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Dizertační práce

Vliv pulzatility krevního toku na vaskulární postižení u pacientů s mechanickou srdeční podporou

PhD Thesis

Effect of pulsatility of blood flow on parametres of vascular damage in patients with mechanical circulatory support

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Key words: mechanical circulatory support, pulsatility, vascular health, microparticles, endothelial progenitor cells, acquired von Willebrad disease

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Seznam použitých zkratek

BTT	přemostění k transplantaci srdce (bridge to transplant)
DT	destinační terapie
ELISA	enzyme-linked immunosorbent assay
EPC	endoteliální progenitorové buňky (endothelial progenitor cells)
HM 3	HeartMate 3
HM II	HeartMate II
HMWMs	multimery von Willebrandova faktoru s vysokou molekulární hmotností
ISHLT	International Society for Heart and Lung Transplantation (Mezinárodní společnost pro transplantaci srdce a plic)
LVAD	levostranná mechanická srdeční podpora (left ventricular assist device)
MP	mikročástice (microparticles)
MSP	mechanická srdeční podpora
NO	oxid dusnatý
OTs	ortotopická transplantace srdce
RiCO	ristocetin kofaktor
RVAD	pravostranná mechanická srdeční podpora
SC	kmenové buňky (stem cells)
V-A ECMO	veno-arteriální extrakorporální membránový oxygenátor
vWD	von Willebrandova nemoc (von Willebrand disease)
VWF	von Willebrandův faktor
vWF Ag	antigen von Willebrandova faktoru

Abstrakt

Mechanické srdeční podpory jsou důležitou terapeutickou modalitou v oblasti pokročilé chirurgické terapie terminálního srdečního selhání. Doposud používaná zařízení generují převážně nepulzatilní tok krve. Navzdory prokazatelným klinickým úspěchům této léčby se setkáváme s komplikacemi specifickými pro zařízení s kontinuálním průtokem. Komplikace jsou připisovány zejména zvýšenému smykovému zatížení a změnám cév, krevních elementů a endotelu. Cílem práce bylo zjistit vliv kontinuálního průtoku na vaskulaturu a krevní elementy pomocí longitudinálního sledování vybraných biomarkerů vaskulárního zdraví. Ve studii byly sledovány cirkulující mikročástice, endoteliální progenitorové buňky a kmenové buňky a byla vyšetřena dynamika degradace von Willebrandova faktoru a jeho funkce.

Výsledky dosažené v naší studii potvrzují stanovenou hypotézu o změnách dynamiky sledovaných markerů v závislosti na změně charakteristiky krevního toku. Ve sledovaném období byl pozorován pravděpodobný negativní vliv kontinuálního průtoku na sledované parametry. Při sledování degradace multimerů von Willebrandova faktoru s vysokou molekulární hmotností byl pozorován pravděpodobný pozitivní vliv arteficiální pulzatility. Další výzkum může poskytnout významné podklady při vývoji specifických charakteristik nových generací mechanických srdečních podpor, zejména v definování míry pulzní amplitudy a její synchronizace s nativním srdečním rytmem.

Abstract

Ventricular assist devices are an important therapeutic modality in advanced surgical therapy of end-stage heart failure. Devices mainly used until recently generate primarily non-pulsatile blood flow. Despite indisputable clinical success of this therapy, we encounter complications specific to the devices with continuous flow. Complications are mostly attributed to increased shear stress and changes in blood vessels, blood elements and endothelium. The aim of this study was to determine the effect of continuous blood flow on the vasculature and blood elements by longitudinal monitoring of selected biomarkers of vascular health. During the study we monitored circulating microparticles, endothelial progenitor cells and stem cells and examined degradation dynamics of von Willebrand factor and its function.

Results obtained in our study confirm the hypothesis of changes in the dynamics of studied markers dependent on the change of characteristics of blood flow. The possible negative effect of continuous flow on monitored parameters was observed in tracked period. In degradation of the high molecular weight von Willebrand factor multimers the probable positive effect of arteficial pulsatility was observed. Further research can provide important data for the development of specific characteristics of new generations of mechanical circulatory support, especially in defining pulse amplitude rate and its synchronisation with native heart rhythm.

Obsah

1 ÚVOD

Syndrom srdečního selhání je charakterizován neschopností srdce pumpovat krev s efektivitou dostačující pro zabezpečení adekvátního zásobování ostatních orgánů, tedy poruchou srdce jako pumpy a důsledků tohoto stavu. Pro pacienty, u kterých onemocnění navzdory extenzivní konzervativní terapii progredovalo do terminální fáze, byla donedávna krajní léčebnou možností transplantace srdce. V posledních letech se modality pokročilé chirurgické terapie terminálního srdečního selhání rozšířili o možnost implantace mechanických srdečních podpor.

Mechanické srdeční podpory (MSP) jsou čerpadla krve, která jsou schopna u pacientů s pokročilým srdečním selháním částečně nebo úplně převzít úlohu srdce v krevním oběhu s cílem obnovení dostatečného srdečního výdeje. Tento typ terapie je nyní již standardní a účinnou metodou umožňující záchranu života pacientů s terminálním srdečním selháním. Dynamický rozvoj terapie pomocí MSP je ovlivněn zvyšujícím se počtem pacientů se srdečním selháním a celosvětovým nepříznivým trendem v počtech vhodných dárcovských srdcí pro srdeční transplantaci.

Používané systémy MSP generují převážně kontinuální průtok. Navzdory absenci pulzní vlny jsou však klinické výsledky terapie více než uspokojivé. Nepulzatilnímu průtoku jsou však připisovány specifické komplikace, kterých příčinou je pravděpodobně omezené cyklické namáhání cévní stěny, zvýšené smykové zatížení a následné změny cév, krevních elementů a endotelu.

V době zahájení projektu bylo problematice pulzatility, vaskulárních parametrů a endoteliální funkce u mechanických srdečních podpor dedikováno jen omezené množství publikací. Sledování dlouhodobějšího vlivu mechanické srdeční podpory na parametry cévního poškození a analýza pulzatility průtoku pravděpodobně ovlivní

možnosti optimalizace dlouhodobého nastavení používaných přístrojů, případně poskytne informace potřebné pro vývoj další generace systémů MSP.

Předkládaná práce slouží k alespoň částečnému objasnění otázek týkajících se omezené pulzatility a jejího vlivu na vaskulaturu. Pro tento účel bylo nutné longitudinální sledování vaskulárních parametrů u pacientů se srdečním selháním a implantovanou mechanickou srdeční podporou.

2 PŘEHLED PROBLEMATIKY

2.1 Srdeční selhání

Srdeční selhání je rostoucí globální epidemií s odhadovanou prevalencí více než 37,7 miliónu pacientů celosvětově, která neustále narůstá (Ziaeian a Fonarow, 2016). Je definováno jako abnormalita srdeční struktury nebo funkce vedoucí k selhání hlavní funkce srdce, tedy adekvátní dodávky kyslíku k pokrytí nároků metabolizujících orgánů. Klinická definice syndromu srdečního selhání zahrnuje typické symptomy (např. dušnost, sníženou výkonnost) a fyzikální znamení (např. zvýšenou náplň krčních žil, poslechové chrůpky na plicích, otoky, nebo posun úderu srdečního hrotu), jako výsledek poruchy funkce nebo struktury srdce, rezultující ve snížený srdeční výdej a/nebo zvýšené intrakardiální tlaky v klidu nebo při zátěži (Ponikowski et al., 2016).

Setrvalý nárůst počtu nemocných se srdečním selháním je nejen odrazem stárnoucí populace ve vyspělých zemích, kde prevalence ve věku nad 70 let je ≥ 10 %, ale také odrazem pokroku v terapii kardiovaskulárních onemocnění (Ziaeian a Fonarow, 2016; Guha a McDonagh, 2016). Navzdory těmto pokrokům v prevenci, léčbě, diagnostice a péči o pacienty se srdečním selháním, je tento syndrom provázený vysokou, 45 – 60%, pětiletou mortalitou (Bui et al., 2011).

Nejčastějšími příčinami srdečního selhání jsou ischemická choroba srdeční, hypertenze, nemoci srdečních chlopní, kardiomyopatie z různých příčin (toxická, dilatační, hypertrofická, arytmogenní, nonkompaktní, restriktivní, atd), myokarditidy, vrozené srdeční vady a také celková onemocnění, například diabetes mellitus, HIV, poruchy štítné žlázy, hemochromatóza, amyloidóza a další.

Rizikové faktory vzniku srdečního selhání zahrnují zvýšený body mass index, akumulaci abdominálního tuku, elevovanou lačnou hladinu glukózy, zvýšený systolický krevní tlak, nebo zvýšený poměr apolipoproteinu B k apolipoproteinu A. Kouření, ischemická choroba srdeční, hypertenze a chlopenní vady předcházejí diagnózu srdečního selhání nejčastěji (Braunwald, 2013).

Terapie srdečního selhání zahrnuje nejen režimová a dietní opatření a farmakologickou léčbu, ale také léčbu chirurgickou, zahrnující základní techniky (revaskularizace, výkony na chlopních, chirurgickou remodelaci levé komory, resynchronizační terapii) a techniky pokročilé (zavedení mechanické srdeční podpory, srdeční transplantace, úplná náhrada srdce - total arteficial heart).

2.2 Pokročilá chirurgická terapie terminálního srdečního selhání, použití mechanických srdečních podpor

Ortotopická transplantace srdce (OTs) je považována za metodu volby chirurgické léčby u pacientů s terminálním srdečním selháním, kteří zůstávají symptomatičtí navzdory optimální medikamentózní terapii (Alraies a Eckman, 2014). První srdeční transplantace u člověka provedena dr. Barnardem v roce 1967 byla následována dalšími 99 transplantacemi v následujícím roce, nicméně, většina týmů opustila tento způsob léčby z důvodu vysoké mortality způsobené zejména rejekcí transplantátu. Objev cyklosporinu v sedmdesátých letech 20. století způsobil renesanci transplantace srdce a od roku 1982 bylo těchto transplantací provedeno přes 121 000, dle mezinárodního registru mezinárodní společnosti pro transplantace srdce a plic (ISHLT). Celosvětový počet srdečních transplantací stoupl až na 5 000 za rok v devadesátých letech, byl však následován výrazným poklesem, způsobeným zejména

úbytkem počtu vhodných dárců srdečního štěpu. Po roce 2010 se počet transplantací srdce následně ustálil na 4 500 za rok (Lund et al., 2015). Narůstající prevalence srdečního selhání v populaci a nepříznivý trend v počtu vhodných dárcovských srdcí postupně vedl k prodlužování čekací doby na čekací listině k transplantaci srdce a s ní spojenou signifikantní morbiditou i mortalitou. Zejména tyto faktory vedly zásadním pokrokům ve vývoji mechanických srdečních podpor.

Koncept použití přístroje umožňujícího dostatečného zásobení orgánů kyslíkem a živinami a udržení hemodynamických parametrů byl uveden do kardiochirurgické praxe nejprve v podobě mimotělního oběhu v roce 1953 (Gibbon, 1954). První mechanická srdeční podpora byla následně použita a implantována v roce 1963 a první úplná mechanická náhrada srdce (total arteficial heart) v roce 1969 (DeBakey, 1971, 2005). Další vývoj technologie umožnil použití mechanických srdečních podpor jako přemostění k transplantaci srdce (Oz et al., 1997).

Historicky rozeznáváme několik generací dlouhodobých mechanických srdečních podpor. První generace MSP byla vyvinuta na pulzatilním principu. Tyto pumpy však obsahují množství pohyblivých součástí a jejich dlouhodobé použití je limitováno zejména vysokým rizikem dysfunkce čerpadla a také rizikem infekcí (Pasque a Rogers, 2002). Druhá generace MSP je charakterizovaná přítomnosti axiálního čerpadla, generujícího kontinuální průtok. Tento technologický průlom umožnil miniaturizaci čerpadla, zmenšení velikosti povrchu, který přichází do kontaktu s krví a zejména snížení počtu pohyblivých součástí pumpy. Tyto vlastnosti umožnily zlepšení implantability MSP druhé generace a vedly k redukci nežádoucích účinků, zejména ve smyslu dysfunkce čerpadla (John et al., 2008). Nejnovějším zdokonalením je použití elektromagnetického nebo hydrodynamického závěsu rotoru, a tedy eliminace přítomnosti ložisek, u třetí generace MSP. Tím se zredukovalo mechanické opotřebení,

traumatizace krevních elementů procházejících čerpadlem a opět se prodloužila životnost pump. Tyto MSP pracují na principu centrifugálního čerpadla a jsou umístěny přímo v perikardu, na rozdíl od předchozích generací, kdy pumpa samotná se umisťuje do preperitoneální kapsy. Poslední mechanická podpora této generace, zařízení HeartMate 3, dokonce vytváří arteficiální pulzatilitu cyklickým zvyšováním a snižováním otáček čerpadla s frekvencí 30 cyklů za minutu.

Specifickým případem je úplná náhrada srdce, kdy srdce, kromě síní, pacienta je excidováno a nahrazeno mechanickou srdeční podporou, našitou na srdeční síně (Cook et al., 2015).

2.3 Strategie terapie pomocí MSP

Mechanické srdeční podpory lze z hlediska délky plánovaného použití rozdělit na krátkodobé, střednědobé a dlouhodobé. Podle konfigurace se MSP dělí na univentrikulární, nejčastěji levostranné, a biventrikulární. Mechanismus, kterým MSP čerpá krev, může být buď pulzatilní, nebo rotační (s kontinuálním průtokem). Naprostá většina typů pump je zaváděna chirurgicky, i když nelze nezmínit nová čerpadla ke krátkodobému použití (Impella, TandemHeart, HeartMate PHP), která jsou implantována perkutánně. Speciálním typem mechanické podpory je extrakorporální membránový oxygenátor (ECMO), používaný zejména v emergentních případech při kardiogenním šoku ve veno-arteriálním zapojení (V-A ECMO) (Truby et al., 2015).

Při úvaze o implantaci mechanické srdeční podpory je kromě pečlivého výběru vhodného kandidáta nutné myslet i na plánovaný výsledek a strategii použití zařízení. Mezi pět základních cílů terapie pomocí mechanických srdečních podpor patří: „bridge

to transplantation“ (most k transplantaci srdce, BTT), destinační terapie (DT), „bridge to recovery“ (most k zotavení), „bridge to decision“(most k rozhodnutí) a „bridge to bridge“ (přemostění k dlouhodobé podpoře pomocí podpory krátkodobé).

V současné době je v České republice hlavní indikací k implantaci MSP přemostění k transplantaci srdce. Výše zmíněné technologické průlomy jako zavedení rotačních čerpadel, minimalizace pohyblivých součástí a miniaturizace celých systémů umožnily nejen zlepšení implantability, ale také snížení míry mechanického selhání systémů MSP. Díky tomu dalším, v zahraničí dnes již běžně používaným postupem, je použití MSP jako tzv. destinační terapie, kdy je umělá podpora implantována jako trvalé řešení u pacientů, u kterých věk, nebo přidružená onemocnění vylučují jejich zařazení do transplantačního programu (Ryan et al., 2015; Hollander et al., 2016). Terapie pomocí mechanické srdeční podpory je v těchto případech nadřazená medikamentózní terapii srdečního selhání, signifikantně zvyšuje kvalitu života i délku přežívání pacientů (Rose et al., 2001; Trivedi et al., 2014; Long et al., 2014). Trend k použití MSP v této indikaci zaznamenáváme již i v České republice, v USA je v indikaci DT implantováno víc než 40 % MSP (Kirklin et al., 2014). Destinační terapie je nejvíce se rozrůstající indikací na poli MSP.

Další možností je zavedení MSP u stavů, kdy je možné předpokládat zotavení srdeční funkce, díky tzv. reverzní remodelaci, charakterizované komplexními molekulárními a buněčnými změnami v srdečním svalu (Birks et al., 2013; Wever-Pinzon et al., 2016). Tyto změny jsou nastartovány po zavedení MSP, která umožní snížení zatížení (unloading) levé srdeční komory (Braunwald, 2015). Stavy, u kterých je možno o této eventualitě uvažovat, jsou nejčastěji myokarditidy, postpartální nebo toxické kardiomyopatie (Topkara et al., 2016). Bohužel pacientů, u kterých je tato strategie úspěšná, je zatím poměrně málo, a proto je tato oblast předmětem intenzivního

výzkumu, zejména v oblasti remodelační a anti-remodelační terapie. Na druhou stranu se možnosti zotavení úspěšně využívá při implantacích pravostranných mechanických podpor pro akutní selhání pravostranných srdečních oddílů po implantaci levostranné mechanické podpory nebo transplantaci srdce (Bhama et al., 2009).

U pacientů v kritickém hemodynamickém stavu je možné použít krátkodobé mechanické srdeční podpory a po stabilizaci a ozřejmění celkového stavu pacienta se rozhodnout („bridge to decision“) o implantaci dlouhodobé MSP („bridge to bridge“).

Pro výběr pacientů k implantaci mechanických srdečních podpor platí obecně zavedená indikační kritéria (Netuka et al., 2008). Důležitý význam pro výsledek implantace MSP má však také správné načasování výkonu, zejména zachycení počátečních stadií multiorgánového selhání (Yoshioka et al., 2012). Dalšími důležitými faktory ovlivňujícími výsledek terapie MSP je sociální zázemí, psychologická způsobilost a schopnost pacienta vykonávat úkony spojeny s péčí o MSP. Z toho důvodu je u části pacientů se speciálními potřebami preferenčně volena transplantace srdce (např. u neurodegenerativních onemocnění) (Segovia et al., 2001).

2.4 Operační technika

Vzhledem k šíři problematiky budou stručně popsány operační techniky nejčastěji používané na pracovišti autora.

Po standardní sterilní přípravě operačního pole v oblasti hrudníku je provedena sternotomie. U MSP druhé generace (HeartMate II) je vytvořena preperitoneální kapsa pro pozdější umístění přístroje, přístroj třetí generace (HeartMate 3) se umísťuje intraperikardiálně. Po zavedení kanyl mimotělního oběhu a jeho zahájení se výkon

provádí na bijícím srdci. Do hrotu levé komory je vytnut otvor, do kterého se po našití objímky umístí vtoková kanyla, výtoková kanyla je následně našita na ascendentní aortu. Během umisťování pumpy je vytvořen podkožní tunel pro transkutánní napájecí kabel. Ten je připojen k řídící jednotce a čerpadlo je za postupného snižování průtoku mimotělním oběhem uvedeno do plného provozu.

V naléhavých situacích je možno implantovat krátkodobou mechanickou srdeční podporu bez použití mimotělního oběhu (CentriMag Levitronix). Tento systém umožňuje biventrikulární nebo univentrikulární zapojení. V případě levostranného zapojení je vtoková kanyla umístěna do levé síně a výtoková kanyla do cévní protézy našité na ascendentní aortu. U pravostranné mechanické srdeční podpory je vtoková kanyla umístěna do pravé síně a výtoková do plicnice. Jedná se o parakorporální systémy, kanyly jsou externalizovány preperitoneálně a přes břišní stěnu.

U pacientů v kritickém kariogenním šoku je zaváděno V-A ECMO k úplné náhradě funkce srdce (a plic). Nejčastěji přístupem je *vena femoralis,* odkud je odčerpávaná krev, která je vháněna čerpadlem do oxygenátoru a následně do *arteria femoralis.*

Hlavním současným trendem v chirurgických implantačních a explantačních technikách mechanických srdečních podpor je snížení invazivity výkonů a zkrácení doby na mimotělním oběhu (Haberl et al., 2014; Cheung et al., 2014; Anyanwu et al., 2014; Makdisi a Wang, 2015).

2.5 Pulzatilní a nepulzatilní průtok

Obecně je přijímán názor, že arteriální pulz je důležitou součástí kardiovaskulárního systému. Pulzní vlna je fyziologický jev, pozorovaný a také měřitelný v arteriálním systému krevního oběhu. Vzniká prostřednictvím objemu krve vypuzeného v systole. Tato krev se jako vlna šíří tepnami v důsledku vzájemné přeměny kinetické energie vyloučeného objemu krve a potenciální energie napnutého segmentu pružné cévní stěny. Buňky lidského těla, zejména endoteliální, jsou adaptovány na cyklické změny tlaku a průtoku. Pro definici pulzatility jsou užívány dvě základní veličiny a to pulzní tlak a pulzní index. Rozdíl nejvyššího systolického a nejnižšího diastolického krevního tlaku se nazývá pulzní tlak. Index pulzatility definuje pulzatilitu pomocí průtoku a je rozdílem mezi nejvyšší a nejnižší rychlostí proudění krve v systole resp. v diastole dělenou střední rychlostí během srdečního cyklu (Soucy et al., 2013; Cheng et al., 2014). U zdravých jedinců je pulzní vlna měřitelná například pomocí ultrazvuku na velkých arteriích. Případem, kdy pulzní vlna není měřitelná vůbec, je použití mimotělního oběhu při kardiochirurgických operacích se srdeční zástavou a při úplné náhradě srdce pomocí podpor s kontinuálním průtokem. U pacientů s levostrannou mechanickou podporou je v některých případech možné detekovat přítomnost pulzní vlny. Jednoznačným důvodem přítomnosti tohoto fenoménu může být zachovalé otevírání aortální chlopně během terapie MSP. Dalšími příčinami můžou být pohyby pravého srdce, reziduální kontraktilita levé komory, která vytváří pulzní vlnu i při zavřené aortální chlopni a to na základě změny tlakového gradientu přes čerpadlo, které je preload senzitivní, tedy při zvýšení tlaku před čerpadlem se zvětší tlakový gradient mezi levou komorou a aortou.

Endoteliální buňky, hladké svalové buňky i fibroblasty v cévách jsou ovlivněny mechanickými sílami, které vytváří pulzatilní průtok. Pulzatilita ovlivňuje signální cesty a buněčné procesy, jako například buněčnou proliferaci a diferenciaci, kontrakci svalových buněk, vasodilataci a vasokonstrikci, produkci oxidu dusnatého (NO) a bradykininu, oxidativní stres a vaskulární remodelaci včetně apoptózy nebo, v konečném důsledku, i aterosklerózy (Templeton et al., 2012; Segura et al., 2013; Prescimone et al., 2014). Nejdůležitějším vazodilatačním mediátorem uvolňovaným právě endotelem je NO. Pulzatilní průtok napomáhá k bazálnímu uvolňování NO endotelem a tím k udržení adekvátního vaskulárního tonu a rezistence (Lerman a Brunett, 1992). V případě přítomnosti kontinuálního průtoku může být jedním z mechanismů vzniku endoteliální dysfunkce a zvýšení vaskulární rezistence právě porucha syntézy NO. Chybějící pulzatilita tedy vyústí ve snížení bazální produkce NO, a je následovaná zvýšenou periferní rezistencí a poruchou endoteliální funkce (Nakano et al., 2000; Hasin et al., 2015). Negativní ovlivnění funkce cévní stěny se v doposud publikovaných pracích přiklání spíše na stranu nepulzatilního průtoku v porovnání s průtokem pulzatilním (Hutcheson et al., 1991; Nishinaka et al., 2001; Gambillara et al., 2008; Thacher et al., 2010). Vzhledem k tomu, že obecně je přijímaný názor o kontinuálním toku v kapilárách, zůstává ovlivnění toku v kapilárách mechanickou srdeční podporu předmětem intenzivního výzkumu. V některých studiích byl prokázán rozdíl při porovnání průtokových vzorů v kapilárách u kontinuálních a pulzatilních MSP (Lee et al. 1994; Baba et al., 2004).

Dnes nejčastěji používané systémy MSP generují převážně kontinuální průtok a počet implantovaných kontinuálních čerpadel daleko převyšuje počet pulzatilních zařízení. Navzdory částečné nebo úplné absenci pulzní vlny je tato převážně non-pulzatilní cirkulace z krátkodobého a střednědobého hlediska až překvapivě dobře

tolerována a orgánové funkce zůstávají zachovány (Radovancevic et al., 2007; Sandner et al., 2009; Slaughter, 2010).

Při použití mechanických podpor s kontinuálním průtokem se však setkáváme se specifickými stavy a komplikacemi, u kterých je vznik přičítán z velké části právě nepulzatilnímu krevnímu toku. Všechny tyto komplikace mají signifikantní negativní vliv na morbiditu i mortalitu pacientů. Kontinuální průtok má jistě vliv na velké arterie, narušení a remodelaci cévních stěn v makrocirkulaci (Westaby et al., 2007; Segura et al., 2013). Postupně dochází i k remodelaci arterií menšího kalibru (Healy et al., 2016) a přenosu této poruchy na kapilární řečiště, co může mít za následek snížení počtu kapilár. Dalšími komplikacemi, které jsou přičítány nepulzatilnímu toku, jsou změny aortální chlopně. U části pacientů dochází ke vzniku komisurální fúze až úplnému uzavření aortální chlopně, nebo naopak, ke vzniku pozdní aortální insuficience (Cowger et al., 2010; Toda et al., 2011; Saito et al., 2016). Míra pulzatility krevního toku má u pacientů s implantovanou MSP také vliv na vznik pozdního, tzv. ne-chirurgického krvácení (Stern et al., 2010; Wever-Pinzon et al., 2013, Mutiah et al., 2016). Jedná se nejčastěji o krvácení do gastrointestinálního traktu, krvácení do gastrointestinálního traktu spojené s otevíráním arterio-venózních malformací, epistaxi, krvácení do močopohlavního traktu a intrakraniální krvácení. Vyšší pulzatilita je spojená s nižším výskytem těchto komplikací (Wever-Pinzon et al., 2013). Ke krvácivým komplikacím přispívá také vznik získané von Willebrandovy nemoci (Uriel et al., 2010; Crow et al., 2010; Meyer et al., 2010, 2014).

2.6 Biomarkery k posouzení stavu vaskulatury

Omezená pulzatilita a snížené cyklické namáhání cév může negativně ovlivnit parametry cévní stěny, zejména ve smyslu endoteliální (dys)funkce. Proto se v poslední době obrátila pozornost na nové potencionální biomarkery vaskulárního zdraví – cirkulující mikročástice a endoteliální progenitorové buňky.

Cirkulující mikročástice jsou uvolňovány za patologických i fyziologických podmínek, jejich zvýšený vznik byl pozorován u mnoha, zejména kardiovaskulárních, onemocnění. Cirkulující kmenové a endoteliální progenitorové buňky jsou markerem buněčné obnovy a regenerační aktivity organismu. Jsou uvolňovány z kostní dřeně při akutním vaskulárním postižení a nutnosti obnovení endotelu.

Nežádoucí procesy také můžou být také reflektovány změnami v koagulačním systému. Změny jsou velmi dobře reprezentované degradací funkce von Willebrandova faktoru a vznikem získané von Willebrandovy nemoci.

2.6.1 Cirkulující mikročástice

Cirkulující mikročástice (microparticles, MP) jsou anukleární fragmenty celulární membrány uvolňované při zvýšeném zatížení nebo poškození buněk velikosti 0,1 – 1,0 μm. Mikročástice jsou uvolňovány ze všech buněk v cirkulaci, nejvyšší procento však z buněk endoteliálních a z trombocytů. Uvnitř mikročástic se nachází cytoplasmatický materiál maternálních buněk. Povrchová membrána MP na svém vnějším povrchu obsahuje fosfatidylserin a fosfatidyletanolamin, které se jinak obvykle nachází na vnitřním povrchu této membrány (Burger a Touyz, 2012). Externalizovaný fosfatidylserin se podílí na endoteliálním poškození při zánětu (Burger et al., 2012;

Amabile et al., 2013) a také na koagulačních pochodech při vaskulárním poranění (Nomura et al., 2001; Sinauridze et al., 2007; Zhou et al., 2010). Mikročástice jsou uvolňovány také jako produkty apoptózy, vaskulárního poškození nebo aktivace. Uvolnění mikročástic je stimulováno fyziologickými i patofyziologickými podněty. Za patologických podmínek jsou mikročástice nejčastěji uvolňovány u kardiovaskulárních onemocnění, metabolických onemocnění, preeklampsie a šokových stavů (VanWijk et al., 2002; Burnier et al., 2009; Schiro et al., 2014; Franca et al., 2015; Zhang et al., 2016).

Zvýšené smykové napětí vytvářené kontinuálním průtokem MSP pravděpodobně může ovlivnit hladinu cirkulujících mikročástic (McGinn et al., 2016). Měření hladin mikročástic, jako markeru stavu vaskulatury může napomoci nejen k porozumění patofyziologie vlivu kontinuálního průtoku na cévní systém, ale také k predikci závažných komplikací spojených s touto terapii (Nascimbene et al., 2014, Sansone et al., 2015).

2.6.2 Endoteliální progenitorové buňky, kmenové buňky

Endoteliální progenitorové buňky (EPC) jsou subpopulací CD34+ mononukleárních kmenových buněk. Připisuje se jim schopnost endoteliální reparace a účast v angiogenezi. Také mohou působit jako rezervoár buněk schopných nahradit poškozený nebo dysfunkční endotel. Změny počtu cirkulujících EPC byly reportované v různých klinických situacích, včetně vaskulárních traumat, fibrilace síní, infarktu myokardu a pravděpodobně při endoteliálním poškození obecně. Snížení množství cirkulujících EPC se vyskytuje u pokročilého srdečního selhání a také predikuje vznik aterosklerózy u zdravé populace. Změny počtů cirkulujících EPC mohou

pravděpodobně reflektovat komplexní, zejména reparativní, změny ve vaskulárním systému (Valgimigli et al., 2004; Werner et al., 2005; Fadini et al., 2006; Leone et al., 2009). EPC jsou považovány za jednu z nejdůležitějších součástí endogenních reparačních mechanismů, působících proti endoteliální dysfunkci (Recchioni et al., 2016). Použití endoteliálních kmenových buněk a kmenových buněk v terapii některých onemocnění, zejména kardiovaskulárních, přináší slibné výsledky (Sun et al., 2016).

Kmenové hematopoetické buňky jsou definované pomocí přítomnosti povrchových glykoproteinů CD 34+, endoteliální progenitorové buňky pomocí dalších znaků (např. CD 31, CD 45, KDR) (Fadini et al., 2006; Blann a Pretorius, 2006; Piťha et al., 2013).

Vzhledem ke svému reparačnímu potenciálu by hladiny endoteliálních progenitorových buněk, kromě jiného, mohly napovědět o míře poškození vaskulatury vlivem mechanické srdeční podpory (Manginas et al., 2009).

2.6.3 Von Willebrandův faktor

Kontinuální průtok a z něho plynoucí snížená pulzatilita, může narušit normální cirkulaci zvýšením smykového zatížení. V těchto podmínkách jsou zaznamenány unikátní reologické změny, manifestující se jako hemolýza z důvodu deformace cirkulujících erytrocytů (Cowger et al., 2014; Hasin et al., 2014) a v plazmě jako rozvoj získané von Willebrandovy nemoci. Patogeneze vzniku získané von Willebrandovy nemoci je obdobná jako u pacientů s aortální stenózou (Casonato et al., 2011; Tamura et al., 2015) a souvisí s degradací vysokomolekulárních multimerů von Willebrandova faktoru a následnou poruchou funkce vWF. Defekty funkce vWF jsou patrné časně po implantaci MSP. K rapidnímu zlepšení všech parametrů funkce vWF dochází po

explantaci MSP nebo po transplantaci srdce, co indikuje závislost mezi tokem generovaným mechanickou srdeční podporou a sníženou funkci vWF (Nascimbene et al., 2016). Degradace je tedy připisována zejména mechanickému poškození a zvýšenému smykovému zatížení při nepulzatilním průtoku. Proto by míra degradace vWF mohla napovědět o další zátěži oběhového systému mechanickou srdeční podporou.

3 HYPOTÉZA

Vzhledem k přítomnosti výrazně snížené pulzatility u pacientů s implantovanou mechanickou srdeční podporou, lze předpokládat změny vaskulatury a cirkulujících krevních elementů.

V práci byla testována hypotéza o změnách koncentrací vybraných recentně zkoumaných cirkulujících biomarkerů vlivem kontinuálního průtoku. Změny byly sledovány pomocí koncentrací cirkulujících mikročástic, cirkulujících kmenových buněk a endoteliálních progenitorových buněk. Nežádoucí procesy také mohou být také reflektovány změnami v koagulačním systému. Změny jsou velmi dobře reprezentované degradací funkce von Willebrandova faktoru, které mizí po obnovení pulzatilního průtoku. Testovali jsme proto také hypotézu o pozitivním vlivu pulzatility na vznik získané von Willebrandovy nemoci.

4 CÍLE PRÁCE

I. Definovat význam nových biomarkerů vaskulárního zdraví (cirkulujících mikročástic) u pacientů s implantovanou mechanickou srdeční podporou.

II. Longitudinálně posoudit vliv mechanické srdeční podpory s kontinuálním průtokem na dynamiku plazmatické koncentrace cirkulujících mikročástic.

III. Longitudinálně posoudit vliv mechanické srdeční podpory s kontinuálním průtokem na dynamiku změn počtu cirkulujících kmentových a endoteliálních progenitorových buněk.

IV. Zhodnotit vliv pulzatility na vznik získané von Willebrandovy nemoci.

5 METODIKA

Detailní popis metodiky je uveden v jednotlivých publikačních výstupech.

Ke zhodnocení koncentrace mikročástic byla použita metoda ELISA (Hyphen Biomed, Francie). Koncentrace byly vyjádřeny v nanomolech na litr vztažených ke koncentraci fosfatidylserinu (nMPS). Cirkulující kmenové buňky a endoteliální progenitorové buňky byly vyšetřeny na expresi povrchových antigenů a měřeny pomocí průtokové cytometrie. Kmenové buňky byly definovány jako CD34+/CD45low+ a endoteliální progenitorové buňky jako CD34+/CD45low+/KDR+ buňky. K analýze multimerů von Willebrandova faktoru s vysokou molekulární hmotností byla použita gelová elektroforéza a chemiluminiscence pomocí Western blot. Další metody analýz vWF jsou podrobně popsané v přiložené publikaci.

Statistické hodnocení je podrobně popsáno v připojených publikacích. Všechna data byla statisticky analyzována pomocí softwaru STATA (STATA Corp LLCC, Texax, USA) a SPSS v. 21 (IBM SPSS Statistics, IBM Corporation, Armonk, New York, USA). V popisných/deskriptivních statistikách byly použity průměry a směrodatné odchylky s výběrem, mediány, případně procenta. Rozdíly mezi jednotlivými skupinami v případě kontinuálních proměnných byly analyzovány nepárovým t-testem (Student) a v případě kategorických proměnných $\chi 2$ testem. Změny sledovaných parametrů (výhradně kontinuální) v čase v jednotlivých skupinách byly analyzovány párovým t-testem. U parametrů s abnormální distribucí bylo použito logaritmických hodnot. Hodnoty p nižší než 0,05 byly považovány za statisticky signifikantní.

6 VÝSLEDKY

[A]

IVÁK, Peter; PIŤHA, Jan; NETUKA, Ivan. Circulating microparticles as a predictor of vascular properties in patients on mechanical circulatory support; hype or hope?. *Physiological Research*. 2016, **65**(5), 727-735. ISSN 0862-8408. **IF: 1.643/2015**.

[B]

IVÁK, Peter; PIŤHA, Jan; WOHLFAHRT, Peter; KRÁLOVÁ LESNÁ, Ivana; STÁVEK, Petr; DORAZILOVÁ, Zora; ŠTĚPÁNKOVÁ, Jitka; MALÝ, Jiří; POKORNÝ, Martin; NETUKA, Ivan. Endothelial dysfunction expressed as endothelial microparticles in patients with end-stage heart failure. *Physiological Research*. 2014, **63**(Suppl. 3), S369-S373. ISSN 0862-8408. **IF: 1.293/2014**.

[C]

IVÁK, Peter; PIŤHA, Jan; WOHLFAHRT, Peter; KRÁLOVÁ LESNÁ, Ivana; STÁVEK, Petr; MELENOVSKÝ, Vojtěch; DORAZILOVÁ, Zora; HEGAROVÁ, Markéta; ŠTĚPÁNKOVÁ, Jitka; MALÝ, Jiří; SEKERKOVÁ, Alena; TURČÁNI, Dominika; NETUKA, Ivan. Biphasic response in number of stem cells and endothelial progenitor cells after left ventricular assist device implantation: A 6 month follow-up. *International Journal of Cardiology*. 2016, **218**(September), 98-103. ISSN 0167-5273. DOI: 10.1016/j.ijcard.2016.05.063. **IF: 4.638/2015**.

[D]

NETUKA, Ivan; KVASNIČKA, Tomáš; KVASNIČKA, Jan; HRACHOVINOVÁ, Ingrid; **IVÁK, Peter**; MAREČEK, František; BÍLKOVÁ, Jana; MALÍKOVÁ, Ivana; JANČOVÁ, Michaela; MALÝ, Jiří; SOOD, Poornima; SUNDARESWARAN, Kartik S.; CONNORS, Jean M.; MEHRA, Mandeep R. Evaluation of von Willebrand factor with a fully magnetically levitated centrifugal continuous-flow left ventricular assist device in advanced heart failure. *The Journal of Heart and Lung Transplantation*. 2016, **35**(7), 860-867. ISSN 1053-2498. DOI: 10.1016/j.healun.2016.05.019. **IF: 7.509/2015**.

PRÁCE A představuje souhrn dosavadních obecných poznatků na poli recentně zkoumaných biomarkerů vaskulárního zdraví, cirkulujících mikročástic. V úvodní části jsou diskutovány potencionální fyziologické regulační úlohy mikročástic a jejich podíl na vzniku a patogeneze u onemocnění, u nichž byly pozorovány změny jejich koncentrace. Cirkulující mikročástice jsou asociovány nejen s patologickými podmínkami, ale připisují se jim také fyziologické funkce. Hrají důležitou roli při regulaci zánětu, u trombózy a při regulaci endoteliální funkce. Změny plazmatických koncentrací těchto markerů jsou u kardiovaskulárních onemocnění popsány jednoznačně. Jejich koncentrace se mění také u dalších onemocnění, nejčastěji metabolických, ale také u šoku, nebo preeklampsie. Proto se změny koncentrací cirkulujících mikročástic staly předmětem intenzivního výzkumu s předpokladem jejich využití nejen jako markeru patologických stavů, ale také jako změny, předcházející kardiovaskulární nežádoucí události. Nejčastěji používanými metodami k detekci mikročástic jsou ELISA a průtoková cytometrie.

Práce komplexně popisuje dosavadní studie zabývající se cirkulujícími mikročásticemi u pacientů s mechanickou srdeční podporou. Změny jejich koncentrace byly popsány prakticky ve všech z nich a to nejen v porovnání proti zdravým kontrolám, ale také v longitudinálním sledování pacientů s MSP. V jedné ze studii byl také popsán možný predikční potenciál u nežádoucích klinických událostí. Měření koncentrací mikročástic se tedy může stát důležitým při určování stavu vaskulatury u pacientů s MSP, má slibný potenciál při predikci nežádoucích událostí a při správné klinické korelaci se může stát nástrojem napomáhajícím při vývoji nových generací mechanických srdečních podpor.

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REVIEW

Circulating Microparticles as a Predictor of Vascular Properties in Patients on Mechanical Circulatory Support; Hype or Hope?

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Summary

Microparticles are small circulating vesicles originating from circulatory system and vascular wall cells released during their activation or damage. They possess different roles in regulation of endothelial function, inflammation, thrombosis, angiogenesis, and in general, cellular stress. Microparticles are the subject of intensive research in pulmonary hypertension, atherosclerotic disease, and heart failure. Another recently emerging role is the evaluation of the status of vasculature in end-stage heart failure patients treated with implantable ventricular assist devices. In patients implanted as destination therapy, assessment of the long-term effect of currently used continuous-flow left ventricular assist devices (LVADs) on vasculature might be of critical importance. However, unique continuous flow pattern generated by LVADs makes it difficult to assess reliably the vascular function with most currently used methods, based mainly on ultrasound detection of changes of arterial dilatation during pulsatile flow. In this respect, the measurement of circulating microparticles as a marker of vascular status may help to elucidate both short- and long-term effects of LVADs on the vascular system. Because data regarding this topic are very limited, this review is focused on the advantages and caveats of the circulating microparticles as markers of vascular function in patients on continuous-flow LVADs.

Key words

Microparticles • Ventricular assist device • Vascular function

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Introduction

Left ventricular assist devices (LVADs) and heart transplantation are the leading therapeutic approaches to manage end-stage heart failure that is unresponsive to medical therapy (Kirklin *et al.* 2012). New developments in design of LVADs have facilitated their durability, effectiveness, and long-term reliability (Holman *et al.* 2013). The number of LVAD implantations for long-term destination therapy continues to increase each year as the acceptance of this therapy increases (Kirklin *et al.* 2014). Consequently, use of the latest generation of LVADs has had positive influence on overall patient survival rates (Rogers *et al.* 2010, Kirklin *et al.* 2013, Kirklin *et al.* 2014). Despite extensive experience in the management of patients with implanted LVADs, the occurrence of device-related complications and their prediction have become a new challenge in patients with long-term mechanical circulatory support therapy. Likewise, the assessment of vasculature and endothelium status by conventionally utilized methods, such as measurement of pulse wave velocity and

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endothelial dysfunction in peripheral vessels, is problematic because these devices generate non-pulsatile flow, thus making these methods much less reliable. Continuous blood flow is generally assumed to be non-physiological and, therefore, may produce increased stress on endothelial and circulating cells. Pathophysiological mechanisms of this phenomenon and impact of continuous-flow LVADs on blood and endothelial cells remain unclear.

To delineate the effect of LVADs on the vasculature, newly detectable biomarkers related to cellular injury, stress, and apoptosis – microparticles – have become an important focus of research.

Microparticles are complex vesicular structures that are anuclear fragments of cellular membrane and cytoplasmic material with a diameter of 0.1 to 1.0 μm. The plasma membrane surface of the microparticle contains phospholipids, and the microparticle itself encompasses proteins and cytoplasmic material from the cell of origin – both blood and endothelial cells (Burger and Touyz 2012). Microparticle formation encompasses loss of physiological phospholipid asymmetry of plasma membrane. Phosphatidylserine (PS) and phosphatidyl-ethanolamine are usually located on the inside surface of plasmatic membrane, while phosphatidylcholine and sphingomyelin are present on outer surface of the membrane. Balance between these substances is sustained by enzymes flippase, floppase and scramblase (McGinn *et al.* 2016). Cell activation followed by increase of cytoplasmic calcium leads to dysregulation of these enzymes causing externalization of phosphatidylserine. Externalized PS can not only induce endothelial damage in inflammatory conditions (Amabile *et al.* 2013, Burger *et al.* 2012), but is also essential in initiating and propagating the coagulation at sites of vascular injury (Nomura *et al.* 2001, Sinauridze *et al.* 2007, Zhou *et al.* 2010).

Considered as the end-product of apoptosis and injury of different cell types, especially platelets, leukocytes, erythrocytes, and endothelial cells, circulating microparticles can act as a strong dysregulators of endothelial function (Dignat-George and Boulanger 2010). Shedding of microparticles is process that can also occur independently of apoptosis (Sapet *et al.* 2006). Microparticle release is stimulated by both physiological and pathological stimuli linked to cell activation, for example in platelets and endothelial cells shear stress represents such stimulus (Mc Ginn *et al.* 2106). Therefore, on one hand, the production of microparticles is believed to be a part of physiologic cell function, but on the other hand, significant increase of microparticles was described in several pathological conditions. Regarding the latter, elevated levels of microparticles correlate with cardiovascular diseases such as atherosclerotic disease (Van Wijk *et al.* 2003, Burnier *et al.* 2009), including coronary artery disease (Bernal Mizrachi *et al.* 2003, Bernal Mizrachi *et al.* 2004, Singh *et al.* 2012), carotid artery disease (Schiro *et al.* 2014), or stroke (Williams *et al.* 2007). Raise of microparticle concentrations is also associated with hypertension (Preston *et al.* 2003), atrial fibrillation (Ederhy *et al.* 2007) or pulmonary hypertension (Diehl *et al.* 2011). Increased levels microparticles were further found in metabolic diseases such as obesity, diabetes (Sabatier *et al.* 2002, Tramontano *et al.* 2010, França *et al.* 2015), and dyslipidemia (Boulanger *et al.* 2006). Furthermore, elevated microparticles could be strong predictor for complications in pregnancy, namely in the case of life threatening preeclampsia (Van Wijk *et al.* 2002, Bretelle *et al.* 2003, Gonzales-Quinteiro *et al.* 2004), but also in septic shock in general population of patients (Zafrani *et al.* 2013, Zhang *et al.* 2016). In addition, levels of circulating microparticles are also affected by various drug therapies, which may also play role in their use as a diagnostic tool. Reduction of microparticle levels was observed in patients treated by aspirin, calcium channel blockers or statins (McGinn *et al.* 2016).

In general, plasma microparticles are increased in most cardiovascular diseases, probably reflecting activation or damage of circulating cells or cells of the vasculature itself (Piccin *et al.* 2007) and this increase might be associated with increased risk of major cardiovascular clinical complications (Bernal Mizrachi *et al.* 2004). Microparticles may also act as an independent predictor of cardiovascular diseases (Amabile *et al.* 2014). Thereby, according to recent scientific literature, microparticles have potential to become diagnostic tool not only in cardiovascular diseases, but also in other pathological states associated with coagulation, endothelial dysfunction and metabolic or infectious diseases, and also complications in pregnancy. However, because of great complexity and variability regarding the origin and structure of microparticles, particular methods used for their determination could be of extreme importance.

Currently there are several methods used to detect microparticles; the two leading methods are flow cytometry and enzyme-linked immunosorbent assay

(ELISA). A key advantage of flow cytometry is its specificity to surface antigens, which allows identification of the maternal cells and, therefore, by this method it is possible to assess the origin of the microparticles. The analysis of plasma samples clearly detects and differentiates microparticles of endothelial, platelet, leukocyte, and erythrocyte origins (Jy *et al.* 2004, Lacroix *et al.* 2010). The greatest advantages of the other technique, ELISA, are its high sensitivity and low cost. This methodology is based on measurement of concentration of phosphatidylserine, and amino-phospholipids typically found on the inner surface of the plasmatic membrane, which is externalized by creation of macrovesicles, by outward blebbing of the membrane (Burger *et al.* 2013, Shah and Kontos 2014). However, the advantages are counterbalanced by the lower specificity of the method (Piccin *et al.* 2007, Jy *et al.* 2004).

Particular assay used for microparticle detection also determines which types of microparticles are detected and measured (Amiral and Seghatchian 2015). Results could be influenced by capture ligand selected, especially in flow cytometry. Previously published studies suggest that capture-based assays such as ELISA method correlate better with disease evolution and severity than flow cytometry (Connor *et al.* 2009, Owen *et al.* 2011, Aleman *et al.* 2011, Amiral and Seghatchian 2015). Design of capture-based assays has also great influence on nature and size of measured microparticles. Flow cytometry assays detect usually microparticles of size over 0.4 µm (Amiral and Seghatchian 2015). However, main advantage of flow cytometry could be its ability to define the cell origin of circulating microparticles more precisely. Such approach is very important, because levels of microparticles of different cell origin are elevated in different pathological states. Platelet microparticle levels increase in states related to bleeding and thrombotic disorders, such as heparin-induced thrombocytopenia (Warkentin *et al.* 1994, Hughes *et al.* 2000). In contrast, the increase of endothelial microparticles has been observed to correlate with loss of flow – mediated dilatation and arterial stiffness (Viera *et al.* 2012) and endothelial dysfunction (Bruyndonckx *et al.* 2014, McGinn *et al.* 2016) as well as with hyperlipidemia (Boulanger *et al.* 2006). Elevations of both platelet and endothelial microparticles were observed in thrombotic diseases, such as venous thromboembolism, antiphospholipid syndrome or thrombotic thrombocytopenic purpura (Burnier *et al.* 2009). This pattern of changes is also frequently found in hypertension and atherosclerotic disease including acute coronary syndromes (Burnier *et al.* 2009), but also in sepsis (Zhang *et al.* 2016). Nevertheless, it should be mentioned that microparticles are also released from other cell types. Leukocyte derived microparticles play role in pathophysiology of cardiovascular diseases (monocytes) and infectious diseases (HIV – lympho-cytes). Erythrocyte derived microparticles are elevated in various clinical situations, particularly in those characterized by hemolysis (Rubin *et al.* 2012). In general, proportions of types of circulating levels of microparticles may differ between various diseases and it could be advantageous to assess not only total levels of circulating microparticles, but also to determine their cellular origin.

Taking all presented observations into account, assessment and especially interpretation of microparticle levels has become a complex issue, which could be particularly interesting to study in unique population of patients with end-stage heart failure, treated with LVADs.

Recent knowledge about microparticles and ventricular assist devices

The role of left ventricular assist devices (LVADs) is rapidly growing beyond the original role of bridging the patient to heart transplantation. The devices have become an alternate permanent therapy known as the destination therapy, which poses the question as to how these devices impact long-term properties of blood components and vessels.

Circulating cells are not only impacted by a milieu of non-pulsatile blood flow but also by the device itself. Mechanical stress produced by the rotary pump can cause possible ongoing disruption of cellular integrity. Therefore, circulating microparticles, as products of cellular damage, apoptosis, and stress, have recently attracted considerable research interest as an important biomarker tool or even a harbinger of cardiovascular adverse events. Table 1 summarizes current knowledge on this subject.

Microparticles as predictor of adverse events in LVAD patients

Aim of the first study of microparticles in patients with implanted LVADs was to establish microparticles as surrogate markers for platelet,

Table 1. Studies analyzing an effect of LVADs on presence and profile of microparticles.

Author, Date, Country	Number of patients	Type of LVAD	Microparticles assessment	Sample collection period	Key results
Diehl *et al.*, 2010, Germany	12	HeartMate II (6) Thoratec VAD (2) Ventrassist (2) Circulite (1) ECMO (1)	Flow cytometry	Not specified	Microparticles levels significantly higher in LVAD patients compared to healthy controls.
Pitha *et al.*, 2012, Czech Republic	8	HeartMate II (axial)	ELISA	Baseline 3 months	No significant changes in concentration of circulating microparticles before and 3 months after LVAD implant.
Nascimbene *et al.*, 2014, USA	20	HeartMate II (axial)	Flow cytometry	Baseline Discharge 3 months after implant	Higher levels of phosphatidylserine positive microparticles in patients before LVAD implant compared to healthy controls. Significantly higher levels of microparticles in patients who developed an adverse event.
Ivak *et al.*, 2014, Czech Republic	30	HeartMate II (axial)	ELISA	Baseline 3 months after implant	Significant decrease in concentration of circulating microparticles before and 3 months after LVAD implant.
Sansone *et al.*, 2015, Germany	14	HeartWare LVAS (centrifugal)	Flow cytometry	3 months after implant	Microparticles levels of both platelet and endothelial origin were significantly increased in LVAD patients when compared to healthy controls and controls with stable coronary artery disease.

leukocyte, and endothelial activation and vascular inflammation (Diehl *et al.*2010). Microparticles of these origins were measured by flow cytometry in 12 patients with LVADs and compared to healthy controls. Patients with LVAD support had significant ($p=0.002$ for platelet and $p<0.001$ for leukocyte and endothelial microparticles) increased concentration of all measured microparticles, suggesting increased vascular inflammation, platelet activation, cellular apoptosis and endothelial dysfunction, which may accelerate pro-atherothrombotic changes in blood vessels and increase risk of thromboembolic and bleeding complications. Importantly, the analysis used in this study implicates a role of platelet specific microparticles, which are believed to give rise to most of released tissue factor as a surrogate factor of pump thrombosis. In summary, this study underscores a need for more origin specific assays to discern vascular and endothelial related properties in contrast to systemic, e.g. pump contact surfaces – blood activation associated microparticle deviations. However, a limitation of this study was that it comprised different LVAD systems (axial, centrifugal, short-term, etc.).

In another study including 20 patients (Nascimbene *et al.* 2014), the concentration of

microparticles was established by flow cytometry and the concentration of aforementioned phosphatidylserine. Results showed that the concentration of microparticles was significantly higher ($p<0.001$) in patients who developed an adverse event as defined by the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) (Kirklin *et al.* 2014). In patients without adverse events, no significant change in the concentration of microparticles was observed after LVAD implantation and at 3 months following implantation. These results suggest that microparticle detection is a promising predictor of LVAD-associated adverse events.

Microparticles as a marker of vascular status

In our pilot study, we did not observe significant changes in concentration of circulating microparticles 3 months after implantation of an axial LVAD (Pitha *et al.* 2012). However, in our expanded study of 30 enrolled patients (Ivak *et al.* 2014), plasma levels of circulating microparticles were significantly decreased after 3 months following implantation ($p=0.003$). No difference was observed between patients with ischemic and non-ischemic etiology of heart failure ($p=0.53$) or between men and women ($p=0.9$). In both aforementioned studies, concentrations of circulating microparticles have been assessed by the ELISA method. Results of this study suggest improvement of vascular function after VAD implantation. The decrease of microparticles at 3 months after VAD implantation reflects potential improvement in vascular function. However, the results are not able to address in detail which specific biological mechanisms connected with the vascular system are involved in these processes. The ELISA assessment method is rather non-specific and cannot reliably determine cell origin of the microparticles. In addition, prediction of adverse events using microparticles was not analyzed in these studies. Another limitation could be the fact, that microparticle levels were not assessed at the time of possible adverse events, as in previously conducted study by Nascimbene *et al*. Observed results could be explained by a wide range of changes, including improved circulatory status and organ perfusion, improved endothelial function and/or alteration of thrombogenic factors, or combination of all these factors. This explanation is also supported by a recently published study, which reported improved microvascular circulatory parameters measured during reactive hyperemia in forearm (Sansone *et al.* 2015). In this study, also microparticles of different origin were assessed by flow cytometry in 14 patients with implanted centrifugal LVADs 3 months after implantation. Results showed higher concentration of blood cell- and endothelium-derived microparticles when compared to healthy controls and controls with stable coronary artery disease. Authors propose explanation, that higher concentrations of blood cell-origin microparticles are most probably the result of lower shear stress accompanied by higher/unique mechanical forces exerted on circulating blood cells. Significantly increased levels of endothelium-derived microparticles ($CD31^+/CD41^-$, $CD62e^+$, $CD144^+$) compared to healthy controls and patients with stable coronary artery disease may suggest possible negative impact of the generated continuous flow on endothelial function. Major limitation of this study was the absence of baseline values for longitudinal comparison. Assumingly, implantation of ventricular assist device led to an activation of endothelium cells and platelets, described as higher plasmatic levels of microparticles of these origins.

When comparing above discussed studies, not only type of used assay, but also the type of ventricular assist device might have effect on concentrations of microparticles.

To the best of our knowledge, there are no published studies, which describe more exactly the representation of specific microparticles by their origin. Therefore, it is not clear, whether status of vasculature or even prediction of clinical adverse events is associated more robustly with overall concentrations of microparticles or rather with concentrations of cell-specific microparticles.

Conclusions

Circulating microparticles play an important role in the regulation of inflammation, thrombosis, or endothelial function. Plasmatic levels of microparticles are increased in most cardiovascular diseases, probably reflecting activation or damage of circulating cells or cells of the vasculature itself. Cellular origin of circulating microparticles may predict their role and potential regulatory function in endothelial regulation, thrombus formation, and inflammatory response. Sparse data exist regarding levels and the role of circulating microparticles in patients with end-stage heart failure, and even less data are available in patients after implantation of a ventricular assist device. Recent

developments in implantable LVADs offer possibilities for long-term use of these devices as an alternative to the heart transplant in the near future. Because most of these devices utilize the principle of continuous flow, long-term impact of this flow pattern on bloodstream and vasculature remains unclear. Several studies describe only small effect of continuous flow on end-organ function (Rogers *et al.* 2010, Bhimaraj *et al.* 2015); however, the impact of LVADs on large arteries is, according to histological findings, mainly unfavorable including decrease in the number of smooth muscle cells in tunica media, higher medial degeneration, fragmentation of elastin fibers, medial fibrosis, and even atherosclerotic changes (Westaby *et al.* 2007, Segura *et al.* 2013).

Measurement of the concentration of circulating microparticles in peripheral blood might be of significant importance in assessment of vasculature status in patients with continuous-flow LVADs. In spite of many questions regarding the routine clinical use of this methodology, such direction of research is highly promising for future management of patients with LVADs and for more detailed understanding of vascular physiology in general. On the basis of continuing intensive research and long-term observations, the clarification of the role of circulating microparticles in assessment of vascular function can be expected in the near future. In particular, the correlation of these parameters with clinical data, including major clinical adverse events, could prove useful in detection and management of adverse events in patients with LVADs.

Conflict of Interest

Dr. Netuka is a proctor and speaker for St. Jude Medical. Other authors – none declared.

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References

ALEMAN MM, GARDINER C, HARRISON P, WOLBERG AS: Differential contributions of monocyte- and platelet-derived microparticles towards thrombin generation and fibrin formation and stability. *J Thromb Haemost* **9**: 2251-2261.

AMABILE N, CHENG S, RENARD JM, LARSON MG, GHORBANI A, MCCABE E, GRIFFIN G, GUERIN C, HO JE, SHAW SY, COHEN KS, VASAN RS, TEDGUI A, BOULANGER CM, WANG TJ: Association of circulating endothelial microparticles with cardiometabolic risk factors in the Framingham Heart Study. *Eur Heart J* **35**: 2972-2979, 2014.

AMABILE N, GUIGNABERT C, MONTANI D, YEGHIAZARIANS Y, BOULANGER CM, HUMBERT M: Cellular microparticles in pathogenesis of pulmonary hypertension. *Eur Respir J* **42**: 272-279, 2013.

AMIRAL J, SEGHATCHIAN J: The diagnostic usefulness of capture assays for measuring global/specific extracellular micro-particles in plasma. *Transfus Apher Sci* **53**: 127-136, 2015.

BERNAL MIZRACHI L, JY W, JIMENEZ JJ, PASTOR J, MAURO LM, HORSTMAN LL, DE MARCHENA E, AHN YS: High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *Am Heart J* **145**: 962-970, 2003.

BERNAL MIZRACHI L, JY W, FIERRO C, MACDONOUGH R, VELASQUES HA, PUROW J, JIMENEZ JJ, HORSTMAN LL, FERREIRA A, DE MARCHENA E, AHN YS: Endothelial microparticles correlate with high-risk angiographic lesions in acute coronary syndromes. *Int J Cardiol* **97**: 439-446, 2004.

BHIMARAJ A, URIBE C, SUAREZ EE: Physiological impact of continuous flow on end-organ function: Clinical implications in the current ERA of left ventricular assist devices. *Methodist DeBakey Cardiovasc J* **11**: 12-17, 2015.

BOULANGER CM, AMABILE N, TEDGUI A: Circulating microparticles: a potential prognostic marker for atherosclerotic vascular disease. *Hypertension* **48**: 180-186, 2006.

BRETELLE F, SABATIER F, DESPREZ D, CAMOIN L, GRUNEBAUM L, COMBES V, D´ERCOLE C, DIGNAT-GEORGE F: Circulating microparticles: a marker of procoagulant state in normal pregnancy and pregnancy complicated by preeclampsia or intrauterine growth restriction. *Thromb Haemost* **89**: 486-492, 2003.

BURGER D, KWART DG, MONTEZANO AC, READ NC, KENNEDY CR, THOMPSON CS, TOUYZ RM: Microparticles induce cell cycle arrest through redox-sensitive processes in endothelial cells: implications in vascular senescence. *J Am Heart Assoc* **1**: e001842, 2012.

BURGER D, TOUYZ RM: Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *J Am Soc Hypertens* **6**: 85-99, 2012.

BURGER D, SCHOCK S, THOMPSON CS, MONTEZANO AC, HAKIM AM, TOUYZ RM: Microparticles: biomarkers and beyond. *Clin Sci* **124**: 423-441, 2013.

BURNIER L, FONTANA P, KWAK BR, ANGELILLO-SCHERRER A: Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost* **101**: 439-451, 2009.

BRUYNDONCKX L, HOYMANS VY, FREDERIX G, DE GUCHTENAERE A, VISSERS DK, VRINTS CJ, RAMET J, CONRAADS VM: Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function. *J Pediatr* **165**: 300-305, 2014.

CONNOR DE, EXNER T, MA DD, JOSEPH JE: Detection of the procoagulant activity of microparticle-associated phosphatidylserine using XACT. *Blood Coagul Fibrinol* **20**: 558-564, 2009.

DIEHL, P, ALEKER M, HELBING T, SOSSONG V, BEYERSDORF F, OLSCHEWSKI M, BODE C, MOSER M: Enhanced microparticles in ventricular assist device patients predict platelet, leukocyte and endothelial cell activation. *Interact Cardiovasc Thorac Surg* **11**: 133-137, 2010.

DIEHL P, ALEKER M, HELBING T, SOSSONG V, GERMANN M, SORICHTER S, BODE C, MOSER M: Increased platelet, leukocyte and endothelial microparticles predict enhanced coagulation and vascular inflammation in pulmonary hypertension. *J Thromb Thrombolysis* **31**: 173-179, 2011.

DIGNAT-GEORGE F, BOULANGER CM: The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol* **31**: 27-33, 2010.

EDERHY S, DI ANGELANTONIO E, MALLAT Z, HUGEL B, JANOWER S, MEUELMAN C, BOCCARA F, FREYSSINET JM, TEDGUI A, COHEN A: Levels of circulating microparticles in nonvalvular atrial fibrillation. *Am J Cardiol* **100**: 989-994, 2007.

FRANÇA CN, IZAR MC, AMARAL JB, TEGANI DM, FONSECA FA: Microparticles as potential biomarkers of cardiovascular disease. *Arq Bras Cardiol* **104**: 169-174, 2015.

GONZALES-QUINTEIRO VH, SMARKUSKY LP, JIMENEZ JJ, MAURO LM, JY W, HORSTMAN LL, O´SULLIVAN MJ, AHN YS: Elevated plasma endothelial microparticles: preeclampsia versus gestational hypertension. *Am J Obstet Gynecol* **191**: 1418-1424, 2004.

HOLMAN WL, NAFTEL DC, ECKERT CE, KORMOS RL, GOLDSTEIN DJ, KIRKLIN JK: Durability of left ventricular assist devices: Interagency registry for mechanically assisted circulatory support (INTERMACS) 2006 to 2011. *J Thorac Cardiovasc Surg* **146**: 437-441, 2013.

HUGHES M, HAYWARD CP, WARKENTIN TE, HORSEWOOD P, CHORNEYKO KA, KELTON JG: Morphological analysis of microparticle generation in heparin-induced thrombocytopenia. *Blood* **96**: 188-194, 2000.

IVAK P, PITHA J, WOHLFAHRT P, KRALOVA-LESNA I, STAVEK P, DORAZILOVA Z, STEPANKOVA J, MALY J, POKORNY M, NETUKA I: Endothelial dysfunction expressed as endothelial microparticles in patients with end-stage heart failure. *Physiol Res* **63**: 369-373, 2014.

JY W, HORSTMAN LL, JIMENEZ JJ, AHN YS, BIRÓ E, NIEUWLAND R, STURK A, DIGNAT-GEORGE F, SABATIER F, CAMOIN-JAU L, SAMPOL J, HUGEL B, ZOBAIRI F, FREYSSINET JM, NOMURA S, SHET AS, KEY NS, HEBBEL RP: Measuring cell-derived microparticles. *J Thromb Heamost* **2**: 1842-1851, 2004.

KIRKLIN JK, NAFTEL DC, PAGANI FD, KORMOS RL, STEVENSON LW, BLUME ED, MILLER MA, TIMOTHY BALDWIN J, YOUNG JB: Sixth INTERMACS annual report: A 10,000-patient database. *J Heart Lung Transplant* **33**: 555-564, 2014.

KIRKLIN JK, NAFTEL DC, KORMOS RL, STEVENSON LW, PAGANI FD, MILLER MA, TIMOTHY BALDWIN J, YOUNG JB: Fifth INTERMACS annual report: Risk factor analysis from more than 6,000 mechanical circulatory support patients. *J Heart Lung Transplant* **32**: 141-156, 2013.

KIRKLIN JK, NAFTEL DC, PAGANI FD, KORMOS RL, STEVENSON LW, MILLER M, YOUNG JB: Long-term mechanical circulatory support (destination therapy): On track to compete with heart transplantation? *J Thorac Cardiovasc Surg* **144**: 584-603, 2012.

LACROIX R, ROBERT S, PONCELET P, DIGNAT-GEORGE F: Overcoming limitations of microparticle measurement by flow cytometry. *Semin Thromb Hemost* **36**: 807-818, 2010.

MCGINN C, MACDONNELL BF, SHAN CX, WALLACE R, CUMMINS PM, MURPHY RP: Microparticles: A pivotal nexus in vascular homeostasis and disease. *Curr Clin Pharmacol* **11**: 28-42, 2016.

NASCIMBENE A, HERNANDEZ R, GEORGE JK, PARKER A, BERGERON AL, PRADHAN S, VIJAYAN KV, CIVITELLO A, SIMPSON L, NAWROT M, LEE VV, MALLIDI HR, DELGADO RM, DONG JF, FRAZIER OH: Association between cell-derived microparticles and adverse events in patients with nonpulsatile left ventricular assist devices. *J Heart Lung Transplant* **33**: 470-477, 2014.

NOMURA S, TANDON NN, NAKAMURA T, CONE J, FUKUHARA S, KAMABAYASHI J: High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. *Atherosclerosis* **158**: 277-287, 2001.

OWEN B, XUE A, HEIT J, OWEN W: Procoagulant activity, but not number, of microparticles increases with age and in individuals after a single venous thromboembolism. *Thromb Res* **127**: 39-46, 2011.

PICCIN A, MURPHY WG, SMITH OP: Circulating microparticles: pathophysiology and clinical implications. *Blood Rev* **21**: 157-171, 2007.

PITHA J, DORAZILOVA Z, MELENOVSKY V, KRALOVA-LESNA I, STAVEK P, STEPANKOVA J, URBAN M, MALY J, NETUKA I: The impact of ventricle assist device on circulating endothelial microparticles – pilot study. *Neuro Endocrinol Lett* **33**: 68-72, 2012.

PRESTON RA, JY W, JIMENEZ JJ, MAURO LM, HORSTMAN LL, VALLE M, AIME G, AHN YS: Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension* **41**: 211-217, 2003.

ROGERS JG, AARONSON KD, BOYLE AJ, RUSSEL SD, MILANO CA, PAGANI FD, EDWARDS BS, PARK S, JOHN R, CONTE JV, FARRAR DJ, SLAUGHTER MS: Continuous flow left ventricular assist device improves functional capacity and quality of life of advanced heart failure patients. *J Am Coll Cardiol* **55**: 1826-1834, 2010.

RUBIN O, CANELLINI G, DELOBEL J, LION N, TISSOT JD: Red blood cell microparticles: Clinical relevance. *Transfus Med Hemoter* **39**: 342-347, 2012.

SABATIER F, DARMON P, HUGEL B, COMBES V, SANMARCO M, VELUT JG, ARNOUX D, CHARPIOT P, FREYSSINET JM, OLIVER C, SAMPOL J, DIGNAT-GEORGE F: Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes* **51**: 2840-2845, 2002.

SAPET C, SIMONCINI S, LORIOD B, PUTHIER B, SAMPOL J, NGUYEN C, DIGNAT-GEORGE F, ANFOSSO F: Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. *Blood* **108**: 1868-1876.

SCHIRO A, WILKINSON FL, WESTON R, SMYTH JV, SERRACINO-INGLOTT F, ALEXANDER MY: Endothelial microparticles as conveyors of information in atherosclerotic disease. *Atherosclerosis* **234**: 295-302, 2014.

SANSONE R, STANSKE B, KEYMEL S, SCHULER D, HORN P, SAEED D, BOEKEN U, WESTENFELD R, LICHTENBERG A, KELM M, HEISS C: Macrovascular and microvascular function after implantation of left ventricular assist devices in end-stage heart failure: Role of microparticles. *J Heart Lung Transplant* **34**: 921-932, 2015.

SEGURA AM, GREGORIC I, RADOVANCEVIC R, DEMIROZU ZT, BUJA LM, FRAZIER OH: Morphologic changes in the aortic wall media after support with a continuous-flow left ventricular assist device. *J Heart Lung Transplant* **32**: 1096-1100, 2013.

SHAH KB, KONTOS MC: Microparticles and left ventricular assist device complications: a causal association? *J Heart Lung Transplant* **33**: 468-469, 2014.

SINGH N, VAN CRAEYVELD E, TJWA M, CIARKA A, EMMERECHTS J, DROOGNE W, GORDTS SC, CARLIER V, JACOBS F, FIEUWS S, VANHAECKE J, VAN CLEEMPUT J, DE GEEST B: Circulating apoptotic endothelial cells and apoptotic endothelial microparticles independently predict the presence of cardiac allograft vasculopathy. *J Am Coll Cardiol* **60**: 324-331, 2012.

SINAURIDZE EI, KIREEV DA, POPENKO NY, PICHUGIN AV, PANTELEEV MA, KRYMSKAYA OV, ATAULLAKHANOV FI: Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thromb Haemost* **97**: 425-434, 2007.

TRAMONTANO AF, LYUBAROVA R, TSIAKOS J, PALAIA T, DELEON JR, RAGOLIA L: Circulating endothelial microparticles in diabetes mellitus. *Mediators Inflam* **2010**: 250476, 2010.

VAN WIJK MJ, NIEUWLAND R, BOER K, VAN DEN POST JA, VAN BAVEL E, STURK A: Microparticle subpopulations are increased in preeclampsia: possible involvement in vascular dysfunction? *J Am Obstet Gynecol* **187**: 450-456, 2002.

VAN WIJK MJ, VAN BAVEL E, STURK A, NIEUWLAND R: Microparticles in cardiovascular diseases. *Cardiovasc Res* **59**: 277-287, 2003.

VIERA AJ, MOOBERRY M, KEY NS: Microparticles in cardiovascular disease pathophysiology and outcomes. *J Am Soc Hypertens* **6**: 243-252, 2012.

WARKENTIN TE, HAYWARD CPM, BOSHKOV LK, SANTOS AV, SHEPPARD JA, BODE AP, KELTON JG: Sera from patients with heparin-induced thrombocytopenia generate platelet-derived microparticles with procoagulant activity: An explanation for the thrombotic complications of heparin-induced thrombocytopenia. *Blood* **84**: 3691-3699, 1994.

WESTABY S, BERTONI GB, CLELLAND C, NISHINAKA T, FRAZIER OH: Circulatory support with attenuated pulse pressure alters human aortic wall morphology. *J Thorac Cardiovasc Surg* **133**: 575-576, 2007.

WILLIAMS JB, JAUCH EC, LINDSELL CJ, CAMPOS B: Endothelial microparticle levels are similar in acute ischemic stroke and stroke mimics due to activation and not apoptosis/necrosis. *Acad Emerg Med* **14**: 685-690, 2007.

ZAFRANI L, INCE C, YUEN PS: Microparticles during sepsis: target, canary or cure? *Intensive Care Med* **39**: 1854-1856, 2013.

ZHANG Y, MENG H, MA R, HE Z, WU X, CAO M, YAO Z, ZHAO L, LI T, DENG R, DONG Z, TIAN Y, BI Y, KOU J, THATTLE HS, ZHOU J, SHI J: Circulating microparticles, blood cells, and endothelium induce procoagulant activity in sepsis through phosphatidylserine exposure. *Shock* **45**: 299-307, 2016.

ZHOU J, SHI J, HOU J, CAO F, ZHANG Y, RASMUSSEN JT, HEEGAARD CW, GILBERT GE: Phosphatidylserine exposure and procoagulant activity in acute promyelocytic leukemia. *J Thromb Haemost* **8**: 773-782, 2010.

PRÁCE B popisuje sledování koncentrací cirkulujících mikročástic u pacientů s implantovanou mechanickou srdeční podporou v období před implantací levostranné mechanické srdeční podpory a 3 měsíce po výkonu. V práci je testovaná hypotéza o longitudinálních změnách v koncentraci mikročástic a vlivu implantace mechanické srdeční podpory na jejich vývoj. Vzhledem k tomu, že hladiny MP se mění u některých chorobných stavů, zejména u kardiovaskulárních onemocnění, cirkulující mikročástice mohou být potencionálním markerem vaskulárního poškození. Dosavadní studie byly prováděny zejména u pacientů s normálním krevním průtokem. V době vzniku této publikace bylo problematice mikročástic u pacientů s implantovanou MSP věnováno jen málo prací. Proto cílem této studie bylo vyjádřit předpokládanou možnou poruchu endoteliální funkce u pacientů s implantovanou mechanickou srdeční podporou s nepulzatilním průtokem pomocí sledování koncentrace mikročástic.

V této studii bylo vyšetřeno 30 pacientů (25 mužů a 5 žen ve věku 54,16±10,03 let) v období před a 3 měsíce po implantaci levostranné mechanické srdeční podpory. Všem pacientům byla implantována mechanická podpora s kontinuálním průtokem HeartMate II v indikaci přemostění k transplantaci srdce. Převažující diagnózou v souboru pacientů byla ischemická etiologie srdečního selhání, diagnostikována u 18 pacientů, u 12 pacientů byla zjištěna kardiomyopatie neischemického původu. Mezi těmito skupinami nebyl signifikantní rozdíl ve věku, BNP před implantací nebo pohlaví. Koncentrace mikročástic byly změřeny pomocí zavedené a komerčně dostupné metody ELISA (Hyphen Biomed, Francie) a vyjádřeny v nanomolech na litr vztažených ke koncentraci fosfatidylserinu (nMPS). V porovnání koncentrací před implantaci mechanické srdeční podpory a 3 měsíce po výkonu byl pozorován signifikantní pokles koncentrací cirkulujících mikročástic ($p=0,03$) u všech pacientů. Nebyl pozorován rozdíl mezi pacienty s ischemickou a neischemickou etiologii srdečního selhání před

implantací MSP (p=0,53) a ani 3 měsíce po ní (p=0,75). Vliv věku (p=0,72) ani pohlaví (p=0,90) na koncentraci cirkulujících mikročástic nebyl statisticky signifikantní.

Výsledky provedené studie neprokázaly jednoznačný negativní efekt kontinuálního průtoku na funkci vaskulatury, vyjádřeného jako koncentrace cirkulujících mikročástic. Navíc také nebyl prokázán rozdíl v poklesu mikročástic při rozdělení pacientů dle etiologie srdečního selhání. Toto pozorování naznačuje, že v krátkodobém horizontu 3 měsíců po implantaci mechanické srdeční podpory, pravděpodobně nedochází k zásadnímu zhoršení funkce vaskulatury vlivem kontinuálního toku. Studie potvrdila hypotézu o změnách koncentrací cirkulujících mikročástic před a po implantaci mechanické srdeční podpory.

Kompletní znění článku je přiloženo v anglickém jazyce.

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Endothelial Dysfunction Expressed as Endothelial Microparticles in Patients With End-Stage Heart Failure

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Summary

Left ventricular assist devices (LVAD), currently used in treatment of terminal heart failure, are working on principle of rotary pump, which generates continuous blood flow. Non-pulsatile flow is supposed to expose endothelial cells to high stress and potential damage. Therefore, we investigated longitudinal changes in concentration of circulating endothelial microparticles (EMP) as a possible marker of endothelial damage before and after implantation of LVAD. Study population comprised 30 patients with end-stage heart failure indicated for implantation of the Heart Mate II LVAD. Concentrations of microparticles were measured as nanomoles per liter relative to phosphatidylserine before and 3 months after implantation. At 3 months after implantation we observed significant decrease in concentration of EMP [5.89 (95 % CI 4.31-8.03) vs. 3.69 (95 % CI 2.70-5.03), p=0.03] in the whole group; there was no difference observed between patients with ischemic etiology of heart failure (n=18) and with heart failure of non-ischemic etiology (n=12). In addition, heart failure etiology had no effect on the rate of EMP concentration decrease with time. These results indicate possibility that LVAD do not cause vascular damage 3 months after implantation. Whether these results suggest improvement of vascular wall function and of endothelium is to be proved in long-term studies.

Key words

Ventricular assist device • Non-pulsatile flow • Circulating endothelial microparticles

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Introduction

Use of continuous left-ventricular assist devices (LVAD) for partial or full support of failing heart have become an important and, at this time, also leading therapeutic option for patients with end-stage heart failure of different etiologies along with heart transplantation (Kirklin *et al.* 2014).

Currently, LVAD are mostly used as a bridge to heart transplant, but the lack of donor organs and also design advancements and increasing reliability of LVAD are widening possibilities of long-term use of these pumps. In the past few years application of mechanical circulatory support continues to rise as permanent therapy/destination therapy. Of interest, in the United States, in 2012 more than 40 % of implants have been designated to destination therapy (Kirklin *et al.* 2014).

Eligibility of long-term use of LVAD was mainly influenced by design advancements. First-generation pulsatile LVAD had higher complication rate than the latest generation of ventricular assist devices working on the principle of rotary pump with continuous-flow (Pagani *et al.* 2009, Slaughter *et al.* 2009).

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Generally it is assumed that the presence of non-physiological continuous blood flow leads to higher stress of endothelial cells, and rises a concern of proatherogenic and prothrombotic changes in endothelium. Surprisingly, this flow pattern of significantly diminished pulsatility is well tolerated in short- and mid-term (Rogers *et al.* 2010).

Studying long-term use of LVAD offers genuine opportunity of more complex understanding of vascular changes in patients with LVAD and revelation of impact of continuous blood flow on endothelial function. Vascular endothelium plays key role in arterial wall integrity and blood flow regulation (Cines *et al.* 1998) and recent studies showed that damage of endothelium cells, their activation and/or apoptosis leads to release of newly detectable biomarkers related to endothelial injury – a complex submicron membrane-shed vesicles called endothelial microparticles (EMP) (Schiro *et al.* 2014).

Microparticles are anuclear fragments of cellular membrane shed from stressed or damaged cells, with a diameter of 0.1 to 1.0 μm. They contain surface proteins and cytoplasmic material of their parental cells (Burger *et al.* 2012).

As possible end products of apoptosis of endothelial cells, circulating microparticles can act as a strong disregulators of endothelial function (Dignat-George *et al.* 2011). The production of microparticles is believed to be a part of normal cell function but elevation of EMP in coronary artery disease (Bernal-Mizrachi *et al.* 2004, Singh *et al.* 2012), stroke (Williams *et al.* 2007) and in carotid artery disease (Schiro *et al.* 2014), was well described in several studies and therefore microparticles may be associated with increased risk of major cardiovascular complications (Bernal-Mizrachi *et al.* 2004).

All these studies were performed in patients with physiological pulsatile flow, and only sparse data are available regarding the role of new potential prognostic cardiovascular risk factor – circulating EMP in patients with end-stage heart failure with continuous-flow LVAD. Almost no data are available describing effect of these devices on EMP in short and long-term perspective. Therefore, the aim of our study was to assess the effect of the mechanical circulatory support on the concentration of circulating EMP after 3 months. In addition, we analyzed whether this effect is different according to etiology of heart failure leading to implantation of LVAD.

Methods

All the patients included in the study underwent LVAD implantation due to end-stage heart failure and received axial continuous-flow device – HeartMate II (Thoratec Corp., Pleasanton, California). LVAD was implanted *via* sternotomy in standard fashion as a bridge to heart transplant in all patients.

Heparin bridge has been implemented in all procedures until target anticoagulation with warfarin has been reached. After implantation, the aim of anticoagulation therapy was to reach international normalized ratio of 1.8-2.2; in patients with trombophilias it was 2.5-3.

Blood samples were collected from peripheral vein during 24-48 h before and 3 months after implantation. Circulating EMP were measured in an audited lipid laboratory under continuous external quality control of CDC Atlanta, USA. The concentration of EMP was determined by ELISA Zymutest MP activity test (Hyphen Biomed, France) according to the methodology established previously (Slavik *et al.* 2010) and expressed as nanomoles per liter relative to phosphatidylserine (nM PS). The microparticles were measured in duplicate and the mean of two measurements was used for further analyses.

Prior to procedure, all patients with ischemic heart disease as a cause of heart failure (IHD) were treated with acetylsalicylic acid and statins in contrast to patients with non-ischemic etiology of heart failure (non-IHD). Of note, acetylsalicylic acid has not been administered as a part of antithrombotic regimen.

Institutional ethics committee approval has been obtained prior to the study initiation and all participants provided their signed informed consent.

Data are expressed as mean ± SD, median (interquartile range-IQR), estimated marginal mean (95 % confidence interval) or number (percentage). Differences between IHD and non-IHD group were analyzed using unpaired t-test in the case of continuous variables and Fischer exact test in the case of categorical variables. In graph estimated marginal mean and standard error of the mean (SEM) is plotted. Longitudinal changes in EMP number were analyzed using generalized linear mixed-effect regression model. In this model intercept was treated as a random factor, while time, heart failure etiology and the interaction term between time and etiology, age and gender were treated as fixed factors. Because EMP concentration was right-skewed, we used

gamma regression (Fitzmaurice *et al.* 2008). Calculations were done using SPSS 21 (IBM Corporation, NY, USA) and STATA. A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 30 patients, 25 males and 5 females were included into the 3 month prospective study. Mean age of participants was 54.16±10.03 years. Eighteen patients were diagnosed with IHD and 12 with non-IHD as a cause of heart failure. Their baseline data are listed in Table 1. We did not find any significant difference between both groups in age, representation of men/women or brain natriuretic factor (BNP) before implantation. The reason of non-significant difference between men and women was caused by a small number of patients in investigated group.

Table 1. Baseline values of selected characteristics of patients treated by left ventricular assist device.

	Ischemic etiology of heart failure	Non-ischemic etiology of heart failure	*p*-value
N	18	12	
Age (years)	54.7 ± 7.99	53.33 ± 13.20	0.36
Men/women (n)	17/1	8/4	0.128
Current smokers (n)	0	0	1.0
BNP before implantation of LVAD (ng/l)	1411 ± 1422 (n=15)	1473 ± 867 (n=11)	0.99

Results are expressed as mean ± SD if not stated differently. LVAD – left ventricular assist device; BNP – Brain Natriuretic Peptide.

In the whole group, we observed a significant decrease of circulating EMP before the implantation of LVAD and three months after the procedure [5.89 (95 % CI 4.31-8.03) and 3.69 (95 % CI 2.70-5.03), p=0.03] (Fig. 1).

Subsequently we analyzed patients with IHD and non-IHD separately, using generalized linear mixed-effect regression model. Prior to implantation, concentrations of circulating EMP in patients with IHD were not significantly different from patients with non-ischemic etiology of heart failure [5.33 (95 % CI 3.60-7.89) and 6.51 (95 % CI 4.02-10.53), p=0.53]. In addition, using this model, heart failure etiology had no robust effect on the rate of decrease of EMP concentration after LVAD implantation (p=0.75). Furthermore, we did not observe any effect of age (p=0.72) or gender (p=0.90) on the rate of decrease of EMP concentration.

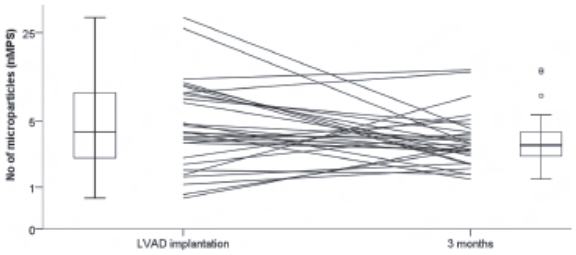

Fig. 1. Concentration of circulating endothelial microparticles before and after implantation of left ventricular assist device (LVAD).

Discussion

In our study we demonstrated that implantation of LVAD led to significant decrease of EMP during 3 months. Additional finding was that this decrease was not different in patients with IHD and non-IHD as a cause of heart failure. Recent study expands our previous pilot study conducted in 8 male patients (Pitha *et al.* 2012), where no significant effect of LVAD on concentration of circulating EMP was described, probably due to low number of participants.

To our knowledge only two other studies were focused on EMP during LVAD therapy. In recent study EMP were correlated with clinical complications in 20 patients with LVAD, and significant association between microparticles levels and subsequent clinical events was observed, levels of microparticles were significantly higher in patients who developed an adverse event than in patients with no events (Nascimbene *et al.* 2014). In another study, levels of microparticles were significantly increased in patients with LVAD compared to healthy controls (Diehl *et al.* 2010).

Miniaturized, both axial and centrifugal, mechanical ventricular assist devices have demonstrated in last decade undisputable benefit in treatment of patients with end-stage heart failure. Nevertheless, an impact of generated continuous blood flow pattern on vasculature and endothelial cells is not yet clear. It is

generally assumed, that non-pulsatile flow could exert negative impact on vascular wall and endothelium and may damage endothelial cell membrane and as a result leads to release of endothelial microparticles. Measurement of concentrations of circulating EMP may therefore help to predict endothelial function and may serve as an important biomarker.

Our longitudinal study demonstrates statistically significant decrease in concentration of EMP in patients with end-stage heart failure three months after implantation of continuous LVAD. This finding may suggest improvement of endothelial function of patients on LVAD. Another potential explanation for significant drop of EMP concentration is an improvement of organ perfusion and microcirculation by restoration of adequate systemic output leading to improved status of vasculature in general.

Intensive research of EMP and their function in several cardiovascular pathophysiological processes and also their potential role as biomarkers was conducted in recent years. Despite this fact more analyses are required to establish precise and standardized methods for clinical use of parameters of these EMP and to determine whether their function could be modified to improve prognosis of our patients.

Certain limitation of our study is possible heterogeneity of EMP and still discussed doubts regarding their origin. Therefore, it is still not clear if EMP really reflect only endothelial damage; however, their detrimental effect was already demonstrated in several studies (Singh *et al.* 2012, Schiro *et al.* 2014). Therefore, results of this study indicate that use of continuous flow LVAD does not exhibit detrimental effect to the endothelium in a short term. Nevertheless, additional long-term observations of the EMP dynamics are needed to clarify a chronic effect on microvasculature and endothelial damage. Moreover, correlations with clinical parameters and outcomes are desirable in order to elucidate potential predictive role of EMP as a biomarker of device related adverse events.

In conclusion, in a short term, LVAD exerted rather favorable effect on the vasculature, defined as decreasing number of circulating endothelial microparticles. If this effect is sustained for longer periods must be confirmed in longer longitudinal studies. Nevertheless, based on our recent results this laboratory method might compensate for some technical problems encountered in examination of the status of the vasculature in patients with LVAD.

Conflict of Interest

There is no conflict of interest.

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References

BERNAL-MIZRACHI L, JY W, FIERRO C, MACDONOUGH R, VELASQUEZ HA, PUROW J, JIMENEZ JJ, HORSTMAN LL, FERREIRA A, DE MARCHENA E, AHN YS: Endothelial microparticles correlate with high-risk angiographic lesions in acute coronary syndromes. *Int J Cardiol* **97**: 439-446, 2004.

BULUT D, BECKER V, MÜGGE A: Acetylsalicylate reduces endothelial and platelet-derived microparticles in patients with coronary artery disease. *Can J Physiol Pharmacol* **89**: 239-244, 2011.

BURGER D, TOUYZ RM: Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells and circulating endothelial cells. *J Am Soc Hypertens* **6**: 85-99, 2012.

CINES DB, POLLAK ES, BUCK CA, LOSCALZO J, ZIMMERMAN GA, MCEVER RP, POBER JS, WICK TM, KONKLE BA, SCHWARTZ BS, BARNATHAN ES, MCCRAE EKR, HUG BA, SCHMIDT AM, STERN DM: Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* **91**: 3527-3561, 1998.

DIEHL P, ALEKER M, HELBING T, SOSSONG V, BEYERSDORF F, OLSCHEWSKI M, BODE C, MOSER M: Enhanced microparticles in ventricular assist device patients predict platelet leukocyte and endothelial cell activation. *Interact Cardiovasc Thorac Surg* **11**: 133-137, 2010.

DIGNAT-GEORGE F, BOULANGER CM: The many faces of endothelial microparticles. *Aterioscler Thromb Vasc Biol* **31**: 27-33, 2011.

FITZMAURICE GM, RAVICHANDRAN C: A primer in longitudinal data analysis. *Circulation* **118**: 2005-2010, 2008.

KIRKLIN JK, NAFTEL DC, PAGANI FD, KORMOS RL, STEVENSON LW, BLUME ED, MILLER MA, TIMOTHY BALDWIN J, YOUNG JB: Sixth INTERMACS annual report: A 10,000-patient database. *J Heart Lung Transplant* **33**: 555-564, 2014.

MOBARREZ F, EGBERG N, ANTOVIC J, BRÖIJERSEN A, JÖRNESKOG G, WALLÉN H: Release of endothelial microparticles in vivo during atorvastatin treatment; a randomized double-blind placebo-controlled study. *Thromb Res* **129**: 95-97, 2012.

NASCIMBENE A, HERNANDEZ R, GEOGE JK, PARKER A, BERGERON AL, PRADHAN S, VIJAYAN KV, CIVITELLO A, SIMPSON L, NAWROT M, LEE VV, MALLIDI HR, DELGADO RM, DONG JF, FRAZIER OH: Association between cell-derived microparticles and adverse events in patients with nonpulsatile left ventricular assist devices. *J Heart Lung Transplant* **33**: 470-477, 2014.

PAGANI FD, MILLER LW, RUSSEL SD, AARONSON KD, JOHN R, BOYLE AJ, CONTE JV, BOGAEV RC, MacGILLIVRAY TE, NAKA Y, MANCINI D, MASSEY HT, CHEN L, KLODELL CT, ARANDA JM, MOAZAMI N, EWALD GA, FARRAR DJ, FAZIER OH: HeartMate II Investigators. Extended mechanical circulatory support with a continuous-flow rotary left ventricular assist device. *J Am Coll Cardiol* **54**: 312-321, 2009.

PITHA J, DORAZILOVA Z, MELENOVSKY V, KRALOVA LESNA I, STAVEK P, STEPANKOVA J, URBAN M, MALY J, NETUKA I: The impact of ventricle assist device on circulating endothelial microparticles – pilot study. *Neuroendocrinol Lett* **33** (Suppl 2): 68-72, 2012.

ROGERS JG, AARONSON KD, BOYLE AJ, RUSSEL SD, MILANO CA, PAGANI FD, EDWARDS BS, PARK S, JOHN R, CONTE JV, FARRAR DJ, SLAUGHTER MS: HeartMate II Investigators. Continuous flow left ventricular assist device improves functional capacity and quality of life of advanced heart failure patients. *J Am Coll Cardiol* **55**: 1826-1834, 2010.

SCHIRO A, WILKINSON FL, WESTON R, SMYTH JV, SERRACINO-INGLOT F, ALEXANDER MY: Endothelial microparticles as conveyors of information in atherosclerotic disease. *Atherosclerosis* **234**: 295-302, 2014.

SINGH N, VAN CRAEYVELD E, TJWA M, CIARKA A, EMMERECHTS J, DROOGNE W, GORDTS SC, CARLIER V, JACOBS F, FIEUWA S, VANHAECKE J, VAN CLEEMPUT J, DE GEEST B: Circulating apoptotic endothelial cells and apoptotic endothelial microparticles independently predict the presence of cardiac allograft vasculopathy. *J Am Coll Cardiol* **60**: 324-331, 2012.

SLAUGHTER MS, ROGERS JG, MILANO CA, RUSSEL SD, CONTE JV, FELDMAN D, SUN B, TATOOLES AJ, DELGADO RM 3RD, LONG JW, WOZNIAK TC, GHUMMAN W, FARRAR DJ, FRAZIER OH: HeartMate II Investigators. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med* **361**: 2241-2251, 2009.

SLAVÍK L: Mikropartikule (in Czech). *In vitro diagnostika* **16**: 19-22, 2010.

WILLIAMS JB, JAUCH EC, LINDSELL CJ, CAMPOS B: Endothelial microparticle levels are similar in acute ischemic stroke and stroke mimics due to activation and not apoptosis/necrosis. *Acad Emerg Med* **14**: 685-690, 2007.

PRÁCE C byla zaměřena na longitudinální sledování počtu cirkulujících kmenových (SC) a zejména endoteliálních progenitorových buněk (EPC), kterým je připisována schopnost endoteliální regenerace a participace v angiogenezi. V práci je testovaná hypotéza o změnách počtu těchto buněk v periferní krvi v závislosti na změně typu krevního průtoku ovlivněného mechanickou srdeční podporou a to za předpokladu, že kontinuální MSP vytvářejí zvýšené smykové zatížení endoteliálních a cirkulujících buněk. Z tohoto důvodu lze u progenitorových buněk předpokládat změny v jejich cirkulujícím počtu. Tyto změny by mohly odrážet míru poškození a komplexnost změn vytvořených nepulzatilním tokem. U pacientů zařazených do studie byla také provedena sub-analýza cirkulujících mikročástic. Hlavním cílem studie byla evaluace paralelních změn u cirkulujících endoteliálních progenitorových buněk, kmenových buněk a cirkulujících mikročástic.

Analýza proběhla u 23 pacientů indikovaných k implantaci mechanické srdeční podpory. Pět pacientů bylo ženského pohlaví, průměrný věk pacientů 50,6±14,1 roku. Všem pacientům byla implantována MSP HeartMate II s kontinuálním průtokem v indikaci přemostění k transplantaci srdce. U 8 pacientů byla MSP implantována z mini invazivního přístupu. Všichni pacienti dosáhli šesti měsíčního sledování bez závažných klinických nežádoucích událostí. Měření bylo provedeno ve třech časových bodech: před, 3 měsíce a 6 měsíců (±15 dní) po implantaci levostranné mechanické srdeční podpory. Pacienti byli rozděleni do skupin dle etiologie srdečního selhání a věku. Cirkulující SC a EPC byly měřeny pomocí zavedené metodiky průtokové cytometrie, kmenové buňky byly definovány jako CD34+/CD45low+ a endoteliální progenitorové buňky jako CD34+/CD45low+/KDR+ buňky. Koncentrace cirkulujících mikročástic byly stanoveny metodou ELISA (Hyphen Biomed, Francie).

Počty cirkulujících kmenových buněk a endoteliálních progenitorových buněk signifikantně poklesly 3 měsíce po implantaci mechanické srdeční podpory při porovnání se stavem před implantací (p=0,01 pro SC a p=0,001 pro EPC). V šestém měsíci došlo k jejich opětovnému nárůstu v porovnání s měřením ve třetím měsíci (p=0,006 pro SC a 0,003 pro EPC) téměř na úroveň stavu před implantací MSP. Proporce endoteliálních progenitorových buněk v populaci mononukleárních buněk se také významně lišila, paralelně se změnami SC a EPC (p=0,001 pro změnu mezi předimplantačním vyšetřením a 3. měsícem a p=0,001 mezi 3. a 6. měsícem). Koncentrace cirkulujících mikročástic se statisticky signifikantně nelišily, i když trendově kopírovaly jejich koncentrace vývoj SC a EPC (p=0,33 pro změnu mezi předimplantačním vyšetřením a 3. měsícem a p=0,38 mezi 3. a 6. měsícem). Pacienti byli rozděleni do skupin dle etiologie srdečního selhání. U deseti pacientů byla diagnostikována ischemická etiologie srdečního selhání, u 13 pacientů etiologie neischemická. Etiologie srdečního selhání neměla statisticky signifikantní vliv na cirkulující biomarkery. Skupina pacientů s ischemickou etiologií srdečního selhání se od skupiny s neischemickou etiologií signifikantně lišila věkem, vyšším výskytem hypertenze, diabetes mellitus a užíváním statinů.

U pacientů starších 55 let byl pozorován efekt věku na cirkulující kmenové buňky, s významným rozdílem v předimplantačním vyšetření. Počet SC byl u této skupiny snížený (p=0,03) a nevykázal dynamiku sledovanou u EPC.

Implantace levostranné mechanické srdeční podpory tedy vedla k signifikantním změnám v počtu cirkulujících SC a EPC a potvrzení zkoumané hypotézy. Vzhledem k vysoce omezenému počtu dostupných dat z jiných studií o těchto změnách, lze jen opatrně spekulovat o důvodech popsané dynamiky. Nejpravděpodobnějším mechanismem poklesu počtu sledovaných buněk ve 3. měsíci po implantaci MSP se zdá

znovuobnovení suficientního krevního toku do tkání. Tímto způsobem je pravděpodobně eliminována ischemie tkání, která se jeví jako jeden ze stimulů, podporujících uvolnění EPC a SC do cirkulace. Následný nárůst hladin sledovaných buněk v 6. měsíci by mohl ukazovat na reakci organismu na pokračující endoteliální dysfunkci a poškození, vyvolané nepulzatilním krevním tokem. Koncentrace cirkulujících mikročástic kopírovaly průběh změn u EPC, k významné statistické korelaci ale nedošlo. Tuto skutečnost přisuzujeme nízkému počtu pacientů ve studii. V této studii jsme tedy pravděpodobně svědky dvou základních mechanismů ovlivňujících koncentrace sledovaných markerů. Na jedné straně stojí snížený vaskulární stres a snížení ischemie díky zavedení MSP, na druhé zvýšený vaskulární stres způsobený nepulzatilním průtokem při dlouhodobé terapii pomocí MSP.

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Biphasic response in number of stem cells and endothelial progenitor cells after left ventricular assist device implantation: A 6 month follow-up☆

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ABSTRACT

Background: Continuous blood flow could have deleterious effects on endothelium and vascular health. This could have serious consequences in patients with heart failure treated with continuous flow left ventricular assist devices (LVAD). Therefore, we studied effect of LVAD on three circulating vascular biomarkers: stem cells (SC), endothelial progenitor cells (EPC) and microparticles (MP).

Methods: In 23 patients (5 women) with end-stage heart failure, SC, EPC and MP were measured before, and 3 and 6 months after implantation of LVAD (HeartMate II). SC were defined using determination of surface antigen expression as mononuclear CD34+/CD45low+ cells and EPC as mononuclear CD34+/CD45low+/KDR+ cells. MP concentrations were determined by ELISA method.

Results: Three months after LVAD implantation numbers of SC and EPC significantly decreased ($p = 0.01$ and $p = 0.001$, respectively). On the contrary, between 3rd and 6th month after implantation they significantly increased ($p = 0.006$ and $p = 0.003$, respectively). MP did not change significantly during the study despite exerting similar trend as SC and EPC.

Conclusions: Observed biphasic changes of SC and EPC might reflect two processes. First, shortly after LVAD implantation, improved tissue perfusion could lead to decrease in ischemic stimuli and ensuing decrease of SC and EPC. Second, continuous flow between 3rd and 6th month produced by LVAD could lead to increase of SC and EPC through activation of endothelium. This explanation could be supported also by similar trend in the changes of concentrations of MP.

1. Introduction

Use of continuous-flow left ventricular assist devices (LVAD) has become a routine and well-established treatment of advanced heart failure [1,2]. LVAD significantly reduced heart transplantation waiting list mortality and improved the quality of life and survival rates in patients with end-stage heart failure [3–6].

Currently, the most commonly used generation of long-term LVAD generates continuous flow. On one hand it is assumed, that the presence of non-physiological continuous blood flow leads to higher stress of endothelial cells and vessel wall and rises a concern of worsening of endothelial function [7]. On the other hand, despite changes that are caused by continuous flow, it is well tolerated by LVAD recipients [8]. However, the change in flow pattern may also contribute to specific clinical adverse events, such as non-surgical bleeding, that occur in patients with LVAD [9]. A deeper understanding of vascular changes in patients with continuous flow LVAD is desirable, as the design of the pumps is evolving towards artificial pulsatility [10]. Furthermore,

Abbreviations: LVAD, left ventricular assist device; SC, stem cells; EPC, endothelial progenitor cells; MP, microparticles.

☆ Authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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a comparison of impact on vasculature between non-pulsatile devices and devices with artificial pulsatility would be convenient, due to potential promise of enhanced hemocompatibility in the latest devices [10,11]. To delineate the effect of LVAD on vasculature, several detectable biomarkers have become an important focus of research. Among them stem cells (SC) and especially endothelial progenitor cells (EPC) are under intensive investigation. EPC are a subpopulation of circulating CD 34+ mononuclear stem cells. EPC are thought to have ability of endothelial repair and they participate in angiogenesis [12,13]. Increase of EPC has been demonstrated in several clinical conditions including vascular trauma, persistent atrial fibrillation, acute myocardial infarction and endothelial damage in general [14]. In addition, reduced levels of EPC have been found in advanced stages of heart failure and also predict atherosclerosis in the general healthy population [15,16]. However, increased levels of endothelial progenitor cells were in some studies associated with reduced risk of death from cardiovascular causes [17] signaling, that changes of their levels could reflect more complex changes in vascular system.

Circulating microparticles (MP) originate from stressed and damaged cells and contain cytoplasmic material and cellular membrane of their maternal cells. Microparticles, with size of 0.1 to 1.0 μm, are elevated in several pathological conditions including coronary artery disease, stroke or carotid artery disease and their increased levels may be associated with increased risk of major cardiovascular complications [18–20]. Circulating microparticles mainly derive from platelets and endothelial cells in response to activation, injury and/or apoptosis.

To our knowledge, there are only sparse data available about the numbers of vascular biomarkers including stem cells, endothelial progenitor cells and circulating microparticles in patients with continuous flow ventricular assist devices. Until now, no study described parallel changes of SC, EPC and MP in mid- and long- term perspective after LVAD implantation.

The main aim of this study was to evaluate in parallel changes of circulating SC, EPC and MP in patients with end-stage heart failure before, three and six months after LVAD implantation.

2. Materials and methods

Study was designed as observational prospective study. The study was conducted in compliance with the Declaration of Helsinki, International Conference on Harmonization/Good Clinical Practices, and the International Organization for Standardization of medical devices for human subjects, known as 14,155:201. Study was approved by institutional regulatory boards and ethics committee. All patients provided written informed consent with participation in the study.

2.1. Study patients

Twenty-three patients (5 females), aged 21–67 years (mean age 50.6 ± 14.1 years) participated in the study. All patients were diagnosed with end-stage heart failure and implanted with continuous-flow axial ventricular assist device HeartMate II (St. Jude Medical, Pleasanton, California) as a bridge to heart transplant. HeartMate II (HM II) was implanted via sternotomy in 15 patients and via a subcostal approach in 8 patients, according to standards of our institution. All patients reached 6-month study follow up without serious clinical adverse events including signs of pump thrombosis. Heparin bridge was implemented in all procedures until target anticoagulation with warfarin was reached. After implantation the aim of anticoagulation therapy was to reach international normalized ratio of 1.8–2.2; in patients with trombophilias it was 2.5–3.0. Thrombophilias were defined as follows: mutation in factor V (Leiden), factor II (prothrombin), homozygosity for methylentetrahydropholate reductase (MTHFR), heterozygosity for MTHFR accompanied by hyperhomocysteinaemia or the presence of lupus anticoagulant antibodies.

2.2. Blood sample collection and assessment of circulating vascular biomarkers

Blood samples for baseline assessments were collected from peripheral vein during 24–48 h before implantation of LVAD. Follow up collection of blood samples was conducted at the 3rd and 6th month (± 15 days) after LVAD implantation. Human endothelial progenitor cells were analyzed for the expression of surface antigens as previously reported [16,21]. Briefly, before staining with specific monoclonal antibodies, cells were treated with 40 μl of fetal serum for 15 min. Then 200 μl of peripheral blood was stained with 40 μl of phycoerytrin conjugated anti CD 34 (Beckman Coulter), 20 μl of fluorescein isothiocyanate conjugated CD 45 (Beckman Coulter) and 10 μl of Alexa Fluor 647 conjugated anti-KDR (e-Bioscience). Analysis was performed with an automated fluorescence-activated cell counter (CyAn, Beckman Coulter) and 500 thousand events for each analysis were counted. Based on population human studies stem cells were defined as mononuclear CD34+/CD45low + cells and endothelial progenitor cells as mononuclear CD34+/CD45low+/KDR + cells. The detection of endothelial progenitor cells and stem cells in peripheral blood was performed using determination of surface antigen expression. Prior to staining with a specific monoclonal antibody, 200 μl of peripheral blood was incubated with 40 μl of fetal serum. Next, monoclonal antibody was added, i.e. 40 μl of antiCD 34 conjugated with phycoerythrin (Beckman Coulter), 20 μl antiCD 45 conjugated with fluorescein isothiocyanate (Beckman Coulter) and 10 μl anti-KDR conjugated with Alexa Fluor 647 (e-Bioscience). The amount of SC and EPC was expressed as number of cells per ml; addition proportion of EPC from circulating mononuclear cells was analyzed (%EPC). (See Fig. 1) Measurement of circulating microparticles was done according to assay protocol. Blood plasma was collected through a frank venipuncture, using citrate anticoagulant and plasma supernatant was decanted in 2 h with following 15 min centrifugation at 1500 g and then again at 13,000 g at room temperature for 2 min. Then the plasma was obtained by collecting the supernatant, avoiding contact with the platelet pellet. Platelet free plasma was used for further analyses.

For measurements of microparticles, blood samples were immediately centrifuged and frozen to −80 degrees of Celsius and their concentrations were determined by ELISA method (Elisa Zymutest MP activity test – Hyphen Biomed, France). Their concentrations were expressed as nanomoles per liter related to phosphatidylserine [20]. All laboratory parameters were measured in an audited lipid laboratory under continuous external quality control of CDC Atlanta, USA. In addition, all laboratory measurements were done by the same methodology and by the same kits throughout the study.

Other analyzed laboratory parameters were measured by standard laboratory methods used for clinical purposes at our institution.

2.3. Statistical analysis

Data are expressed as mean ± standard deviation, median (interquartile range) or numbers (percents). In graphs, estimated marginal mean and standard error is shown. Because data from the same patient are correlated, longitudinal changes were assessed using generalized mixed-effect models. In these models, intercept was treated as a random effect, whereas time was treated as a fixed effect. To assess the effect of age and gender, interaction between change in time and these variables was tested. Median of age was used to separate the population. For right-skewed data, we used gamma regression. Results from mixed effect models are reported as estimated marginal mean (95% confidence interval). A two-sided p value <0.05 was considered statistically significant. To decrease the risk of falsely positive results, p value was adjusted for multiple comparisons. Calculations were performed using SPSS version 21 (IBM SPSS Statistics, IBM Corporation, Armonk, New York).

Table 2
Changes of laboratory and pump characteristics in patients with left ventricular assist devices. (Estimated marginal means and 95% confidence interval are presented. P value is adjusted for multiple comparisons.)

	Baseline	3rd month	6th month	p^1	p^2	p^3
Microparticles (nM PS*)	7.9 (5.5–11.5)	6.1 (4.3–8.8)	7.7 (5.3–11.2)	0.33	0.38	0.92
Stem cells (cells/ml)	2420 (1912–3063)	1770 (212–1394)	2499 (295–1974)	0.01	0.006	0.78
Endothelial progenitor cells (cells/ml)	159.7 (112.2–227.3)	54.3 (38.2–77.3)	114.0 (80.7–161.1)	0.001	0.003	0.13
%EPC**	0.008 (0.006–0.011)	0.002 (0.002–0.003)	0.006 (0.004–0.008)	0.001	0.001	0.10
Brain natriuretic peptide (ng/l)	1917 (1537–2389)	377 (301–473)	288 (223–372)	0.001	0.12	0.0001
Lactate dehydrogenase (ukat/l)	6.76 (5.45–8.39)	5.42 (4.41–6.67)	5.98 (4.84–7.38)	0.10	0.45	0.36
Hemoglobin (g/l)	121.0 (113.9–128.2)	121.3 (114.2–128.5)	132.7 (125.6–139.9)	0.95	0.02	0.01
Creatinine (umol/l)	100.8 (89.3–112.3)	85.6 (74.1–97.2)	91.8 (80.3–103.3)	0.006	0.26	0.096
Urea (mmol/l)	11.0 (9.2–13.2)	5.7 (4.8–6.9)	6.2 (5.2–7.4)	0.001	0.51	0.001
Total bilirubin (umol/l)	30.0 (24.5–36.8)	12.8 (10.4–15.6)	13.7 (11.2–16.7)	0.001	0.53	0.001
Alanine aminotransferase (ukat/l)	1.89 (1.16–3.08)	0.53 (0.32–0.86)	0.67 (0.41–1.09)	0.003	0.43	0.008
Aspartate aminotransferase (ukat/l)	2.58 (1.38–4.85)	0.53 (0.28–1.00)	0.66 (0.35–1.26)	0.02	0.63	0.03
Total cholesterol (mmol/l)	3.5 (3.0–4.0)	5.6 (5.1–6.1)	5.2 (4.6–5.8)	0.001	0.40	0.001
C-reactive protein (mg/l)	27.7 (18.2–42.0)	14.2 (9.4–21.5)	9.7 (6.4–14.7)	0.02	0.15	0.002
Leukocytes (×10^9/L)	9.7 (8.6–10.8)	7.3 (6.2–8.4)	7.4 (6.4–8.5)	0.001	0.88	0.001
Pump Parameters						
Pump speed (RPM)	*NA*	9000 (8840–9150)	8985 (8840–9130)	*NA*	0.66	*NA*
Pump flow (LPM)	*NA*	5.1 (4.5–5.6)	5.1 (4.5–5.6)	*NA*	0.99	*NA*
Pulse index	*NA*	6.3 (5.6–7.0)	6 (5.7–6.4)	*NA*	0.34	*NA*
Pump power (W)	*NA*	5.3 (4.8–5.8)	5.3 (5.0–5.6)	*NA*	0.99	*NA*

p^1 difference between baseline and 3rd month; p^2 difference between 3rd and 6th month; p^3 difference between baseline and 6th month.
NA not applicable.
* nM PS – nanomoles per liter related to phosphatidylserine.
** %EPC – proportion of endothelial progenitor cells from mononuclear cells.

affect changes of SC, EPC, and MP throughout the study period (all interactions between time and etiology of heart failure $p > 0.05$).

Regarding other parameters under study, we observed significant decrease in levels of brain natriuretic peptide, plasma creatinine, urea, total bilirubin, ALT, AST, and C-reactive protein, before and 3 months after LVAD implantation, while total cholesterol in this period significantly increased. In addition, between 3rd and 6th month no differences in other laboratory parameters of interest were observed with the exception of increase in hemoglobin (Table 2). Regarding LVAD parameters, mean pump flow and speed, pulse index and pump power assessed in the third and sixth month with no significant changes of these parameters ($p > 0.05$ for all parameters; Table 2).

4. Discussion

Implantation of LVAD led to significant changes of circulating vascular biomarkers represented by stem cells and endothelial progenitor cells. These changes had biphasic character. In 3 months after implantation, numbers of stem cells and endothelial progenitor cells significantly decreased and between 3rd and 6th month significantly increased to levels similar with their pre-implant values. Nevertheless, third vascular biomarker under study, circulating microparticles, did not change significantly during the whole study period, despite similar trend of changes was observed as in stem cells and endothelial progenitor cells (Fig. 2). Despite very sparse data are available regarding changes of these parameters in such unique population, they could add valuable information regarding short and long term effects of LVAD on vascular health. This could be very important especially for patients selected for destination therapy with LVAD. In the most recent study Manginas et al. [14] described transient increase of stem cells (CD34+) in 5 patients 15 days after placement of ventricular assist device and their decrease at 60 days after implantation. Despite also biphasic response in five patients studied by Manginas et al. was detected, findings from abovementioned study are not completely comparable with our data, because of different number of participants, and different follow-up periods after LVAD implantation. Another reason for different findings could be use of pulsatile LVAD in majority of patients in this study in contrast to non-pulsatile device used in patients in our study. In addition, in shorter interval after LVAD implantation studied by Manginas et al. the possibility of early activation of bone marrow with operative trauma after the LVAD placement could not be excluded, as discussed by the authors. In our study we intended to avoid this potential effect by postponing first follow-up to 3 months after LVAD implantation. In addition, significant decrease of C-reactive protein and white blood cells count at 3rd and especially at 6th month (Table 2) was observed, and, therefore, it is unlikely that later increase of stem cells and endothelial progenitor cells was influenced by inflammatory activation or surgical trauma after the LVAD placement. Robust decrease of circulating stem cells and endothelial progenitor cells at the 3rd month might be caused by restoration of sufficient blood flow to most of organs after LVAD implantation and attenuation of ischemia as a stimulant for their release. The mechanism of the effect of ischemia on the proliferation of progenitor endothelial cells was recently described and offers biologically plausible explanation also of our findings [22]. This explanation is further supported by our own previous findings in patients after renal transplantation undergoing exercise program [23]. In this population, stem cells and endothelial progenitor cells significantly decreased after exercise compared to control group. One of possible explanations was the alleviation of chronic activation of reparative mechanisms of vascular system in subjects exposed to multiple cardiovascular risk factors by lifestyle intervention. Similar explanation may be plausible for our recent results which could also reflect different mode of mobilization of stem cells and endothelial progenitor cells in patients with different stages of heart failure as was previously described [13,16]. On the contrary, the increase of stem cells and endothelial progenitor cells in the circulation between 3rd and 6th month after LVAD implantation may be explained as a response to non-pulsatile blood flow and inappropriate activation of endothelium caused by the lack of cyclic straining of the vessel wall. This presumption is further supported by the evidence from recent studies that detected compromised function of peripheral vessels in patients with non-pulsatile LVAD despite improvement of central hemodynamics [24,25]. These findings really rise concerns of proatherogenic and prothrombotic changes in the vessel wall after LVAD implantation. In general, we might witness two main mechanisms operating in opposite directions. First, decreased vascular stress and ischemic insults shortly after LVAD implantation caused by improvement in circulation and, second, increased vascular stress caused by non-pulsatile blood flow later after LVAD implantation.

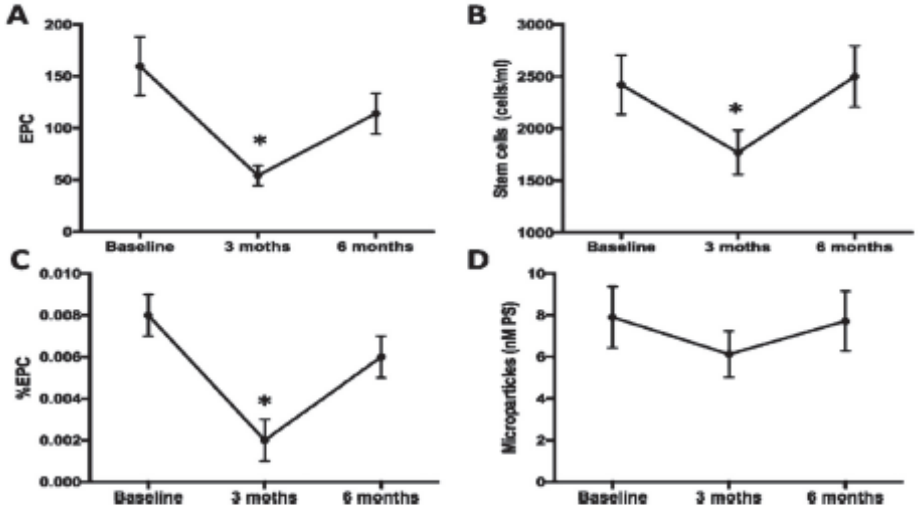

Fig. 2. Changes of endothelial progenitor cells (EPC), stem cells (SC), proportion of endothelial progenitor cells from mononuclear cells (%EPC) and microparticles between baseline, 3rd and 6th month. *p < 0.05 for the difference between baseline and 3rd month and 3rd and 6th month. Estimated marginal mean and standard error is shown in graphs.

Differences in numbers of stem cells were observed between older and younger participants. Patients older than 55 years had significantly lower number of stem cells at baseline and over the study period and no significant changes in stem cells were observed during follow-up in this group in contrast to patients younger than 55 years (Fig. 3). This impact of age is in accordance with already published results [26,27]. In addition, no significant differences between patients with ischemic and non-ischemic etiology were detected regarding changes of vascular biomarkers under study. Therefore, we do not suppose that the etiology of heart failure could have any robust modifying effect on our findings. Additionally, parameters of the ventricular assist devices did not change significantly over time, therefore, obtained results were not modified by changes in pump flow, pump speed, pulse index or pump power.

Regarding circulating microparticles, their changes were moderate and did not reach statistical significance. Their lower concentrations at the 3rd month after LVAD implantation support the above mentioned hypothesis that vascular status of patients firstly improves due to improved end-organ perfusion. This is supported also by our early study which revealed significant decrease of circulating microparticles at the 3rd month after LVAD implantation [7]. Nevertheless, in our yet earlier study focused on circulating microparticles we did not observe any significant effect of LVAD on concentrations of microparticles during 3-month follow-up [28]. However, this negative finding was potentially caused by low number of participants in the study. Finally, higher concentrations of microparticles at the 6th month might reflect already discussed higher shear stress and cell damage caused by continuous flow and probably by the pump itself. Furthermore, as was recently reported, microparticles may virtually play important role in predicting LVAD-associated adverse events and may be of assistance in assessing microvascular function in LVAD patients [29,30]. However, based on low number of patients and, more importantly, on low incidence of serious clinical events we are not able to confirm these findings.

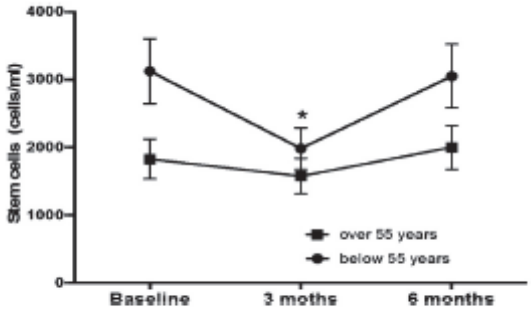

Fig. 3. Stem cells changes by age groups. Estimated marginal mean and standard error is shown in graph. In the group over 55 years, levels of stem cells did not significantly change during follow up, while there was a significant decrease in stem cells at the 3rd month in the group under 55 years (p for interaction between groups = 0.03).

The limitation of the study is the absence of control group. Therefore, longitudinal changes in the number of stem cells, endothelial progenitor cells and microparticles could not be compared with their longitudinal changes in the control group. However, the main aim of the study was to study changes of vascular biomarkers caused by implantation and subsequent function of LVAD. Therefore, we do not expect to gain valuable information from control population represented either by patients with heart failure not indicated to LVAD implantation or even by healthy controls. Another limitation is possible heterogeneity of circulating microparticles and still discussed doubts about their real origin when analyzed by ELISA methods. However, despite some limitations of our study, several previous studies suggest important role of stem cells, endothelial progenitor cells and other similar biomarkers as a valuable predictors of regenerative capacity of the organism and evaluation of their levels had shown close relationship to cardiovascular risk factors and clinical adverse events [17,31]. Therefore, measurement of stem cells, endothelial progenitor cells, and concentrations of circulating microparticles might be of significant importance in assessment of vascular status also in patients with ventricular assist device. To the best of our knowledge, after initial report on the topic [14], this is the first study focused on the effect of LVAD on several vascular biomarkers in a higher number of patients and with several months of follow up.

In conclusion, vascular biomarkers used in our study might be important tool for prediction of adverse events in LVAD patients and future research in this field including study of their association with clinical data could be very rewarding. Additionally, assessment of vascular function by circulating and well established vascular biomarkers may also be of great importance for studying potential effects of newer devices with artificial pulse.

Conflict of interest

Dr. Netuka is a consultant, proctor, and speaker for St. Jude Medical. Other authors – none declared.

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References

[1] J.K. Kirklin, D.C. Naftel, F.D. Pagani, et al., Seventh INTERMACS annual report: 15,000 patients and counting, J. Heart Lung Transplant. 34 (2015) 1495–1504.
[2] J. Parameshwar, J. Wallwork, Left ventricular assist devices: current status and future applications, Int. J. Cardiol. 62 (Suppl. 1) (1997) 23–27.
[3] J.K. Kirklin, D.C. Naftel, F.D. Pagani, et al., Sixth INTERMACS annual report: a 10,000 patient database, J. Heart Lung Transplant. 33 (2014) 555–564.
[4] M. Urban, A. Slavcev, T. Gazdic, P. Ivak, J. Besik, I. Netuka, The impact of angiotensin type 1 receptor antibodies on post-heart transplantation outcome in heart mate II bridged recipients, Interact. Cardiovasc. Thorac. Surg. 15 (Dec 2015) (Epub ahead of print).
[5] A. Clarke, R. Pulikottil-Jacob, M. Connock, et al., Cost-effectiveness of left ventricular assist devices (LVADs) for patients with advanced heart failure: analysis of the British NHS bridge to transplant (BTT) program, Int. J. Cardiol. 171 (2014) 338–345.
[6] J.R. Trivedi, A. Cheng, R. Singh, M.L. Williams, M.S. Slaughter, Survival on the heart transplant waiting list: impact of continuous flow left ventricular assist device as bridge to transplant, Ann. Thorac. Surg. 98 (2014) 830–834.
[7] P. Ivak, J. Pitha, P. Wohlfahrt, et al., Endothelial dysfunction expressed as endothelial microparticles in patients with end-stage heart failure, Physiol. Res. 63 (2014) 369–373.
[8] J.G. Rogers, K.D. Aaronson, A.J. Boyle, et al., Continuous flow left ventricular assist device improves functional capacity and quality of life of advanced heart failure patients, J. Am. Coll. Cardiol. 55 (2010) 1826–1834.
[9] O. Wever-Pinzon, C.H. Selzman, S.G. Drakos, et al., Pulsatility and the risk of nonsurgical bleeding in patients supported with the continuous-flow left ventricular assist device HeartMate II, Circ. Heart Fail. 6 (2013) 517–526.
[10] I. Netuka, P. Sood, Y. Pya, et al., Fully magnetically levitated left ventricular assist system for treating advanced HF: a multicenter study, J. Am. Coll. Cardiol. 66 (2015) 2579–2589.
[11] N. Uriel, S. Adatya, M.R. Mehra, Evolution in mechanical circulatory support: are we the precipice of a disruptive innovation? J. Am. Coll. Cardiol. 66 (2015) 2590–2593.
[12] M. Hristov, W. Erl, P.C. Weber, Endothelial progenitor cells: mobilization, differentiation, and homing, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1185–1189.
[13] A.M. Leone, M. Valgimigli, M.B. Giannico, et al., From bone marrow to the arterial wall: the ongoing tale of endothelial progenitor cells, Eur. Heart J. 30 (2009) 890–899.
[14] A. Manginas, A. Tsiavou, P. Sfyrakis, et al., Increased number of circulating progenitor cells after implantation of ventricular assist devices, J. Heart Lung Transplant. 28 (2009) 710–717.
[15] M. Valgimigli, G.M. Rigolin, A. Fucili, et al., CD34+ and endothelial progenitor cells i patients with various degrees of congestive heart failure, Circulation 110 (2004) 1209–1212.
[16] G.P. Fadini, A. Coracina, I. Baesso, et al., Peripheral blood CD34+/KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in middle-aged general population, Stroke 37 (2006) 2277–2282.
[17] N. Werner, S. Kosiol, P. Ahlers, et al., Circulating endothelial progenitor cells and cardiovascular outcomes, N. Engl. J. Med. 353 (2005) 999–1007.
[18] A. Schiro, F.L. Wilkinson, R. Weston, et al., Endothelial microparticles as conveyors of information in atherosclerotic disease, Atherosclerosis 23 (2014) 295–302.
[19] D. Burger, S. Schock, C.S. Thompson, A.C. Montezano, A.M. Hakim, R.M. Touyz, Microparticles: biomarkers and beyond, Clin. Sci. (Lond.) 124 (2013) 423–441.
[20] J. Amiral, J. Seghatchian, The diagnostic usefulness of capture assays for measuring global/specific extracellular micro-particles in plasma, Transfus. Apher. Sci. 53 (2015) 127–136.
[21] J. Pitha, K. Lesná, A. Sekerkova, et al., Menopausal transition enhances the atherogenic risk of smoking in middle aged women, Int. J. Cardiol. 168 (2013) 190–196.
[22] J. Zhang, Q. Liu, Z. Fang, et al., Hypoxia induces the proliferation of endothelial progenitor cells via upregulation of Apelin/APLNR/MAPK signaling, Mol Med Rep (Dec 17 2015) (Epub ahead of print).
[23] J. Pit'ha, I. Králová Lesná, P. Stávek, A. Mahrová, J. Racek, A. Sekerková, V. Teplan, M. Štollová, Effect of exercise on markers of vascular health in renal transplant recipients, Physiol. Res. 64 (2015) 945–949.
[24] M.A. Witman, R.S. Garten, J.R. Giffort, et al., Further peripheral vascular dysfunction in heart failure patients with a continuous-flow left ventricular assist device: the role of pulsatility, JACC Heart Fail. 3 (2015) 703–711.
[25] T. Hasin, Y. Matsuzawa, R.R. Guddeti, et al., Attenuation in peripheral endothelial function after continuous flow left ventricular assist device therapy is associated with cardiovascular adverse events, Circ. J. 79 (2015) 770–777.
[26] R.G. Shaffer, S. Greene, A. Arshi, et al., Flow cytometric measurement of circulating endothelial cells: the effect of age and peripheral arterial disease on baseline levels of mature and progenitor cells, Cytometry B Clin. Cytom. 70 (2006) 56–62.
[27] R.J. Scheubel, H. Zorn, R.E. Silber, et al., Age-dependent depression in circulating endothelial progenitor cells undergoing coronary artery bypass grafting, J. Am. Coll. Cardiol. 42 (2003) 2073–2080.
[28] J. Pitha, Z. Dorazilova, V. Melenovsky, et al., The impact of left ventricle assist device on circulating endothelial microparticles – pilot study, Neuro Endocrinol. Lett. 33 (2012) 68–72.
[29] R. Sansone, B. Stanske, S. Keymel, et al., Macrovascular and microvascular function after implantation of left ventricular assist devices in end-stage heart failure: role of microparticles, J. Heart Lung Transplant. 34 (2015) 921–932.
[30] A. Nascimbene, R. Hernandez, J.K. George, et al., Association between cell-derived microparticles and adverse events in patients with nonpulsatile left ventricular assist devices, J. Heart Lung Transplant. 33 (2014) 470–477.
[31] A.C. Alba, S.D. Lalonde, V. Rao, et al., Changes in circulation progenitor cells are associated with outcome in heart failure patients: a longitudinal study, Can. J. Cardiol. 29 (2013) 1657–1664.

PRÁCE D popisuje vznik získané von Willebrandovy nemoci (vWD) u pacientů s implantovanou mechanickou podporou s kontinuálním průtokem a MSP s arteficiální pulzatilitou. Současné mechanické srdeční podpory s kontinuálním průtokem jsou asociované s degradací multimerů von Willebrandova faktoru (vWF) s vysokou molekulární hmotností (HMWMs). Tyto jsou důležité pro správnou funkci trombocytů. Zvýšené degradaci HMWMs a vzniku získané von Willebrandovy nemoci se připisuje spoluúčast na vzniku krvácivých komplikací při léčbě pomocí MSP. Po odstranění mechanické srdeční podpory při transplantaci srdce a znovuobnovení pulzatilního průtoku se však výše zmíněné komplikace již nevyskytují. Nejnovější typ mechanické srdeční podpory HeartMate 3 (HM 3) je čerpadlem s arteficiální pulzatilitou. Lze předpokládat, že vytvořením arteficiální pulzní vlny se sníží cirkulační smykové zatížení a tím bude pozitivně ovlivněna funkce studovaných hemostatických parametrů. Práce je první studií porovnávající čerpadlo s kontinuálním průtokem a s arteficiální pulzatilitou s ohledem na vznik získané von Willebrandovy nemoci. Hypotéza testovaná v této práci předpokládá rozdílnou degradaci HMWMs u MSP s kontinuálním průtokem a arteficiální pulzatilitou.

Primárním cílem této prospektivní studie bylo určit efekt čerpadla HeartMate 3 na smykové zatížení krevních elementů, vyjádřených jako míra degradace multimerů von Willebrandova faktoru s vysokou molekulární hmotností. Sekundárním cílem bylo porovnat degradaci s kontrolní skupinou pacientů s implantovanou mechanickou srdeční podporou HeartMate II (HM II) s kontinuálním průtokem.

Během studie bylo vyšetřeno 15 pacientů (3 ženy) s implantovanou MSP HM 3 s arteficiální pulzatilitou a 11 pacientů (1 žena) s implantovanou MSP HM II s kontinuálním průtokem. Ve skupině HM 3 byl průměrný věk pacientů 67,3±1,4 let a ve skupině HM II 52,8±2,5 roku. Pacientům byly odebrány krevní vzorky před implantací

mechanické srdeční podpory a následně 2., 7., 30., a 45. pooperační den. Hlavní analýza byla zaměřená na změny multimerů von Willebrandova faktoru s vysokou molekulární hmotností, měření ristocetin kofaktoru, vWF antigenu a aktivitu metaloproteinázy ADAMTS 13. Během studie byly vyšetřené také další parametry související se získanou vWD – aktivita ristocetin-kofaktoru (RiCO), vyšetření antigenu von Willebrandova faktoru (vWF Ag) a jejich poměr (RiCO:vWF Ag ratio). Sub-analýzy zahrnovaly analýzu vlivu programované rychlosti otáček MSP, indikátorů hemolýzy a otevírání aortální chlopně na sledované parametry. U hlavních sledovaných parametrů byla také provedena komparativní analýza náhodných vzorků (n=10) pro obě skupiny pacientů a to porovnáním primární analýzy se zaslepenou expertní vizuální analýzou a počítačem asistovanou metodologií.

U skupiny s implantovaným čerpadlem s arteficiální pulzatilitou (HM 3) byla potvrzena signifikantně nižší degradace HMWMs v porovnání se skupinou s implantovanou MSP bez arteficiální pulzatility, s maximálním rozdílem ve druhém pooperačním dni (všechny $p<0,001$). Další statisticky významné rozdíly nebyly pozorované, navzdory přítomnosti trendových rozdílů v aktivitě RiCO. U ostatních sledovaných parametrů a sub-analýz nebyl mezi sledovanými skupinami prokázán statisticky signifikantní rozdíl.

Komparativní analýza potvrdila rozdílný vzorec degradace HMWMs mezi sledovanými skupinami ($p<0,05$ pro všechna vyšetření).

Ve studii byla potvrzena hypotéza, že efekt čerpadla s arteficiální pulzatilitou pravděpodobně redukuje poškození plasmatických elementů, včetně HMWMs. Toto pozorování je podpořeno také nižším výskytem hemolýzy u tohoto zařízení. Ve studii se neprokázaly signifikantní rozdíly ve funkčních analýzách aktivity von Willebrandova

faktoru mezi sledovanými skupinami. U obou zařízení bylo pozorováno podobné zachování funkčních atributů vWF.

Studie byla limitována nízkým počtem pacientů, pacienti nebyli randomizovaní a pacienti ve skupině HM 3 navíc užívali antiagregační terapii. Z těchto důvodů je nutná opatrná interpretace prezentovaných výsledků, které však potvrzují vyšší prezervaci HMWMs při použití nového typu čerpadla s arteficiální pulzatilitou. Pulzatilita však není jedinou pokrokovou součástí čerpadla HeartMate3 a proto podíl pulzatility na zachovávání krevních elementů bude předmětem dalšího zkoumání.

Kompletní znění článku je přiloženo v anglickém jazyce.

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FEATURED PAPERS

Evaluation of von Willebrand factor with a fully magnetically levitated centrifugal continuous-flow left ventricular assist device in advanced heart failure

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KEYWORDS:
shear stress;
hemolysis;
von Willebrand syndrome;
multimers;
left ventricular assist device;
magnetic levitation;
hemocompatibility

BACKGROUND: Contemporary continuous-flow left ventricular assist devices (CF-LVADs) are associated with degradation of von Willebrand factor (vWF) high-molecular-weight multimers (HMWMs), a critical factor supporting platelet function. We hypothesized that the HeartMate 3 fully magnetically levitated LVAD, designed to reduce circulatory shear stress, favorably influences these hemostatic parameters.

METHODS: Fifteen consecutive HeartMate 3 LVAD patients were compared with 11 consecutive HeartMate II controls. Serial plasma samples were collected pre-implant and on Days 2, 7, 30 and 45 post-operatively. Changes in vWF HMWMs were evaluated by 2 independent, study-blind hematologists and confirmed using densitometry-based computerized software. Ristocetin cofactor (RiCO) and vWF antigen (vWF Ag) were measured using standard protocols with enzyme-linked immunosorbent assay.

RESULTS: HeartMate 3 patients and HeartMate II controls had a mean age of 67.3 ± 1.4 and 52.8 ± 2.5 years, respectively (INTERMACS Profiles 2 to 4 in 93.3% and 91%, respectively). HeartMate 3 group demonstrated a significantly greater preservation of HMWMs compared with the HeartMate II group, with the most prominent decrease occurring by Day 2 post-operatively and sustained through 45 days (71.94% vs 31.16%, $p = 0.001$). Laboratory values (normalized to baseline) for RiCO activity, vWF Ag and RiCO: vWF Ag ratio remained in the functional range with no statistically significant differences observed between groups.

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CONCLUSION: The HeartMate 3 LVAD is associated with enhanced hemocompatibility compared with the HeartMate II LVAD, as demonstrated by the improved preservation of vWF HMWMs. In contrast, effects on HMWM degradation appeared to be dissociated from functional attributes. Further confirmation of these findings in randomized clinical trials is warranted.

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Contemporaneous continuous-flow left ventricular assist devices (CF-LVADs) are a mainstay in the therapeutic armamentarium in selected patients with advanced heart failure. Although favorable 1- and 2-year survival rates of 80% and 70% have been reported,[1–3] significant morbidity related to major bleeding, neurologic complications and device thrombosis limits broader clinical acceptance of this therapy.[1,4,5]

Continuous flow, and consequent low pulsatility, can disrupt the normal circulation by increasing circulatory shear stress. In such settings, unique rheologic changes are noted, manifested as hemolysis due to deformation of circulating red blood cells[6,7] and, within plasma, as the development of an acquired von Willebrand syndrome (avWS).[8,9] Furthermore, these physiologic changes are associated with thrombosis and bleeding complications, which are reversed once heart transplantation restores a more normal pulsatile circulatory state.

The HeartMate 3 LVAD (St. Jude Medical, Minneapolis, MN) is engineered to enhance hemocompatibility by reducing shear stress on circulating blood elements. The centrifugal device rotor is fully magnetically levitated, allowing for consistently wide blood flow paths, and also features an intrinsic pulse intended to enhance washout.[10,11] Early data from the Conformité Européene (CE) Mark Trial[12] suggest that these design features are associated with absence of clinically relevant hemolysis and lower levels of lactate dehydrogenase (LDH) and plasma free hemoglobin (PHGB). However, the impact of these device characteristics on von Willebrand factor (vWF) remain unknown.

The primary purpose of our prospective investigation was to assess the effect of the HeartMate 3 on clinical measures of shear stress by serial evaluation of vWF high-molecular-weight multimers (HMWMs), and functional activity. Furthermore, we sought to compare and contrast these effects of the HeartMate 3 by using the HeartMate II (St. Jude Medical) axial-flow assist device as a control.

Methods

Patients

The study was approved by the institutional ethics committee and informed consent was obtained from all patients before LVAD implantation.

Patients were enrolled at a single center within the HeartMate 3 CE Mark Trial and sequentially within the HeartMate 3 LIS (less invasive study) along with a consecutive control cohort of HeartMate II patients (Figure 1). The procedure was performed either via median sternotomy or as a less invasive implant via left lateral mini-thoracotomy combined with upper partial hemi-sternotomy, both on cardiopulmonary bypass. The inflow cannula was placed into the LV apex and the outflow graft anastomosed to ascending aorta. Unfractionated heparin was used in all procedures until target anti-coagulation with warfarin was reached. Based on our institutional standard-of-care anti-thrombotic regimen, all patients were maintained on warfarin therapy with a target international normalized ratio (INR) of 2.0 to 3.0. HeartMate 3 recipients were also prescribed an anti-platelet agent (acetylsalicylic acid 100 mg) as required by the study protocol, whereas HeartMate II patients were not treated with anti-platelet therapy in accordance with our institutional practice standard. If INR dropped below the therapeutic range, low-molecular-weight heparin was used for bridging.

Sample collection and laboratory assessment

Patients' characteristics, medical history, laboratory assessments, anti-coagulation, anti-platelet medications, device programming, echocardiographic aortic valve opening parameters and clinical outcomes were assessed over the course of LVAD support. Serial hematologic indices and blood plasma sample collection were conducted pre-implant and on Days 2, 7, 30 and 45 post-operatively in all patients (Figure 1). All enrolled patients completed the follow-up.

HMWM analysis

Multimeric structure was determined by separation of plasma vWF multimers with 1.6% sodium dodecylsulfate (SDS) agarose gel electrophoresis,[13] with minor modifications. Samples were diluted between 1:20 and 1:60, based on vWF antigen concentration to obtain commensurate samples for vWF multimer analysis (buffer of 10 mmol/liter Tris-HCl, 1 mmol/liter ethylene-diamine tetra-acetic acid, 2% SDS [pH 8.0]) and subjected to overnight electrophoresis. Separated multimers were then transferred onto nitrocellulose by electroblotting with 50 mmol/L phosphate buffer (pH 7.4), containing 0.04% SDS, and incubated sequentially with a

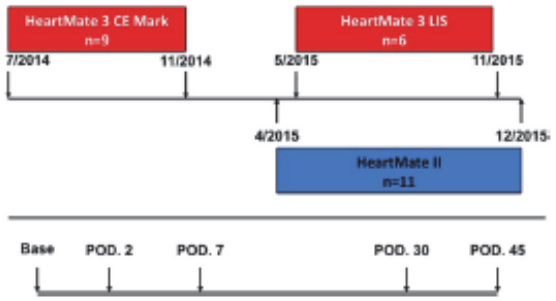

Figure 1 Study enrollment and sample collection timeline.

rabbit anti–vWF antibody–horseradish peroxidase conjugate (Dako, Copenhagen, Denmark). Detection of vWF was performed by chemiluminiscence (ECL chemiluminescence Western blotting detection reagent; GE Healthcare, UK) with a CCD camera (Syngene G:Box Chemi-XT4; GeneSys-Synoptics, UK). Data were converted to jpeg format and analyzed.

All samples were subject to visual quantitative analysis by an expert hematologist blinded to both patient and device. Triplets 1 to 5 from the dye front of the electrophoretic strip were classified as low, 6 to 10 as intermediate and >10 as HMWM, similar to previous publications[14] (Figure 2). To further validate the findings, a set of randomly selected samples ($n = 10$) underwent identical protocol evaluation by a second blinded hematologist at a different institution (Brigham & Women's Hospital, Boston, MA). To avoid subjective bias, another analysis was performed with the densitometry-based software IMAGE J (NIH, Bethesda, MD; http://dx.doi.org/http://rsbweb.nih.gov/ij/), widely used to quantify bands in Western blots. This method converted the bands to a curve with multiple peaks representing each triplet of multimers, and the temporal change in the number of peaks from baseline to the specific time-points in the HMWM domain was analyzed (Figure 3).

Other vWF Assays

Key functional indices of vWF expressed as a relative percentage of standardized reference controls were assayed and represented by the following:

- Ristocetin cofactor activity (RiCO) — measures the ability of a patient's plasma to agglutinate platelets in the presence of the antibiotic ristocetin. The rate of ristocetin-induced agglutination is related to the concentration and functional activity of the plasma vWF (Berichrom von Willebrand Reagent, Siemens, Germany).
- Plasma vWF antigen concentration (vWF Ag) — measures the protein level of vWF in plasma, which is analyzed by enzyme-linked immunosorbent assay (ELISA; von Willebrand Factor Antigen Test Kit; Corgenix, USA).
- vWF ristocetin activity/antigen ratio (RiCO:vWF Ag) — a comparison of platelet binding activity to the protein concentration, utilized for qualitative defects analysis of Types 2A, 2B and 2M which demonstrate a decrease of functional activity compared to antigen concentration.

Baseline metalloprotease ADAMTS 13 activity in the HeartMate 3 and HeartMate II groups was measured by fluorogenic ELISA (Technoclone, Austria).

Statistical analysis

Analysis was performed using GraphPad PRISM 5.0 (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA) and SAS (SAS Institute, Inc., Cary, NC) software. Continuous data are reported as mean ± SEM, and categorical data are presented as proportions. Differences between the groups were compared using the Mann-Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables. For temporally varying continuous data, a linear mixed effects model was utilized, with time, pump type and interaction between time and pump type as predictor variables. Post-hoc comparisons (if differences were statistically significant) were performed using the Scheffé test. Differences were considered statistically significant at $p < 0.05$.

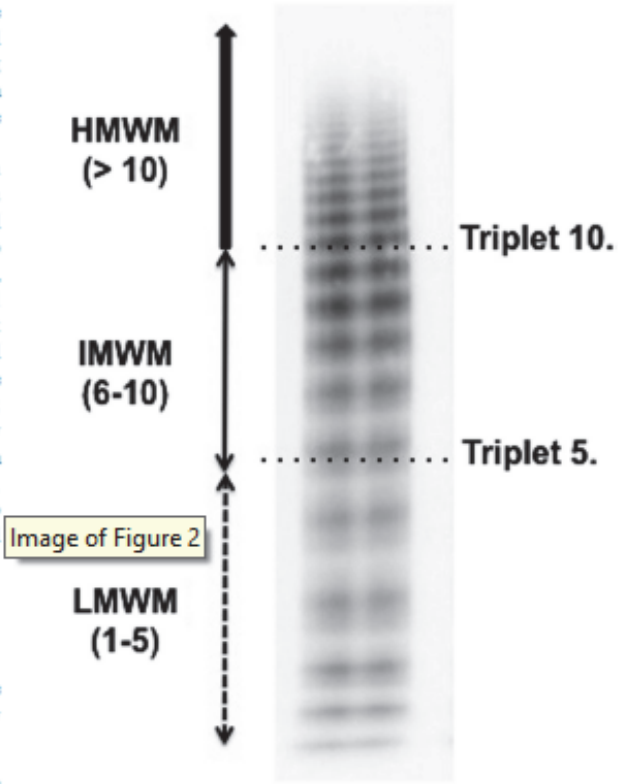

Image of Figure 2

Figure 2 Classification of the multimers on agar gel electrophoresis. Triplets 1 to 5 from the dye front of the electrophoretic strip were classified as low (LMWMs), 6 to 10 as intermediate (IMWMs) and all those >10 as high-molecular-weight multimers (HMWMs).

Results

Patients

The cohorts comprised 15 HeartMate 3 patients and 11 HeartMate II controls, with mean ages of 67.3 ± 1.4 years and 52.8 ± 2.5 years, respectively. Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles were typical of severe advanced heart failure patients receiving LVAD therapy, with 93.3% and 91% of patients, respectively, in Profiles 2 to 4. Detailed baseline characteristics, including indication, mean INTERMACS profile, blood type, cardiovascular history and risk factors, are presented in Table 1. Patient-device interface characteristics, including programming speed, hemolysis indicators and echocardiographic aortic valve opening, are presented in Table 2. Activity levels of metalloprotease ADAMTS 13, expressed as percent of normal control, were 76 ± 22.1% for HeartMate II and 81.9 ± 14.8% for HeartMate 3 (p = not statistically significant).

HMWM analysis

The primary analysis demonstrated that the HeartMate 3 is associated with a significantly lower level of HMWM

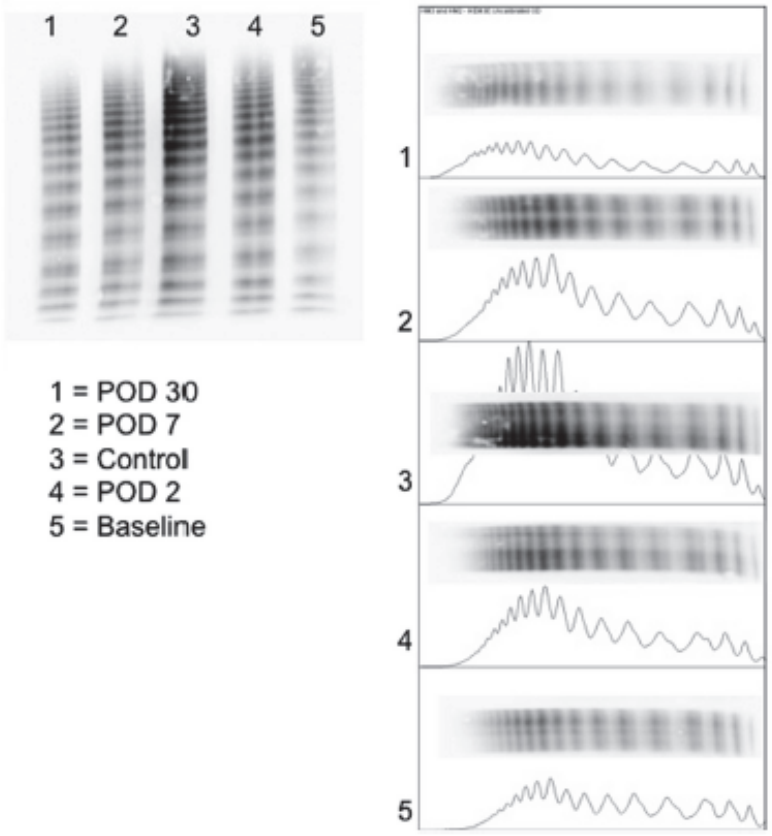

Figure 3 An example of computer-assisted development of curves with comparative calculation of number of peaks at different time-points in the HMWM domain.

Table 1 Baseline Characteristics Including Indication, Mean INTERMACS Profile, Blood Type, Cardiovascular History and Risk Factors

Characteristics	HeartMate II ($n = 11$)	HeartMate 3 ($n = 15$)	p-value
Age (years)	52.8 ± 2.8	67.3 ± 1.3	0.0001
Male [n (%)]	10 (91)	12 (80)	NS
Indication BTT [n (%)]	10 (91)	2 (13)	0.0002
INTERMACS profile [n (%)]			
Profile 1	1 (9)	0 (0)	
Profile 2	4 (36)	1 (7)	
Profile 3	4 (36)	8 (53)	NS
Profile 4	2 (18)	4 (27)	
Profile 5	0 (0)	2 (13)	
Diabetes mellitus [n (%)]	2 (18)	3 (20)	NS
Hypertension [n (%)]	3 (27)	10 (67)	NS
Dilated cardiomyopathy [n (%)]	9 (82)	6 (40)	0.0506
Ischemic cardiomyopathy [n (%)]	2 (18)	9 (60)	0.0506
COPD [n (%)]	0 (0)	2 (13)	NS
Blood type [n (%)]			
0	4 (36)	6 (40)	
A	3 (27)	6 (40)	
B	4 (36)	2 (13)	NS
AB	0 (0)	1 (7)	
Major thrombophilias[a]	3 (20)	1 (9)	NS

BTT, bridge to transplant; COPD, chronic obstructive pulmonary disease; INTERMACS, ; NS, not statistically significant.
[a]Leiden Factor V and Prothrombin Factor II mutations.

Table 2 Patient-Device Interface Characteristics: Hemolysis, Device Speed and Aortic Valve Opening

	HeartMate II ($n = 11$)	HeartMate 3 ($n = 15$)
Hemolysis		
LDH (U/liter) at baseline[a]	293.4	238.9
LDH (U/liter) at follow-up[b]	365.9	245.5
ΔLDH (U/liter)	+72.5	+6.6
Average device speed (rpm)	8,800	5,200
Aortic valve opening[c]		
1:1	2 (18)	5 (33.3%)
1:2	0 (0)	1 (6.6)
None	9 (82)	9 (60%)

LDH, lactate dehydrogenase.
[a]Within 7 days before implant.
[b]During stable phase between Days 30 to 60 post-operatively.
[c]Aortic valve opening was adjudicated by echocardiography at Days 30 to 60 post-operatively; 1:1 refers to opening with each beat, whereas 1:2 denotes every other beat aortic valve opening.

degradation compared with the HeartMate II subset, with the most prominent decrease by Day 2 post-operatively (Figure 4).

Figure 5 demonstrates a comparative analysis of random samples ($n = 10$) for both device groups by primary analysis, second blinded visual expert quantification and the third, peak-to-peak amplitude computer-assisted methodology. Both additional analyses support the divergent pattern of HMWM degradation with substantial preservation within the HeartMate 3 subset compared with HeartMate II ($p < 0.05$ for all 3 analyses for preservation of HMWMs in the HeartMate 3 group vs the HeartMate II group).

Other vWF assays

The laboratory values (normalized to baseline) for RiCO activity, vWF antigen and RiCO:vWF Ag ratios are depicted at Figure 6A-C. Despite the trend in slope for RiCO activity, no statistically significant differences were observed for any of the 3 variables between groups.

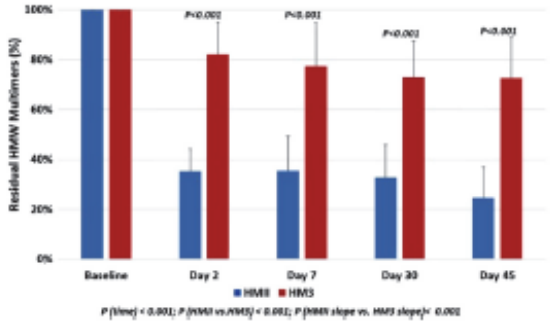

Figure 4 Preservation of HMWM in the primary analysis expressed as a percentage of large multimers normalized to baseline, separated by device type.

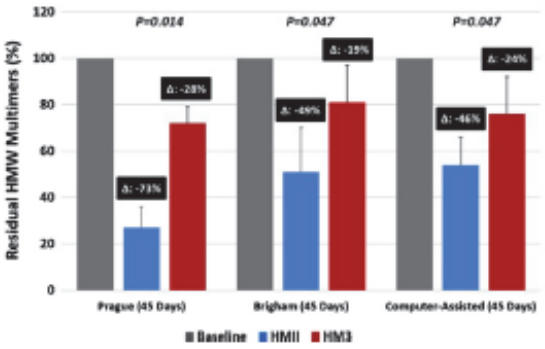

Figure 5 Side-by-side correlation of independent blinded expert evaluations and computer-assisted interpretations of the HMWMs in a random sample ($n = 10$).

Effects of aortic valve opening

In the HeartMate II group, 2 of 11 (18.1%) patients demonstrated any aortic valve opening at 45 ± 15 days of follow-up, whereas 6 of 15 (40%) had opened aortic valves during the cardiac cycle in the HeartMate 3 group. The average preservation of vWF HMWMs in those who had any aortic valve opening in the HeartMate II group was 41% compared with 21% in those who did not; in the HeartMate 3 group, these values were 72.8% for those with any aortic valve opening, compared with 69.22% in those without aortic pulsatility (p-values not calculated due to small numbers and potential for Type II error).

Clinical outcomes

All patients were followed to 180 days. During this period, we observed no episodes of right heart failure (requiring long-term inotropic support or a secondary right heart support device) in either group. Any neurologic complication (transient ischemic attack [TIA] or seizures of disabling strokes) was observed in 0% in the HeartMate II group and 20% of patients in the HeartMate 3 group (3 events, including 1 TIA, 2 seizures and 0 stroke episodes). Any instance of bleeding (based on INTERMACS definitions) was 63% ($n = 7$) and 60% ($n = 9$) in the HeartMate II and HeartMate 3 groups, respectively. Gastrointestinal bleeding occurred in 27.3% ($n = 3$) and 6.9% ($n = 1$) of those the HeartMate II and HeartMate 3 groups, respectively. No episodes of clinical hemolysis or pump thrombosis were noted in either group. One death occurred in this series of 26 patients (traumatic accidental death in 1 HeartMate 3 patient).

Discussion

In this comparative analysis we have demonstrated significant differences in the degree of vWF HMWM degradation between the HeartMate 3 and HeartMate II CF-LVADs. Notably, the effects of the HeartMate 3 on preserving vWF HMWMs provide further corroboration that this device reduces blood element disruption in plasma,

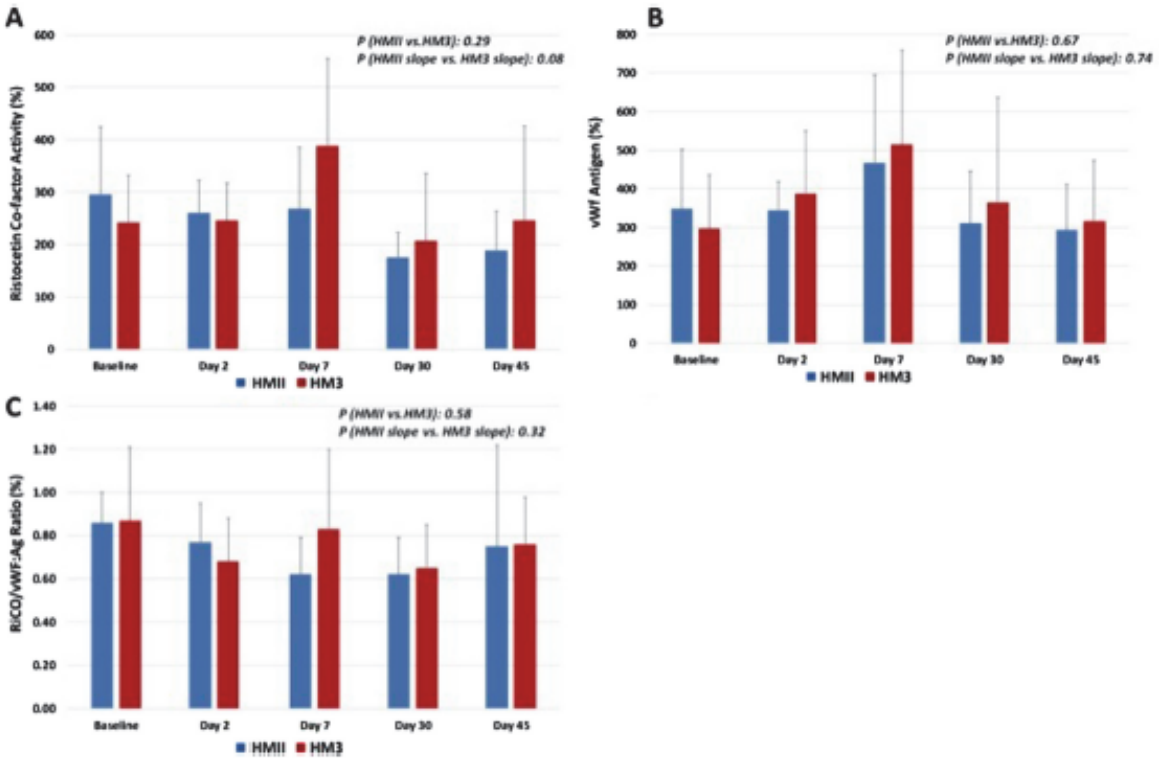

Figure 6 Serial outcomes in functional assays of vWF: ristocetin cofactor (RiCO) (A); vWF Ag (B); and ratio of ristocetin to vWF Ag (C).

similar to observations of reduced red blood cell-related hemolysis. Alterations in HMWMs occur as early as 48 hours and these changes are sustained through 6 weeks. Serial studies by Crow and colleagues[15,16] have also shown this pattern of a significant degradation at the first post-operative sample collection, which they performed at 7 days. Although our findings suggest that the HeartMate 3 better preserves vWF HMWMs compared with the HeartMate II, we were unable to demonstrate downstream functional differences in VWF activity between these devices.

A cross-sectional study by Meyer et al[17] of 102 devices with either axial- or centrifugal-flow characteristics showed a similar degree of residual HMWMs: 30 ± 14% for the axial-flow HeartMate II and 34 ± 13% for the centrifugal-flow HVAD. They used an HMWM assessment approach similar to that used in our study. Thus, it is likely that the unique design characteristics of the HeartMate 3 device principally account for these observed differences in vWF HMWMs rather than the directional flow characteristics of the devices.

It has been hypothesized that a critical shear force of 500 pN is required to unfold the HMWMs and expose the sites for cleavage.[18] If so, then one explanation for the difference in vWF HMWM degradation may lie in the absolute or average revolution-per-minute (rpm) speeds at which a particular device is programmed. We have found that this is less likely to be the case as studies of the lower speed HVAD demonstrated a similar degradation of vWF HMWMs to that of the higher speed HeartMate II.[17] The HeartMate 3 is typically clinically programmed at an intermediate speed (range 4,900 to 5,400 rpm), between those clinical settings of an HVAD (range 2,200 to 3,200 rpm) or the higher speed HeartMate II (8,600 to 9,800 rpm). This suggests that the wider consistent flow paths in the HeartMate 3 device and more stable operation at wide speed ranges with full magnetic levitation may result in significantly less damage to hemostatic components.

We were unable to show significant differences in functional assays of vWF activity between the HeartMate 3 and HeartMate II LVAD groups; however, we did find preservation of functional attributes of vWF with both devices. This is in contrast to findings by Crow and colleagues,[15,16] who identified a significant decrease in functional assays of vWF with the HeartMate II device by Day 30. Curiously, in that analysis, the HMWMs were degraded early (Day 7); however, functional attributes (RiCO:vWF Ag ratio) changed later, suggesting some temporal dissociation with this observed effect. Intermediate weight multimers of vWF could mediate sufficient preservation in functional attributes to account for these findings in our study. Furthermore, the absence of anti-platelet therapy in the HeartMate II cohort in our series could explain these discrepant findings, but both of these contentions remain speculative.

An era effect, exemplified by differences in device programming, may have also contributed to these differences. Previous studies included a strategy of higher pump speed management with the HeartMate II LVAD.[19]

Subsequently, the devices were programmed to maintain some degree of aortic valve opening in an effort to reduce complications of gastrointestinal bleeding, aortic regurgitation and valve leaflet fusion.[20–23] Thus, the average speed of the HeartMate II in the study by Meyer et al[17] was 9,741 rpm, whereas our series used a markedly lower average speed for the device at 8,800 rpm. Differences in unloading, maintained pulsatility and facilitated aortic valve opening may also allow for the dissociation in HMWMs and subsequent functional activity, as highlighted in our study where numerically less degradation in the HMWMs was observed in both device groups in the presence of any aortic valve opening. We consider these trends to be hypothesis-generating. However, differences between the devices in degree of vWF degradation persisted, irrespective of the presence or absence of an aortic valve opening. We also detected numerical trends of a lower rate of gastrointestinal bleeding in the HeartMate 3 group and, although these findings need to be confirmed in larger trials, there is some doubt regarding the usefulness of functional assays as correlates for this complication.

Limitations

There are obvious limitations to this single-center, prospective, observational study with relatively small numbers of patients. We urge caution in interpreting these findings as the patients were not randomized, which resulted in unmatched subsets of patients who were significantly older, with more destination therapy indications and instances of ischemic cardiomyopathy seen in the HeartMate 3 cohort. In addition, the anti-platelet strategy was markedly different between the groups. It is unclear whether these changes influenced functional parameters in one or both arms. Furthermore, it remains uncertain whether the artificial intrinsic pulsatility in the HeartMate 3 device played a role in our findings. There were important imbalances in the INTERMACS profiles and age of patients between the groups, with more patients in the HeartMate II arm demonstrating greater disease severity (45% in INTERMACS Profiles 1 or 2) compared with 7% in the HeartMate 3 group. Despite limited patient numbers, we did not find any trend in HMWM degradation as a function of initial disease severity. It is also possible that the ethnic homogeneity of the study population could limit broader interpretation of the findings, and additional unmeasured confounders may have influenced the observed outcomes.

We conclude that the HeartMate 3 LVAD design may translate to enhanced hemocompatibility as demonstrated by greater preservation of vWF HMWMs when compared with the HeartMate II LVAD. In contrast, effects on vWF HMWM degradation appeared dissociated from the functional attributes. Further evaluation of the impact of the HeartMate 3 device on vWF properties, and their clinical impact, requires an adequately powered, prospective, randomized clinical trial, such as the ongoing MOMENTUM 3 study.

Disclosure statement

I.N. is a consultant and advisory board member at St. Jude Medical, Inc. J.M. is a consultant for St. Jude Medical, Inc. J.M.C. is a consultant for St. Jude Medical, Inc. M.R.M. is a consultant for St. Jude Medical, Boston Scientific, Teva, Johnson and Johnson and Medtronic. P.S. and K.S.S. are employees of St. Jude Medical, Inc. The other authors have no conflicts of interest to disclosure.

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References

1. Kirklin JK, Naftel DC, Pagani FD, et al. Seventh INTERMACS annual report: 15,000 patients and counting. J Heart Lung Transplant 2015;34:1495-504.
2. Jorde UP, Kushwaha SS, Tatooles AJ, et al. Results of the Destination Therapy Post-Food and Drug Administration Approval Study With a Continuous Flow Left Ventricular Assist Device: a prospective study using the INTERMACS Registry (Interagency Registry for Mechanically Assisted Circulatory Support). J Am Coll Cardiol 2014;63:1751-7.
3. Starling RC, Moazami N, Silvestry SC, et al. Unexpected abrupt increase in left ventricular assist device thrombosis. N Engl J Med 2014;370:33-40.
4. Mehra MR, Stewart GC, Uber PA. The vexing problem of thrombosis in long-term mechanical circulatory support. J Heart Lung Transplant 2014;33:1-11.
5. Boyle AJ, Jorde UP, Sun B, et al. Pre-operative risk factors of bleeding and stroke during left ventricular assist device support: an analysis of more than 900 HeartMate II outpatients. J Am Coll Cardiol 2014;63:880-8.
6. Cowger JA, Romano MA, Shah P, et al. Hemolysis: a harbinger of adverse outcome after left ventricular assist device implant. J Heart Lung Transplant 2014;33:35-43.
7. Hasin T, Deo S, Maleszewski JJ, et al. The role of medical management for acute intravascular hemolysis in patients supported on axial flow LVAD. ASAIO J 2014;60:9-14.
8. Klovaite J, Gustafsson F, Mortensen SA, et al. Severely impaired von Willebrand factor-dependent platelet aggregation in patients with a continuous-flow left ventricular assist device (HeartMate II). J Am Coll Cardiol 2009;53:2162-7.
9. Uriel N, Pak SW, Jorde UP, et al. Acquired von Willebrand syndrome after continuous-flow mechanical device support contributes to a high prevalence of bleeding during long-term support and at the time of transplantation. J Am Coll Cardiol 2010;56:1207-13.
10. Schmitto JD, Hanke JS, Rojas SV, et al. First implantation in man of a new magnetically levitated left ventricular assist device (HeartMate III). J Heart Lung Transplant 2015;34:858-60.
11. Heatley G, Sood P, Goldstein D, et al. Clinical trial design and rationale of the Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy With HeartMate 3 (MOMENTUM 3) investigational device exemption clinical study protocol. J Heart Lung Transplant 2016;35:528-36.
12. Netuka I, Sood P, Pya Y, et al. Fully magnetically levitated left ventricular assist system for treating advanced HF: a multicenter study. J Am Coll Cardiol 2015;66:2579-89.
13. Budde U, Schneppenheim R, Plendl H, et al. Luminographic detection of von Willebrand factor multimers in agarose gels and on nitrocellulose membranes. Thromb Haemost 1990;63:312-5.
14. Meyer AL, Malehsa D, Bara C, et al. Acquired von Willebrand syndrome in patients with an axial flow left ventricular assist device. Circ Heart Fail 2010;3:675-81.
15. Crow S, John R, Boyle A, et al. Gastrointestinal bleeding rates in recipients of nonpulsatile and pulsatile left ventricular assist devices. J Thorac Cardiovasc Surg 2009;137:208-15.
16. Crow S, Chen D, Milano C, et al. Acquired von Willebrand syndrome in continuous-flow ventricular assist device recipients. Ann Thorac Surg 2010;90:1263-9.

17. Meyer AL, Malehsa D, Budde U, et al. Acquired von Willebrand syndrome in patients with a centrifugal or axial continuous flow left ventricular assist device. JACC Heart Fail 2014;2:141-5.
18. Joyce D, Crow S, Li Z, et al. Pilot investigation of a novel testing strategy for bleeding in ventricular assist device recipients. J Heart Lung Transplant 2012;31:750-6.
19. Slaughter MS, Pagani FD, Rogers JG, et al. Clinical management of continuous-flow left ventricular assist devices in advanced heart failure. J Heart Lung Transplant 2010;29(suppl):S1-39.
20. Uriel N, Morrison KA, Garan AR, et al. Development of a novel echocardiography ramp test for speed optimization and diagnosis of device thrombosis in continuous-flow left ventricular assist devices: the Columbia ramp study. J Am Coll Cardiol 2012;60:1764-75.
21. Jorde UP, Uriel N, Nahumi N, et al. Prevalence, significance, and management of aortic insufficiency in continuous flow left ventricular assist device recipients. Circ Heart Fail 2014;7:310-9.
22. Saeed O, Maybaum S, Alessandro DD. Aortic valve opening and thrombotic events with continuous-flow left ventricular assist devices. J Heart Lung Transplant 2014;33:109-12.
23. Wasson LT, Yuzefpolskaya M, Wakabayashi M, et al. Hypertension: an unstudied potential risk factor for adverse outcomes during continuous flow ventricular assist device support. Heart Fail Rev 2015;20:317-22.

7 DISKUZE

Zavedení a použití mechanických srdečních podpor (MSP) v poslední dekádě prokázalo nesporný přínos pro pacienty s terminálním srdečním selháním. Jejich použití se stalo rutinním a dobře zavedeným léčebným postupem. Do nedávné doby byla používaná zejména čerpadla generující nepulzatilní průtok. Vliv generovaného kontinuálního toku na vaskulaturu však navzdory vynikajícím klinickým výsledkům zůstává nejasný. Předkládaná práce byla proto zaměřena zejména na změny cirkulujících biomarkerů vaskulárního zdraví způsobené mechanickou srdeční podporou. Zjišťování vlivu nepulzatilního toku na vaskulaturu se stalo důležitou součástí výzkumu a hraje i významnou roli v přípravě designu nových generací mechanických srdečních podpor.

Hlavním cílem práce bylo potvrzení hypotézy o vlivu kontinuálního toku na vaskulaturu a cirkulující elementy. K tomuto účelu bylo použito sledování změn koncentrací cirkulujících vaskulárních biomarkerů a stanovení degradace a funkce von Willebrandova faktoru. Do studie byli zařazeni pacienti, kterým byla implantována mechanická srdeční podpora HeartMate II a HeartMate 3 (St. Jude Medical, Pleasanton, Kalifornie) z důvodu pokročilého srdečního selhání.

Během studie byly detekovány změny koncentrací cirkulujících biomarkerů – mikročástic, endoteliálních progenitorových buněk a kmenových CD 34+ buněk. Publikované výsledky práce referují o snížení koncentrací cirkulujících mikročástic ve třetím měsíci po implantaci mechanické srdeční podpory. Při použití metody detekce mikročástic, která byla použita v této studii, literatura uvádí u zdravých pacientů

referenční hodnoty pod 10 nM PS; tento předpoklad se potvrdil i v námi vyšetřené kontrolní skupině.

Za příčinu pozorované snížené koncentrace cirkulujících mikročástic ve 3. měsíci může být považováno zlepšení perfuze orgánů po zvýšení srdečního výdeje do adekvátních hodnot. S restaurací adekvátního srdečního výdeje se pravděpodobně sníží stres vyvolávající zvýšení počtu mikročástic i zatížení endoteliální vrstvy v cévách. Dále se eliminuje relativní ischemie cílových orgánů. Navzdory tomu, že naprostá většina námi detekovaných mikročástic má původ v endoteliální vrstvě cév a trombocytech, bude v dalším výzkumu nutná více specifická detekce mikročástic dle původu z jednotlivých buněk pomocí cytoflowmetrie. Důležitým zjištěním ve studii bylo, že koncentrace mikročástic se nestaly prediktorem nežádoucích klinických událostí nebo úmrtí pacienta. Porovnání výsledků s doposud publikovanými studiemi není jednoduché, vzhledem k použití ELISA metodiky v této práci oproti použití průtokové cytometrie v jiných pracích (Diehl et al., 2010; Nascimbene et al., 2014; Sansone et al., 2015). Dalším rozdílem jsou odlišné typy MSP zkoumané v těchto publikacích. Navzdory tomu, hlavním zjištěním všech autorů byl rozdíl v hodnotách u zdravých jedinců a pacientů s MSP, což bylo potvrzeno i v naší studii. V jedné z prací (Nascimbene et al., 2015) byly pozorovány vyšší hladiny mikročástic u pacientů s klinickou komplikací, ale vzhledem k tomu, že MP byly odebrány až po vzniku těchto komplikací, lze obtížně posoudit, zda by se koncentrace MP mohly stát prediktorem vzniku nežádoucích událostí. Důvodem pro toto tvrzení je fakt, že zvýšené koncentrace mikročástic byly pozorovány u mnoha kardiovaskulárních a dalších onemocnění. V naší studii se koncentrace mikročástic jako prediktoru nežádoucích klinických událostí neprokázala, vzhledem k limitaci protokolární pravidelnosti náběrů, které nekorelovaly

s výskytem těchto příhod. U našich pacientů také nebyla potvrzena souvztažnost mezi trendy koncentrací cirkulujících mikročástic jako prediktoru nežádoucích příhod.

Hodnoty absolutního počtu kmenových buněk (SC, CD 34+) a subpopulace endoteliálních progenitorových buněk (EPC) v porovnání s obdobím před implantací MSP signifikantně poklesly ve třetím měsíci. V dalším sledování v 6. měsíci hodnoty stoupaly k původním hodnotám. Zejména EPC jsou považovány za ukazatel regenerační schopnosti organizmu a jejich zvýšení poukazuje na zvýšenou nutnost obnovy endoteliální vrstvy cévní stěny. Proto je obnovení adekvátního srdečního výdeje ve spojení s adekvátní dodávkou kyslíku a ostatních živin po implantaci MSP charakterizováno snížením počtu EPC ve třetím měsíci. V kontrastu, při dlouhodobém nepulzatilnímu průtoku jsou stěny cév vystaveny většímu stresu, nejsou cyklicky namáhány a prohlubuje se endoteliální dysfunkce. Proto se zvyšuje nutnost obnovy endoteliálních buněk, která se odráží zvýšením hladin EPC i kmenových buněk v šestém měsíci po implantaci MSP. V jediné doposud publikované studii (Manginas et al., 2009) byla pozorována bifázická odpověď u kmenových buněk, ale v časovém rozmezí 15 a 60 dnů od implantace MSP. Navzdory podobné odpovědi v koncentracích kmenových buněk nejsou tato data porovnatelná s naší studií a to z důvodu odlišného počtu zkoumaných subjektů, odlišných časových intervalů odběru a použití pulzatilních typů MSP ve studii Manginas et al. Dále jsou limitací této studie časné pooperační odběry a tedy možný vliv doznívajícího operačního traumatu, aktivace zánětlivé odpovědi, i časných pooperačních komplikací. Tyto faktory byly ve zde předkládané práci eliminovány tříměsíčním časovým odstupem od implantace MSP.

Dle našeho dalšího zjištění se hodnoty kmenových buněk však u skupiny pacientů nad 55 let signifikantně neměnily. Nejpravděpodobnějším vysvětlením se jeví snížená kapacita produkce a předpokládané vyčerpání rezerv kmenových buněk u

starších pacientů, zejména v terénu chronického srdečního selhání. Vliv věku na hodnoty CD 34+ byl popsán i v dalších studiích (Scheubel et al., 2003; Schaffer et al., 2006). Sledování koncentrací mikročástic popsané v práci [C], bylo pravděpodobně ovlivněno velikostí sledované skupiny, při které výsledky nedosáhly statistické významnosti.

V další části práce byla prováděna komparativní analýza pacientů s MSP s kontinuálním průtokem a MSP s arteficiální pulzatilitou z hlediska funkce von Willebrandova faktoru a degradace jeho multimerů s vysokou molekulární hmotností (HMWMs). Zde byly demonstrovány signifikantní rozdíly mezi degradací řetězců von Willebrandova faktoru u odlišných typů čerpadel. Nižší poškození HMWMs a dalších krevních elementů u MSP s arteficiální pulzatilitou bylo dále potvrzeno i nižším výskytem hemolýzy u tohoto typu MSP (Netuka et al., 2015). Navzdory tomu, že výsledky naznačují vyšší prezervaci HMWMs u přístroje HeartMate 3 s arteficiální pulzatilitou, toto pozorování nebylo asociováno s funkční diferencí parametrů von Willebrandova faktoru při porovnání s kontinuálním čerpadlem HeartMate II. V doposud publikovaných studiích předchozích typů MSP (axiální, centrifugální), nebyl mezi nimi pozorován rozdíl v degradaci HMWMs. To naznačuje, že unikátní charakteristiky MSP HeartMate 3 s arteficiální pulzatilitou se odrážejí na vyšší prezervaci HMWMs. Při porovnání s dosavadními studiemi nebyl potvrzen vliv rychlosti otáček čerpadla (revolutions-per-minute, rpm), což opět podporuje hypotézu o vyšším zachování krevních elementů díky konstrukčním a funkčním vlastnostem této pumpy, mezi které patří i arteficiální pulzatilita. Vzhledem k tomu, že otevírání aortální chlopně dále zvyšuje pulzatilitu, může se také podílet na nižší degradaci HMWMs, proto v další sub-analýze obou skupin byl posouzen vliv otevírání aortální chlopně na zachování HMWMs. V obou skupinách byl pozorován trend k nižší degradaci HMWMs

v případě přítomnosti otevírání aortální chlopně, a tedy pozitivní vliv vyšší pulzatility na sledované HMWMs.

Studie byla limitována tím, že byla prováděna jako observační, unicentrická a na relativně malém počtu pacientů. Tyto faktory mohou mít vliv na výslednou interpretaci výsledků, která musí být nanejvýš obezřetná. Na druhou stranu pozorování potvrzují vyšší hemokompatibilitu při použití čerpadla s arteficiální pulzatilitou.

Celkově bylo v předkládané práci pozorováno pravděpodobné zhoršení endoteliální funkce u pacientů s implantovanou mechanickou srdeční podporou. Dokladovatelná změna koncentrací cirkulujících biomarkerů se jeví jako pravděpodobné reakce na aberantní zatížení cévní stěny kontinuálním tokem. Koncentrace cirkulujících mikročástic, které jsou pravděpodobně uvolňovány při poškození krevních elementů a endotelu statisticky významně poklesly. Pozorování lze vysvětlit buď obnovením srdečního výdeje a odstraněním povšechné ischemie, nebo působením mechanických sil v čerpadle samotném a z toho rezultujícímu poškození mikročástic a jejich degradaci, která byla měřena jako jejich signifikantní pokles. Toto vysvětlení podporuje hypotézu o zvýšeném smykovém zatížení krevních elementů proudících přes turbínu čerpadla. Zjištění zvýšené degradace multimerů von Willebrandova faktoru činí tuto možnost pravděpodobnou, avšak u čerpadel s arteficiální pulzatilitou k této degradaci dochází v menším měřítku. Hypotéza o patologickém zatížení cévní stěny je také podpořena změnou koncentrací cirkulujících endoteliálních progenitorových buněk a kmenových buněk. Změny jejich koncentrace s vlivem kontinuálního toku naznačují zvýšenou potřebu obnovy endotelu v dlouhodobém horizontu a tedy jeho zvýšené poškození a pravděpodobnou dysfunkci vlivem nepulsatilního průtoku. I když analýza mikročástic, EPC a SC u MSP s arteficiální pulzatilitou zatím neproběhla, lze usuzovat, že více fyziologický pulzatilní

tok, může být z dlouhodobého hlediska významným faktorem ovlivňujícím pozitivně funkci vaskulatury. Rozšíření všech sledovaných skupin pacientů bude mít neocenitelný význam v další analýze výsledků.

V roce 2016 byla průměrná čekací doba na čekací listině transplantace srdce 275 dní. Vzhledem k tomu je v našich podmínkách obtížné provádět dlouhodobější sledování. Další validní výsledky bude možné pravděpodobně dosáhnout při sledování pacientů indikovaných k implantaci MSP v indikaci destinační terapie.

Lze konstatovat, že se jedná o jedny z prvních systematických pozorování u jedinečné skupiny pacientů. Výsledky mohou osvětlit unikátní patofyziologii průtoku krve vaskulaturou u tohoto typu terapie. Zároveň mohou poskytnout zpětnou vazbu při vývoji dalších generaci mechanických srdečních podpor určených k dlouhodobému použití s cílem maximální hemokompatibility a eliminace nežádoucích událostí.

8 ZÁVĚRY

I. V práci byly identifikovány a zhodnoceny moderní plasmatické biomarkery, zejména cirkulující mikročástice, a dynamika jejich koncentrací v souhrnu recentních publikací a vlastních výsledků. Souhrn podtrhuje jejich změny v přítomnosti mechanické srdeční podpory a potenciál markerů ve vztahu k nežádoucím klinickým příhodám.

II. Longitudinální posouzení plasmatické koncentrace mikročástic prováděné metodou ELISA vykazuje dlouhodobý pokles koncentrací mikročástic, který lze vysvětlit jako úpravu funkce vaskulatury ve 3. měsíci po implantaci MSP. Nelze však vyloučit podíl mechanických sil reprezentovaných zvýšeným smykovým třením.

III. Počty endoteliálních progenitorových a kmenových buněk vykázaly signifikantní bifázickou dynamiku. Na poklesu ve 3. měsíci po implantaci MSP se pravděpodobně dominantně podílí zlepšení funkce vaskulatury a zejména tkáňové perfuze. Následný vzestup přisuzujeme negativnímu účinku kontinuálního toku krve a prohloubení endoteliální dysfunkce, které aktivují reparační mechanismy endotelu.

IV. Degradace multimerů von Willebrandova faktoru s vysokou molekulární hmotností byla signifikantně redukována u mechanické srdeční podpory s arteficiální pulzatilitou v porovnání s MSP s kontinuálním průtokem. Tento efekt naznačuje přínos arteficiální pulzatility pro zachování krevních elementů a pozitivní vliv na hemokompatibilitu.

Navzdory výraznému zlepšení orgánové perfuze díky znovuobnovení adekvátního srdečního výdeje naše pozorování naznačují, že ve sledovaném období přetrvává převážně negativní vliv kontinuálního průtoku na parametry vaskulárního zdraví a cirkulující krevní elementy. Toto tvrzení je doloženo zejména změnou koncentrace mikročástic, počtů endoteliálních progenitorových buněk a vyšetřením degradace multimerů von Willebrandova faktoru s vysokou molekulární hmotností. Při sledování degradace multimerů von Willebrandova faktoru s vysokou molekulární hmotností se potvrdil statisticky signifikantní potencionální pozitivní vliv arteficiální pulzatility. Další výzkum může poskytnout významné podklady při vývoji specifických charakteristik nových generací mechanických srdečních podpor, zejména v definování míry pulzní amplitudy a její synchronizace s nativním srdečním rytmem.

9 SEZNAM PUBLIKACÍ DOKTORANDA

1. publikace *in extenso,* které jsou podkladem dizertace

a) s IF

1. **IVÁK, Peter;** PIŤHA, Jan; NETUKA, Ivan. Circulating microparticles as a predictor of vascular properties in patients on mechanical circulatory support; hype or hope?. *Physiological Research*. 2016, **65**(5), 727-735. ISSN 0862-8408. **IF: 1.643/2015**.

2. **IVÁK, Peter;** PIŤHA, Jan; WOHLFAHRT, Peter; KRÁLOVÁ LESNÁ, Ivana; STÁVEK, Petr; MELENOVSKÝ, Vojtěch; DORAZILOVÁ, Zora; HEGAROVÁ, Markéta; ŠTĚPÁNKOVÁ, Jitka; MALÝ, Jiří; SEKERKOVÁ, Alena; TURČÁNI, Dominika; NETUKA, Ivan. Biphasic response in number of stem cells and endothelial progenitor cells after left ventricular assist device implantation: A 6 month follow-up. *International Journal of Cardiology*. 2016, **218**(September), 98-103. ISSN 0167-5273. DOI: 10.1016/j.ijcard.2016.05.063. **IF: 4.638/2015**.

3. NETUKA, Ivan; KVASNIČKA, Tomáš; KVASNIČKA, Jan; HRACHOVINOVÁ, Ingrid; **IVÁK, Peter**; MAREČEK, František; BÍLKOVÁ, Jana; MALÍKOVÁ, Ivana; JANČOVÁ, Michaela; MALÝ, Jiří; SOOD, Poornima; SUNDARESWARAN, Kartik S.; CONNORS, Jean M.; MEHRA, Mandeep R. Evaluation of von Willebrand factor with a fully magnetically levitated centrifugal continuous-flow left ventricular assist device in advanced heart failure. *The Journal of Heart and Lung Transplantation*. 2016, **35**(7), 860-867. ISSN 1053-2498. DOI: 10.1016/j.healun.2016.05.019. **IF: 7.509/2015**.

4. **IVÁK, Peter;** PIŤHA, Jan; WOHLFAHRT, Peter; KRÁLOVÁ LESNÁ, Ivana; STÁVEK, Petr; DORAZILOVÁ, Zora; ŠTĚPÁNKOVÁ, Jitka; MALÝ, Jiří; POKORNÝ, Martin; NETUKA, Ivan. Endothelial dysfunction expressed as endothelial microparticles in patients with end-stage heart failure. *Physiological Research*. 2014, **63**(Suppl. 3), S369-S373. ISSN 0862-8408. **IF: 1.293/2014**.

2. publikace *in extenso,* bez přímého vztahu k tématu dizertace

a) s IF

1. **IVÁK, Peter**; ZUMROVÁ, Alena; NETUKA, Ivan. Friedreich's ataxia and Advanced Heart Failure: An Ethical Conundrum in Decision MakingClinical Decision Making in Friedreich's ataxia. *The Journal of Heart and Lung Transplantation*. 2016, **35**(9), 1144-1145. ISSN 1053-2498. DOI: 10.1016/j.healun.2016.06.021. **IF: 7.509/2015**.

2. URBAN, Marian; SLAVČEV, Antonij; GAZDIČ, Tomáš; **IVÁK, Peter**; BEŠÍK, Josef; NETUKA, Ivan. The impact of angiotensin II type 1 receptor antibodies on post-heart transplantation outcome in Heart Mate II bridged recipients. *Interactive Cardiovascular and Thoracic Surgery*. 2016, **22**(3), 292-297. ISSN 1569-9293. DOI: 10.1093/icvts/ivv344. **IF: 1.329/2015**.

3. URBAN, Marian; SLAVČEV, Antonij; GAZDIČ, Tomáš; **IVÁK, Peter;** NETUKA, Ivan. The impact of Angiotensin II Type 1 Receptor antibodies on morbidity and mortality in Heart Mate II supported recipients. *Biomedical Papers*. 2016, **160**(4), 518-523. ISSN 1213-8118. DOI: 10.5507/bp.2016.025. **IF: 0.924/2015**.

4. NETUKA, Ivan; **IVÁK, Peter**; SZARSZOI, Ondřej; URBAN, Marian; NOVOTNÝ, Jiří; BEŠÍK, Josef; MALÝ, Jiří. Minimally Invasive Removal of a Temporary RVAD. *ASAIO Journal*. 2015, **61**(2), 202-204. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000184. **IF: 2.291/2015**.

10 LITERATURA

1. ALRAIES, M. C.; ECKMAN, P. Adult heart transplant: indications and outcomes. *Journal of Thoracic Disease*. 2014, **6**(8), 1120-1128. ISSN 2072-1439. DOI: 10.3978/j.issn.2072-1439.2014.06.44.

2. AMABILE, N.; GUIGNABERT, C.; MONTANI, D.; YEGHIAZARIANS, Y.; BOULANGER, C. M.; HUMBERT, M. Cellular microparticles in pathogenesis of pulmonary hypertension. *The European Respiratory Journal.* 2013, **42**(1), 272-279. ISSN 0903-1936. DOI: 10.1183/09031936.00087212.

3. ANYANWU, A. C.; ITAGAKI, S.; PINNEY, S.; ADAMS, D. H. Initial experience with routine less invasive implantation of HeartMate II left ventricular assist device without median sternotomy. *European Journal of Cardio-Thoracic Surgery*. 2014, **46**(6), 985-990. ISSN 1010-7940. DOI: 10.1093/ejcts/ezu044.

4. BABA, A.; DOBSAK, P.; SAITO, I.; ISOYAMA, T.; TAKIURA, K.; ABE, Y.; CHINZEI, T.; VAŠKŮ, J.; IMACHI, K. Microcirculation of the bulbar conjunctiva in the goat implanted with a total artificial heart: Effects of pulsatile and nonpulsatile flow. *ASAIO Journal*. 2004, **50**(4), 321-327. ISSN 1058-2916. DOI: 10.1097/01.MAT.0000129320.57362.db.

5. BHAMA, J. K.; KORMOS, R. L.; TOYODA, Y.; TEUTEBERG, J. J.; MCCURRY, K. R.; SIEGENTHALER, M. P. Clinical Experience Using the Levitronix CentriMag System for Temporary Right Ventricular Mechanical Circulatory Support. *The Journal of Heart and Lung Transplantation*. 2009, **28**(9), 971-976. ISSN 1053-2498. DOI: 10.1016/j.healun.2009.04.015.

6. BIRKS, E. J. Molecular Changes After Left Ventricular Assist Device Support for Heart Failure. *Circulation Research*. 2013, **113**(6), 777-791. ISSN 0009-7330. DOI: 10.1161/CIRCRESAHA.113.301413.

7. BLANN, A. D.; PRETORIUS, A. Circulating endothelial cells and endothelial progenitor cells: Two sides of the same coin, or two different coins?. *Atherosclerosis*. 2006, **188**(1), 12-18. ISSN 0021-9150. DOI: 10.1016/j.atherosclerosis.2005.12.024.

8. BRAUNWALD, E. Heart Failure. *JACC: Heart Failure*. 2013, **1**(1), 1-20. ISSN 2213-1779. DOI: 10.1016/j.jchf.2012.10.002.

9. BRAUNWALD, E. The war against heart failure: the Lancet lecture. *The Lancet*. 2015, **385**(9970), 812-824. ISSN 0140-6736. DOI: 10.1016/S0140-6736(14)61889-4.

10. BUI, A. L.; HORWICH, T. B.; FONAROW, G. C. Epidemiology and risk profile of heart failure. *Nature Reviews: Cardiology*. 2011, **8**(1), 30-41. ISSN 1759-5002. DOI: 10.1038/nrcardio.2010.165.

11. BURGER, D.; TOUYZ, R.M. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. *Journal of the American Society of Hypertension.* 2012, **6**(2)**,** 85–99. ISSN 1933-1711. DOI: 10.1016/j.jash.2011.11.003.

12. BURGER, D.; KWART, D. G.; MONTEZANO, A. C.; READ, N.C.; KENNEDY, C. R.; THOMPSON, C. S.; TOUYZ, R. M. Microparticles induce cell cycle arrest through redox-sensitive processes in endothelial cells: implications in vascular senescence. *Journal of the American Heart Association.* 2012, **1**(3), e001842. ISSN 2047-9980. DOI: 10.1161/JAHA.112.001842.

13. BURNIER, L.; FONTANA, P.; KWAK, B. R.; ANGELILLO-SCHERRER, A. Cell-derived microparticles in haemostasis and vascular medicine. *Thrombosis and Haemostasis*. 2009, **101**(3), 439-451. ISSN 0340-6245. DOI: 10.1160/TH08-08-0521.

14. CASONATO, A.; SPONGA, S.; PONTARA, E.; CATTINI, M. G.; BASSO, C.; THIENE, G.; CELLA, G.; DAIDONE, V.; GEROSA, G.; PAGNAN, A. von Willebrand factor abnormalities in aortic valve stenosis: Pathophysiology and impact on bleeding. *Thrombosis and Haemostasis*. 2011, **106**(1), 58-66. ISSN 0340-6245. DOI: 10.1160/TH10-10-0634.

15. COOK, J. A.; SHAH, K. B.; QUADER, M. A.; COOKE, R. H.; KASIRAJAN, V.; RAO, K. K.; SMALLFIELD, M. C.; TCHOUKINA, I.; TANG, D. G. The total artificial heart. *Journal of Thoracic Disease*. 2015, **7**(12), 2172-2180. ISSN 2072-1439. DOI: 10.3978/j.issn.2072-1439.2015.10.70.

16. COWGER, J. A.; ROMANO, M. A.; SHAH, P.; SHAH, N.; MEHTA, V.; HAFT, J. W.; AARONSON, K. D.; PAGANI, F. D. Hemolysis: A harbinger of adverse outcome after Left ventricular assist device implant. *The Journal of Heart and Lung Transplantation*. 2014, **33**(1), 35-43. ISSN 1053-2498. DOI: 10.1016/j.healun.2013.08.021.

17. CROW, S.; CHEN, D.; MILANO, C.; THOMAS, W.; JOYCE, L.; PIACENTINO, V.; SHARMA, R.; WU, J.; AREPALLY, G.; BOWLES, D.; ROGERS, J.; VILLAMIZAR-ORTIZ, N. Acquired von Willebrand Syndrome in Continuous-Flow Ventricular Assist Device Recipients. *The Annals of Thoracic Surgery*. 2010, **90**(4), 1263-1269. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2010.04.099.

18. DEBAKEY, M. E. Development of mechanical heart devices. *The Annals of Thoracic Surgery*. 2005, **79**(6), S2228-S2231. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2005.03.029.

19. DEBAKEY, M. E. Left ventricular bypass pump for cardiac assistance: Clinical experience. *The American Journal of Cardiology*. 1971, **27**(1), 3-11. ISSN 0002-9149.

20. DIEHL, P.; ALEKER, M.; HELBING, T.; SOSSONG, V.; BEYERSDORF, F.; OLSCHEWSKI, M.; BODE, C.; MOSER, M. Enhanced microparticles in ventricular assist device patients predict platelet, leukocyte and endothelial cell activation. *Interactive Cardiovascular Thoracic Surgery*. 2010, **11**(2): 133–137. ISSN 1569-9293. DOI: 10.1510/icvts.2010.232603.

21. FADINI, G. P.; CORACINA, A.; BAESSO, I.; AGOSTINI, C.; TIENGO, A.; AVOGARO, A.; DE KREUTZENBERG, S. V. Peripheral Blood CD34+KDR+ Endothelial Progenitor Cells Are Determinants of Subclinical Atherosclerosis in a Middle-Aged General Population. *Stroke*. 2006, **37**(9), 2277-2282. ISSN 0039-2499. DOI: 10.1161/01.STR.0000236064.19293.79.

22. FRANÇA, C. N.; IZAR, M. C.; AMARAL, J. B.; TEGANI, D. M.; FONSECA, F. A. Microparticles as potential biomarkers of cardiovascular disease. *Arquivos*

Brasileiros de Cardiologia. 2015, **104**(2), 169-174. ISSN 0066-782X. DOI: 10.5935/abc.20140210.

23. GAMBILLARA, V.; THACHER, T.; SILACCI, P.; STERGIOPULOS, N. Effects of reduced cyclic stretch on vascular smooth muscle cell function of pig carotids perfused Ex vivo. *American Journal of Hypertension*. 2008, **21**(4), 425-431. ISSN 0895-7061. DOI: 10.1038/ajh.2007.72.

24. GIBBON, J. H. Application of a mechanical heart and lung apparatus to cardiac surgery. *Minnesota Medicine*. 1954, **37**(3), 171-185. ISSN 0026-556X.

25. GUHA, K.; MCDONAGH, T. Heart failure epidemiology: European perspective. *Current Cardiology Reviews*. 2013, **9**(2), 123-127. ISSN 1573-403X.

26. HABERL, T.; RIEBANDT, J.; MAHR, S.; LAUFER, G.; RAJEK, A.; SCHIMA, H.; ZIMPFER, D. Viennese approach to minimize the invasiveness of ventricular assist device implantation. *European Journal of Cardio-Thoracic Surgery*. 2014, **46**(6), 991-996. ISSN 1010-7940. DOI: 10.1093/ejcts/ezu051.

27. HASIN, T.; DEO, S.; MALESZEWSKI, J. J.; TOPILSKY, Y.; EDWARDS, B. S.; PEREIRA, N. L.; STULAK, J. M.; JOYCE, L.; DALY, R.; KUSHWAHA, S. S.; PARK, S. J. The Role of Medical Management for Acute Intravascular Hemolysis in Patients Supported on Axial Flow LVAD. *ASAIO Journal*. 2014, **60**(1), 9-14. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000012.

28. HASIN, T.; MATSUZAWA, Y.; GUDDETI, R. R.; AOKI, T.; KWON, T.-G.; SCHETTLE, S.; LENNON, R. J.; CHOKKA, R. G.; LERMAN, A.; KUSHWAHA, S. S. Attenuation in Peripheral Endothelial Function After Continuous Flow Left Ventricular Assist Device Therapy Is Associated With Cardiovascular Adverse Events. *Circulation Journal*. 2015, **79**(4), 770-777. ISSN 1346-9843. DOI: 10.1253/circj.CJ-14-1079.

29. HEALY, A. H.; MCKELLAR, S. H.; DRAKOS, S. G.; KOLIOPOULOU, A.; STEHLIK, J.; SELZMAN, C. H. Physiologic effects of continuous-flow left ventricular assist devices. *Journal of Surgical Research*. 2016, **202**(2), 363-371. ISSN 0022-4804. DOI: 10.1016/j.jss.2016.01.015.

30. HOLANDER, S. A.; RIZZUTO, S.; HOLANDER, A. M.; LIN, A.; LIU, E.; MURRAY, J. M.; ALMOND, C. S.; ROSENTHAL, D. N. Obesity and Premature Loss of Mobility in Two Adolescents with Becker Muscular Dystrophy After HeartMate II Implantation. *ASAIO Journal*. 2016, **62**(1), e5-e7. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000292.

31. HUTCHENSON, I. R.; GRIFFITH, T. M. Release of endothelium-derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. *American Journal of Physiology*. 1991, **261**(1), H257-H262. ISSN 0002-9513.

32. CHENG, A.; WILLIAMITIS, C. A.; SLAUGHTER, M. S. Comparison of continuous-flow and pulsatile-flow left ventricular assist devices: is there an advantage to pulsatility?. *Annals of Cardiothoracic Surgery*. 2014, **3**(6), 573-581. ISSN 2225-319X. DOI: 10.3978/j.issn.2225-319X.2014.08.24.

33. CHEUNG, A.; SOON, J.-L.; BASHIR, J.; KAAN, A.; IGNASZEWSKI, A. Minimal-access left ventricular assist device implantation. *Innovations*. 2014, **9**(4), 281-285. ISSN 1556-9845. DOI: 10.1097/IMI.0000000000000086.

34. JOHN, R.; KAMDAR, F.; LIAO, K.; COLVIN-ADAMS, M.; BOYLE, A.; JOYCE, L. Improved survival and decreasing incidence of adverse events with the HeartMate II left ventricular assist device as bridge-to-transplant therapy. *The Annals of Thoracic Surgery*. 2008, **86**(4), 1227-1235. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2008.06.030.

35. KIRKLIN, J. K.; NAFTEL, D. C.; PAGANI, F. D.; KORMOS, R. L.; STEVENSON, L. W.; BLUME, E. D.; MILLER, M. A.; BALDWIN, J. T.; YOUNG, J. B. Sixth INTERMACS annual report: A 10,000-patient database. *The Journal of Heart and Lung Transplantation*. 2014, **33**(6), 555-564. ISSN 1053-2498. DOI: 10.1016/j.healun.2014.04.010.

36. LEE, J. J.; TYML, K.; MENKIS, A. H.; NOVICK, R. J.; MCKENZIE, F. N. Evaluation of Pulsatile and Nonpulsatile Flow in Capillaries of Goat Skeletal Muscle Using Intravital Microscopy. *Microvascular Research*. 1994, **48**(3), 316-327. ISSN 0026-2862. DOI: 10.1006/mvre.1994.1058.

37. LEONE, A. M.; VALGIMIGLI, M.; GIANNICO, M. B.; ZACCONE, V.; PERFETTI, M.; D´AMARIO, D.; REBUZZI, A. G.; CREA, F. From bone marrow to the arterial wall: the ongoing tale of endothelial progenitor cells. *European Heart Journal.* 2009, **30**(8):890-899. ISSN 0195-668X. DOI: 10.1093/eurheartj/ehp078.

38. LERMAN, A.; BURNETT, J. C. Intact and altered endothelium in regulation of vasomotion. *Circulation*. 1992, **86**(Suppl. 6), III12-III19. ISSN 0009-7322.

39. LONG, E. F.; SWAIN, G. W.; MANGI, A. A. Comparative Survival and Cost-Effectiveness of Advanced Therapies for End-Stage Heart Failure. *Circulation: Heart Failure*. 2014, **7**(3), 470-478. ISSN 1941-3289. DOI: 10.1161/CIRCHEARTFAILURE.113.000807.

40. LUND, L. H.; EDWARDS, L. E.; KUCHERYAVAYA, A. Y.; BENDEN, CH.; CIPCHAND, A. I.; GOLDFARB, S.; LEWEY, B. J.; MEISER, B.; ROSSANO, J. W.; YUSEN, R. D.; STEHLIK, J. The Registry of International Society for Heart and Lung Transplantation: Thirty-second Official Adult Heart Transplantation Report-2015; Focus Theme: Early Graft Failure. *The Journal of Heart and Lung Transplantation.* 2015, **34**(10), 1244-1254. ISSN 1053-2498. DOI: 10.1016/j.healun.2015.08.003.

41. MAKDISI, G.; WANG, I.-W. Minimally invasive is the future of left ventricular assist device implantation. *Journal of Thoracic Disease*. 2015, **7**(9), e283-e288. ISSN 2072-1439. DOI: 10.3978/j.issn.2072-1439.2015.08.30.

42. MANGINAS, A.; TSIAVOU, A.; SFYRAKIS, P.; GIAMOUZIS, G.; TSOURELIS, L.; LEONTIADIS, E.; DEGIANNIS, D.; COKKINOS, D. V.; ALIVIZATOS, P. A. Increased number of circulating progenitor cells after implantation of ventricular assist devices. *The Journal of Heart and Lung Transplantation*. 2009, **28**(7), 710-717. ISSN 1053-2498. DOI: 10.1016/j.healun.2009.04.006.

43. MCGINN, C. M.; MACDONNELL, B. F.; SHAN, C. X.; WALLACE, R.; CUMMINS, P. M.; MURPHY, R. P. Microparticles: A Pivotal Nexus in Vascular

Homeostasis and Disease. *Current Clinical Pharmacology*. 2016, **11**(1), 28-42. ISSN 1574-8847.

44. MEYER, A. L.; MALEHSA, D.; BARA, C.; BUDDE, U.; SLAUGHTER, M. S.; HAVERICH, A.; STRUEBER, M. Acquired von Willebrand Syndrome in Patients With an Axial Flow Left Ventricular Assist Device. *Circulation: Heart Failure*. 2010, **3**(6), 675-681. ISSN 1941-3289. DOI: 10.1161/CIRCHEARTFAILURE.109.877597.

45. MEYER, A. L.; MALEHSA, D.; BUDDE, U.; BARA, C.; HAVERICH, A.; STRUEBER, M. Acquired von Willebrand Syndrome in Patients With a Centrifugal or Axial Continuous Flow Left Ventricular Assist Device. *JACC: Heart Failure*. 2014, **2**(2), 141-145. ISSN 2213-1779. DOI: 10.1016/j.jchf.2013.10.008.

46. MUTHIAH, K.; CONNOR, D.; LY, K.; GARDINER, E. E.; ANDREWS, R. K.; QIAO, J.; RUTGERS, D.; ROBSON, D.; LOW, J.; JARVIS, S.; MACDONALD, P.; DHITAL, K.; JANSZ, P.; JOSEPH, J.; HAYWARD, C. S. Longitudinal changes in hemostatic parameters and reduced pulsatility contribute to non-surgical bleeding in patients with centrifugal continuous-flow left ventricular assist devices. *The Journal of Heart and Lung Transplantation*. 2016, **35**(6), 743-751. ISSN 1053-2498. DOI: 10.1016/j.healun.2015.12.024.

47. NAKANO, T.; TOMINAGA, R.; NAGANO, I.; OKABE, H.; YASUI, H. Pulsatile flow enhances endothelium-derived nitric oxide release in the peripheral vasculature. *American Journal of Physiology: Heart and Circulatory Physiology*. 2000, **278**(4), H1098-H1104. ISSN 0363-6135.

48. NASCIMBENE, A.; HERNANDEZ, R.; GEORGE, J. K.; PARKER, A.; BERGERON, A. L.; PRADHAN, S.; VIJAYAN, K. V.; CIVITELLO, A.; SIMPSON, L.; NAWROT, M.; LEE, V.-V.; MALLIDI, H. R.; DELGADO, R. M.; DONG, J. F.; FRAZIER, O. H. Association between cell-derived microparticles and adverse events in patients with nonpulsatile Left ventricular assist devices. *The Journal of Heart and Lung Transplantation*. 2014, **33**(5), 470-477. ISSN 1053-2498. DOI: 10.1016/j.healun.2014.01.004.

49. NASCIMBENE, A.; NEELAMEGHAM, S.; FRAZIER, O. H.; MOAKE, J. L.; DONG, J.-F. Acquired von Willebrand syndrome associated with left ventricular assist device. *Blood*. 2016, **127**(25), 3133-3141. ISSN 0006-4971. DOI: 10.1182/blood-2015-10-636480.

50. NETUKA, I.; MALÝ, J.; SZÁRSZOI, O. Mechanické srdeční podpory v terapii terminálního srdečního selhání. *Cor et Vasa.* 2008, **50**(5), 207-214. ISSN 0010-8650.

51. NETUKA, I.; SOOD, P.; PYA, Y.; ZIMPFER, D.; KRABATSCH, T.; GARBADE, J.; RAO, V.; MORSHUIS, M.; MARASCO, S.; BEYERSDORF, F.; DAMME, L.; SCHMITTO, J. D. Fully Magnetically Levitated Left Ventricular Assist System for Treating Advanced HF: A Multicenter Study. *Journal of the American College of Cardiology*. 2015, **66**(23), 2579-2589. ISSN 0735-1097. DOI: 10.1016/j.jacc.2015.09.083.

52. NISHINAKA, T.; TATSUMI, E.; NISHIMURA, T.; SHIOYA, K.; OHNISHI, H.; TAENAKA, Y.; TAKANO, H. Change in vasoconstrictive function during prolonged nonpulsatile left heart bypass. *Artificial Organs*. 2001, **25**(5), 371-375. ISSN 0160-564X. DOI: 10.1046/j.1525-1594.2001.025005371.x.

53. NOMURA, S.; TANDON, N. N.; NAKAMURA, T.; CONE, J.; FUKUHARA, S.; KAMABAYASHI, J. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. *Atherosclerosis.* 2001, **158**(2), 277-287. ISSN 0021-9150. DOI: 10.1016/S0021-9150(01)00433-6

54. OZ, M. C.; ARGENZIANO, M.; CATANESE, K. A.; GARDOCKI, M. T.; GOLDSTEIN, D. J.; ASHTON, R. C.; GELIJNS, A. C.; ROSE, E. A.; LEVIN, H. R. Bridge experience with long-term implantable left ventricular assist devices - Are they an alternative to transplantation?. *Circulation*. 1997, **95**(7), 1844-1852. ISSN 0009-7322.

55. PASQUE, M. K.; ROGERS, J. G. Adverse events in the use of HeartMate vented electric and Novacor left ventricular assist devices: Comparing apples and

oranges. *The Journal of Thoracic and Cardiovascular Surgery*. 2002, **124**(6), 1063-1067. ISSN 0022-5223. DOI: 10.1067/mtc.2002.123520.

56. PIŤHA, J.; LESNÁ KRÁLOVÁ, I.; SEKERKOVÁ, A.; POLEDNE, R.; KOVÁŘ, J.; LEJSKOVÁ, M.; DVOŘÁKOVÁ, H.; ADAMKOVÁ, S.; LÁNSKÁ, V.; BOBÁK, M. Menopausal transition enhances the atherogenic risk of smoking in middle aged women. *International Journal of Cardiology*. 2013, **168**(1), 190-196. ISSN 0167-5273. DOI: 10.1016/j.ijcard.2012.09.095.

57. PONIKOWSKI, P.; VOORS, A. A.; ANKER, S. D.; BUENO, H.; CLELAND, J. G. F.; COATS, A. J. S.; FALK, V.; GONZÁLEZ-JUANATEY, J. R.; HARJOLA, V.-P.; JANKOWSKA, E. A.; JESSUP, M.; LINDE, C.; NIHOYANNOPOULOS, P.; PARISSIS, J. T.; PIESKE, B.; RILEY, J. P.; ROSANO, G. M. C.; RUILOPE, L. M.; RUSCHITZKA, F.; RUTTEN, F. H.; VAN DER MEER, P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European Heart Journal*. 2016, **37**(27), 2129-2200. ISSN 0195-668X. DOI: 10.1093/eurheartj/ehw128.

58. PRESCIMONE, T.; MASOTTI, S.; D'AMICO, A.; CARUSO, R.; CABIATI, M.; CASELLI, C.; VIGLIONE, F.; VERDE, A.; DEL RY, S.; GIANNESSI, D. Cardiac molecular markers of programmed cell death are activated in end-stage heart failure patients supported by left ventricular assist device. *Cardiovascular Pathology*. 2014, **23**(5), 272-282. ISSN 1054-8807. DOI: 10.1016/j.carpath.2014.04.003.

59. RADOVANCEVIC, B.; VRTOVEC, B.; DE KORT, E.; RADOVANCEVIC, R.; GREGORIC, I. D.; FRAZIER, O. H. End-organ function in patients on long-term circulatory support with continuous- or Pulsatile-flow assist devices. *The Journal of Heart and Lung Transplantation*. 2007, **26**(8), 815-818. ISSN 1053-2498. DOI: 10.1016/j.healun.2007.05.012.

60. RECCHIONI, R.; MARCHESELLI, F.; ANTONICELLI, R.; LAZZARINI, R.; MENSÀ, E.; TESTA, R.; PROCOPIO, A. D.; OLIVIERI, F. Physical activity and

progenitor cell-mediated endothelial repair in chronic heart failure: Is there a role for epigenetics?. *Mechanisms of Ageing and Development*. 2016, **159**(October), 71-80. ISSN 0047-6374. DOI: 10.1016/j.mad.2016.03.008.

61. ROSE, E. A.; GELIJNS, A. C.; MOSKOWITZ, A. J.; HEITJAN, D. F.; STEVENSON, L. W.; DEMBITSKY, W.; LONG, J. W.; ASCHEIM, D. D.; TIERNEY, A. R.; LEVITAN, R. G.; WATSON, J. T.; MEIER, P.; RONAN, N. S.; SHAPIRO, P. A.; LAZAR, R. M.; MILLER, L. W.; GUPTA, L.; FRAZIER, O. H.; DESVIGNE-NICKENS, P.; OZ, M. C.; POIRIER, V. L. Long-term use of a left ventricular assist device for end-stage heart failure. *The New England Journal of Medicine*. 2001, **345**(20), 1435-1443. ISSN 0028-4793. DOI: 10.1056/NEJMoa012175.

62. RYAN, T. D.; JEFFERIES, J. L.; SAWNANI, H.; WONG, B. L.; GARDNER, A.; DEL CORRAL, M.; LORTS, A.; MORALES, D. L.S. Implantation of the HeartMate II and HeartWare Left Ventricular Assist Devices in Patients With Duchenne Muscular Dystrophy: Lessons Learned From the First Applications. *ASAIO Journal*. 2014, **60**(2), 246-248. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000050.

63. SAITO, Tomohiro; WASSILEW, Katharina; GORODETSKI, Boris; STEIN, Julia; FALK, Volkmar; KRABATSCH, Thomas; POTAPOV, Evgenij. Aortic Valve Pathology in Patients Supported by Continuous-Flow Left Ventricular Assist Device. *Circulation Journal*. 2016, **80**(6), 1371-1377. ISSN 1346-9843. DOI: 10.1253/circj.CJ-15-1188.

64. SANDNER, S. E.; ZIMPFER, D.; ZRUNEK, P.; RAJEK, A.; SCHIMA, H.; DUNKLER, D.; GRIMM, M.; WOLNER, E.; WIESELTHALER, G. M. Renal Function and Outcome After Continuous Flow Left Ventricular Assist Device Implantation. *The Annals of Thoracic Surgery*. 2009, **87**(4), 1072-1078. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2009.01.022.

65. SANSONE, R.; STANSKE, B.; KEYMEL, S.; SCHULER, D.; HORN, P.; SAEED, D.; BOEKEN, U.; WESTENFELD, R.; LICHTENBERG, A.; KELM, M.; HEISS, C. Macrovascular and microvascular function after implantation of left ventricular assist devices in end-stage heart failure: Role of microparticles.

The Journal of Heart and Lung Transplantation. 2015, **34**(7), 921-932. ISSN 1053-2498. DOI: 10.1016/j.healun.2015.03.004.

66. SEGOVIA, J.; ALONSO-PULPON, L.; BURGOS, R.; SILVA, L.; SERRANO, S.; CASTEDO, E.; JIMÉNEZ, J.; FUENTES, R.; CAÑAS, A.; UGARTE, J. Heart transplantation in Friedreich's ataxia and other neuromuscular diseases. *The Journal of Heart and Lung Transplantation*. 2001, **20**(2), 169. ISSN 1053-2498.

67. SEGURA, A. M.; GREGORIC, I.; RADOVANCEVIC, R.; DEMIROZU, Z. T.; BUJA, L. M.; FRAZIER, O. H. Morphologic changes in the aortic wall media after support with a continuous-flow left ventricular assist device. *The Journal of Heart and Lung Transplantation*. 2013, **32**(11), 1096-1100. ISSN 1053-2498. DOI: 10.1016/j.healun.2013.07.007.

68. SHAFFER, R. G.; GREENE, S.; ARSHI, A.; SUPPLE, G.; BANTLY, A.; MOORE, J. S.; MOHLER, E. R. 3RD. Flow cytometric measurement of circulating endothelial cells: the effect of age and peripheral arterial disease on baseline levels of mature and progenitor cells. *Cytometry. Part B, Clinical Cytometry*. 2006, **70**(2), 56-62. ISSN 1552-4949. DOI: 10.1002/cyto.b.20085.

69. SCHEUBEL, R. J.; ZORN, H.; SILBER, R. E.; KUSS, O.; MORAWIETZ, H.; HOLTZ, J.; SIMM, A. Age-dependent depression in circulating endothelial progenitor cells undergoing coronary artery bypass grafting. *Journal of the American College of Cardiology.* 2003, **42**(12), 2073-2080. ISSN 0735-1097. DOI 10.1016/j.jacc.2003.07.025

70. SCHIRO, A.; WILKINSON, F. L.; WESTON, R.; SMYTH, J. V.; SERRACINO-INGLOTT, F.; ALEXANDER, M. Y. Endothelial microparticles as conveyors of information in atherosclerotic disease. *Atherosclerosis*. 2014, **234**(2), 295-302. ISSN 0021-9150. DOI: 10.1016/j.atherosclerosis.2014.03.019.

71. SINAURIDZE, E. I.; KIREEV, D. A.; POPENKO, N. Y.; PICHUGIN, A. V.; PANTELEEV, M. A.; KRYMSKAYA, O. V.; ATAULLAKHANOV, F. I. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thrombosis and Haemostasis.* 2007, **97**(3): 425-434. ISSN 0340-6245. DOI: 10.1160/TH06-06-0313

72. SLAUGHTER, M. S. Long-Term Continuous Flow Left Ventricular Assist Device Support and End-Organ Function: Prospects for Destination Therapy. *Journal of Cardiac Surgery*. 2010, **25**(4), 490-494. ISSN 0886-0440. DOI: 10.1111/j.1540-8191.2010.01075.x.

73. SOUCY, K. G.; KOENIG, S. C.; GIRIDHARAN, G. A.; SOBIESKI, M. A.; SLAUGHTER, M. S. Rotary Pumps and Diminished Pulsatility: Do We Need a Pulse?. *ASAIO Journal*. 2013, **59**(4), 355-366. ISSN 1058-2916. DOI: 10.1097/MAT.0b013e31829f9bb3.

74. STERN, D. R.; KAZAM, J.; EDWARDS, P.; MAYBAUM, S.; BELLO, R. A.; D'ALESSANDRO, D. A.; GOLDSTEIN, D. J. Increased Incidence of Gastrointestinal Bleeding Following Implantation of the HeartMate II LVAD. *Journal of Cardiac Surgery*. 2010, **25**(3), 352-356. ISSN 0886-0440. DOI: 10.1111/j.1540-8191.2010.01025.x.

75. SUN, B. C.; CATANESE, K. A.; SPANIER, T. B.; FLANNERY, M. R.; GARDOCKI, M. T.; MARCUS, L. S.; LEVIN, H. R.; ROSE, E. A.; OZ, M. C. 100 long-term implantable left ventricular assist devices: The Columbia Presbyterian Interim Experience. *The Annals of Thoracic Surgery*. 1999, **68**(2), 688-694. ISSN 0003-4975. DOI: 10.1016/S0003-4975(99)00539-1.

76. TAMURA, T.; HORIUCHI, H.; IMAI, M.; TADA, T.; SHIOMI, H.; KURODA, M.; NISHIMURA, S.; TAKAHASHI, Y.; YOSHIKAWA, Y.; TSUJIMURA, A.; AMANO, M.; HAYAMA, Y.; IMAMURA, S.; ONISHI, N.; TAMAKI, Y.; ENOMOTO, S.; MIYAKE, M.; KONDO, H.; KAITANI, K.; IZUMI, C.; KIMURA, T.; NAKAGAWA, Y. Unexpectedly High Prevalence of Acquired von Willebrand Syndrome in Patients with Severe Aortic Stenosis as Evaluated with a Novel Large Multimer Index. *Journal of Atherosclerosis and Thrombosis*. 2015, **22**(11), 1115-1123. ISSN 1340-3478.

77. TEMPLETON, D. L.; MOSSER, K. H. H.; CHEN, C. N.; STONE, M. D.; JOHN, R.; DENGEL, D. R.; THOMPSON, L. V. Effects of Left Ventricular Assist Device (LVAD) Placement on Myocardial Oxidative Stress Markers. *Heart, Lung and Circulation*. 2012, **21**(9), 586-597. ISSN 1443-9506. DOI: 10.1016/j.hlc.2012.04.016.

78. THACHER, T.; GAMBILLARA, V.; DA SILVA, R. F.; SILACCI, P.; STERGIOPULOS, N. Reduced cyclic stretch, endothelial dysfunction, and oxidative stress: an ex vivo model. *Cardiovascular Pathology*. 2010, **19**(4), e91-e98. ISSN 1054-8807. DOI: 10.1016/j.carpath.2009.06.007.

79. TODA, K.; FUJITA, T.; DOMAE, K.; SHIMAHARA, Y.; KOBAYASHI, J.; NAKATANI, T. Late Aortic Insufficiency Related to Poor Prognosis During Left Ventricular Assist Device Support. *The Annals of Thoracic Surgery*. 2011, **92**(3), 929-934. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2011.04.115.

80. TOPKARA, V. K.; GARAN, A. R.; FINE, B.; GODIER-FURNEMONT, A. F.; BRESKIN, A.; CAGLIOSTRO, B.; YUZEFPOLSKAYA, M.; TAKEDA, K.; TAKAYAMA, H.; MANCINI, D. M.; NAKA, Y.; COLOMBO, P. C. Myocardial Recovery in Patients Receiving Contemporary Left Ventricular Assist Devices Results From the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS). *Circulation: Heart Failure*. 2016, **9**(7), e003157. ISSN 1941-3289. DOI: 10.1161/CIRCHEARTFAILURE.116.003157.

81. TRIVEDI, J. R.; CHENG, A.; SINGH, R.; WILLIAMS, M. L.; SLAUGHTER, M. S. Survival on the Heart Transplant Waiting List: Impact of Continuous Flow Left Ventricular Assist Device as Bridge to Transplant. *The Annals of Thoracic Surgery*. 2014, **98**(3), 830-834. ISSN 0003-4975. DOI: 10.1016/j.athoracsur.2014.05.019.

82. TRUBY, L.; MUNDY, L.; KALESAN, B.; KIRTANE, A.; COLOMBO, P. C.; TAKEDA, K.; FUKUHARA, S.; NAKA, Y.; TAKAYAMA, H. Contemporary Outcomes of Venoarterial Extracorporeal Membrane Oxygenation for Refractory Cardiogenic Shock at a Large Tertiary Care Center. *ASAIO Journal*. 2015, **61**(4), 403-409. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000225.

83. URIEL, N.; PAK, S.-W.; JORDE, U. P.; JUDE, B.; SUSEN, S.; VINCENTELLI, A.; ENNEZAT, P.-V.; CAPPLEMAN, S.; NAKA, Y.; MANCINI, D. Acquired von Willebrand Syndrome After Continuous-Flow Mechanical Device Support Contributes to a High Prevalence of Bleeding During Long-Term Support and at the Time of Transplantation. *Journal of the American College of Cardiology*. 2010, **56**(15), 1207-1213. ISSN 0735-1097. DOI: 10.1016/j.jacc.2010.05.016.

84. VALGIMIGLI, M.; RIGOLIN, G. M.; FUCILI, A.; PORTA, M. D.; SOUKHOMOVSKAIA, O.; MALAGUTTI, P.; BUGLI, A. M.; BRAGOTTI, L. Z.; FRANCOLINI, G.; MAURO, E.; CASTOLDI, G.; FERRARI, R. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. *Circulation*. 2004, **110**(10), 1209-1212. ISSN 0009-7322. DOI: 10.1161/01.CIR.0000136813.89036.21.

85. VAN WIJK, M. J.; VAN BAVEL, E.; STURK, A.; NIEUWLAND, R. Microparticles in cardiovascular diseases. *Cardiovascular Research*. 2003, **59**(2), 277-287. ISSN 0008-6363. DOI: 10.1016/S0008-6363(03)00367-5.

86. WERNER, N.; KOSIOL, S.; AHLERS, P.; WALENTA, K.; LINK, A.; BÖHM, M.; NICKENIG, G. Circulating endothelial progenitor cells and cardiovascular outcomes. *The New England Journal of Medicine.* 2005, **353**(10), 999-1007. ISSN 0028-4793. DOI: 10.1056/NEJMoa043814.

87. WESTABY, S.; BERTONI, G. B.; CLELLAND, C.; NISHINAKA, T.; FRAZIER, O. H. Circulatory support with attenuated pulse pressure alters human aortic wall morphology. *The Journal of Thoracic and Cardiovascular Surgery*. 2007, **133**(2), 575-576. ISSN 0022-5223. DOI: 10.1016/j.jtcvs.2006.10.014.

88. WEVER-PINZON, O.; DRAKOS, S. G.; MCKELLAR, S. H.; HORNE, B. D.; CAINE, W. T.; KFOURY, A. G.; LI, D. Y.; FANG, J. C.; STEHLIK, J.; SELZMAN, C. H. Cardiac Recovery During Long-Term Left Ventricular Assist Device Support. *Journal of the American College of Cardiology*. 2016, **68**(14), 1540-1553. ISSN 0735-1097. DOI: 10.1016/j.jacc.2016.07.743.

89. WEVER-PINZON, O.; SELZMAN, C. H.; DRAKOS, S. G.; SAIDI, A.; STODDARD, G. J.; GILBERT, E. M.; LABEDI, M.; REID, B. B.; DAVIS, E. S.; KFOURY, A. G.; LI, D. Y.; STEHLIK, J.; BADER, F. Pulsatility and the Risk of Nonsurgical Bleeding in Patients Supported With the Continuous-Flow Left Ventricular Assist Device HeartMate II. *Circulation: Heart Failure*. 2013, **6**(3), 517-526. ISSN 1941-3289. DOI: 10.1161/CIRCHEARTFAILURE.112.000206.

90. YOSHIOKA, D.; SAKAGUCHI, T.; SAITO, S.; MIYAGAWA, S.; NISHI, H.; YOSHIKAWA, Y.; FUKUSHIMA, S.; SAITO, T.; DAIMON, T.; UENO, T.;

KURATANI, T.; SAWA, Y. Predictor of Early Mortality for Severe Heart Failure Patients With Left Ventricular Assist Device Implantation - Significance of INTERMACS Level and Renal Function. *Circulation Journal*. 2012, **76**(7), 1631-1638. ISSN 1346-9843. DOI: 10.1253/circj.CJ-11-1452.

91. ZHANG, Y.; MENG, H.; MA, R.; HE, Z.; WU, X.; CAO, M.; YAO, Z.; ZHAO, L.; LI, T.; DENG, R.; DONG, Z.; TIAN, Y.; BI, Y.; KOU, J.; THATTE, H. S.; ZHOU, J.; SHI, J. Circulating Microparticles, Blood Cells, and Endothelium Induce Procoagulant Activity in Sepsis Through Phosphatidylserine Exposure. *Shock*. 2016, **45**(3), 299-307. ISSN 1073-2322. DOI: 10.1097/SHK.0000000000000509.

92. ZHOU, J.; SHI, J.; HOU, J.; CAO, F.; ZHANG, Y.; RASMUSSEN, J. T.; HEEGAARD, C. W.; GILBERT, G. E.; Phosphatidylserine exposure and procoagulant activity in acute promyelocytic leukemia. *Journal of Thrombosis and Haemostasis.* 2010, **8**(4):773-782. ISSN 1538-7933. DOI: 10.1111/j.1538-7836.2010.03763.x.

93. ZIAEIAN, B.; FONAROW, G. C. Epidemiology and aetiology of heart failure. *Nature Reviews: Cardiology*. 2016, **13**(6), 368-378. ISSN 1759-5002. DOI: 10.1038/nrcardio.2016.25.

11 PŘÍLOHY

Další publikace autora práce.

1. NETUKA, Ivan; **IVÁK, Peter**; SZARSZOI, Ondřej; URBAN, Marian; NOVOTNÝ, Jiří; BEŠÍK, Josef; MALÝ, Jiří. Minimally Invasive Removal of a Temporary RVAD. *ASAIO Journal*. 2015, **61**(2), 202-204. ISSN 1058-2916. DOI: 10.1097/MAT.0000000000000184. **IF: 2.291/2015**.

2. **IVÁK, Peter**; ZUMROVÁ, Alena; NETUKA, Ivan. Friedreich's ataxia and Advanced Heart Failure: An Ethical Conundrum in Decision MakingClinical Decision Making in Friedreich's ataxia. *The Journal of Heart and Lung Transplantation*. 2016, **35**(9), 1144-1145. ISSN 1053-2498. DOI: 10.1016/j.healun.2016.06.021. **IF: 7.509/2015**.

3. URBAN, Marian; SLAVČEV, Antonij; GAZDIČ, Tomáš; **IVÁK, Peter**; BEŠÍK, Josef; NETUKA, Ivan. The impact of angiotensin II type 1 receptor antibodies on post-heart transplantation outcome in Heart Mate II bridged recipients. *Interactive Cardiovascular and Thoracic Surgery*. 2016, **22**(3), 292-297. ISSN 1569-9293. DOI: 10.1093/icvts/ivv344. **IF: 1.329/2015**.

4. URBAN, Marian; SLAVČEV, Antonij; GAZDIČ, Tomáš; **IVÁK, Peter;** NETUKA, Ivan. The impact of Angiotensin II Type 1 Receptor antibodies on morbidity and mortality in Heart Mate II supported recipients. *Biomedical Papers*. 2016, **160**(4), 518-523. ISSN 1213-8118. DOI: 10.5507/bp.2016.025. **IF: 0.924/2015**.

Minimally Invasive Removal of a Temporary RVAD

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We describe a minimally invasive technique for the removal of a temporary right ventricular assist device (RVAD) that provided support concomitant with durable left ventricular assist device support. The RVAD cannulas are mobilized through a small subxiphoid incision at the cannula exit site. Both cannulas are transected subcutaneously, then occluded with plugs made of rolled bovine pericardium, and the skin is closed. The cannula remnants are left in place until heart transplantation is accomplished. To minimize risk of thrombus formation at the cannula tips and subsequent embolization into the right atrium or pulmonary artery, anticoagulation is increased to achieve an international normalized ratio (INR) in the range of 2.5–3.0. *ASAIO Journal* 2015; 61:202–204.

Keywords: right heart failure, RVAD, LVAD, explantation.

The latest generations of axial- and centrifugal-flow left ventricular assist devices (LVADs) have been very effective in providing long-term support of patients with advanced stage heart failure. Patients receiving this therapy have experienced substantial improvements in morbidity, mortality, and quality of life. Nevertheless, complications inherent with advanced heart failure and major implant surgery persist. Right ventricular failure remains an important perioperative management issue despite optimized pharmacological management and a better understanding of LVAD implant timing. Temporary right ventricular assist device (RVAD) support is needed in approximately 3% of cases following durable continuous-flow LVAD implantation to stabilize hemodynamics and to avoid end-organ dysfunction.[1–3] Removal of the RVAD after sufficient recovery of right heart function is ideally associated with minimal morbidity, allowing patients to continue recovery until transplantation. We report a case using our novel minimally invasive technique for removal of RVAD support while leaving both inflow and outflow cannulas "in situ" until transplantation.

Methods

A 59 year old man with ischemic cardiomyopathy presented with severe bilateral ventricular dysfunction, including low cardiac output syndrome and incipient multiorgan failure. The patient was promptly taken to the operating room for implantation of HeartMate II LVAD (Thoratec Corporation, Pleasanton, California) and temporary RVAD implant with a CentriMag (also Thoratec) extracorporeal system. After the expected 2–4 weeks of RVAD support, there were no signs of right heart recovery and serious infectious complications compromised further attempts of testing for RVAD weaning. After 105 days of biventricular support, signs of right ventricular recovery indicated that an RVAD weaning attempt was prudent. Weaning was gradual; after initiating milrinone therapy, the RVAD flow rate was decreased in increments of 0.5 L/min every 12 hours with careful monitoring of the central venous pressure (CVP) and arterial blood pressure. After 36 hours with the RVAD set at 2.0 L/min, the patient was taken to the operating room for RVAD explant. Preoperatively, anticoagulation was maintained at an INR of 2.0 and was not reversed. Intraoperatively, 10,000 units of heparin were given and the RVAD flow rate was decreased to 0.5 L/min. Hemodynamic monitoring (CVP and arterial blood pressure), transesophageal echocardiography (left atrial size), LVAD flow rate and the occurrence of suction events were used to assess the ability to remove the RVAD. After marking the exact location of both cannulas with the aid of portable X-ray, small subxiphoid incision was made and the cannulas were mobilized. The cannulas were cross-clamped and the pumps were stopped. After another period of assessing cardiac function and the patient's ability to maintain adequate cardiac output with the LVAD alone, the cannulas were transected subcutaneously as deep as possible. A plug made of rolled bovine pericardium secured with 2-0 polypropylene suture was inserted into the end of each cannula (**Figure 1**). The ends of each cannula were then oversewn with a 2-0 polypropylene suture, and the skin incisions were closed (**Figure 2**). The cannula remnants will remain in place until

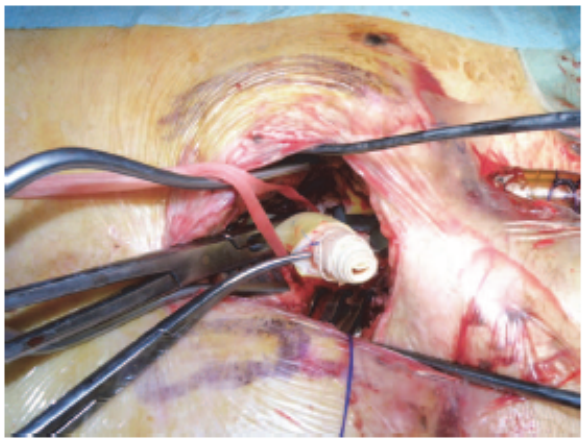

Figure 1. Intraoperative picture showing outflow cannula partially obturated by preformed bovine pericardial cylinder.

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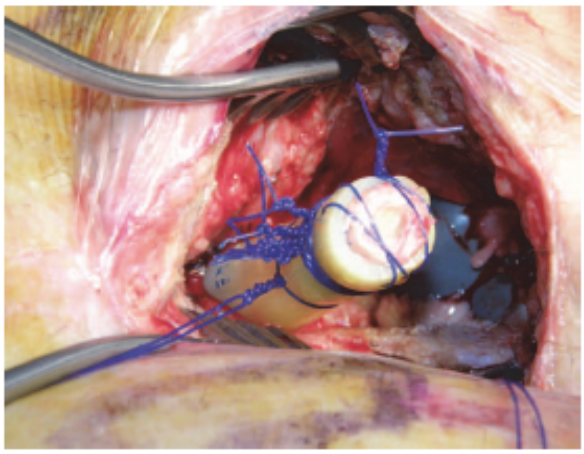

Figure 2. Intraoperative picture showing secured and oversewn plug *in situ*.

heart transplantation. Heparin was reversed with protamine, and the patient was taken from the operating room in good condition. Thrombosis within the cannulas was confirmed by computed tomography scan (**Figure 3**), and there have been no signs of pulmonary embolism. Warfarin anticoagulation with a target INR of 2.5–3.0 was resumed due to continuing LVAD support. After 10 days of recovery, the patient was discharged from the hospital and he has undergone successful heart transplant 50 days after RVAD removal. At transplant, no thrombi were found on the cannula tips or within the right atrium and pulmonary artery.

Discussion

The approach described here for the removal of temporary RVAD support has the potential to minimize morbidity in patients waiting for heart transplant while being supported by an LVAD. Although we performed this explant in the cardiovascular operating room for safety reasons, the amount of surgery was minimal, reducing the risk of common complications such as excessive bleeding. Resternotomy in chronically anticoagulated patients is a high-risk procedure, and reversal of vitamin K antagonist anticoagulation increases the risk of LVAD thrombosis; both problems are minimized by our approach. Furthermore, removal of RVAD after prolonged RVAD support is challenging due to the presence of significant adhesions requiring extensive surgical dissection when the sternotomy approach is used. By avoiding an extensive operation, this patient recovered from the procedure, was effectively treated for infection, and underwent safe heart transplant. Thromboembolic complications from the remnant cannulas did not occur in this case.

Prolonged RVAD support (many days to a few months) with subsequent explant following durable LVAD implantation has resulted in favorable outcomes and may be more cost effective than long-term biventricular support with a fully implantable device.[4] Use of the CentriMag for long-duration RVAD support appears to be safe and effective, owing to its durability and lack of thrombogenicity.[5] Percutaneous cannulation with a flexible outflow cannula placed from the right internal jugular vein to the pulmonary artery and an inflow cannula placed from the femoral vein to the right atrium[6] or minimally invasive short-term VAD implant from right sided thoracotomy[7] in high risk cases for postoperative right heart failure are appropriate

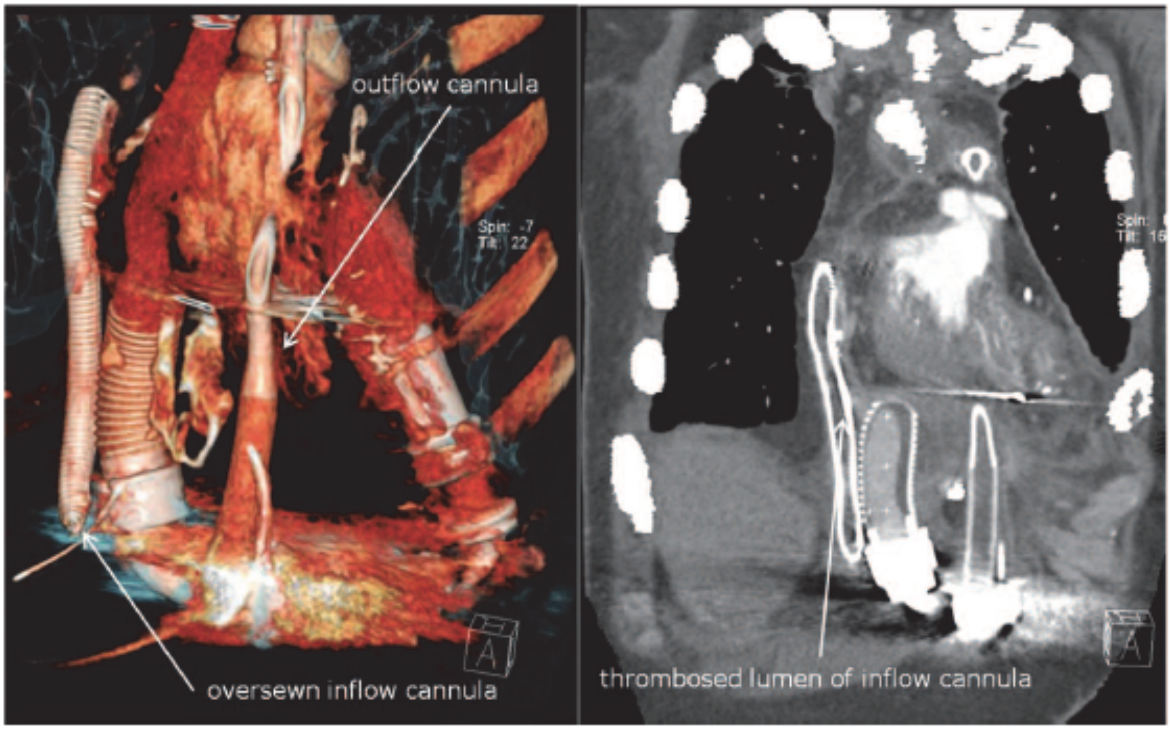

Figure 3. CT scan showing early cannula thrombosis and the location of the cannula tips. CT, computed tomography.

approaches for patients who are expected to have a short recovery time. Extracorporeal membrane oxygenation is not a good option for patients who are at high risk of needing extended support. RVAD implant with a subsequent need for resternotomy[8] carries a risk of injury to cardiac structures due to adhesions. Modified technique with a pulmonary chimney prosthetic graft with subcutaneous ligation at a time of RVAD termination still requires right thoracotomy to address explantation of the right atrial cannula.[5] In addition, infection and thromboembolic complications due to a need of full reversal of anticoagulation at a time of procedure are potential lethal complications of longer term RVAD support. These drawbacks can be effectively ameliorated by the use of our suggested approach.

In summary, limited subxiphoid explantation of a temporary RVAD after delayed right ventricular recovery can be performed safely to minimize perioperative risk connected to conventional explants, and it facilitates early rehabilitation and discharge. Additional research is necessary to determine patient populations that may benefit from this technique.

References

1. Kirklin JK, Naftel DC, Kormos RL, *et al*: Fifth INTERMACS annual report: Risk factor analysis from more than 6,000 mechanical circulatory support patients. *J Heart Lung Transplant* 32: 141–156, 2013.
2. John R, Long JW, Massey HT, *et al*: Outcomes of a multicenter trial of the Levitronix CentriMag ventricular assist system for short-term circulatory support. *J Thorac Cardiovasc Surg* 141: 932–939, 2011.
3. Bhama JK, Kormos RL, Toyoda Y, Teuteberg JJ, McCurry KR, Siegenthaler MP: Clinical experience using the Levitronix CentriMag system for temporary right ventricular mechanical circulatory support. *J Heart Lung Transplant* 28: 971–976, 2009.
4. Potapov EV, Kaufmann F, Stepanenko A, *et al*: Pump exchange for cable damage in patients supported with HeartMate II left ventricular assist device. *ASAIO J* 58: 578–582, 2012.
5. Griffith KE, Jenkins E, Stulak J, Paugh T, Pagani FD. Long-term use of the CentriMag® Ventricular Assist System as a right ventricular assist device: A case report. *Perfusion* 27: 65–70, 2012.
6. Takayama H, Naka Y, Kodali SK, *et al*: A novel approach to percutaneous right-ventricular mechanical support. *Eur J Cardiothorac Surg* 41: 423–426, 2012.
7. Gregoric ID, Cohn WE, Akay MH, La Francesca S, Myers T, Frazier OH: CentriMag left ventricular assist system: Cannulation through a right minithoracotomy. *Tex Heart Inst J* 35: 184–185, 2008.
8. Loforte A, Montalto A, Lilla Della Monica P, Musumeci F: Simultaneous temporary CentriMag right ventricular assist device placement in HeartMate II left ventricular assist system recipients at high risk of right ventricular failure. *Interact Cardiovasc Thorac Surg* 10: 847–850, 2010.

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References

1. Kadavath S, Zapantis E, Zolty R, Efthimiou P. A novel therapeutic approach in pulmonary arterial hypertension as a complication of adult-onset Still's disease: targeting IL-6. Int J Rheum Dis 2014;17:336-40.
2. Padilla-Ibarra J, Sanchez-Ortiz A, Sandoval-Castro C, Ramos-Remus C. Rituximab treatment for pulmonary arterial hypertension in adult-onset Still's disease. Clin Exp Rheumatol 2013;31:657-8.
3. Campos M, Schiopu E. Pulmonary arterial hypertension in adult-onset Still's disease: rapid response to anakinra. Case Rep Rheumatol 2012;2012:537613.
4. de Siqueira MEM, Rodrigues RP, Kormann APM. Adult-onset Still's disease and pulmonary arterial hypertension. Respir Med CME 2009;2:70-2.
5. Muhashir E, Ahmed MM, Hayat S, Heldmann M, Berney SM. Pulmonary hypertension in a patient with adult-onset Stills disease. Clin Rheumatol 2007;26:1359-61.
6. Khatri S, Barland P. Pulmonary hypertension in a patient with adult-onset Still disease. J Musculoskelet Med 2011;28:388-90.
7. Gerfaud-Valentin M, Jamilloux Y, Iwaz J, Seve P. Adult-onset Still's disease. Autoimmun Rev 2014;13:708-22.
8. Zen A, Yamashita N, Ueda M, et al. [A case of adult Still's disease with pulmonary hypertension]. Ryumachi [Rheumatism] 1990;30:45-52.
9. Chen CH, Chen HA, Wang HP, et al. Pulmonary arterial hypertension in autoimmune diseases: an analysis of 19 cases from a medical center in northern Taiwan. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi 2006;39:162-8.
10. Thakare M, Habibi S, Agrawal S, Narsimulu G. Pulmonary arterial hypertension complicating adult-onset Still's disease. Clinical rheumatology 2013;32(Suppl 1):S1-2.

Friedreich's ataxia and advanced heart failure: An ethical conundrum in decision-making

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Friedreich's ataxia (FA) is a neurodegenerative disorder with an autosomal recessive pattern of inheritance, resulting from amplified intronic GAA repeats in FXN domain[1] on chromosome 9q13–q21.1. The defect disrupts production of frataxin, which regulates iron transfer in the mitochondria and results in excessive free radical generation with subsequent neuronal degeneration. FA is a common (1:50,000) inherited ataxia and presents with progressive neurologic symptoms (spinocerebellar ataxia, arreflexia, pyramidal signs, dysarthria and nystagmus). Notably, cardiac involvement is frequent in this disorder and most patients succumb to heart failure.[2] There is controversy in choosing the appropriate therapeutic strategy in such patients and prevalent guidelines do not specifically address this dilemma.[3]

We performed a heart transplant in a 23-year-old woman with FA, first diagnosed at age 16 years (symptoms of vertigo, worsening upper extremities ataxia and saccadic speech disorder), and conclusively confirmed by genetic testing at 18 years of age. At age 21 years, she developed progressive heart failure in the setting of dilated cardiomyopathy with frequent hospitalizations. This coincided with neurologic deterioration and the conversation turned to consideration of advanced therapy for heart failure, because optimal medical therapy was also failing. After much deliberation, a multidisciplinary team approved transplant listing, as the prognosis for heart failure was considered worse than that from the neurologic disease, particularly in the setting of strong family support for the patient's care.

After transplant, the post-operative course was uneventful and allograft function remained without rejection with preserved function through the follow-up at 100 months. Notably, her neurologic status improved, and at 8 years it stabilized with favorable scores compared with pre-transplant baseline (Table 1). Importantly, she became pregnant and subsequently delivered a healthy baby boy in June 2016 by planned cesarean section at Week 38 of gestation. The course has been uneventful

Table 1 Serial Neurologic Status Evaluation

FARS evalution	Pre-transplant	6 mo	1 y	2 y	3 y	4 y	5 y	6 y	7 y	8 y*
Functional staging of ataxia	6	2	2	2	3	3	4	3	3	4
Activities of daily living	32	8	9	12	16	18	19	18	18	20
Neurologic examination score	88	30	38	39	45	58	60	68	66	74
Total score	126	40	49	53	74	79	83	89	84	98

Data are based on a Friedreich's Ataxia Rating Scale (FARS), which is available online at: http://www.ataxia-study-group.net/html/about/ataxiascales/fars (the lower score, the better neurologic/clinical status). mo, months; y, years.
*At time of evaluation, the patient was in Week 35 of pregnancy, with a weight gain of 16 kg and with moderate swelling of lower extremities.

Table 2 Baseline Characteristics and Outcomes of Reported Cases in Patients Treated With Advanced End-stage Heart Therapies (Heart Transplant or LVAD Implant)

	Gender	FA diagnosed before transplant (age)	CM	LVEF before transplant	Documented survival (months)	Last documented graft function	Neurologic status after transplant
Leonard et al[6]	Male	No (NA)	Dilated	NA ("poor function")	6	"Excellent function"	Stationary
Sedlak et al[7]	Male	Yes (in teens)	Dilated	10% to 15%	23	"Excellent function"	Improved
Silva Sieger et al[8]	Male	No (NA)	Dilated	<10%	96	"Excellent function"	Symptoms progression
Yoon et al[4]	Male	Yes (11)	Dilated	23%	20	Normal function	Symptoms progression
Yoda et al[5]	Male	Yes (13)	Dilated	30%	12	NA	Stationary
Segovia et al[9] (Patient 1)	Male	NA	Dilated	NA	43	NA	NA
Segovia et al[9] (Patient 2)	Male	NA	Dilated	NA	8	NA	NA
Segovia et al[9] (Patient 3)	Male	NA	Dilated	NA	17	NA	NA
Present study	Female	Yes (16)	Dilated	20%	100	LVEF 60%	Gradual, mild progression of symptoms, successful childbirth

CM, cardiomyopathy; FA, Friedreich's ataxia; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; NA, not available.

and her cardiac and neurologic function remains stable, without evidence of deterioration.

Ethical concerns about cardiac transplantation in patients with neurodegenerative disease stem from a need for rational allocation of scarce resource as it should be reserved for those who most likely benefit from survival and quality of life.[4] The choices center around providing palliative care for those with late-stage neurologic disease to consideration of transplantation in those likely to have longer survival from allograft transplantation with a slower course of progression of the neurologic disease. Similarly, a strategy of LVAD destination therapy[5] remains contentious because management of device peripherals may be self-limiting for these patients with impaired manual dexterity and thus may hinder optimal rehabilitation. Because both neurologic and cardiac symptoms present with FA in early adulthood, it may be posited that life expectancy would rather be limited by longevity of the allograft than by natural neurologic decline. In addition, post-transplant immunosuppression itself may modify the immunology of FA and warrants further study.

Table 2 summarizes published outcomes with cases and survival up to 8 years, and we have presented our case within this context. Curiously, our case is the first report of cardiac transplantation in a woman with FA, particularly one who was successfully bridged through a pregnancy.

This case anecdote exemplifies the need for a stratified approach among various neurodegenerative disease treatments, with individual adjudication.

Disclosure statement

The authors have no conflicts of interest to disclose.

References

1. Campuzano V, Montermini L, Molto MD, et al. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 1996;271:1423-7.
2. Weidemann F, Rummey C, Bijnens B, et al. The heart in Friedreich ataxia: definition of cardiomyopathy, disease severity, and correlation with neurological symptoms. Circulation 2012;125:1626-34.
3. Mehra MR, Canter CE, Hannan MM, et al. International Society for Heart Lung Transplantation (ISHLT) Infectious Diseases Council; International Society for Heart Lung Transplantation (ISHLT) Pediatric Transplantation Council; International Society for Heart Lung Transplantation (ISHLT) Heart Failure and Transplantation Council. The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: a 10-year update. J Heart Lung Transplant 2016;35:1-23.
4. Yoon G, Soman T, Wilson J, et al. Cardiac transplantation in Friedreich ataxia. J Child Neurol 2012;27:1193-6.
5. Yoda M, El-Banayosy A, Arusoglu L, et al. Permanent use of a ventricle assist device for dilated cardiomyopathy in Friedreich's ataxia. J Heart Lung Transplant 2006;25:251-2.
6. Leonard H, Forsyth R. Friedreich's ataxia presenting after cardiac transplantation. Arch Dis Child 2001;84:167-8.
7. Sedlak TL, Chandavimol M, Straatman L. Cardiac transplantation: a temporary solution for Friedreich's ataxia–induced dilated cardiomyopathy. J Heart Lung Transplant 2004;23:1304-6.
8. Silva Sieger FA, Díaz Silva GA, Ardila Vera MA, et al. Cardiac transplantation for dilated cardiomyopathy in patient with Friedreich's ataxia: a case report. International Journal of Case Reports and Images 2012;3:30-3.
9. Segovia J, Alonso-Pulpon L, Burgos R, et al. Heart transplantation in Friedreich's ataxia and other neuromuscular diseases. J Heart Lung Transplant 2001;20:169.

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The impact of angiotensin II type 1 receptor antibodies on post-heart transplantation outcome in Heart Mate II bridged recipients

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Abstract

OBJECTIVES: Antibodies targeting angiotensin II type 1 receptor (AT1R) have been associated with malignant hypertension, autoimmune diseases and acute rejection and graft loss in solid organ transplantation. The aim of our study was to assess the impact of anti-AT1R antibodies on survival and incidence of acute cellular rejection (ACR) and pathology antibody-mediated rejection (pAMR) in a population of heart transplant recipients who were bridged to transplantation with a durable mechanical assist device Heart Mate II.

METHODS: Sera of 69 consecutive heart transplant recipients transplanted between October 2008 and August 2014 were tested for the presence of angiotensin II type 1 receptor antibodies before Heart Mate II device implantation and at the time of transplantation. Overall survival and post-transplant rejection-free survival were compared between antibody-negative and antibody-positive recipients using Kaplan–Meier and log-rank tests.

RESULTS: Anti-AT1R antibodies were present in 8 patients (11.6%) before Heart Mate II implantation. During the left ventricular assist device (LVAD) bridging, 44 patients (63.8%) who were initially anti-AT1R antibody-negative became positive, leaving 17 (24.6%) anti-AT1R antibody-negative patients at the time of transplantation for all comparisons. One- and 5-year survival was 88 ± 8 and 76 ± 10% for anti-AT1R antibody-negative and 87 ± 5 and 81 ± 7% for anti-AT1R antibody-positive patients, respectively ($P = 0.582$). Freedom from ACR at 1 year was 68 ± 12% for anti-AT1R-negative and 75 ± 6% for anti-AT1R-positive recipients ($P = 0.218$). None of the anti-AT1R-negative patients developed AMR 1 year post-transplantation, whereas freedom from pAMR in anti-AT1R-positive recipients was 98 ± 2% ($P = 0.198$).

CONCLUSIONS: Our data showed no difference in the overall post-heart transplant survival and freedom from acute cellular and antibody-mediated rejection between anti-AT1R-negative and anti-AT1R-positive recipients. Further research is needed to assess the role of anti-AT1R antibodies in the risk stratification of LVAD-bridged recipients on the post-heart transplantation outcomes.

Keywords: Heart transplantation • Mechanical circulatory support • Angiotensin II type 1 receptor

INTRODUCTION

Left ventricular assist devices (LVADs) have reduced heart transplantation waiting list mortality and improved the quality of life and survival in patients with end-stage heart failure [1, 2]. One of the proposed limitations of mechanical device support therapy is a higher degree of sensitization among LVAD recipients. Apart from antibodies directed against human leucocyte antigen (HLA), several non-HLA antibodies like major histocompatibility class I-related chain, autoantibodies against angiotensin II type 1 receptor (AT1R) and endothelin receptor A as well as antibodies to cardiac self-antigens (myosin and vimentin) have been associated with an LVAD use [3–6]. AT1R differs from all other non-HLA antigenic targets in the mechanism of action. Binding of antibodies to AT1R induces unique physiological effects that mimic those of receptor ligand binding (angiotensin II in the renin–angiotensin system [7]). Anti-AT1R antibodies exert their pathological effects by binding to extracellular loops of vascular receptors, and via intracellular signalling lead to proinflammatory and procoagulatory responses. Anti-AT1R antibodies have also been associated with systemic sclerosis, pre-eclampsia and malignant hypertension [8–10]. There is growing body of evidence of a negative impact of these antibodies on the graft survival in renal transplantation [11–13]. The objective of our study was to compare the survival and freedom from acute cellular- and antibody-mediated rejection in heart transplant recipients bridged with Heart Mate II assist device stratified according to the pretransplant presence of anti-AT1R antibodies.

MATERIALS AND METHODS

Patients

Between October 2008 and August 2014, we prospectively evaluated sera of 69 patients implanted with durable continuous-flow axial mechanical support Heart Mate II who subsequently underwent heart transplantation. A cut-off of 17 U/ml was used to determine anti-AT1R positivity/negativity. Hospital database and medical records were searched for clinical data on the survival and incidence of acute cellular- and antibody-mediated rejection. Identification and classification of rejection episodes was based on histopathology and immunohistochemistry evaluation of endomyocardial biopsy specimens and followed the International Society for Heart and Lung Transplantation guidelines [14, 15]. Patients with acute cellular rejection (ACR) ≥2R and pathology antibody-mediated rejection (pAMR) of any grade were included in the time-to-event analyses. As per our institutional protocol, all heart transplant recipients received induction therapy with antithymocyte globulin (1.5 mg/kg body weight). Maintenance immunosuppression comprised a combination of calcineurin inhibitor with either cyclosporine (trough level 200 mg/dl) or tacrolimus (trough level 3–8 ng/dl), antiproliferative agent (mycophenolate mofetil) and steroids (tapering regimen). The median follow-up was 39 months (24–54 months), was 100% complete, totalled 2587 patient-months and ended on 5 April 2015.

Antibody analysis

The first sample was collected before implanting the device. The second sample was obtained at the time of transplantation. Anti-AT1R antibodies were assayed by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Thermo Fisher Scientific–One lambda, Waltham, MA, USA).

Coagulated blood was drawn into sterile 10-ml serum separator tubes. Samples were centrifuged at 1000 g for 15 min; serum was collected and stored at −20°C until the day of measurement. The concentration of anti-AT1R IgG antibody in serum was measured by ELISA according to the manufacturer's instructions. The samples were assayed on angiotensin II type 1-receptor-precoated microtiter plates. Standards and diluted 1:100 samples were added into the wells and incubated for 2 h at 2–8°C. After washing steps, anti-AT1R antibody was detected with POD-labelled anti-human IgG antibody (1:100) followed by colour development with tetramethylbenzidine (TMB) substrate solution and, measured at 450 nm, with the correction wavelength set at 630 nm. Optical densities were then converted into concentration by the use of a standard curve. The detection range of the test was >2, 5 U/ml with positive value set at 17 U/ml and negative value set at ≤17 U/ml.

Statistical analysis

Continuous variables are presented as median with 25th and 75th percentile interval. Categorical variables are shown as the percentage of the sample. Fisher's exact test was used to evaluate the difference between categorical baseline demographic and clinical characteristics. Continuous variable comparisons were performed using Mann–Whitney *U*-test for two study groups and Kruskal–Wallis one-way analysis of variance test for multiple group analysis. Post-transplant survival and freedom from rejection were assessed by Kaplan–Meier method (with the date of transplant as the time origin for the analysis) and the log-rank test was used for comparison. Univariable analysis was performed to identify risk factors associated with overall post-transplant survival. Variables with $P < 0.2$ on univariable analysis were entered into multivariable logistic regression with a forward conditional selection model. A *P*-value of less than 0.05 was considered significant. The statistical analyses were performed with IBM SPSS 18 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Altogether, 69 patients were transplanted with the Heart Mate II device at our institution during the study period. The mean time of mechanical support before heart transplantation was 11 months (range 1–53 months). Anti-AT1R antibodies were present in 8 (11.6%) and anti-HLA antibodies in 3 (4.3%) patients before Heart Mate II implantation. During the support, 44 patients (63.8%) who were initially anti-AT1R-negative became anti-AT1R-positive and 17 (24.6%) remained anti-AT1R antibody-negative until transplantation. Of the 67 patients who were not sensitized against HLA antigens before HM II implantation, 6 (9%) developed anti-HLA antibodies during the support. At the time of transplantation, there were 13 patients who were antibody-negative for both HLA and AT1R antigens (AT1R−HLA−), 3 patients who were anti-AT1R antibody-negative and anti-HLA antibody-positive (AT1R−HLA+), 47 patients who were anti-AT1R antibody-positive and anti-HLA antibody-negative (AT1R+HLA−) and 4 patients who were sensitized against both AT1R and HLA antigens (AT1R+HLA+). Basic demographic and clinical characteristics of patients stratified according to the presence of anti-AT1R antibodies are presented in Table 1.

Survival

Of the 69 transplanted patients, 8 did not survive until discharge. Primary graft dysfunction was the leading cause of death, followed by sepsis and neurological complications (Table 2). Four additional patients died after being discharged from the hospital during the follow-up period.

Of the 42 clinical, demographic, haemodynamic and echocardiographic recipient, donor and perioperative variables, only 11 with $P < 0.2$ (Table 3) on univariable analysis were entered into multivariable logistic regression model. Serum blood urea nitrogen level at the time of transplantation was identified as a sole predictor for post-transplantation death (odds ratio 1.459, 95% confidence interval: 1.010–2.107, $P = 0.044$). Survival analysis of recipients stratified according to the presence of anti-AT1R antibodies before transplantation revealed 1- and 5-year survival of 88 ± 8 and 76 ± 10% for anti-AT1R antibody-negative and 87 ± 5 and 81 ± 7% for anti-AT1R antibody-positive patients, respectively ($P = 0.582$) (Fig. 1).

Acute cellular rejection

Of the 67 heart transplant recipients who had biopsy results available, 14 (20.9%) were diagnosed with ACR with ISHLT grade ≥2R (12 patients 2R and 2 patients 3R). Patient stratification according to the pretransplant presence of antibodies against AT1R and HLA antigens with respect to subsequent post-transplant ACR is depicted in Table 4. Both recipients with grade 3R rejection

Table 1: Basic demographic and clinical characteristics of patients stratified according to the presence of anti-AT1R antibodies before Heart Mate II implantation and throughout the support

	AT1R-positive before HMII implantation ($n = 8$)	AT1R-positive during HMII support ($n = 44$)	AT1R-negative ($n = 17$)	P-value
Age (years)	49 (41, 58)	51 (39, 59)	48 (41, 58)	0.875*
BSA (m^2)	1.81 (1.66, 2.03)	1.97 (1.81, 2.10)	2.01 (1.91, 2.13)	0.118*
BMI	21 (20, 25)	25 (23, 27)	27 (24, 29)	0.054*
Female gender (%)	1 (12.5)	7 (15.9)	1 (5.9)	0.580†
Diabetes (%)	1 (12.5)	8 (18.2)	3 (17.6)	0.926†
COPD (%)	1 (12.5)	7 (15.9)	2 (11.8)	0.905†
Previous stroke (%)	2 (25)	10 (22.7)	2 (11.8)	0.596†
INTERMACS I,II (%)	2 (25)	27 (61.4)	10 (58.9)	0.158†
Ischaemic aetiology of HF (%)	2 (25)	16 (36.4)	5 (29.4)	0.902†
HLA sensitized (%)	0	3 (8.6)	0	0.354†
Previous sternotomy (%)	1 (12.5)	8 (18.2)	4 (23.5)	0.792†
Previous VA ECMO	0	4 (9.1)	0	0.299†
After HMII implantation				
Concomitant procedure (%)		14 (31.8)	2 (11.8)	0.110†
PRBC (units)		10 (7, 14)	9 (7, 17)	0.863**
Platelets (units)		4 (3, 6)	3 (2, 5)	0.159**
FFP (units)		24 (18, 35)	26 (16, 31)	0.700**
Major bleeding (%)		6 (13.6)	0	0.173†
Major infection (%)		11 (25)	5 (29.4)	0.725†
Neurological dysfunction (%)		1 (2.3)	0	0.531†
Device malfunction (%)		1 (2.3)	1 (5.9)	0.478†
ARBs during support (%)		8 (18.2)	1 (5.9)	0.206†
HLA sensitized during support (%)		13 (30.2)	7 (41.2)	0.418†
Mean BP on support (mmHg)		85 (80, 90)	90 (81, 99)	0.048**
Duration of support (months)		9 (5, 16)	11 (5, 16)	0.705**

BSA: body surface area; BMI: body mass index; COPD: chronic obstructive pulmonary disease; INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support; HF: heart failure; HLA: human leucocyte antigen; HMII: Heart Mate II; VA ECMO: veno-arterial extracorporeal membrane oxygenation; PRBC: pure red blood cell; FFP: fresh frozen plasma; ARB: angiotensinogen receptor blocker; BP: blood pressure.
*Kruskal–Wallis test.
**Mann–Whitney U-test.
†Fisher's exact test.

Table 2: Survival in days and causes of death of individual patients

Patient	Survival in days	Anti-AT1R antibody at transplantation	Cause of death
Patient 1	1	Negative	PGD
Patient 2	58	Positive	Sepsis
Patient 3	19	Positive	Ischaemic stroke, Sepsis, MOF
Patient 4	26	Positive	PGD, ACR
Patient 5	6	Positive	PGD, small bowel ischaemia
Patient 6	1	Positive	PGD
Patient 7	67	Positive	Sepsis
Patient 8	16	Negative	PGD
Patient 9	672	Negative	CAV
Patient 10	830	Negative	Unknown
Patient 11	1417	Positive	Ischaemic stroke
Patient 12	176	Positive	Unknown

AT1R: angiotensin II type 1 receptor; PGD: primary graft dysfunction; MOF: multi-organ failure; ACR: acute cellular rejection; CAV: cardiac allograft vasculopathy.

presented with an associated graft dysfunction. The first patient was successfully treated with 1 g of intravenous solumedrol administered daily for 3 days. The second patient required veno-arterial extracorporeal membrane oxygenation (ECMO) implanted centrally for severe biventricular graft dysfunction on top of pulse steroid therapy. After 12 days of support, the graft function recovered and ECMO was successfully explanted. The median time to ACR episode was 147 days (43 606) in anti-AT1R antibody-negative and 46 days (17 264) in anti-AT1R antibody-positive recipients ($P = 0.306$). Freedom from ACR at 1 year was 68 ± 12% for anti-AT1R-negative and 75 ± 6% for anti-AT1R-positive recipients ($P = 0.218$) (Fig. 2).

Pathologicy antibody-mediated rejection

Four patients' endomyocardial biopsy specimens yielded histology and/or immunohistochemistry signs of antibody-mediated rejection (Table 5). Only the patient with Grade 3 pAMR was positive for donor-specific antibodies against HLA and had concomitant graft dysfunction. Acute rejection was treated with a pulse of steroid that consisted of 1 g of intravenous solumedrol administered for 3 consecutive days, 10 cycles of therapeutic plasma exchange and intravenous immunoglobulins at 100 mg/kg. After multimodality treatment, this patient is now symptom free, showing no signs of rejection in the latest endomyocardial biopsies and the graft function assessed with transthoracic echocardiography is satisfactory. None of the anti-AT1R-negative patients presented with pAMR at

Table 3: Univariable analysis for an overall post-heart transplantation survival.

Variables	Survivors ($n = 57$)	Non-survivors ($n = 12$)	P-value
Age (years)	50 (41, 58)	55 (42, 61)	0.103
Creatinine (μmol/l)	79 (89, 104)	99 (93, 139)	0.014
BUN (mmol/l)	5.8 (4.3, 6.7)	8.1 (6.1, 9.8)	0.018
GFR (ml/1.72 m^2)	110 (88, 129)	107 (67, 112)	0.053
PASP before HMII implantation (mmHg)	58 (45, 67)	66 (53, 69)	0.002
TPG before HMII implantation (mmHg)	10 (9, 14)	14 (11, 19)	0.023
CVP before HMII implantation (mmHg)	10 (6, 14)	15 (10, 19)	0.026
HMII concomitant procedure (%)	13 (22.8)	6 (50)	0.077
HMII AVR (%)	3 (5.3)	5 (41.7)	0.003
HLA sensitized on HMII (%)	22 (38.6)	1 (8.3)	0.048
AT1R antibody conversion during HMII (%)	39 (68.4)	5 (41.7)	0.149

BUN: blood urea nitrogen; GFR: glomerular filtration rate; PASP: pulmonary artery systolic pressure; TPG: trans-pulmonary gradient; CVP: central venous pressure; AVR: aortic valve replacement; HLA: human leucocyte antigen; AT1R: angiotensin II type 1 receptor; HMII: Heart Mate II.

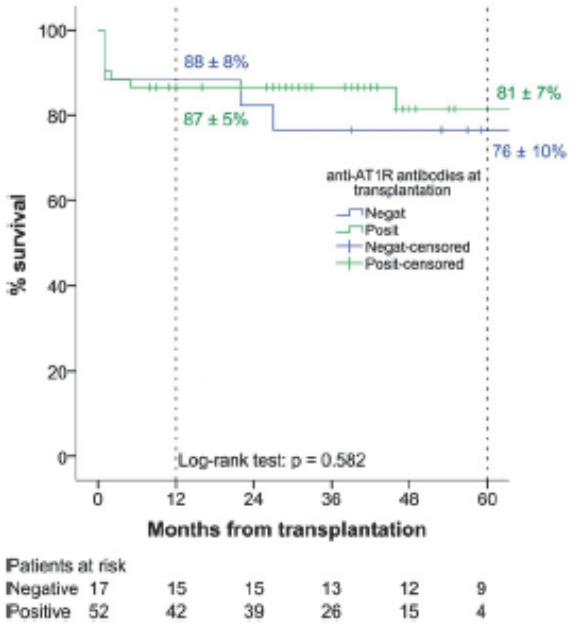

Figure 1: Overall post-heart transplant survival stratified according to the presence of anti-AT1R antibodies before transplantation. AT1R: angiotensin II type 1 receptor.

Table 4: Acute cellular rejection rate stratified by grade and the presence of anti-AT1R antibodies and anti-HLA antibodies

ACR ISHLT grade	AT1R−HLA− ($n = 13$)	AT1R−HLA+ ($n = 3$)	AT1R+HLA− ($n = 47$)	AT1R+HLA+ ($n = 4$)
0 ($n = 31$)	7 (53.8%)	3 (100%)	19 (40.4%)	2 (50%)
1R ($n = 22$)	3 (23.1%)	0	18 (38.3%)	1 (25%)
2R ($n = 12$)	3 (23.1%)	0	9 (19.1%)	0
3R ($n = 2$)	0	0	1 (2.1%)	1 (25%)

AT1R: angiotensin II type 1 receptor; HLA: human leucocyte antigen; ACR: acute cellular rejection.

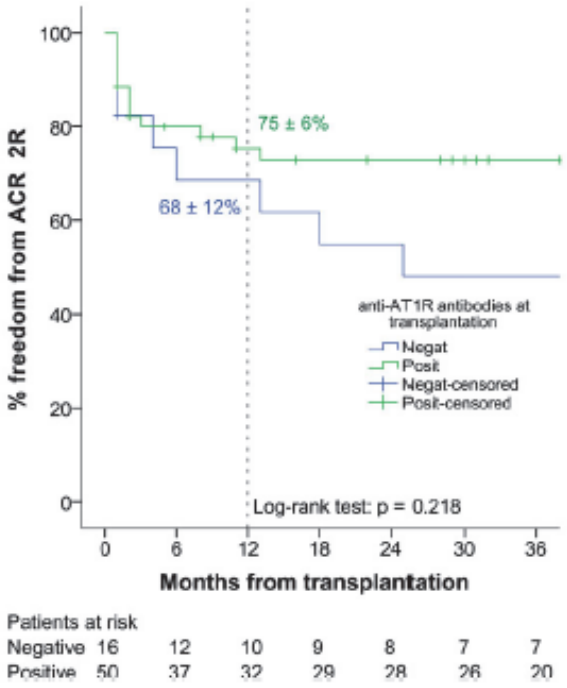

Figure 2: Freedom from ACR ≥2R. AT1R: angiotensin II type 1 receptor; ACR: acute cellular rejection.

1 year post transplantation, whereas freedom from pAMR in anti-AT1R-positive recipients was 98 ± 2% ($P = 0.198$) (Fig. 3).

DISCUSSION

The use of mechanical circulatory support to bridge patients to transplant increased to 41% in 2012, predominantly in the form of LVADs [16]. These patients now constitute a substantial proportion of the heart transplant recipients. They present unique challenges for the healthcare professionals in the perioperative as well as postoperative period. One of the shortcomings of the mechanical

Table 5: Pathological antibody-mediated rejection rate stratified by grade and the presence of anti-AT1R antibodies and anti-HLA antibodies

pAMR ISHLT grade	AT1R−HLA− (*n* = 13)	AT1R−HLA+ (*n* = 3)	AT1R+HLA− (*n* = 47)	AT1R+HLA+ (*n* = 4)
0 (*n* = 63)	13 (100%)	2 (66.7%)	45 (95.7%)	3 (75%)
1i (*n* = 1)	0	0	1 (2.1%)	0
1 h (*n* = 0)	0	0	0	0
2 (*n* = 2)	0	0	1 (2.1%)	1 (25%)
3 (*n* = 1)	0	1 (33.3%)	0	0

pAMR: pathology antibody-mediated rejection; AT1R: angiotensin II type 1 receptor; HLA: human leucocyte antigen.

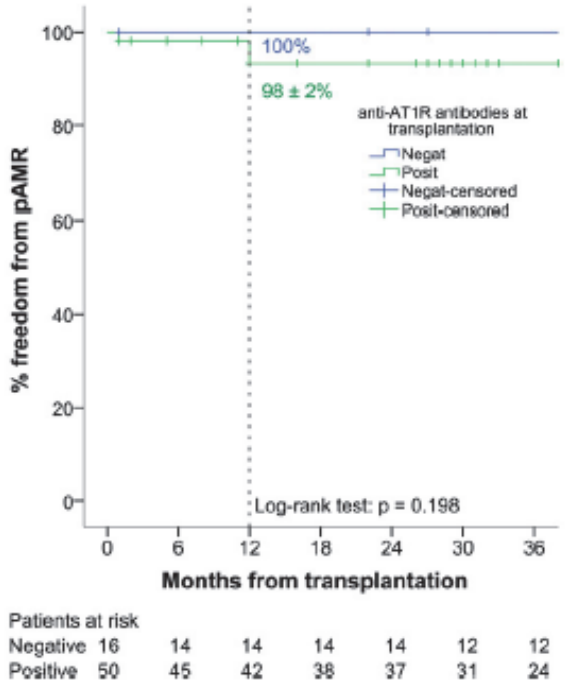

Figure 3: Freedom from pathology antibody-mediated rejection of any grade. AT1R: angiotensin II type 1 receptor.

assist devices is the overproduction of antibodies. The main finding of our study is that more than 60% of patients with the end-stage heart failure who were bridged to transplantation with Heart Mate II device developed antibodies against AT1R. There are multiple pathways by which these antibodies may appear before transplantation in mechanically supported patients. Protein antigenic determinants may become accessible after injury or surgical stress associated with an LVAD implantation. Inflammatory events might lead to de novo expression of these auto antigens [17, 18]. Anti-AT1R antibodies may also develop through similar pathways as those observed for HLA-specific antibodies: transfusions, pregnancies and previous solid organ transplantations. From our cohort, 12% of patients tested positive for the presence of anti-AT1R antibodies before Heart Mate II implantation. During the support, 64% of the initially negative AT1R patients became positive. We observed no association between preoperative demographics, blood product use or duration of mechanical support and conversion of AT1R-negative to AT1R-positive status. Barten *et al.* [6] found in their study of 29 VAD recipients that 65.5% were positive for anti-AT1R antibodies. Of note, most of the patients showed extremely high antibody titres up to 1000 U. In contrast to our own observation, they noted higher amount of blood transfusions in AT1R positive compared with AT1R-negative VAD recipients.

Although anti-AT1R antibodies may belong to complement fixing IgG subclasses (IgG1 and IgG3 isotypes), C4d-positive staining was found not to be very frequent in the biopsies of renal transplant recipients with anti-AT1R antibody-mediated rejections [7, 19] implicating complement independent mechanism of injury. This would explain the lack of association between anti-AT1R antibody status and pAMR in our series. We found that pAMR as defined by ISHLT guidelines was an extremely rare event after heart transplantation in our cohort. During the follow-up, we detected only one clinically significant antibody-mediated rejection which was accompanied with graft dysfunction. Our results also showed no statistically significant difference in the freedom from ACR ≥2R between anti-AT1R antibody-negative and -positive recipients. Given the putative mechanism of action of these antibodies that primarily act on vascular endothelium causing non-specific, non-complement-mediated microvascular damage, these results are not surprising. When we stratified the patients by the presence of anti-AT1R antibodies combined with the anti-ALA antibodies status, our results showed that none of the transplant recipients who were both anti-AT1R and anti-HLA antibody negative experienced pAMR or Grade 3R ACR. Conversely, 25% of recipients who were sensitized against both AT1R and HLA antigens presented post-transplantation with high grade ACR with associated graft dysfunction and another 25% with pAMR similarly with graft dysfunction. This leads us to believe that knowing the anti-AT1R antibody status on top of standard evaluation of anti-HLA antibodies pretransplantation adds an incremental value in a risk stratification of post-heart transplantation immunological related adverse events.

Although there is a substantial amount of literature on the deleterious effects of anti-AT1R antibodies on post-renal transplantation outcomes, we were only able to find one manuscript in reference to heart transplantation. Whereas we studied the effect of anti-AT1R antibodies as detected before transplantation, Hiemann *et al.* [20] evaluated the impact of anti-AT1R antibodies detected immediately post-transplantation and during 1 year of follow-up. The relevant clinical end-points included ACR of any grade, antibody-mediated rejection and microvasculopathy. Evaluating the results of 30 heart transplant recipients, the authors concluded that elevated post-transplantation levels of anti-AT1R antibodies (cut-off >16.5 U/ml) are associated with cellular- and antibody-mediated rejection and early onset of microvasculopathy and should be routinely monitored after heart transplantation. Apart from the difference in the time frame of anti-AT1R antibody evaluation, all our patients were bridged to transplantation with an LVAD and 75% were antibody-positive before transplantation. Also, ISHLT standardization of nomenclature of pAMR [14] was published only 1 year after the study. We believe there are fundamental differences about how the clinical end-points were defined and the results of those two studies are therefore difficult to compare. We nevertheless find the concept of increasing titres of anti-AT1R antibodies after transplantation very intriguing and plan to expand on the results of our study by evaluating the post-

transplantation sera of all our patients. Another noteworthy aspect of the study by Hiemann *et al*. [20] is the suggestion of a potential association between anti-AT1R antibodies and post-transplant microvasculopathy. There is also increasing evidence for the active role of AT1R itself in the pathogenesis of chronic allograft rejection explaining the link between acute rejection and subsequent long-term clinical outcome [21]. Yamani *et al*. [22] observed an increase in mRNA of AT1R in 14 heart transplant recipients who had recurrent ACR in comparison with controls. In our study cohort, we only had the results of 41 coronary angiograms available and for that reason we did not include cardiac allograft vasculopathy (CAV) among the outcome measures in our study. We nevertheless acknowledge the compelling evidence for the immunoregulatory function of the renin–angiotensin system and its role in the pathogenesis of chronic allograft rejection. Comparing the incidence of CAV between groups of patients stratified by the presence of anti-AT1R antibodies and increased expression of AT1 receptor is a challenge for future studies.

Strengths and limitations

This study is the first of its kind to investigate the impact of anti-AT1R antibodies on post-heart transplantation outcome of LVAD-bridged recipients. It includes a homogenous group of patients supported with the same device. All recipients received identical immunosuppression as per our institutional protocol. The study has several limitations inherent to the retrospective nature of a single-centre observational study. Another limitation is a relatively small number of patients with relatively low event rates increasing the probability of Type II error. The study is meant as a pilot and no power analysis was performed. Another drawback of our study is the fact that all our patients received Heart Mate II device, thus limiting the generalization of our results to other types of mechanical devices. Future studies will need to address the question of whether newer generation of devices would show the same high degree of sensitization against AT1R and assess the role of these antibodies in post-transplantation outcome of mechanically bridged recipients.

In conclusion, although our data failed to demonstrate the association of pretransplant level of anti-AT1R antibodies with overall survival and acute cellular- and antibody-mediated rejection, we believe our study to be a valuable contribution. We consider it to be a first step in further research on the impact of these non-HLA-specific antibodies on post-heart transplantation outcome, especially in the era of mechanical circulatory support devices, improved diagnostic tools and increased awareness of antibody-mediated rejection.

Conflict of interest: none declared.

REFERENCES

[1] Trivedi JR, Cheng A, Singh R, Williams ML, Slaughter MS. Survival on the heart transplant waiting list: impact of continuous flow left ventricular assist device as bridge to transplant. Ann Thorac Surg 2014;98:830–4.

[2] Long EF, Swain GW, Mangi AA. Comparative survival and cost-effectiveness of advanced therapies for end-stage heart failure. Circ Heart Fail 2014;7:470–8.

[3] Askar M, Hsich E, Reville P, Daghstani J, Nowacki A, Zhang A *et al*. Comparison of HLA & MICA allosensitization patterns among patients supported by ventricular assist devices (VAD) and patients with no devices. J Heart Lung Transplant 2013;32:S74.

[4] Askar M, Hsich E, Reville P, Nowacki AS, Baldwin W, Bakdash S *et al*. HLA and MICA allosensitization patterns among patients supported by ventricular assist devices. J Heart Lung Transplant 2013;32:1241–8.

[5] Banan B, Phelan D, Medhat A, Ewald G, Mohanakumar T. (47) - Increased sensitization to HLA and to cardiac self-antigens (myosin and vimentin) in patients waiting for cardiac transplantation with left ventricular assisting device (LVAD). J Heart Lung Transplant 2014;33:S25.

[6] Barten MJ, Dragun D, von Salisch S, Dieterlen MT, Garbade J, Klein S *et al*. 112 Identification of non-HLA antibodies in ventricular assist device recipients. J Heart Lung Transplant 2012;31:S46.

[7] Dragun D, Muller DN, Bissen JH, Fritsche L, Nieminen-Kehla M, Dechend R *et al*. Angiotensin II type 1 receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005;352:558–69.

[8] Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A *et al*. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest 1999;103:945–52.

[9] Riemekasten G, Philippe A, Nather M, Slowinski T, Muller DN, Heidecke H *et al*. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. Ann Rheum Dis 2011;70:530–6.

[10] Fu ML, Herlitz H, Schulze W, Wallukat G, Micke P, Eftekhari P *et al*. Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension. J Hypertens 2000;18:945–53.

[11] Taniguchi M, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE *et al*. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. Am J Transplant 2013;13:2577–89.

[12] Giral M, Foucher Y, Dufay A, Van Huyen JP, Renaudin K, Moreau A *et al*. Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. Am J Transplant 2013;13:2567–76.

[13] Banasik M, Boratynska M, Koscielska-Kasprzak K, Kaminska D, Bartoszek D, Zabinska M *et al*. The influence of non-HLA antibodies directed against angiotensin II type 1 receptor (AT1R) on early renal transplant outcomes. Transpl Int 2014;27:1029–38.

[14] Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC *et al*. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. J Heart Lung Transplant 2013;32:1147–62.

[15] Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J *et al*. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant 2005;24:1710–20.

[16] Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI *et al*. The Registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report—2014; focus theme: retransplantation. J Heart Lung Transplant 2014;33:996–1008.

[17] Dragun D. Humoral responses directed against non-human leucocyte antigens in solid-organ transplantation. Transplantation 2008;86:1019–25.

[18] Dragun D, Philippe A, Catar R. Role of non-HLA antibodies in organ transplantation. Transplantation 2012;17:440–5.

[19] Reinsmoen NL, Lai CH, Heidecke H, Haas M, Cao K, Ong G *et al*. Anti-angiotensin type 1 receptor antibodies associated with antibody-mediated rejection in donor HLA antibody negative patients. Transplantation 2010;90:1473–7.

[20] Hiemann NE, Meyer R, Wellnhofer E, Schoenemann C, Heidecke H, Lachmann N *et al*. Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation. Transplantation 2012;94:919–24.

[21] Yousufuddin M, Cook DJ, Starling RC, Abdo A, Paul P, Tuzcu EM *et al*. Angiotensin II receptors from peritransplantation through first-year-post transplantation and the risk of transplant coronary artery disease. J Am Coll Cardiol 2004;43:1565–73.

[22] Yamani MH, Cook DJ, Rodrigues ER, Thomas DM, Gupta S, Alster J *et al*. Incresed expression of Angiotensin II Type 1 Receptor (AGTR1) in heart transplant recipients with acute rejection. J Heart Lung Transplant 2006;25:1283–9.

The impact of Angiotensin II Type 1 Receptor antibodies on morbidity and mortality in Heart Mate II supported recipients

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Aims. One of the proposed limitations of left ventricular assist device (LVAD) therapy is high degree of sensitization. Apart from human leukocyte antigen (HLA), antibodies against Angiotensin II Type 1 Receptor (AT1R) have been associated with adverse outcomes. The purpose of this study was to compare complications and survival of anti – AT1R positive versus negative Heart Mate II (HMII) recipients.
Methods. Altogether 96 patients received HMII at our institution between 2008 and 2012. These were stratified into three groups: antibody positive before implantation (AT1R+), antibody conversion during support (AT1R-/+) and patients who remained antibody negative (AT1R-). Survival, major on-device adverse events and post-transplant rejections were assessed with Kaplan-Meier and log-rank tests.
Results. Two year on-device and overall survival was 78 ± 12% and 75 ± 10% in AT1R-, 60 ± 23% and 60 ± 15% in AT1R+ and 92 ± 6% and 87 ± 5% in AT1R-/+ group ($P = 0.409$, $P = 0.185$). Freedom from major adverse event at two years for AT1R-, AT1R+ and AT1R-/+ was 49 ± 14%, 53 ± 16% and 41 ± 11% ($P = 0.875$). Freedom from rejection was 63 ± 17% in patients who were both anti-AT1R and HLA negative and 65 ± 13% in those who were antibody positive ($P = 0.788$).
Conclusion. Patients who were anti-AT1R antibody positive had similar on-device survival and rate of complications in comparison to those who were antibody negative. In transplanted patients, there were no differences in the overall survival and rejection between the groups.

Key words: Heart Mate II; LVAD; Angiotensin II Type 1 Receptor; heart transplantation

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INTRODUCTION

Left ventricular assist devices (LVAD) reduce heart transplant waiting list mortality and improve the quality of life and survival in selected group of patients with end – stage heart failure[1]. One of the proposed limitations of mechanical support therapy is a higher degree of sensitization among LVAD recipients. Apart from antibodies directed against human leukocyte antigen (HLA), several non-HLA antibodies such as autoantibodies against Angiotensin II type 1 receptor (AT1R) have been associated with an LVAD use[2]. AT1R differs from all other non-HLA antigenic targets in the mechanism of action. The binding of antibodies to AT1R induces physiological effects that mimic those of natural ligand in the renin-Angiotensin system[3]. Anti-AT1R antibodies exert their damaging effect by binding to the second extracellular loop of AT1R receptors present in endothelial and vascular smooth muscle cells, inducing endothelial activation and dysfunction. Previous reports have identified heart transplant recipients who developed anti-AT1R antibodies to be at increased risk of post-transplant rejection and cardiac allograft vasculopathy[4,5]. Apart from an effect on the vascular tone, these antibodies also lead to pro – inflammatory and pro – coagulatory responses. The objectives of our study were to evaluate the degree of sensitization against AT1R among our LVAD recipients and also to assess whether the presence of these antibodies could cause a higher incidence of thromboembolic and infectious complications.

MATERIALS AND METHODS

Patients

We prospectively evaluated the presence of anti-AT1R antibodies in 96 consecutive Heart Mate II recipients at our institution between 2008 and 2012. After excluding 13 patients who died within 60 days of implantation, 83 patients with a mean duration of 375 ± 34 days of support were left for the analysis. Out of a total of 83 patients, 69 eventually underwent heart transplantation, 9 died on support, three were explanted for recovery and two were still alive on support at the last day of follow-up. Follow-up of all transplanted patients ended on 5 April 2015, was 100% complete, and totalled 2587 patient-months.

Antibody Analysis

Serum samples were collected before implanting the device and at the pre-determined time points throughout

the support. Anti-AT1R antibodies were assayed by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (CellTrend, Luckenwalde, Germany).

Peripheral blood was obtained into sterile 10-ml serum separator tubes. Samples were centrifugated at 1000 g for 15 min; serum was collected and stored at – 20 °C until the day of measurement. The concentration of anti-AT1R IgG antibody in serum was measured by ELISA according to the manufacturer's instructions. The samples were assayed on Angiotensin II type 1-receptor-precoated microtiter plates. Standards and diluted 1:100 samples were added into the wells and incubated for two hours at 2 – 8 °C. After washing steps, anti-AT1R antibody was detected with POD-labelled anti-human IgG antibody (1:100) followed by color development with TMB substrate solution and, measured at 450 nm, with correction wavelength set at 630 nm. Optical densities were then converted into concentration by standard curve. The detection range of the test was > 2, 5 U/mL with positive value set at 17 U/mL and negative ≤ 17 U/mL.

Adverse Events Definitions

Various adverse clinical events during the support were compared between antibody positive and antibody negative recipients. Standard INTERMACS definitions were used to classify individual post Heart Mate II implantation adverse events[6].

Statistical Analysis

Continuous variables are presented as median with 25th and 75th percentile interval. Categorical variables are shown as the percentages. The $\chi 2$-test and Fisher's exact test were used to evaluate categorical variables. The data were analysed using the Mann-Whitney U test and Kruskal Wallis one – way analysis of variance for multiple group analysis. Survival and time-to-event analyses were assessed by Kaplan-Meier method and the log-rank test was used for comparisons. Heart Mate II recipients were censored for transplantation and LVAD explantation after recovery to calculate estimated on-device survival. For overall survival analysis, all patients were censored on the date of death or at conclusion of the study. Only patients surviving the first 60 days post Heart Mate II implantation were included in the on-device survival analysis. Date of Heart Mate II implantation was set as the time origin for survival and freedom from LVAD associated adverse event analyses and the date of transplantation as the time origin for freedom from rejection analysis. The linearized rate for each adverse event was calculated as total number of observed events divided by total patient-years of follow-up and expressed as episodes per one patient – year (eppy). A P value < 0.05 was considered significant. The statistical analyses were performed with IBM SPSS 18 (SPSS Inc., Chicago, Il, USA).

Table 1. Basic characteristics of AT1R antibody negative versus positive HeartMate II recipients before implantation.

	AT1R positive (N = 13)	AT1R negative (N = 70)	*P*
Age, years	50 (40, 59)	45 (33, 58)	0.607
BMI	25.4 (22.9, 27.8)	22.6 (20.3, 25.9)	0.021
Male gender, %	11 (85)	60 (86)	0.918
Ischemic etiology of heart failure, %	3 (23)	24 (34)	0.766
HLA sensitized, %	0	4 (6)	0.477
Previous mechanical support, %	2 (14)	8 (11)	0.822
Previous sternotomy, %	2 (14)	15 (21)	0.660

BMI, body mass index; HLA, human leukocyte antigen

Table 2. Comparison of AT1R negative patients versus those who became AT1R positive during HeartMate II support.

	AT1R negative (N = 20)	AT1R positive (N = 50)	*P*
Age, years	47 (41, 57)	51 (36, 59)	0.969
BMI	26.5 (23.3, 28.8)	25.0 (22.0, 27.0)	0.326
HMII duration of support, days	324 (137, 470)	246 (129,416)	0.907
PRBC during implantation, units	9 (6, 18)	10 (8, 14)	0.608
FFP, units	26 (15, 32)	26 (22, 34)	0.856
Platelets, units	3 (2, 4)	4 (3, 6)	0.277
Ischemic etiology of heart failure, %	6 (30)	18 (36)	0.696
Previous mechanical support, %	1 (5)	6 (12)	0.730
Previous sternotomy, %	5 (25)	10 (20)	0.800
HLA sensitized, %	8 (40)	15 (30)	0.545
Male gender, %	18 (90)	42 (84)	0.713
Driveline infection, %	4 (20)	13 (26)	0.761

BMI, body mass index; HMII, Heart Mate II; PRBC, pure red blood cells; FFP, fresh frozen plasma; HLA, human leukocyte antigen

RESULTS

Anti-AT1R antibodies were observed in 13/83 (16%) of the recipients before Heart Mate II implantation (Table 1). Four of these patients (6%) were also sensitized against HLA antigens. During the support, 50 patients (71%) who were initially anti-AT1R negative became positive (AT1R-/+) and 20 (29%) remained negative (AT1R-). Total amount of Heart Mate II support for all 83 patients was 86.7 patient-years. There were no differences in the duration of support or the amount of the blood products used between LVAD recipients who remained negative and those who became positive. Basic demographic and clinical characteristics of both patients groups are summarized in Table 2. Out of 20 patients who remained negative on the mechanical device, 8 became sensitized to HLA antigens. In a cohort of 50 LVAD recipients who developed anti – AT1R antibodies during the support, 15 recipients also developed concurrent anti – HLA antibodies.

Survival

Out of 83 LVAD recipients who survived 60 days post-implantation, 9 additional patients died after a mean duration of support of 462 (minimum 82, maximum 1123) days. Two year estimated on – device survival was 78 ± 12% in AT1R-, 60 ± 23% in AT1R+ and 92 ± 6% in AT1R-/+ group (P = 0.409) (Fig. 1). Overall survival for AT1R-, AT1R+ and AT1R-/+ was 75 ± 10%, 60 ± 15% and 87 ± 5% at two years and 70 ± 10%, 60 ± 15% and 82 ± 6% at four years from Heart Mate II implantation (P = 0.185) (Fig. 2).

Major adverse events

Freedom from device malfunction, major infection, major bleeding and neurologic dysfunction at two years for AT1R-, AT1R+ and AT1R-/+ was 49 ± 14%, 53 ± 16% and 41 ± 11% (P = 0.875) (Fig. 3).

Device malfunction

Altogether 5 patients (6%) experienced device malfunction in our cohort (0.06 eppy). All episodes were related to pump failure (pump thrombosis in four patients and kinked outflow graft in one patient) and resulted in pump exchange in two patients and death in two patients. One patient with pump thrombosis was successfully treated conservatively and subsequently transplanted. Freedom from device malfunction at 2 years in AT1R+, AT1R- and AT1R-/+ was 100%, 95 ± 5% and 86 ± 8% (P = 0.487).

Major bleeding

Our institutional protocol for patients supported with HeartMate II device is anticoagulation with Warfarin (target INR of 1.8 – 2.2) without antiplatelet therapy. Out of 83, three patients (4%) experienced major bleeding episode after 7 days post implantation (0.03 eppy). The reasons for readmissions for bleeding were epistaxis, retroperitoneal bleeding and GI bleeding. All patients were discharged home following their bleeding episode

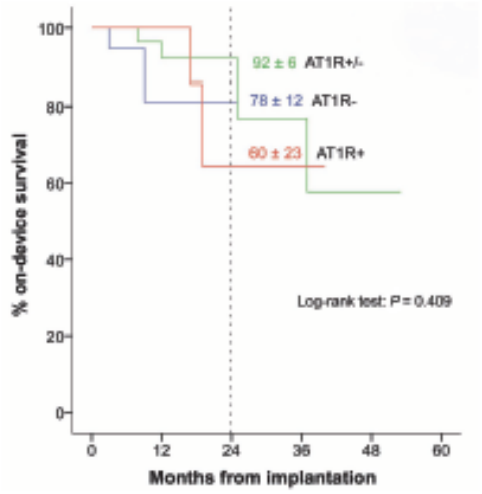

Fig. 1. On-device survival of HeartMate II recipients stratified according the presence of anti-AT1R antibodies.

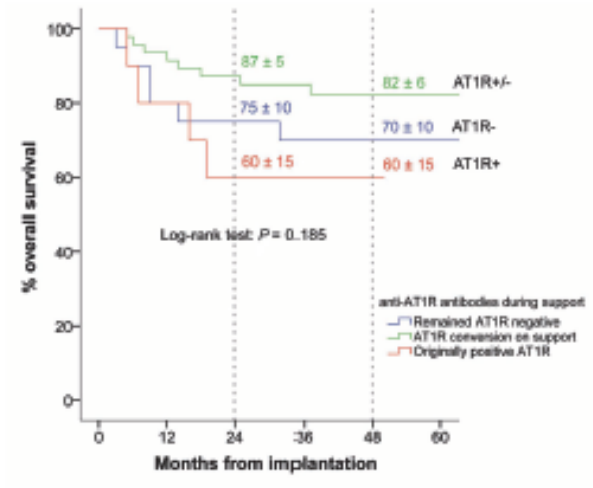

Fig. 2. Overall survival of HeartMate II recipients stratified according the presence of anti-AT1R antibodies.

and all three eventually underwent heart transplantation. Freedom from major bleeding at 2 years in AT1R+, AT1R- and AT1R-/+ was 100%, 100% and 90 ± 5% (P = 0.232).

Major infection

More than one third (27 patients, 33%) of our patients were readmitted due to infection during the course of their mechanical support (0.3). These patients fell into two major categories: infection of a drive - line site (21 patients) and deep sternal wound infection (6 patients). Two patients experienced both drive – line and deep sternal wound infections. One patient with deep sternal wound infection developed sepsis, multi – organ failure and subsequently died as a direct consequence of LVAD infection. Freedom from major infection at 2 years in AT1R+, AT1R- and AT1R-/+ was 54 ± 16%, 62 ± 13% and 51 ± 11% (P = 0.594).

Neurological dysfunction

Altogether six (7%) patients experienced neurological dysfunction. Four patients suffered from hemorrhagic CVA (0.05 eppy) and two from ischemic CVA (0.02 eppy). Two of the patients recovered and were subsequently transplanted, four died as a result of CVA. Freedom from neurologic dysfunction at 2 years in AT1R+, AT1R- and AT1R-/+ was 87 ± 12%, 93 ± 7% and 92 ± 6% (P = 0.997).

Post transplantation rejection

Out of 69 transplanted patients 8 did not survive to discharge and had no biopsy results available. Of the 61 transplant survivors, 44 patients were anti – AT1R positive and 17 were anti – AT1R antibody negative at the time of transplant. There was no difference in freedom from rejection (ACR ≥ 2R and/or pAMR ≥ 1) among transplant survivors based on the pre-transplant presence of anti-HLA and anti-AT1R antibodies (Fig. 4).

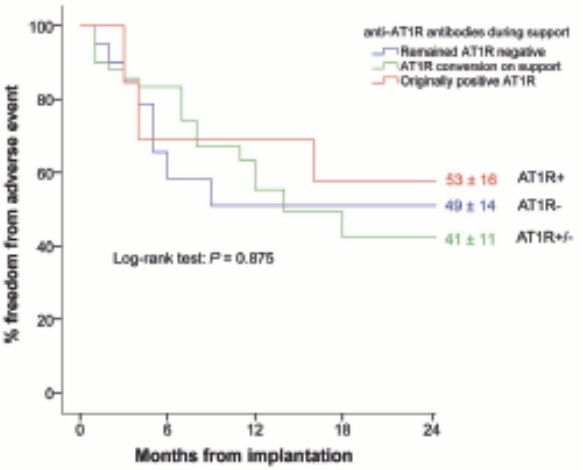

Fig. 3. Freedom from Heart Mate II post – implantation adverse events (device malfunction, major bleeding, major infection and neurologic dysfunction).

DISCUSSION

Left ventricular assist devices are a recognized risk factor for sensitization of patients awaiting cardiac transplantation[7-9]. The negative impact of anti – HLA antibodies on post – transplant allograft function and survival has now been well documented. Recently, there has been accumulating evidence of various non – HLA antibodies involvement in decreased allograft and recipient survival[5,10]. While anti – HLA antibodies exert their negative effect via complement activation and antibody – mediated cytotoxicity, antibodies against AT1R, act as a natural allosteric receptor agonist. Angiotensin type 1 receptor is a G protein-coupled receptor (GPCR) that mediates physiologic actions of Angiotensin II. Binding of agonistic antibodies to AT1R causes activation of the phosphatidylinositol-calcium second messenger system, phosphorylation of extracellular signal-regulated kinase 1/2 (Erk 1/2), activator protein 1 (AP-1) activation, and increase DNA-binding activity of nuclear factor-κB (NF-κB) pro-inflammatory target genes[11]. Anti-AT1R antibodies also trigger tissue factor induction, as evidenced by intense diffuse tissue staining of epithelial, endothelial and mesangial cells in the renal transplant biopsy specimens obtained at the time of AT1R antibody mediated rejection in the absence of complement activation[3]. Anti-AT1R antibodies derived from preeclamptic patients enhanced promoter activity of tissue factor, an initiator of extrinsic coagulation pathway and a target gene for AP-1 and NF-κB in vitro[12]. Anti-AT1R antibodies developed during pregnancy cause both maternal and fetal pathology via pro-inflammatory, vasoconstrictive, pro-coagulatory and pro-apoptotic actions on the placenta[13]. There is also evidence that anti-AT1R antibodies promote endothelial micro particles formation through activating p38 mitogen-activated protein kinase pathway. The "injured" endothelial micro particles trigger reactive oxygen species production and reduce nitric oxide synthesis in vitro experiments[14]. Zhang et al.[15] investigated in an animal model the association between autoantibodies against AT1 receptor and endothelial dysfunction in vivo. The investigators demonstrated an increased activity of lactate dehydrogenase (LDH) in anti-AT1R positive rats which was regarded as an indicator of cell necrotic death. Functional assessment revealed a decline in the endothelium – dependent relaxation and up – regulation of endothelial intracellular adhesion molecule – 1 (ICAM-1) suggesting that endothelial cells may have inflammatory lesions in anti-AT1R positive rats. Given the known potential of these antibodies to activate inflammatory and coagulation cascade we hypothesized that mechanically bridged patients with raised levels of anti – AT1R antibodies may experience increased rate of thromboembolic and infectious complications while on support.

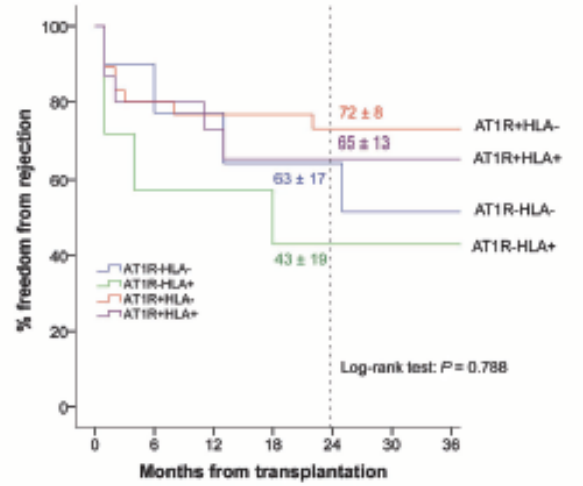

Fig. 4. Freedom from rejection of transplanted patients stratified according the presence of anti-AT1R antibodies and anti-HLA antibodies.

Our results showed that 16% of our patients with end – stage heart-failure were already anti – AT1R positive before LVAD implantation. This finding is in agreement

with Du et al.[16], who showed that anti-AT1R antibodies exist in the sera of congestive heart failure patients with ischemic cardiomyopathy and hypertension. The authors suggested that these antibodies may play an important role in the pathogenesis and myocardial remodelling of heart failure. Anti-AT1R antibodies develop through similar pathways as those observed for HLA specific antibodies: transfusions, pregnancies and prior transplant. We did not find any association between basic demographic and clinical characteristics (female gender/ previous pregnancy, history of surgery) and sensitization against AT1R before LVAD implantation.

There is accumulating evidence that LVAD support may be associated not only with an increased anti-HLA but also various anti non-HLA antibodies. Hiemann et al.[5] reported in their pilot study that patients on assist device support before heart transplantation were more likely to develop high anti – AT1R antibody levels (43% of supported versus 18% of non – supported patients, $P = 0.021$) within 24 h after heart transplantation, implicating pre – transplant sensitization. Barten el al.[2] found in their study of 29 VAD recipients that 65.5% were positive for anti-AT1R antibodies. Our results confirmed these findings. During the support 71% of the initially negative AT1R patients became positive. There are multiple pathways by which the production of antibodies against AT1R in patients supported with mechanical devices may be initiated. Protein antigenic determinants from targets may become accessible after injury or surgical stress. Inflammatory events might lead to de novo expression of autoantigens[17]. These autoantibodies are generally of the IgG class requiring T cell help[18]. T cell self-tolerance may be broken by an inflammatory event or hypoxia. We observed no association between pre-operative demographics, blood product peri-operative use or duration of mechanical support and conversion of AT1R negative to AT1R positive status.

Apart from longer waiting times with associated increased morbidity and mortality, there have been no reports linking anti HLA or anti non-HLA antibodies in mechanically bridged recipients to post-LVAD adverse outcomes. Our theory that anti-AT1R antibodies with their proinflamatory and procoagulation properties and their ability to cause endothelial dysfunction may lead to an increased rate of thromboembolic and infectious complications in LVAD recipients was not borne out in our results. There was no difference in the overall survival among patients who were anti-AT1R antibody negative before Heart Mate II implantation and patients who either became positive or remained negative during the support. The incidence of device malfunction, bleeding, infection and neurological dysfunction was not influenced by the presence of anti-AT1R antibodies. There are several possible explanations for the lack of negative impact of AT1R activating antibodies on survival and adverse LVAD related complications in our cohort. Biological impetus regulating At1R antibody injury is fairly complex. Level of AT1R and induction of specific conformations is dependent on individual genetic polymorphisms and the state of local tissue expression influenced by various stressors. AT1R gene has 14 described polymorphisms, and some of them act as gain or loss of function mutations implicated in receptor activation[19]. The most extensively studied A1166C polymorphism is associated with increased responsiveness to Angiotensin II and various cardiovascular and renal pathologies[20]. It is plausible that mechanical circulatory support with the continuous flow creates a unique microenvironment resulting in lower AT1R expression, potentially less susceptible to anti-AT1R antibody mediated actions. There is compelling evidence that the AT1R may also be activated by mechanical stress without the involvement of Angiotensin II (ref.[21]). The AT1R is the first recognized mechanosensitive GPCR (ref.[22]). It is plausible that in the situation when the heart is fully unloaded with mechanical assist device AT1R would be down regulated. There may also be other factors that influence the features of anti-AT1R antibodies, changing their agonistic affinity. The tissue damage caused by certain mechanisms prior to anti-AT1R binding may affect the level of AT1R expression, resulting in different degree of anti-AT1R binding efficiency. Several modifiers have been identified thus far: ischemia, inflammatory events, and microbiome[23,24].

Limitations

Our study has several limitations inherent to the single centre observational study. Due to the small sample size and high correlation between variables, no multivariable models were fitted. Another implication of a small sample size with is a potential for Type II error. To counterbalance relatively small number of adverse events we combined several events into one composite outcome for the time to event analysis.

AT1R gene (located on chromosome 3) has 14 described polymorphisms. We did not perform a genetic analysis of our LVAD recipients and it is conceivable that the differences in expression and activation of AT1R based on genetic mutations could account for variability in AT1R – antibody mediated action.

CONCLUSIONS

The primary finding of this study is that patients who received a long term LVAD developed a high degree on sensitization against AT1R after implantation. The data showed no impact of anti-AT1R antibodies in Heart Mate II recipients on the overall survival and incidence of LVAD related complications. With the growing population of LVAD supported patients, increasing periods of support times and improved survival, attention is now shifting to the complications of mechanical support. We believe that determining the anti-AT1R antibody profile may prove valuable in risk assessment of mechanically assisted patients and serve as a novel biomarker for the detection of LVAD recipients at risk of an adverse outcome. The impact of anti-AT1R antibodies on the post-heart transplantation outcome will have to be evaluated in further studies.

Author contributions: MU: manuscript writing, literature search, data analysis; AS: literature search; TG: literature search, data analysis; PI: data analysis; IN: final approval.
Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. Trivedi JR, Cheng A, Singh R, Williams ML, Slaughter MS. Survival on the heart transplant waiting list: impact of continuous flow left ventricular assist device as bridge to transplant. Ann Thorac Surg 2014;98:830-4.
2. Barten MJ, Dragun D, von Salisch S, Dieterlen MT, Garbade J, Klein S, Dhein S, Mohr FW, Bittner HB. Identification of Non-HLA Antibodies in Ventricular Assist Device Recipients. J Heart Lung Transplant 2012;31:S46.
3. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kehla M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schonemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G. Angiotensin II type 1 receptor activating antibodies in renal-allograft rejection. N Engl J Med 2005;352:558-69.
4. Reinsmoen NL, Lai CH, Mirocha J, Cao K, Ong GD, Naim M, Wang Q, Riega S, Rafiei M, Patel J, Kobashigawa J. Increased Negative Impact of Anti-Angiotensin Type 1 Receptor Antibodies Together with De Novo Donor HLA Specific Antibodies on Graft Outcome in Heart Transplant Recipients. J Heart Lung Transplant 2013;32:S113.
5. Hiemann NE, Meyer R, Wellnhofer E, Schoenemann C, Heidecke H, Lachmann N, Hetzer R, Dragun D. Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation. Transplantation 2012;94:919-24.
6. Kirklin JK, Naftel DC, Pagani FD, Kormos RL, Stevenson LW, Blume ED, Miller MA, Baldwin JJ, Young JB. Sixth INTERMACS annual report: A 10,000-patient database. J Heart Lung Transplant 2014;33:555-64.
7. Drakos SG, Stringham JC, Long JW, Gilbert EM, Fuller TC, Campbell BK, Horne BD, Hagan ME, Nelson KE, Lindblom JM, Meldrum PA, Carlson JF, Moore SA, Kfoury AG, Renlund DG. Prevalence and risks of allosensitization in HeartMate left ventricular assist device recipients: the impact of leukofiltered blood product transfusions. J Thorac Cardiovasc Surg 2007;133:1612-19.
8. McKenna DH, Eastlund T, Segall M, Noreen HJ, Park SJ. HLA alloimmunization in patients requiring ventricular assist device support. J Heart Lung Transplant 2002;21:1218-24.
9. Massad MG, Cook DJ, Schmitt SK, Smedira NG, McCarthy JF, Vargo RL, McCarthy PM. Factors influencing HLA sensitization in implantable LVAD recipients. Ann Thorac Surg 1997;64:1120-25.
10. Barten MJ, Dragun D, Dieterlen MT, Salisch Sv, Garbade J, Klein S, Dhein S, Mohr FW, Bittner HB. Non-HLA Antibody Screening after Heart Transplantation Identifies High Risk for Cardiac Allograft Vasculopathy. J Heart Lung Transplant 2012;31:S107.
11. Dragun D. The role of angiotensin II type 1 receptor -activating antibodies in renal allograft vascular rejection. Pediatr Nephrol 2007;22:911.
12. Dechend R, Homuth V, Wallukat G, Kreuzer J, Park JK, Theuer J, Juepner A, Gulba DC, Mackman N, Haller H, Luft FC. AT(1) receptor agonistic antibodies from preeclamptic patients cause vascular cells to express tissue factor. Circulation 2000;101:2382-7.
13. Xia Y, Zhou CC, Ramin SM, Kellems RE. Angiotensin receptors, autoimmunity, and preeclampsia. J Immunol 2007;179:3391-5.
14. Yang S, Zhong Q, Qiu Y, Chen X, Chen F, Mustafa K, Ding D, Zhou Y, Lin J, Yan S, Deng Y, Wang M, Zhou Y, Liao Y, Zhou Z. Angiotensin II receptor type 1 autoantibodies promote endothelial microparticles formation through activating p38 MAPK pathway. J Hypertens 2014;32:762-70.
15. Zhang SL, Du YH, Wang J, Yang LH, Yang XL, Zheng RH, Wang YWK, Zhang MS, Liu HR. Endothelial dysfunction induced by antibodies against angiotensin AT1 receptor in immunized rats. Acta Pharmacol Sin 2010;31:1381-8.
16. Du Q, Wu J, Wang H, Wang X, Xu L, Zhang Z, Liu J, Zhang J, Chen J, Hakonarson H, Hu A, Zhang L. Perindopril treatment promote left ventricle remodeling in patients with heart failure screened positive for autoantibodies against angiotensin II type 1 receptor. BMC Cardiovasc Disord 2013;13:94.
17. Dragun D, Philippe A, Catar R. Role of non-HLA antibodies in organ transplantation. Curr Opin Organ Transplant 2012;17:440-5.
18. Win TS, Pettigrew GJ. Humoral autoimmunity and transplant vasculopathy: when allo is not enough. Transplantation 2010;90:113.
19. Mogi M, Iwai M, Horiuchi M. New insights into the regulation of angiotensin receptors. Curr Opin Nephrol Hypertens 2009;18:138-43.
20. van Geel PP, Pinto YM, Voors AA, Bulkema H, Oosterga M, Crijns HJ, van Gilst WH. Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension 2000;35:717-21.
21. Wu J, You J, Wang S, Zhang L, Gong H, Zou Y. Insights into the activation and inhibition of Angiotensin II Type 1 Receptor in the mechanically loaded heart. Circ J 2014;78:1283-9.
22. Storch U, Mederos Y, Schnitzler M, Gudermann T. G protein-mediated stretch reception. American Journal of Physiology. Am J Physiol Heart Circ Physiol 2012;302:H1241-9.
23. Dragun D, Hegner B. Non-HLA antibodies post-transplantation: clinical relevance and treatment in solid organ transplantation. Contrib Nephrol 2009;162:129.
24. Kaminska D, Tyran B, Mazanowska O, Rabczynski J, Szyber P, Patrzalek D, Chudoba P, Polak W, Klinger M. Cytokine gene expression in kidney allograft biopsies after donor brain death and ischemia-reperfusion injury using in situ reverse-transcription polymerase chain reaction analysis. Transplantation 2007;84:1118.