infiltration), patient risk factors). SLNM and perioperative examinations of SLN are a new method that gives more precise information about the most risk LN. With high sensitivity, FS allows the detection of clinically important metastases (bigger than 2 mm) in lymph nodes (FS missed only micrometastases or ITCs). Intraoperative selection of SLN-positive patients enables modification of treatment during the procedure (terminate surgery and refer the patient for chemoradiotherapy or to perform more radical surgery). When FS is negative in early cervical cancer, individually tailored less radical surgery can be performed. Sentinel node biopsy allowed more precise histopathology evaluation of the 'high risk' nodes because serial sections and ultra-staging of whole material were impossible. SLN mapping is not still standard of care and would be used only in the studies. Surgical options are currently a complex decision-making process. Stage IB1 is constituted from prognostically different subgroups. Ultraconservative surgery (hysterectomy type A) is an experimental approach used for patients with stage IA2 and for 'small' IB1 cervical cancer with favourable prognostic factors and negative SLN. Fertility-sparing procedures (e.g. RVT or less radical simple trachelectomy or large cone with pelvic lymphadenectomy) in tumours less than 2 cm in diameter and less than half of stromal invasion are now considered safe surgical procedures. We still need to develop approaches for tumours larger than 2 cm in diameter but for now, the NAC approach seems to be the most promising. The incorporation of fertility-sparing surgery for cervical cancer in young women is one of the most important breakthroughs in onco-gynaecology in the past 20 years. For tumours with infiltration more than one half of stromal invasion, the concept of preservation of autonomic nerves during radical hysterectomy becomes standard in many onco-gynaecological centres. Tumours infiltrated more than two thirds of the cervical stroma, tumours with low uterine segment, and tumours that extend to the pericervical fascia are the most controversial. These tumours are not yet staged but are very similar with respect to risk factors or management. The key question today is to select patients that will benefit from surgery or chemoradiotherapy.

Declaration of interest: This work is supported by grant IGA MZ CR NS9914-4. The authors alone are responsible for the content and writing of the paper.

References

1. Arbyn M, Autier P, Ferlay J. Burden of cervical cancer in the 27 member states of the European Union: Estimates for 2004. Ann Oncol 2007;18:1423–1425.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–2916.
3. Rob L, Halaska M, Robova H. Nerve-sparing and individually tailored surgery for cervical cancer. Lancet Oncol 2010;11:292–301.
4. Stegeman M, Louwen M, van der Velden J, ten Kate FJ, den Bakker MA, Burger CW, et al. The incidence of parametrial tumor involvement in select patients with early cervix cancer is too low to justify parametrectomy. Gynecol Oncol 2007;105:475–480.
5. Frumovitz M, Sun CHC, Schmeler KM, Deavers MT, Dos Reis R, Levenback CF, et al. Parametrial involvement in radical hysterectomy specimens for women with early-stage cervical cancer. Obstet Gynecol 2009;114:93–99.
6. Watson M, Saraiya M, Benard V, Conghlin SS, Flowers L, Cokkinides V, et al. Burden of cervical cancer in the United States, 1998–2003. Cancer 2008;113:2855–2864.
7. Raspagliesi F, Ditto A, Fontanelli R, Zanaboni F, Solima E, Spatti G, et al. Type II versus type III nerve-sparing radical hysterectomy: Comparison of lower urinary tract dysfunctions. Gynecol Oncol 2006;102:256–262.
8. Bergmark K, Avall-Lundquist E, Dickman PW, Henningsohn L, Steineck G. Vaginal changes and sexuality in women with a history of cervical cancer. New Engl J Med 1999;340:1383–1389.
9. Brown JS, Sawaya G, Thom DH, Grady D. Hysterectomy and urinary incontinence: A systematic review. Lancet 2000;356:535–539.
10. Margina JF, Goodrich MA, Weaver AL, Podratz KC. Modified radical hysterectomy: Morbidity and mortality. Gynecol Oncol 1995;59:277–282.
11. Spirtos NM, Schlaerth JB, Kimball RE, Leiphart VM, Ballon SC. Laparoscopic radical hysterectomy (type III) with aortic and pelvic lymphadenectomy. Am J Obstet Gynecol 1996;174:1767–1768.
12. Zakashansky K, Chuang L, Gretz H, Nagarshetn NP, Rahaman J, Nezhat FR. A case controlled study of total laparoscopic radical hysterectomy with pelvic lymphadenectomy versus radical abdominal hysterectomy in a fellowship training program. Int J Gynecol Cancer 2007;17:1075–1082.
13. Possover M, Quakernack J, Chiantera V. The LANN technique to reduce postoperative functional morbidity in laparoscopic radical pelvic surgery. J Am Coll Surg 2005;201:913–917.
14. Soliman PT, Frunovitz M, Sun CC, Dos Reis R, Smeler KM, Westin SN, et al. Radical hysterectomy: A comparison of

surgical approaches after adoption of robotic surgery in gynaecologic oncology. Gynecol Oncol 2011;123:333–336.
15. Tinelli R, Malzoni M, Cosentino F, Perone C, Fusco A, Cicinelli E, et al. Robotic versus laparoscopic radical hysterectomy with lymphadenectomy in patients with early cervical cancer: A multicenter study. Ann Surg Oncol 2011;18:2622–2628.
16. Maas CP, Trimbos JB, De Ruiter MC, van de Velde CJH, Kenter GG. Nerve sparing radical hysterectomy: Latest developments and historical perspective. Crit Rev Oncol 2003;48:271–279.
17. Höckel M, Konerding M, Heussel CP. Liposuction-assisted nerve-sparing extended radical hysterectomy: Oncologic rationale, surgical anatomy, and feasibility study. Am J Obstet Gynecol 1998;178:971–976.
18. Fujii S, Takakura K, Matsumura N, Higuchi T, Yura S, Mandai M, et al. Anatomic identification and functional outcomes of the nerve sparing Okabayashi radical hysterectomy. Gynecol Oncol 2007;107:4–13.
19. Sakuragi N, Todo Y, Kudo M, Yamamoto R, Sato T. A systematic nerve-sparing radical hysterectomy technique in invasive cervical cancer for preserving postsurgical bladder function. Int J Gynecol Cancer 2005;15:389–397.
20. Trimbos JB, van den Tillaart HM, Maas CP, Peters AAW, Gaarenstroom KN, Kenter GG, et al. The swift operation: A modification of the Leiden nerve-sparing radical hysterectomy. Gynecol Surg 2008;5:193–198.
21. Raspagliesi F, Ditto A, Fontanelli R, Solima E, Hanzozet F, Zanaboni F, et al. Nerve-sparing radical hysterectomy: A surgical technique for preserving the autonomic hypogastric nerve. Gynecol Oncol 2004;93:307–314.
22. Höckel M, Horn LC, Hentschel B, Höckel S, Naumann G. Total mesometrial resection: High resolution nerve-sparing radical hysterectomy based on developmentally defined surgical anatomy. Int J Gynecol Cancer 2003;13:791–803.
23. Kato T, Murakami G, Yabuki Y. A new prospective on nerve-sparing radical hysterectomy: Nerve topography and over-preservation of the cardinal ligament. Jpn J Clin Oncol 2003;33:589–591.
24. Mantzaris G, Rodolakis A, Vlachos G, Athanasiou S, Theocharis S, Sotiripoulou CHM, et al. Magnifying lenses assisted nerve-sparing radical hysterectomy and prevention of nerve plexus trauma. Int J Gynecol Cancer 2008;18:868–75.
25. Querleu D, Narducci F, Poulard V, Lacaze S, Occeli B, Leblanc E, et al. Modified radical vaginal hysterectomy with or without laparoscopic nerve-sparing dissection: A comparative study. Gynecol Oncol 2002;85:154–158.
26. Sonoda Y, Abu-Rustum NR, Gemignani ML, Nadeem R, Mary L, Dennis SCHI, et al. A fertility sparing alternative to radical hysterectomy: How many patients may be eligible? Gynecol Oncol 2004;95:534–538.
27. Dargent D, Burn JL, Roy M, Remi I. Pregnancies following radical trachelectomy for invasive cervical cancer (Abstract 14). Gynecol Oncol 1994;52:105.
28. Shepherd JH, Crawford RAF, Oram DH. Radical trachelectomy: A way to preserve fertility in the treatment of early cervical cancer. Br J Obstet Gynaecol 1998;105:912–916.
29. Covens A, Shaw P, Murphy J, Petrillo D, Lickrish G, Rosen B, et al. Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA–B carcinoma of the cervix? Cancer 1999;86:2273–2279.
30. Smith JR, Boyle DC, Corless DJ, Lawson AD, Ungar L, Del Priore G, et al. Abdominal radical trachelectomy: A new surgical technique for the conservative management of cervical carcinoma. Br J Obstet Gynaecol 1997;104:1196–1200.
31. Kim JH, Park JY, Kim DY, Kim YM, Kim YT, Nam JH. Fertility-sparing laparoscopic radical trachelectomy for young women with early stage cervical cancer. Br J Obstet Gynaecol 2010;117:340–347.
32. Ramirez PT, Schmeler KM, Malpiga A, Soliman PT. Safety and feasibility of robotic radical trachelectomy in patients with early-stage cervical cancer. Gynecol Oncol 2010;116:512–515.
33. Persson J, Kannisto P, Bossmar T. Robot-assisted abdominal laparoscopic radical trachelectomy. Gynecol Oncol 2008;111:564–567.
34. Rob L, Charvát M, Robová H, Pluta M, Strnad P, Hrehorcak M, et al. Less radical fertility-sparing surgery than radical trachelectomy in early cervical cancer. Int J Gynecol Cancer 2007;17:304–310.
35. Landoni F, Parma G, Peiretti M, Zanagnolo V, Sideri M, Colombo N, et al. Chemo-conization in early cervical cancer. Gynecol Oncol 2007;107:S125–126.
36. Plante M, Lau S, Brydon L, Swenerton K, LeBlanc R, Roy M. Neoadjuvant chemotherapy followed by vaginal radical trachelectomy in bulky stage 1B1 cervical cancer: Case report. Gynecol Oncol 2006;101:367–370.
37. Robova H, Pluta M, Hrehorcak M, Skapa P, Rob L. High dose density chemotherapy followed by simple trachelectomy: Full-term pregnancy. Int J Gynecol Cancer 2008;18:1367–1371.
38. Maneo A, Chiari S, Bonazzi C, Mangioni C. Neoadjuvant chemotherapy and conservative surgery for stage 1B1 cervical cancer. Gynecol Oncol 2008;111:438–443.
39. Plante M. Vaginal radical trachelectomy: An update. Gynecol Oncol 2008;111:S105–110.
40. Shepherd JH, Spencer C, Herod J, Te I. A radical vaginal trachelectomy as a fertility-sparing procedure in women with early-stage cervical cancer cumulative pregnancy rate in a series of 123 women. Br J Obstet Gynaecol 2006;113:719–724.
41. Diaz JP, Sonoda Y, Leitaio MM, Zivanovic O, Brown CL, Chi DS, et al. Oncologic outcome of fertility-sparing radical trachelectomy versus radical hysterectomy for stage IB1 cervical carcinoma. Gynecol Oncol 2008;111:255–260.
42. Smith AL, Frumowitz M, Schmeler KM, dos Reis R, Nick AM, Coleman RL, et al. Conservative surgery in early-stage cervical cancer: What percentage of patients may be eligible for conization and lymphadenectomy? Gynecol Oncol 2010;119:183–186.
43. Kinney MM, Hodge DO, Egorshin EV, Ballard DJ, Podratz KC. Identification of low risk subset of patients with stages IB invasive squamous cancer of the cervix possibly suited to less radical surgical treatment. Gynecol Oncol 1995;57:3–6.
44. Benedetti-Panici P, Angioli R, Palaia I, Muzii L, Zullo MA, Manci N, et al. Tailoring the parametrectomy in stages IA2–IB1 cervical carcinoma: Is it feasible and safe? Gynecol Oncol 2005;96:792–798.
45. Landoni F, Bocciolone L, Perego P, Maneo A, Bratina G, Mangioni C. Cancer of cervix, FIGO stages IB and IIA: Patterns of local growth and paracervical extension. Int J Gynecol Oncol 1995;5:329–334.
46. Covens A, Rosen B, Murphy J, Laframboise S, DePetrillo AD, Lickrish G, et al. How important is removal of the parametrium at surgery for carcinoma of the cervix? Gynecol Oncol 2002;84:145–149.
47. Steed H, Capstick V, Schepansky A, Honore L, Hiltz M, Faught W. Early cervical cancer and parametrial involvement: Is it significant? Gynecol Oncol 2006;103:53–57.
48. Puente R, Guzman S, Israel E, Poblete MT. Do the pelvic lymph nodes predict the parametrial status in cervical cancer stages IB-IIA? Int J Gynecol Cancer 2004;14:832–840.

49. Rob L, Charvat M, Robova H, Strnad P, Pluta M, Halaska M, et al. Sentinel lymph node identification (SLNI) in the management of conservative surgery in early cervical cancer: Is it acceptable? Gynecol Oncol 2005;99:S147–S148.
50. Strnad P, Robova H, Skapa P, Pluta M, Hrehorcak M, Halaska M, et al. A prospective study of sentinel lymph node status and parametrial involvement in patients with small tumour volume cervical cancer. Gynecol Oncol 2008;109:280–284.
51. Signorelli M, Guerra L, Montanelli L, Crivellaro C, Buda A, Dell'Anna T, et al. Preoperative staging of cervical cancer: Is 18-FDG-PET/CT really effective with early stage disease? Gynecol Oncol 2011;123:236–240.
52. Fischerova D, Cibula D, Stehnova H, Vondrichova H, Calda P, Zikan M, et al. Transrectal ultrasound and magnetic resonance imaging in staging of early cervical cancer. Int J Gynecol Cancer 2008;18:766–772.
53. Singh N, Arif S. Histopathologic parameters of prognosis in cervical cancer – A review. Int J Gynecol Cancer 2004;14:741–750.
54. Zivanovic O, Leitao MM, Park JK, Zhao H, Diaz JP, Konner J, et al. Small cell neuroendocrine carcinoma of the cervix: Analysis of outcome, recurrence pattern and the impact of platinum-based combination chemotherapy. Gynecol Oncol 2009;112(3):590–593.
55. Selman TJ, Mann C, Zamora J, Appleyard TL, Khan K. Diagnostic accuracy of test for lymph node status in primary cervical cancer: A systematic review and meta-analysis. CMAJ 2008;178(7):855–862.
56. Bernardini MQ, Covens A. Imaging of lymph node metastases in cervical cancer. CMAJ 2008;178:67–69.
57. Wright JD, Dehdashti F, Herzog TJ, Much DG, Huettner PC, Rader JS, et al. Preoperative lymph node staging of early-stage cervical carcinoma by /18F/-Fluoro-2-Deoxy-D-Glucose-Positron Emission Tomography. Cancer 2005;104:2484–2491.
58. De Souza NM, Dina R, McIndoe A, Soutter WP. Cervical cancer: Value of an endovaginal coil magnetic resonance imaging technique in detecting small volume disease and assessing parametrial extension. Gynecol Oncol 2006;102:80–85.
59. Sahdev A, Sohaib SA, Wenaden AE, Shepherd JH, Reznek RH. The performance of magnetic resonance imaging in early cervical carcinoma: A long-term experience. Int J Gynecol Cancer 2007;17:629–636.
60. Rockall AG, Sohaib SA, Harisinghani NG, Barbar SA, Singh N, Oram DH, et al. Diagnostic performance of nano particle-enhanced MRI in the diagnosis of lymph node metastasis in patients with endometrial and cervical cancer. J Clin Oncol 2005;23:2813–2821.
61. Rob L, Strnad P, Robova H, Charvat M, Pluta M, Schlegerova D, et al. Study of lymphatic mapping and sentinel node identification in early stage cervical cancer. Gynecol Oncol 2005;98:281–288.
62. Marnitz S, Köhler C, Bongardt S, Braig U, Hertel H, Schneider A, et al. Topographic distribution of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol 2006;103:35–44.
63. Altgassen C, Hertel H, Brandstädt A, Köhler CH, Dürst M, Schneider A. Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO study group. J Clin Oncol 2008;26:2943–2951.
64. Sobin LH, Gosporarowicz MK, Wittekind CH. TNM classification of malignant tumours, 7th edition. Wiley-Blackwell 2099; ISBN 978-1-4443-3241-4: 167-171.
65. Cserni G. A model for determining the optimum histology of sentinel lymph nodes in breast cancer. J Clin Pathol 2004;57:467–471.
66. Cserni G, Amendoeira I, Apostolikas N, Bellocq JP, Bianchi S, Boecker W, et al. Discrepancies in current practice of pathological evaluation of sentinel lymph nodes in breast cancer. Results of a questionnaire based survey by the European Working Group for Breast Screening Pathology. J Clin Pathol 2004;57:695–701.
67. Moore RG, Granai CO, Gajewski W, Gordinier M, Steinhoff MM. Pathologic evaluation of inguinal sentinel lymph nodes in vulvar cancer patients: A comparison of immunohistochemical staining versus ultrastaging with hematoxylin and eosin staining. Gynecol Oncol 2003;91:378–382.
68. Euscher ED, Malpica A, Atkinson EN, Levenback CF, Frumovitz M, Deavers MT. Ultrastaging improves detection of metastases in sentinel lymph nodes of uterine cervix squamous cell carcinoma. Am J Surg Pathol 2008;32:1336–1343.
69. Pluta M, Rob L, Charvat M, Chmel R, Halaska M, Skapa P, et al. Less radical surgery than radical hysterectomy in early stage cervical cancer: A pilot study. Gynecol Oncol 2009;113:181–184.
70. Fader AN, Edwards RP, Cost M, Shakir AK, Kelley JL, Shwartz B, et al. Sentinel lymph node biopsy in early-stage cervical cancer: Utility of intraoperative versus postoperative assessment. Gynecol Oncol 2008;111:13–17.
71. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynecol Obstet 2009;105:107–108.
72. Bisseling KC, Bekkers RL, Rome RM, Quinn MA. Treatment of microinvasive adenocarcinoma of the uterine cervix: A retrospective study and review of the literature. Gynecol Oncol 2007;107:424–430.
73. Rogers LJ, Lusley DM. Stage IA2 Cervical carcinoma. How much treatment is enough? Int J Gynecol Cancer 2009;19:1620–1624.
74. van Meurs H, Visser O, Buist MR, ten Kate FJW, van der Velden J. Frequency of pelvic lypmh node metastases and parametrial involvement in stage IA2 cervical cancer. Int J Gynecol Cancer 2009;19:21–26.
75. Baalbergen A, Smedts F, Helmerhorst TJM. Conservative therapy in microinvasive adenocarcinoma of the uterine cervix is justified. An Analysis of 59 cases and a review of the literature. Int J Gynecol Cancer 2011;21:1640–1645.
76. Rob L, Pluta M, Strnad P, Hrehorcak M, Chmel R, Skapa P, et al. A less radical treatment option to the fertility-sparing radical trachelectomy in patients with stage I cervical cancer. Gynecol Oncol 2008;111:S116–S120.
77. Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. Lancet Oncol 2011;12:192–200.
78. Butler-Manuel SA, Buttery LD, A'Hern RP, Polak JM, Barton DP. Pelvic nerve plexus trauma at radical hysterectomy and simple hysterectomy. Cancer 2000;89:834–841.
79. Piver MS, Rutledge F, Smith JP. Five classes of extended hysterectomy for women with cervical cancer. Obstet Gynecol 1974;44:265–272.
80. Querleu D, Morrow CP. Classification of radical hysterectomy. Lancet Oncol 2008;9:297–303.
81. Landoni F, Maneo A, Cormio G, Perego P, Milani R, Caruso O, et al. Class II versus class III radical hysterectonomy in stage IB-IIA cervical cancer: A prospective randomised study. Gynecol Oncol 2001;80:3–12.
82. Benedetti Panici PL, Bellati F, Pastore M, Manci N, Musella A, Pauselli S, et al. An update in neoadjuvant chemotherapy in cervical cancer. Gynecol Oncol 2007;107:S20–22.
83. Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration. Neoadjuvant chemotherapy for locally advanced cervical cancer: A systematic

review and meta-analysis of individual patient data from 21 randomised trials. Eur J Cancer 2003;39:2470–2486.
84. Robova H, Halaska M, Pluta M, Skapa P, Strnad P, Lisy J, et al. The role of neoadjuvant chemotherapy and surgery in cervical cancer. Int J Gynecol Cancer 2010;20:S42–46.
85. Eddy GL, Manetta A, Alvarez RD, Williams L, Creasman WT. Neoadjuvant chemotherapy with vincristine and cisplatin followed by radical hysterectomy and pelvic lymphadenectomy for FIGO stage IB bulky cervical cancer: A Gynecologic Oncology Group pilot study. Gynecol Oncol 1995;57:412–416.

Příloha XIV.

Robová H., Halaška M., Pluta M., Škapa P., Matěcha J., Lisý J., Rob L. (2014) Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer. ***Gynecol Oncol*** (v tisku)

IF 3,687

Gynecologic Oncology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer

Helena Robova [a], Michael J. Halaska [a], Marek Pluta [a], Petr Skapa [b], Jan Matecha [a], Jiri Lisy [c], Lukas Rob [a,*]

[a] Department of Obstetrics and Gynecology, 2nd Medical Faculty Charles University Prague, Czech Republic
[b] Department of Pathology and Molecular Medicine, 2nd Medical Faculty Charles University Prague, Czech Republic
[c] Department of Radiology, 2nd Medical Faculty Charles University Prague, Czech Republic

HIGHLIGHTS

- Prospective study on neoadjuvant chemotherapy in cervical cancer larger then 2 cm before fertility sparing surgery, oncologic and fertility results.
- Evaluation of oncological results: adverse events, response for neoadjuvant chemotherapy, recurrence rate.
- Evaluation of pregnancy results after neoadjuvant chemotherapy followed by laparoscopoic pelvic lymphadenectomy and simple trachelectomy.

ARTICLE INFO

Article history:
Received 19 April 2014
Accepted 15 August 2014
Available online xxxx

Keywords:
Fertility-sparing surgery
Neoadjuvant chemotherapy
Trachelectomy
Pregnancy outcome
Fertility outcome
Cervical cancer

ABSTRACT

Objective. 28 women under 35 years with early-stage cervical cancer and strong desire for fertility preservation that do not fulfil standard criteria for fertility-sparing surgery (tumour larger than 2 cm or with deep of infiltration more than half of stroma) were included in prospective study.

Methods. Dose-dense neoadjuvant chemotherapy (NAC) was performed on all 28 patients in 10-day intervals: cisplatin plus ifosfamide in squamous cell cancer (15 women—53.6%) or cisplatin plus doxorubicin in adenocarcinoma (13 women—46.3%). Patients underwent laparoscopic lymphadenectomy and vaginal simple trachelectomy after NAC. Patients with positive lymph nodes or inadequate free surgical margins underwent radical hysterectomy.

Results. No residual disease was found in 6 women (21.4%), microscopic disease was observed in 11 women (39.3%) and macroscopic tumour in was observed in 11 women (39.3%). Ten women (35.7%) lost fertility. Four women (20%) after fertility-sparing surgery recurred, two died of the disease (10%). Fertility was spared in 20 (71.4%) women and 10 of them became pregnant (50%). Eight women delivered ten babies (6 term and four preterm deliveries). There were two miscarriages in second trimester (in one woman) and one in first trimester. One woman underwent four unsuccessful cycles of IVF, one failed to become pregnant and one recurred too early. Two women underwent chemoradiotherapy for recurrence and lost chance for pregnancy.

Conclusions. Downstaging by NAC in IB1 and IB2 cervical cancer before fertility-sparing surgery is still an experimental procedure, but shows some promise. Long-term results in relation to oncological outcome for this concept are still needed.

Introduction

The mean age of women at the time of first delivery has increased in the past decade, which, inadvertently, has led to an increase in the number of women with cervical cancer who wish to preserve their reproductive potential to achieve pregnancy in the future. Vaginal radical trachelectomy (VRT), abdominal radical trachelectomy (ART) or simple trachelectomy are safe fertility procedures for the treatment of women with lymph node-negative early-stage cervical cancer. One of the limitations of fertility-preserving surgery is deep stromal invasion and a tumour larger than 2 cm [1,2]. The recurrence rate in women with a tumour larger than 2 cm is 20.8% after VRT and 20% after ART [3]. Cervical cancer is usually chemosensitive on a platinum-based combination of agents. Some centres use neoadjuvant chemotherapy (NAC) in "bulky" cervical cancer to downstage tumour before radical surgery. The response rate of NAC in advanced cervical cancer is between 60 and 95% [4–6]. NAC can decrease tumour volume before surgery, which enables the complete removal of the tumour with negative margins whilst

* Corresponding author at: Department of Obstetrics and Gynecology, 2nd Medical Faculty, Charles University, V uvalu 84, 150 00 Prague 5, Czech Republic.
E-mail address: lukas.rob@lfmotol.cuni.cz (L. Rob).

http://dx.doi.org/10.1016/j.ygyno.2014.08.021
0090-8258/

preserving an adequate amount of cervical tissue. Such a procedure improves the chances for successful pregnancy. This is a prospective study of women with early-stage cervical cancer with tumours that exceeded 2 cm in the largest diameter or infiltrated more than half of the cervical stroma and who had undergone high-dose density neoadjuvant chemotherapy.

Material and methods

Patients with previously untreated histologically confirmed invasive squamous cell carcinoma, adenocarcinoma or adenosquamous cervical carcinoma IB infiltrating more than half and less than two thirds of the cervical stroma and larger than 2 cm in the largest diameter were eligible. All (small and large cell) neuroendocrine or adenocarcinoma with neuroendocrine components were excluded. All women were in fertility age (40 years and younger) and expressed a strong desire to spare their fertility. All women underwent magnetic resonance imagining (MRI) and vaginal ultrasonography (US) examination to evaluate tumour size and volume as well as to assess pelvic lymph node status. Patients with clinically (MRI or US) positive lymph nodes and with tumour that infiltrated more than two thirds of cervical stroma were excluded from the study. In addition, a chest radiograph, blood count and biochemistry (liver function, creatinine clearance and iontogram) were performed. The study was approved by the local ethical committee. Informed consent was obtained from each patient before the initiation of study procedures.

The patients were treated with three cycles of dose-dense NAC using an interval of 10–14 days. A combination of cisplatin (75 mg/m^2) and ifosfamide (2 g/m^2, maximal total dose 3 g) was used in squamous cell carcinoma and cisplatin (75 mg/m^2) plus doxorubicine (35 mg/m^2) in all adenocarcinomas. Blood count and biochemistry were done before each cycle of chemotherapy and before surgery. Adverse effects of chemotherapy were graded with the WHO classification. US or MRI, or both were performed after a 3rd cycle of chemotherapy to evaluate response to chemotherapy.

Laparoscopy with sentinel lymph node (SLN) mapping was performed in all patients. SLNs were sent for frozen section analysis. If the sentinel nodes were negative on frozen section, laparoscopic pelvic lymphadenectomy was completed. If the definitive histopathological evaluation after 7 days did not detect metastases in the lymph nodes, simple trachelectomy was performed. At least two thirds of cervical stroma was removed by simple trachelectomy in all cases (8–10 mm of cervical stroma was preserved). Frozen section was not performed during trachelectomy to identify close margins. Ultramicrostaging of SLNs and standard evaluation of other nodes were performed. In case of positive SLNs or closed margins (less than 8 mm of tumour free tissue) in the trachelectomy specimens radical hysterectomy type C2 with pelvic and low paraaortal lymphadenectomy was performed. A pathologist with experience in oncogynaecology evaluated all histopathological specimens.

The patients remained on a regular follow-up every third month that included clinical examination colposcopy and Pap smear and every six months HPV-HR testing was performed. In addition, vaginal US and MRI were performed every six months and if any abnormalities were detected, MRI is indicated.

Results

Written informed consent was signed by the 28 women between April 2005 and December 2013. Nine patients previously published were included into study and pregnancy data was actualised [4]. Of these 28 women, 26 were nulliparous. The mean age of the women was 28.6 years (range 15–34 years). Fifteen women (53.6%) were diagnosed with squamous cell cancer and 13 (46.4%) were diagnosed with adenocarcinoma. Lymphovascular space involvement (LVSI) presented in 11 women (39.3%), negative LVSI in 7 (25%) and in 10 (37.7%) LVSI was not possible to determine because only punch biopsy was done as the means of diagnosis. Of the 28 women, 21 had stage IB1 cervical cancer (75%) and 7 had stage IB2 cancer (25%) that infiltrated no more than two thirds of the cervical stroma. The average interval between the first day of chemotherapy and surgery was 42.7 days (range 36–53 days). Grade 3 neutropenia was found in five women (17.9%); no other haematological toxicity grade 3 and 4 was diagnosed. Grade 3 neutropenia occurred in all women after the third course of chemotherapy and thus it was not necessary to postpone chemotherapy. Grade 4 of alopecia was found in all women with a regimen of doxorubicin. No other toxicity grade 3 or 4 was seen. Within 6 weeks after surgery, one patient developed inflammatory lymphocyst that was accompanied by fever, elevated C-reactive protein and procalcitonin. She was treated conservatively by antibiotics. Further, one woman had lymph oedema of the legs. Stenosis of the cervical canal after trachelectomy was diagnosed in 5 women of 20 (25%). All of these women underwent dilatation of the cervical canal under general anaesthesia because it was impossible to take an endocervical Pap smear during follow-up. All women had regular menstruation period within four months from last cycle of chemotherapy. No hormonal treatment was necessary.

Median follow-up was 42 months (range 5–103). Complete response (no residual disease after NAC) on the definitive histopathological examination was presented in six women (21.4%) and microscopic residual disease (tumours smaller than 3 mm in the largest diameter) was noted in 11 women (39.3%). Macroscopic residual disease after NAC was diagnosed in 11 women (39.3%). Twenty women (71.4%) retained their fertility. Analysis of frozen sections of SLNs was positive in two women (7.1%), who immediately underwent radical hysterectomy type C2. Positive or close margins on trachelectomy specimens were found in six women (21.4%) with negative lymph nodes. These six women underwent radical hysterectomy. Eight women that underwent radical hysterectomy were excluded from study. The recurrence rate was 20% (4 of 20 women in whom fertility was preserved). Local recurrence in the cervix was diagnosed in three patients. One of these patients underwent radical hysterectomy and she is without evidence of disease. The other two underwent chemoradiotherapy: one is without evidence of disease and one died of disease. Distant recurrence (ovary) was diagnosed in one woman, who later died of disease. The mortality rate was 10% (2 of 20 women with preserved fertility), both of whom had squamous cell cancer. Two women with successfully treated recurrence had adenocarcinoma (Table 1).

Our fertility-sparing procedure was completed in 20 women and ten of them (50%) became pregnant (Table 2). Eight women (40%) delivered ten babies. There were four premature deliveries: one occurred during the 24th week of pregnancy, one during 28th week of pregnancy and two, two were between 34 and 36 weeks of pregnancy. In all four premature deliveries the cause was premature rupture of the membrane (PROM) due to infection. All babies are healthy. Any disability like mental, motoric, hearing lost or vision impairment was not diagnosed in premature delivered babies. One woman had a missed abortion in first trimester. One woman miscarried twice in second trimester, and in both cases intrauterine infection was the cause of the abortion. One woman underwent unsuccessful in vitro fertilisation on four occasions and three women went on to become pregnant. Three women underwent some of the assisted reproduction methods (two women had undergone successful intrauterine insemination and one unsuccessful in vitro fertilisation). Two women recurred and underwent chemoradiotherapy and lost chance for pregnancy. For different reasons (age, partner), 4 women had no plans to become pregnant.

Discussion

The recurrence rate after fertility-sparing surgery without NAC in tumours exceeding 2 cm is 20% after ART and 20.8% after VRT [2]. When a tumour is larger than 2 cm and completely removed with adequate free margins, there is usually enough stromal tissue remaining

Please cite this article as: Robova H, et al, Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer, Gynecol Oncol (2014), http://dx.doi.org/10.1016/j.ygyno.2014.08.021

Table 1
Recurrences.

Histopathology	Tumour volume	Residuum	Site of recurrence	Therapy	Survival
SCC LVSI + G3	IB1 15 × 17 × 24 mm	Macro −13 mm, invasion 6 mm	Distant (Ovary)	Surgery adjuvant RT + CHT	Died of disease
AC LVSI unknown G1	IB1 25 × 25 × 24 mm	Macro −10 mm, Invasion 9 mm	Local	Surgery adjuvant RT	Alive without evidence of disease
SCC LVSI + G3	IB1 26 × 30 × 10 mm	Micro −1 × 2 mm	Local	CHRT	Died of disease
AC LVSI unknown G2	IB2 36 × 40 × 27	Micro 2 × 3 mm	Local	CHRT	Alive without evidence of disease

SCC—squamous cell carcinoma, AC—adenocarcinoma, LVSI—lymphovascular space involvement, G—grade, RT—radiotherapy, CHT—chemotherapy, CHRT—chemoradiotherapy.

for successful pregnancy, especially in nulliparous women. NAC should decrease tumour volume, which would enable the complete removal of the tumour with negative margins whilst preserving an adequate amount of cervical tissue. Such a procedure improves the chance of successful pregnancy. The recurrence and mortality rates of our group of patients after NAC are 20% and 10%, respectively. These figures are quite high when compared with 4.8% (recurrence rate) and 1.6% (mortality rate) in patients with a tumour smaller than 2 cm that underwent VRT without NAC [7]. Tumour characteristics of our group of women are generally unfavourable. All tumours in our group of women exceeded 2 cm in the largest diameter compared with 14%. Negative LVSI was observed in only 25% of the patients compared with only 71% and grade 2 and 3 was diagnosed in 82.1% of the patients compared with 55%. Tumour size, tumour grade and LVSI are important prognostic factors in women with early cervical cancers [8]. Mortality rate in our fertility-sparing group of node-negative patients was 10%. It does not exist any paper about similar aged women with cervical cancer IB1 bigger than 2 cm. Mortality in node-negative patients with bulky tumour after NAC and standard radical hysterectomy is 8.6% [5].

Some studies shows that adenocarcinoma is an independent prognostic factor in early stage cervical cancer with intermediate risk factors (deep stromal invasion, LVSI and a tumour larger than 4 cm) [9]. In Europe, adenocarcinomas of the cervix currently account for approximately 18% of all cervical cancers [10]. Adenocarcinomas represented 46.4% of the tumours in our group of patients. Adenocarcinomas are less chemosensitive and radiosensitive than squamous cell cancers. The high number of patients with bulky adenocarcinoma could have affected the recurrence rate. In our group of patients recurrent rate is the same (two and two women) and only women with squamous cell cancer died.

Current data are not sufficient to identify the optimal procedure after NAC (ART or VRT or simple trachelectomy). Fertility results are satisfactory only after VRT and simple trachelectomy [2]. When we perform VRT, the same amount of cervical stroma as in simple trachelectomy is removed; additionally only parametrial tissue is resected. Three of four recurrences in our group of patients were localised in the residual part of cervix. One of our patients had haematological dissemination that is not dependent on the type of procedure.

When the same NAC was used in IB bulky tumours followed by radical hysterectomy, the optimal response rate was 78.8% [5]. The optimal response rate in our fertility-sparing group of patients was only 60.7%. However, the criteria for an optimal response rate differ. Reduction of tumour volume of 50% and more was defined as an optimal response before radical hysterectomy. Complete response and microscopic residual disease were defined as an optimal response before fertility-sparing surgery. In macroscopic residual disease, it is more difficult to preserve an adequate amount of cervical stroma to increase the chance of pregnancy and simultaneously attain safe free margins. Macroscopic residual disease after NAC and fertility-sparing surgery is more risky for local tumour recurrence.

We did not use adjuvant treatment in our patient group after trachelectomy, some kind of adjuvant treatment is usually used after NAC followed by radical hysterectomy when any risk factor is present [5]. Adjuvant chemotherapy after NAC followed by radical hysterectomy is indicated at our department in all lymph node negative patients that respond to NAC and have macroscopic residual disease [4,5]. In the future, adjuvant chemotherapy after NAC followed by trachelectomy in women with residual disease would reduce the number of recurrences [5].

Lymph node involvement is the most important risk factor in early cervical cancer [8]. Lymphadenectomy before NAC is probably a safer procedure, mainly because fertility-sparing surgery is offered only to "true" lymph node negative patients [12]. NAC reduced the number of lymph node positive patients [5,13]. Lymph node positive women remain at higher risk for recurrence than node negative women, especially when adjuvant treatment is not performed. The problem arises when some complications during or after this procedure occur (e.g., bleeding or lymphocytes); in such an event, blood support to the tumour is poor and chemotherapy is less effective.

Table 2
Pregnancy outcome.

Pregnancy	Termination of pregnancy	Weeks of termination	Notes
Spont. pregnancy	Spont. delivery	24 weeks (650 g)	PROM—cause of premature delivery
Spont. pregnancy 2×	Spont. deliveries 2×	40 weeks(3700 g) and 34 weeks (2460 g)	PROM—cause of premature delivery
Spont. pregnancy	Spont. delivery	36 weeks (2510 g)	PROM—cause of premature delivery
Spont. pregnancy 2×	Spont. deliveries 2×	41 weeks (3340 g) and 40 weeks (3440 g)	
Pregnancy after IUI 2×	Spont. miscarriages 2×	16 weeks and 19 weeks	PROM—cause of both miscarriage-infection
Spont. pregnancy	Caesarean section	37 weeks (2910 g)	Acute hypoxia of foetus
Spont. pregnancy	Spont. delivery	37 weeks (2450 g)	
Spont. pregnancy	Missed abortion	10 weeks	
Pregnancy after IUI		Ongoing 21 weeks	
Spont. pregnancy		Ongoing 30 weeks	

IUI—intrauterine insemination, PROM—premature rupture of membrane.

Please cite this article as: Robova H, et al, Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer, Gynecol Oncol (2014), http://dx.doi.org/10.1016/j.ygyno.2014.08.021

A variety of chemotherapeutic protocols have been used followed by fertility-sparing surgery. The first-line combination of TIP (paclitaxel, Ifosfamide and cisplatin) or TEP (paclitaxel, epirubicin and cisplatin) is currently most often used [7,11,12,14,15]. There are more complete responses, but the interval between chemotherapy normally varies between 21 and 28 days. These triplets are more toxic and it is not possible to use them in dose-dense schemes. The interval between cycles of chemotherapy prolongs the interval between initiation of chemotherapy and surgery, which can result in tissue changes and surgery that is more difficult (increase in complications such as blood loss and cervical stenosis). In our group, the median interval between the first day of the first cycle of NAC and surgery was 42.7 days. This is not only important for surgery but also from a quality of life perspective. Toxicity of TIP/TEP chemotherapy is higher than in doublet regimens if it is used before radical hysterectomy [4,5,16]. When a TIP/TEP combination is used before fertility-sparing surgery, toxicity is similar to that in a doublet regimen. The younger age of women indicated for NAC followed by fertility-sparing surgery would account for the lower toxicity.

Our pregnancy results are excellent and comparable with pregnancy results after VRT or simple trachelectomy [2,7]. Pregnancy rate was 50% (10 women out of 20) in our group of patients in comparison with 52.3% in VRT group [7]. First trimester loss was seen in 7.7% of cases (1 out of 13 pregnancies) compared with 20% in VRT group. Second trimester miscarriage occurred in 15.3% of cases (2 out of 13 pregnancies) compared to 3% after VRT. Seventy seven percent of pregnancies of were terminated by delivery compared with 73% in VRT group. Pregnancy results after NAC are better in general due to Maneo study inclusion. Only large cone was performed after NAC in this study.

In conclusion, there is no doubt about the safety and successful pregnancy outcome of fertility-sparing surgery in tumours smaller than 2 cm and infiltrating less than half of the stroma (laparoscopic lymphadenectomy followed by VRT or simple trachelectomy or large cone). Downstaging by NAC in IB1 and IB2 cervical cancer before fertility-sparing surgery is still an experimental procedure, but shows some promise. Patients with only microscopic disease after NAC seem to be best candidates for fertility-sparing surgery. Current data are not sufficient to identify the optimal procedure after NAC (ART or VRT or simple trachelectomy). Pregnancy outcome seems better after simple trachelectomy than after radical trachelectomy or abdominal trachelectomy [2]. Long-term results in relation to oncological outcome for this concept are still needed.

Conflict of interest statement

None of the authors has any conflict of interest to declare.

Acknowledgement

This work is supported by grant MZ CR NT 13166.

References

[1] Schneider A, Erdemoglu E, Chiantera V, Reed N, Morice P, Rodolakis A, et al. Clinical recommendation radical trachelectomy for fertility preservation in patients with early-stage cervical cancer. Int J Gynecol Cancer 2012;22(4):659–66.
[2] Rob L, Skapa P, Robova H. Fertility-sparing surgery in patients with cervical cancer. Lancet Oncol 2011;12:192–200.
[3] Rob L, Pluta M, Skapa P, Robova H. Advanced in fertility-sparing surgery for cervical cancer. Expert Rev Anticancer Ther 2010;10(7):1101–14.
[4] Robova H, Halaska M, Pluta M, Skapa P, Strnad P, Lisy J, et al. The role of neoadjuvant chemotherapy and surgery in cervical cancer. Int J Gynecol Cancer 2010;20:S42–6.
[5] Robova H, Rob L, Halaska MJ, Pluta M, Skapa P, Strnad P, et al. High-dose density neoadjuvant chemotherapy in bulky IB cervical cancer. Gynecol Oncol 2013;128:49–53.
[6] Benedetti-Panici P, Greggi S, Colombo A, Amoroso M, Smaniotto D, Giannarelli D, et al. Neoadjuvant chemotherapy and radical surgery versus exclusive radiotherapy in locally advanced squamous cell cervical cancer: result from the Italian multicenter randomized study. J Clin Oncol 2002;20(1):179–88.
[7] Plante M, Gregoire J, Renaud MC, Roy M. The vaginal radical trachelectomy: an update of series of 125 cases and 106 pregnancies. Gynecol Oncol 2011;121:290–7.
[8] Delgado G, Bundy B, Zaino R, Sevin BV, Creasman WT, Major F. Prospective surgical-pathological study of disease-free interval in patients with stage IB squamous cell carcinoma of the cervix: a Gynecology Oncology Group study. Gynecol Oncol 1990;38:352–7.
[9] Mabuchi S, Okazawa M, Matsuo K, Kawano M, Suzuki O, Miyatake T, et al. Impact of histopathological subtype on survival of patients with surgically-treated stage IA2-IIA cervical cancer: adenocarcinoma versus squamous cell carcinoma. Gynecol Oncol 2012;127:114–20.
[10] Tjalma WA, Fiander A, Reich O, Powell N, Nowakowski AM, Kirschner B, et al. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. Int J Cancer 2013;132(4):854–67.
[11] Eddy GL, Bundy BN, Creasman WT, Spirtos NM, Mannel RS, Hannigan E, et al. Treatment of ("bulky") stage IB cervical cancer with or without neoadjuvant vincristine and cisplatin prior to radical hysterectomy and pelvic/para-aortic lymphadenectomy: A phase III trial of gynecologic oncology group. Gynecol Oncol 2007;106:362–9.
[12] Lanowska M, Mangler M, Speiser D, Bockholdt C, Schneider A, Köhler C, et al. Radical vaginal trachelectomy after laparoscopic staging and neoadjuvant chemotherapy in women with early-stage cervical cancer over 2 cm. Oncologic fertility, and neonatal outcome in a series of 20 patients. Int J Gynecol Cancer 2014;23(3):586–93.
[13] Bader AA, Winter R, Moinfar F, Petru E, Pristauz G, Scholz HS, et al. Is intraoperative frozen section analysis of pelvic lymph nodes accurate after neoadjuvant chemotherapy in patients with cervical cancer? Gynecol Oncol 2006;103:106–12.
[14] Maneo A, Chiari S, Bonazzi C, Mangioni C. Neoadjuvant chemotherapy and conservative surgery for stage IB1 cervical cancer. Gynecol Oncol 2008;111:438–43.
[15] Marchiole P, Tigaud JD, Costantini S, Mammoliti S, Buenerd A, Moran E, et al. Neoadjuvant chemotherapy and vaginal radical trachelectomy for fertility-sparing treatment in women affected by cervical cancer (FIGO IB-IIA1). Gynecol Oncol 2011;122:484–90.
[16] Lissoni AA, Colombo N, Pellegrino A, Parma G, Zola P, Katsaros D, et al. A phase II, randomized trial of neo-adjuvant chemotherapy comparing a three drug combination of paclitaxel, ifosfamide and cisplatin (TIP) versus paclitaxel and cisplatin followed by radical surgery in patients with locally advanced squamous cell carcinoma: the Snap-02 Italian Collaborative Study. Ann Oncol 2009;20(4):660–5.

Please cite this article as: Robova H, et al, Oncological and pregnancy outcomes after high-dose density neoadjuvant chemotherapy and fertility-sparing surgery in cervical cancer, Gynecol Oncol (2014), http://dx.doi.org/10.1016/j.ygyno.2014.08.021