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Vliv mechanické srdeční podpory na cévní systém

Effect of mechanical circulatory support on the vascular systém

Disertační práce

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ABSTRAKT

Mechanické srdeční podpory jsou čerpadla krve schopná částečně nebo úplně nahradit funkci srdce k dosažení adekvátního srdečního výdeje. Dlouhodobé implantabilní levostranné mechanické srdeční podpory jsou zlatým standartem v terapii terminálních fází srdečního selhání. Zlepšují přežívání i kvalitu života jak pacientů na čekací listině k transplantaci srdce, tak i těch, u nichž se jedná o trvalou doživotní terapii. Zároveň je však tato skupina pacientů ohrožena specifickými komplikacemi souvisejícími s terapií. Mezi ty nejzávažnější s významným vlivem na morbiditu a mortalitu patří nežádoucí události spojené s hemokompatibilitou, tedy krvácení, trombotické komplikace a cévní mozkové příhody. Jednou z příčin těchto stavů může být skutečnost, že současné mechanické srdeční podpory generují převážně nepulsatilní tok krve. Tato práce se proto zabývá posouzením vlivu dlouhodobých implantabilních mechanických srdečních podpor s kontinuálním krevním tokem na cévy a cévní systém. Ve studii byly posuzovány funkční a morfologické změny cév a a taktéž změny v oblasti biomarkerů vaskulárního poškození. Výsledky studie potvrdily hypotézu, že nepulsatilní krevní tok ovlivňuje hladiny sledovaných markerů a vede ke změnám vaskulárního systému jak morfologickým a funkčním, tak na úrovni genové exprese. Získané poznatky mohou přispět k predikci a včasnějšímu řešení komplikací a mohou být také přínosné pro vývoj dalších generací mechanických srdečních podpor.

Klíčová slova: Mechanická srdeční podpora, srdeční selhání, hemokompatibilita, pulsatilita, vaskulární poškození

ABSTRACT

Ventricular assist devices are blood pumps capable of partially or completely replacing the function of the heart to achieve adequate cardiac output. Long-term fully implantable left-ventricular assist devices became the gold standard in the treatment of terminal stages of heart failure. They improve survival and quality of life of the patients on the waiting list for heart transplantation and those implanted as destination therapy. At the same time, this specific group of patients is at risk of a number of complications specific to this therapeutic modality. Among the most serious belong haemocompatibility related adverse events with a significant impact on morbidity and mortality. One of the aspects influencing the occurrence of these complications may be the non-physiological, non-pulsatile blood flow generated by current generation of devices. Therefore, the aim of this study was to assess the impact of long-term implantable mechanical circulatory support with continuous blood flow on the vasculature and vascular system. The functional and morphological changes in blood vessels as well as changes in biomarkers of vascular injury were analyzed. The results of our study supported the hypothesis that non-pulsatile blood flow affects the vascular system at morphological and functional level, as well as at the level of gene expression. The findings may contribute to the prediction and more timely management of serious complications and could be also essential for the development of the next generations of the devices**.**

Key words: mechanical circulatory support, heart failure, hemocompatibility, pulsatility, vascular damage

SEZNAM ZKRATEK

MSP	mechanická srdeční podpora
ICHS	ischemická choroba srdeční
HM3	HeartMate 3
HMII	HeartMate II
LVAD	*left ventricular assist device*, levostranná mechanická srdeční podpora
IABK	intraaortální balónková kontrapulsace
BTT	*bridge to transplant*, most k transplantaci
DT	*destination therapy*, destinační terapie
ICD	implantabilní kardioverter-defibrilátor
PI	pulsatilní index
PSV	*peak systolic velocity*, vrcholová rychlost v systole
EDV	*end-diastolic velocity*, rychlost na konci diastoly
MV	*mean velocity*, průměrná rychlost
RI	*resistive index*, odporový index
AI	augmentační index
INTERMACS	Interagency Registry for Mechanically Assisted Circulatory Support
CMP	cévní mozková příhoda

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1 ÚVOD

Implantace mechanických srdečních podpor (MSP) se stala standartní terapeutickou modalitou pro pacienty se srdečním selháním, u nichž i přes maximalizovanou konzervativní terapii nemoc progredovala do terminální fáze. Donedávna byla jedinou možností léčby u těchto pacientů transplantace srdce, avšak vzhledem k narůstajícímu počtu nemocných se srdečním selháním je zásadním limitem nedostatek vhodných dárců. Další velmi početnou skupinou jsou pacienti, kteří z různých důvodů (nejčastěji pokročilý věk spolu s přidruženými nemocemi) již nejsou k transplantaci srdce indikováni. Pro obě tyto skupiny jsou mechanické srdeční podpory cestou k výraznému zlepšení kvality života i přežívání.

V současnosti používané MSP generují převážně nepulsatilní, kontinuální tok krve. Historicky sice vývoj čerpadel nahrazujících funkci srdce směřoval k pulsatilním systémům, tyto však byly vysoce poruchové vzhledem k množství pohyblivých elementů a další vývoj proto směřoval k nepulsatilním pumpám. I přes absenci pulsatility mají dnešní čerpadla velmi positivní výsledky v klinické praxi.

Terapie pomocí MSP je však také spojena s výskytem mnoha komplikací, z nichž nejzávažnější jsou nežádoucí události spojené s hemokompatibilitou. Je stále předmětem studií, jak moc jsou tyto komplikace ovlivněny nebo přímo způsobeny dlouhodobou absencí pulsové vlny se sníženým cyklickým namáháním cévní stěny, zvýšených smykovým zatížením a následnými změnami endotelu.

Tato práce se proto věnovala posouzení vlivu absence pulsatility na cévní řečiště dlouhodobým sledováním morfologie a funkce cévní stěny a posouzením změn biomarkerů vaskulárního poškození. Výsledky práce by měly pomoci porozumět problematice specifických komplikací u pacientů s MSP a pomoci optimálnímu nastavení stávajících podpor a vývoji nových generací MSP.

2 PŘEHLED PROBLEMATIKY

2.1 Srdeční selhání

2.1.1 Definice srdečního selhání

Srdeční selhání je charakterizováno jako stav, kdy srdce není schopno plnit svou základní funkci a zajistit adekvátní perfuzi tkání potřebnou k pokrytí energetických nároků. Typickými příznaky jsou dušnost, nevýkonnost, nechutenství, kachektizace, otoky a známky hypoperfuze orgánových systémů. Jedná se o onemocnění s rostoucí prevalencí více než 64 miliónů pacientů na světě (Lippi et al, 2020). Nárůst výskytu tohoto onemocnění je způsoben stárnutím populace (výskyt u 10% populace nad 75 let) a zlepšujícími se možnostmi terapie akutních i chronických kardiovaskulárních onemocnění (Metra, Teerlink, 2017). Jedná se však o onemocnění s přetrvávající vysokou mortalitou i přes všechny pokroky moderní medicíny, kdy 5-leté přežívání se pohybuje pouze kolem 50% (Braunwald, 2013).

Srdeční selhání můžeme rozdělit dle dynamiky na akutní a chronické, dále dle selhávajícího úseku srdce na levostranné, pravostranné nebo oboustranné.

2.1.2 Příčiny srdečního selhání

Příčiny srdečního selhání jsou ve většině případů multifaktoriální. Zásadní roli hraje genetická dispozice ve spojení s rizikovými faktory ze strany nemocného.

Nejčastější příčinou srdečního selhání je neléčená nebo neřešitelná ischemická choroba srdeční, mnohdy v koincidenci s dilatační kardiomyopatií. Druhou nejčastější příčinou jsou kardiomyopatie – dilatační (familiární, idiopatická, toxonutritivní), restriktivní (např. stavy po radioterapii), hypertrofická nebo arytmogenní. Z dalších vyvolávajících onemocnění můžou vést k srdečnímu selhání hypertenze, nemoci srdečních chlopní, záněty srdečního svalu nebo vrozené srdeční vady. Etiologicky se mohou na srdečním selhání podílet i celková onemocnění jako diabetes mellitus, HIV, amyloidoza, nemoci štítné žlázy, hemochromatózy a další (Pirk et al 2019).

Vznik srdečního selhání dále potencují mnohé rizikové faktory. Mezi nejčastější patří kouření a dále důsledky metabolického syndromu, jako obezita, diabetes mellitus, hypertenze nebo dyslipidémie. Ovlivnění těchto rizikových faktorů má však vliv pouze na

počáteční fáze srdečního selhání a je velmi závislé na spolupráci pacienta s léčbou a režimovými opatřeními.

2.2 Možnosti terapie srdečního selhání

Terapie srdečního selhání závisí na tom, jak dalece je odstranitelná primární příčina kardiální insuficience a také jaká je tíže srdečního selhání v době diagnózy. Základem terapie jsou režimová a dietní opatření spolu s farmakologickou léčbou. V této oblasti se medicína neustále vyvíjí a nalézá stále účinnější látky ovlivňující nejen symptomatologii, ale také prognózu pacienta (např. sacubitril/valsartan). Dalším pilířem v terapii, potažmo v primární prevenci náhlé srdeční smrti je implantace ICD (implantailní kardioverter-defibrilátor) a resynchromizační terapie. Pokud lze odstranit primární příčinu onemocnění (nejčastěji v rámci ischemické choroby srdeční nebo chlopenních vad), přichází na řadu invazivní kardiologické intervence (PCI - perkutánní koronární intervence, TAVI – transkutánní implantace aortální chlopně apod.) nebo chirurgické řešení. Chirurgická léčba srdečního selhání zahrnuje revaskularizační operace, výkony na chlopních a v případech jinak neřešitelného onemocnění v terminální fázi potom transplantaci srdce, zavedení mechanické srdeční podpory nebo úplnou náhradu srdce srdcem umělým.

2.2.1 Transplantace srdce

Myšlenka nahradit selhávající srdce zdravým orgánem od jiného pacienta se datuje již do počátku 20. století, kdy byly učiněny první pokusné výměny orgánů v experimentu na zvířecím modelu. První takovýto pokus byl proveden již v roce 1905, kdy se jednalo o heterotopickou auxiliární transplantaci – srdce ze psa bylo implantováno jako pomocné do oblasti krku jiného psa. Postupně vývoj směřoval až k první ortotopické transplantaci srdce u člověka, která byla provedena v roce 1967 Christiaanem Barnardem. V následujícím roce bylo provedeno hned 102 transplantací v 52 centrech po celém světě. Prvotní nadšení však brzy vymizelo vzhledem k velmi vysoké mortalitě těchto pacientů vlivem časné rejekce. Tato situace positivně přispívala k rozvoji programu mechanických srdečních podpor v tomto období. V 70. letech 20. století byl aktivní transplantační program pouze v několika málo centrech na světě. Až objev cyklosporinu v roce 1978 umožnil renesanci této zásadní terapeutické modality. V současné době se provádí více než 6000 transplantací srdce ročně po celém světě (Kiran et al, 2021). V České republice se každý rok transplantuje 70-90 srdcí.

Jednoleté přežívání pacientů se pohybuje kolem 80-90 % v závislosti na věku pacienta, pětileté přežívání pak činí přes 70% (Kiran et al, 2021).

Významnou limitací této terapie je však nedostatečný počet vhodných dárců. I přesto, že je celosvětově vyvíjena významná aktivita pro zvýšení počtu dárcovských srdcí, například pomocí systémů pro ex-vivo perfuzi srdce, je stále počet orgánů nedostačující stále se zvětšující poptávce. Zde proto mají svou významnou úlohu mechanické srdeční podpory.

2.2.2 Mechanické srdeční podpory

2.2.2.1 Historie MSP

První nástin teorie mechanické srdeční podpory dočasné i dlouhodobé představil již v 19. století Le Gallois (Frasier et al, 1997). Zásadním krokem v moderní éře mechanických srdečních podpor bylo představení mimotělního oběhu v klinické praxi Gibbonem v roce 1953 (Gibbon, 1954). Původní myšlenkou bylo sice použít tento koncept k léčbě plicní embolie, nakonec však výsledkem byl počátek nové éry kardiovaskulární chirurgie i vývoje mechanických srdečních podpor (Helman et al, 2000). První a dosud stále klinicky využívanou mechanickou srdeční podporou byla intraaortální balónková kontrapulsace (IABK), představená v roce 1962 Moulopoulosem a poprvé využitá v klinické praxi Kantrowitzem v roce 1968 (Kantrowitz et al, 1968). Tato však byla nedostatečná pro úplnou náhradu funkce selhávajícího srdce a vzhledem ke stagnaci programů srdečních transplantací v tomto období z důvodu neznalosti imunosupresivní terapie byla velká snaha o vývoj úplné mechanické náhrady srdečního svalu. První úplná náhrada srdce (total arteficial heart) byla implantována v roce 1969, pacient byl následně po 64 hodinách transplantován (DeBakey, 2005). Koncem 70. let 20. století vývoj směřoval postupně k dlouhodobým levostranným srdečním podporám. První použití LVAD jako přemostění k transplantaci bylo představeno v roce 1980 Normanem (Norman et al, 1978). První úplná srdeční náhrada pro dlouhodobé použití Jarvik-7 byla implantována v roce 1982 DeVriesem a pacient ji měl následně po dobu 112 dní (DeVries et al, 1984). Od druhé poloviny osmdesátých let jsou již k dispozici komerčně vyráběné systémy.

S vývojem dalších, modernějších srdečních podpor se k původní indikaci přemostění k transplantaci srdce přidala ještě indikace destinační terapie, kdy je přístroj implantován pacientovi nesplňujícímu kritéria k transplantaci srdce a ponechán jako trvalé řešení onemocnění.

V průběhu postupného vývoje srdečních podpor se vystřídalo několik generací těchto přístrojů. První generace byla vyvinuta v souladu se snahou o co nejvíce fyziologickou náhradu srdce, tedy na pulzatilním principu. Tento typ srdečních podpor však obsahoval velké množství pohyblivých komponent, což velice omezovalo dlouhodobou funkci čerpadla (Pasque a Rogers, 2002). Druhou generací MSP byly pumpy axiálního typu (pracující na principu Archimedova šroubu), které generovaly nepulsatilní, kontinuální tok krve. Bylo také možno přístroj minimalizovat a tím zlepšit jeho implantabilitu a také byla redukována plocha, která přicházela do kontaktu s krevními elementy, což mělo positivní vliv zejména na komplikace spojené s trombosou čerpadla (John et al, 2008). Prozatím poslední a nejnovější generace MSP jsou čerpadla centrifugálního typu s použitím elektromagnetického nebo hydrodynamického závěsu rotoru, což umožnilo další snížení kontaktu s krevními elementy a také další zmenšení velikosti pumpy. Díky tomu ji je možno umístit přímo v perikardu, zatímco předchozí generace bylo nutné umístit do speciálně vytvořené preperitoneální kapsy. Nejnovější pumpa (a prakticky jediná používaná v současné klinické praxi) je HeartMate 3 (HM3, Abbott, Abbott Park, Illinois, USA). Toto čerpadlo má tři zásadní charakteristiky, díky nimž je maximalizována jeho hemokompatibilita – elektromagnetický závěs rotoru bez ložisek a pohyblivých částí, široké krevní cesty a arteficiální pulsatilitu způsobenou periodickým zvyšováním a snižováním rychlosti čerpadla, umožňující lepší promývání pumpy. I díky tomuto principu se u tohoto typu téměř nevyskytuje de-novo trombóza pumpy.

2.2.2.2 Typy MSP

MSP je možné rozdělit podle několika různých kritérií. Dle předpokládané délky terapie je rozdělujeme na krátkodobé, se kterými pacienti zpravidla zůstavají v nemocnici a doba terapie trvá řádově dny nebo týdny, nebo dlouhodobé, s nimiž pacienti odcházejí do domácí péče. Podle srdečního oddílu, který nahrazují, se srdeční podpory rozdělují na levostranné, pravostranné, biventrikulární nebo úplnou náhradu srdce (total arteficial heart – TAH). Rozdíl mezi posledními dvěma zmíněnými podporami je ten, že u biventrikulární MSP je srdce ponecháno in situ, pouze jsou na jeho oddíly našity kanyly jednotlivých podpor, zatímco u TAH je srdce nebo jeho větší část odstraněno a plně nahrazeno přístrojem. Dle charakteru toku krve generovaného podporami je dále rozdělujeme na pulsatilní a nepulsatilní. Co se týče způsobu implantace MSP, historicky byly všechny zaváděny

chirurgickou cestou a i dnes zatím tato metoda převažuje, nicméně některé dočasné srdeční podpory lze zavést také perkutánně.

Všechny typy mechanických srdečních podpor je možno implantovat v těchto základních indikacích: „bridge to transplant“ (most k transplantaci srdce, BTT), destinační terapie (DT), „bridge to recovery“ (most k zotavení, BTC), „bridge to decision“ (most k rozhodnutí, BTD) a „bridge to bridge“ (přemostění k dlouhodobé MSP pomocí krátkodobé podpory). U dlouhodobých implantabilních levostranných MSP v současnosti nejvíce narůstají počty pacientů implantovaných v indikaci destinační terapie, kdy je MSP pacientům implantována jako permanentní terapie srdečního slehání. V České republice stále převažuje indikace BTT, ale například v USA jsou dlouhodobé MSP implantovány jako destinační terapie již v nadpoloviční většině případů (Molina et al, 2021).

2.2.2.3 Chirurgická technika implantace MSP

Implantace dlouhodobé mechanické srdeční podpory je možná z několika různých přístupů. Nejčastěji používaným chirurgickým přístupem je podélná střední sternotomie, z méně invazivních přístupů je to buď kombinace levostranné anterolaterální minitorakotomie a horní parciální sternotomie nebo použití levostranné a pravostranné minitorakotomie. Implantace se nejčastěji provádí za použití mimotělního oběhu, u selektovaných rizikových pacientů indikovaných čistě k implantaci LVAD je tato možná i bez jeho použití (tzv. off-pump), případně za využití již předtím implantované krátkodobé MSP (např. ECMO apod.). Na srdci je následně nejprve za pomoci peroperační jícnové echokardiografie lokalizován hrot levé komory srdeční a následně je na něj našita objímka. Do hrotu je poté vyříznut dedikovaným nástrojem otvor a po aspekci vnitřku levé komory a případné extrakci buď trombu nebo obturujících papilárních svalů je do objímky zasunut samotný přístroj a zafixován fixačním mechanismem. V menším procentu případů lze postup obrátit – tedy nejprve udělat otvor do hrotu levé komory a poté našít prstenec. Tento způsob byl používán spíše dříve, v současné době již pouze v některých indikacích (např. aneurysma hrotu LK). V případě velmi limitované velikosti perikardiálního vaku je nutné provést takzvanou V-incizi v dlouhé ose levé komory tak, aby bylo možno přístroj do perikardiální dutiny uložit (Netuka, 2018). Výtoková kanyla (cévní protéza) je následně vedena paralelně s bránicí a kolem pravé komory tak, aby jednak nedocházelo k útlaku PK a také aby neležela přímo pod sternem (minimalizace rizika poranění při resternotomii např. během transplantace srdce) a našita na vzestupnou aortu. V případě, že se jedná v době implantace již o reoperaci, je

s výhodou vést výtokovou kanylu pleurálním prostorem a nemuset tak preparovat ze srůstů celé srdce (Netuka 2018). U všech pacientů je aplikována goretexová membrána kolem přístroje LVAD tak, aby se minimalizoval vznik srůstů pro případné další operace. U pacientů implantovaných jako „BTT, bridge to transplant" je do této membrány obaleno i srdce a překryta výtoková kanyla MSP. Tento mechanismus výrazně urychluje následnou transplantaci srdce a zejména je pak zásadně sníženo difuzní krvácení ze srůstů. Napájecí kabel LVAD je veden v břišní stěně metodou „C-shape" – nejprve se kabel vyvede ven v předem připraveném řezu v pravém horním kvadrantu břicha, aby byl následně opět vnořen do břišní stěny a vyveden v definitivním místě v levém horním kvadrantu. Tato metoda se ukázala jako velmi efektivní v blokádě přenosu případné infekce okolí vyústění perkutánního vodiče až do oblasti mediastina a samotné pumpy. Zásadní je následně pečlivá péče o vyústění kabelu a správná fixace k zamezení pohybu kabelu (Yarboro 2014, Schibilsky 2012)

2.2.2.4 Komplikace terapie pomocí MSP

Mechanické srdeční podpory významně zlepšují kvalitu života i přežívání jak pacientů čekajících na transplantaci srdce, tak indikovaných k destinační terapii. Tito pacienti jsou však ohroženi řadou komplikací. Nejčastější jsou komplikace infekční, které postihují více než polovinu pacientů v prvních 2 letech od implantace MSP (Mehra 2019). Zde se vedle klasických lokalizací infekce (plicní infekce, infekce urogenitálního traktu, infekce operačních ran atd.) setkáváme i s lokalitami výlučně souvisejícími s implantovaným přístrojem. Je to buď častější a lépe řešitelná infekce vyústění napájecího kabelu srdeční podpory z podkoží v oblasti epigastria, nebo výrazně závažnější infekce okolí přístroje v perikardu (pump pocket infection), mnohdy řešitelná pouze explantací systému nebo urgentní transplantací srdce.

Neméně závažnou komplikací je pravostranné srdeční selhání, které vyplývá z podstaty primárního onemocnění. Možnosti terapie jsou od režimových opatření přes inotropní medikaci po implantaci dočasné nebo i dlouhodobé pravostranné srdeční podpory.

Velmi specifickou kapitolou pro pacienty s MSP jsou nežádoucí události spojené s hemokompatibilitou. Mezi ně se řadí krvácení, cévní mozkové příhody a trombotické komplikace včetně trombózy čerpadla. Cévní mozkové příhody (CMP), ať již ischemické nebo hemoragické, jsou závažnou komplikací vyskytující se až u 10% pacientů (Mehra 2019, Molina 2020). Zvláště hemoragické CMP mají výrazný vliv na prognózu a kvalitu

života nemocných, proto v současné době probíhá celosvětově několik studií s cílem zajistit optimální nastavení antikoagulační a antiagregační terapie po implantaci MSP.

Další častou komplikací u této skupiny pacientů je krvácení. Pomineme-li krvácení spojené s vlastní implantací čerpadla, pacienti trpí zejména pozdnějšími krvácivými komplikacemi nejčastěji v podobě krvácení do trávicího traktu, epistaxe, hematurie nebo silného gynekologického krvácení. Tato problematika zčásti souvisí s užívanou antikoagulační a antiagregační terapií, ale určitý a dost možná významný vliv mají právě cévní změny související s kontinuálním, nepulsatilním tokem krve. (Mehra 2019, Netuka 2018).

Nežádoucí příhoda přímo spojená s MSP je trombóza pumpy. Tato komplikace byla relativně častá u předchozích generací čerpadel, kdy se může vyskytovat u 12-14% pacientů (Mehra 2019). U nyní používaného typu podpory s magnetickým levitujícím rotorem HeartMate3 je výskyt de-novo trombózy čerpadla prakticky eliminován.

2.2.2.5 MSP a hemokompatibilita

V současnosti je prakticky jedinou klinicky používanou dlouhodobou mechanickou srdeční podporou centrifugální čerpadlo HeartMate3. Dle výsledků dosud největší randomizované studie týkající se MSP (MOMENTUM 3 - Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3) je patrné jednoznačné snížení nežádoucích událostí spojených s hemokompatibilitou (hemocompatibility related adverse events, HRAEs) u centrifugální MSP HM3 v porovnání s předchozí generací čerpadla s axiálním pohonem HeartMate II. Další typ centrifugálního čerpadla třetí generace, HeartWare HVAD (Medtronic Inc., Minnesota, MN, USA), byl na základě nerandomizovaných studií a klinických výsledků stažen z trhu výrobcem právě pro zvýšený výskyt HRAEs (Uriel et al, 2017).

Pokles výskytu HRAEs u HM3 je podmíněn zejména sníženou incidencí trombózy pumpy a cévních mozkových přihod, výskyt krvácivých komplikací však zůstává poměrně vysoký v obou skupinách (Uriel et al, 2017). Snížení výskytu zejména trombotických komplikací poukazuje na tromborezistenci čerpadla HM3, podmíněnou jeho konstrukčními charakteristikami. K dalšímu snížení výskytu komplikací, zejména krvácivých, by mohla přispět optimalizace a antikoagulační a antiagregační terapie, která se na těchto komplikacích zcela jistě podílí. Standardní terapie zahrnuje warfarinizaci s cílovým INR 2,0-3,0 a podávání kyseliny acetylicylové v dávce 100mg/den. Studie MAGENTUM 1

(prospektivní monocentrická studie se sníženým antikoagulačním protokolem u pacientů s HM3) (Netuka et al, 2018) provedená na pracovišti autorky s jejím přispěním prokázala, že u vybraných pacientů je bezpečné snížení cílového rozmezí INR z původního 2,0-3,0 na 1,5-1,9, kdy 93% pacientů dosáhlo primárního endpointu přežití bez trombozy pumpy, devastující cévní mozkové příhody nebo krvácení 6 měsíců po implantaci. Recentně publikované výsledky studie ARIES, mezinárodní, randomizované, dvojitě zaslepené, placebem kontrolované studie porovnávající efekt aspirinu u pacientů s MSP ukázaly, že absence aspirinu nezvyšuje trombotické komplikace a naopak je sníženo riziko krvácení (Mehra et al, 2023).

V současné době probíhá celosvětově více studií zaměřených na optimalizaci antikoagulační a antiagregační terapie u pacientů s MSP (Mehra 2021). Cílem všech těchto studií je dosažení rovnováhy mezi krvácivými a trombotickými komplikacemi.

2.3 Patofyziologie cévní stěny

2.3.1 Vliv pulsatility krevního toku na cévní stěnu

Pulsatilitu nebo také pulsní vlnu můžeme definovat jako fyziologický jev detekovatelný v tepenném řečišti. Pulzní vlna vzniká při srdeční revoluci tokem krve vypuzené v systole z levé komory do aorty a dále do periferních tepen. Rychlost pulsní vlny je mnohem větší než rychlost toku krve. Pulsní vlna má dvě hlavní součásti. První z nich je systolická tlaková vlna, tvořená vypuzenou krví z levé komory přes aortální chlopeň do aorty, druhou tvoří tlaková vlna odražená zpět do aorty ze spodní poloviny těla pokračující do horních končetin. Tvar pulsové vlny je určen mnoha vlivy včetně věku, pohlaví, tělesné výšky, tělesné zdatnosti a srdečního pulsu. Buňky cévní stěny a zejména pak endoteliální buňky jsou adaptovány na opakující se změny tlaku a průtoku v arteriálním řečišti. Pulsatilita může být definována pulsním tlakem a pulsním indexem (indexem pulsatility, PI). Pulsní tlak je rozdílem nejvyššího systolického a nejnižšího diastolického tlaku, pulsní index určuje pulsatilitu dle průtoku a je dán rozdílem mezi nejvyšší a nejnižší rychlosti proudění krve v systole (ev. v diastole).

Současné implantabilní levostranné dlouhodobé mechanické srdeční podpory generují průtok krve buď plně nepulsatilní (HM II), nebo kontinuální s arteficiální pulsatilitou způsobenou periodickým zvyšováním a snižováním otáček pumpy s cílem promývaní čerpadla a prevenci trombózy (HM3). Výsledný krevní tok je u většiny pacientů převážně,

někteří pacienti však mají jasně patrnou systolicko-diastolickou diferenci (pokud se například otevírá aortální chlopeň a pacient generuje svůj vlastní příspěvek krevnímu průtoku).

Pulsatilitě jako takové se věnovalo již nespočet studií počínaje Aristotelem či Avicennou. Již v roce 1954 Burton prokázal, že průtok kapilárním řečištěm ustává po poklesu arteriálního tlaku pod kritický uzavírací tlak a že pulz prodlužuje dobu otevření kapilár (Burton et al, 1954). V roce 1960 Tekeda prokázal na zvířecím experimentu, že nepulsatilní tok krve vede ke kolapsu kapilární struktury, snížení krevního průtoku a zvýšení kapilárních zkratů bez ohledu na střední arteriální tlak (Tekeda et al, 1960). Později Prior představil hypotézu, že profil pulzního tlaku na kapilární úrovni je spolu se středním krevním tlakem a extracelulárním osmotickým tlakem hlavním faktorem odpovědným za udržování rovnováhy tekutin a výměnu živin na buněčné úrovni (Prior et al, 1995).

I recentní studie zaměřené na mechanické srdeční podpory naznačují, že vlivem ztráty pulsatility dochází k mnohým morfologickým a funkčním změnám stěny cév (Ambardekar et al, 2018, , Ivak et al, 2016), což následně může vést k endoteliální dysfunkci a dysregulaci. Přesný popis patofyziologických procesů je však stále předmětem mnoha studií, stejně jako teoretický positivní vliv zachované residuální pulsatility na riziko vzniku a vývoje komplikací. (Wever-Pinzon et al. 2013)

2.3.2 Možnosti neinvazivního posouzení stavu vaskulatury

Změny cévního systému lze posuzovat jak na morfologické, tak na funkční úrovni. Nejjednodušším způsobem je nezatěžující ultrazvukové vyšetření arteriálního řečiště. Pro posouzení stavu velkých cév se velmi dobře hodí dobře dostupné karotické nebo femorální tepny. Na morfologické úrovni lze sledovat zejména stupeň preklinické ateroskleróz pomocí ultrasonografických kritérií zvaných Belcaro skóre, kdy stupeň I je normální vzhled a tloušťka intimy i médie a nejvyšší stupeň IV je aterosklerotický plát podmiňující stenósu nad 50%. Belcaro ve své publikaci z roku 1996 uvádí, že během 6ti letého sledování nezaznamenal žádnou kardiovaskulární příhodu u pacientů s Belcaro skóre I, zato ji zaznamenal u 17% populace se skóre II-IV (Belcaro et al, 1996).

Funkční sonografické vyšetření velkých tepen zahrnuje zejména stanovení maximální systolické (peak systolick velocity, PSV), end-diastolické (end-diastolic velocity, EDV) a průměrné rychlosti (mean velocity, MV) a následné stanovení pulsatilního indexu (PI) dle vzorce PI= (PSV-EDV)/MV k posouzení pulsatility arteriálního řečiště. Dalším parametrem

je rezistenční index (RI), definovaný jako RI= (PSV-EDV)/PSV. Tento údaj vypovídá o pružnosti a odporu cévní stěny. Obecně je vyšší v tepnách zásobujících svalovou hmotu než v těch zásobujících mozek a parenchymové orgány.

Studií věnujících se posouzení těchto parametrů u pacientů s mechanickými srdečními podporami je velmi málo. Stöhr například publikoval, že arteficiální pulsatilita LVAD HM3 je detekovatelná v makro- i mikrocirkulaci, ale bez významných změn pulsatilního indexu oproti pacientům s plně kontinuálním tokem HM II (Stöhr et al, 2023). Další práce poukázala na to, že preexistující ateroskleróza v kombinaci s LVAD může vést ke zvýšenému riziku nežádoucích komplikací (Kyiatkin et al, 2021). K plnému pochopení dané problematiky je však ještě třeba vyčkat výsledku dalších studií.

Neméně důležitým parametrem, který je možné neinvazivně monitorovat, je tuhost (rigidita) cévní stěny. Snížená poddajnost cév má negativní vliv na krevní tlak a perfuzi cílových orgánů. Tuhost cévní stěny obecně zvyšují onemocnění jako hypertenze, diabetes mellitus či chronické onemocnění ledvin nebo obecně rostoucí věk. Etiologie zvýšené tuhosti cév u pacientů s mechanickou srdeční podporou není jasně vysvětlena, předpokládá se ale vliv absence pulsní vlny a tím způsobené ztráty elasticity cév.

Tuhost cévní stěny společně s endoteliální dysfunkcí je možno neinvazivně monitorovat pomocí systému EndoPat 2000 (Endo-PAT 2000®, Itamar Medical, Israel). V této metodice je pomocí sondy lokalizované na prstu horní končetiny možné měřit změny v periferním cevním systému doprovázející pulsní vlnu. Pomocí software je následně dopočítán tzv. augmentační index (AI), přičemž nižší hodnota tohoto indexu značí lepší elasticitu a menší tuhost cévní stěny. Dále je touto metodou možno určit tzv. reaktivní hyperemický index (RHI), který je ukazatelem endoteliální funkce. Měření se provádí v klidném prostředí za stabilní teploty 21-25 st. Celsia, kdy jsou prováděna měření systémem EndoPat nejprve v úplném klidu, poté po 5-minutové okluzi přítoku krve brachiální tepnou a následně po 5 minutách reperfuze. Jedná se o neinvazivní, na vyšetřujícím nezávislé měření velmi dobře použitelné k posouzení funkce endotelu a stavu periferního cévního řečiště.

2.3.3 Biomarkery vaskulárního poškození

Biomarkery vaskulárního poškození jsou dalšími z možných ukazatelů stavu cévního systému a zejména endotelu. Jak již bylo zmíněno, ztráta pulsní vlny a periodického namáhání stěny cév může mít negativní vliv na funkci endotelu a tuhost cévní stěny. Cirkulující biomarkery vaskulárního poškození, mezi které patří cirkulující mikročástice,

endoteliální progenitorové buňky nebo cirkulující mikroRNA, je možné detekovat ve vzorcích krevní plasmy a dle změny jejich hladin před a v různých časových intervalech po implantaci LVAD poté posuzovat přítomnost endoteliálního poškození.

Cirkulující mikročástice jsou fragmenty buněčné membrány uvolňované při zvýšeném namáhání nebo poškození buněk. Jsou uvolňovány ze širokého spektra buněk, nejvíce však z endotelu a trombocytů. Zvýšená detekce mikročástic je popisována u kardiovaskulárních onemocnění, metabolických onemocnění, preeklampsie a šokových stavů (VanWijk et al, 2003, Burnier et al 2009). Zvýšení smykového napětí při kontinuálním toku krve může ovlivňovat hladinu cirkulujících mikročástic (McGinn et al, 2016). Dosavadní studie prokázaly, že hladiny cirkulujících mikročástic jsou vyšší u pacientů, u nichž byly zaznamenány nežádoucí komplikace (Nascimbene et al., 2014). Dále bylo prokázáno, že hladiny mikročástic nejprve těsně po implantaci mechanické srdeční podpory klesnou, což může odpovídat zlepšené perfuzi orgánů než v předchozí fázi srdečního selhání, aby následně hladiny opět stoupaly v čase od implantace MSP (Ivak et al., 2016). Tento jev je dán patrně narůstajícím poškozením endotelu vlivem nefyziologického, kontinuálního toku krve.

Endoteliální progenitorové buňky (EPC) se řadí mezi subpopulaci CD34+ mononukleárních kmenových buněk. Mají schopnost endoteliální reparace a účastní se angiogeneze, působí proti endoteliální dysfunkci (Recchioni et al, 2016). Zároveň se podílejí na různých fyziologických procesech, jako je vazomotorický tonus, pohyb buněk nebo vrozená a adaptivní imunita (Yan F. et al, 2021). Mnohé studie se v posledním desetiletí věnovaly možnostem terapeutického využití EPC u různých kardiovaskulárních onemocnění, jako akutní infarkt myokardu nebo srdeční selhání (Prasad et al, 2020). Výsledky těchto studií jsou však zatím velmi limitovány omezeným počtem vysoce selektovaných pacientů. Další studie věnující se využití EPC jako biomarkerů vaskulárního poškození ukázaly jejich zvýšený počet u nemocí spojených s endoteliální dysfunkcí jako je ischemická choroba srdeční, srdeční selhání, diabetes mellitus, fibrilace síní nebo hypertenze. Byla prokázána i asociace zvýšených hladin EPC s onemocněním mozkových tepen (Huang ZX. Et al, 2021). Hladiny EPC jsou proto studovány jako další možné ukazatele míry poškození endotelu působením MSP. Dle zatím dostupných studií je jejich dynamika po implantaci srdeční podpory podobná, jako u cirkulujících mikročástic (Ivak et al., 2016), tedy nejprve dochází k poklesu hladin díky vyřešení stavu těžkého srdečního selhání a poté opět k elevaci působením MSP na endotel cév.

Cirkulující mikroRNA jsou jednovláknové řetězce nekódující RNA o délce 21-23 nukleotidů, která vzniká z dlouhého primárního transkriptu (pri-miRNA) a vlásenkové prekurzorové struktury (pre-miRNA) účinkem ribonukleáz v jádře a cytoplazmě a jejíž hlavní funkcí je post-transkripční regulace genové exprese. MiRNA vznikají transkripcí genů v DNA, kdy poté nedochází k jejich translaci na bílkovinu. Jsou to vysoce stabilní molekuly, jejichž poločas dosahuje mnoho hodin, a dokonce i dnů. Změny v expresi miRNA mohou potencionálně ovlivňovat funkci endoteliálních buněk a hladkých svalových buněk cév, což může následně vést k zánětu a progresi aterosklerotických změn. Mnohé typy miRNA jsou exprimovány nespecificky různými typy buněk. Avšak některé podtypy jsou exprimovány pouze během patologických procesů, například při nádorovém bujení nebo právě v případě poškození endotelu. Jejich přítomnost v krevní plasmě pak může plnit funkci biomarkerů vaskulárního poškození. V dříve publikovaných studiích byl prokázána možnost použití specifických subtypů miRNA (miR-30a-5p, miR-654-5p) v rámci diagnostiky pokročilých fází srdečního selhání a positivního efektu terapie (Quian Lu et al, 2022). V jiné studii byla porovnávána exprese rozdílných typů miRNA u mechanických srdečních podpor s kontinuálním a pulsatilním tokem krve (Lok et al, 2015). Efekt a využití tohoto velmi specifického biomarkeru k posouzení vlivu mechanických srdečních podpor na cévní systém je však v současnosti stále ne zcela jasný a je předmětem dalších studií a publikací.

3. CÍLE PRÁCE A HYPOTÉZY

3.1 Hypotézy

V práci byla testována hypotéza, že vliv kontinuálního, nepulsatilního toku krve generovaného mechanickou srdeční podporou lze pozorovat přímo na cévním řečišti neinvazivně jak pomocí sonografického vyšetření karotických tepen, tak sledováním cirkulujících biomarkerů vaskulárního poškození. Dále byla testována hypotéza, že posouzením důsledků těchto změn lze predikovat u konkrétního pacienta míru rizika úmrtí a vzniku závažných nežádoucích příhod.

3.2 Cíle práce

I. Definovat jednotlivé parametry krevního toku a strukturální změny cévního řečiště, které vznikají v důsledku terapie pomocí mechanických srdečních podpor a posoudit vliv změn těchto parametrů na následné riziko nežádoucích příhod.

II. Posoudit vliv arteficiální pulsatility nejnovějšího typu MSP – HeartMate 3 - na periferní vaskulaturu.

III. Posoudit vliv terapie dlouhodobou mechanickou srdeční podporou na hladiny mikroRNA.

IV. Zhodnotit dlouhodobé změny mRNA/mikroRNA u pacientů s implantovanou dlouhodobou mechanickou srdeční podporou.

V. Poskytnout souhrn současných poznatků o vlivu současných MSP na endotel a cévní systém.

4. METODIKA

Detailní popis metodiky je uveden v jednotlivých publikačních výstupech.

Sonografické vyšetření karotického řečiště bylo prováděno pomocí přístroje Toshiba APLIO 50bXV (Tochibi, Japan). Pacienti byli vyšetřováni v supinační poloze s krkem rotovaným 45% na kontralaterální stranu. Přítomnost aterosklerózy byla posuzována dle Belcaro score (Belcaro et al, 1996).

Tuhost cévní stěny byla měřena pomocí systému Endo-PAT 2000 (Endo-PAT 2000®software, Itamar Medical, Israel). Tento systém je nezávislý na vyšetřujícím a měří tuhost cévní stěny za použití augmentačního indexu (AI) (Yang et al, 2011). Pacienti byli vyšetřováni za standartních podmínek v ranních hodinách v tiché místnosti o stálé teplotě. Vyšetření probíhalo u všech pacientů po pětiminutové fázi odpočinku v supinační pozici. Následně byla pomocí sondy systému Endo-PAT umístěné na jeden prst každé ruky změřena úvodní pulsová amplituda. Následně po dalších pěti minutách byla nastolena ischemie levé horní končetiny pomocí tlakové manžety, zatímco pravá horní končetina sloužila jako kontrola. Periferní arteriální tonus byl hodnocen na jednom prstu každé končetiny. Po pěti minutách tlaku v manžetě 200mmHg byla manžeta povolena k nastolení reaktivní hyperémie dané končetiny. Pomocí automatického algorytmu byl následně změřen augmentační index s pomocí periferního arteriálního tonu změřeného před začátkem testování. Čím je výsledný augmentační index nižší, tím je vyšší elasticita cévní stěny.

RNA, včetně mikroRNA byla extrahována z krevní plasmy nebo tkáně aortální stěny pomocí miRCURY^{TM} RNA isolačního kitu (pro plasmu Exiqon, Vedbaek, Denmark, pro tkáně Qiagen GmBH Strasse 1, Hilden, Germany). Dále bylo provedeno kvantitativní PCR pomocí systému ABI 7300 v případě krevní plasmy a pomocí miRNome Panelů (Qiagen GmBH Strasse 1, Hilden, Germany) v případě aortální tkáně. Pasivní reference Dye (ROX^{TM}) byla zahrnuta ve všech PCR reakcích.

Principy statistických analýz jsou podrobně popsány v jednotlivých publikacích. Data jsou vyjádřena jako průměr +/- směrodatná odchylka, medián (interquartile range) nebo četnost (procenta). Průběžné změny hemodynamických parametrů byly analyzovány pomocí párového T-testu. Rozdíly mezi jednotlivými typy mechanických srdečních podpor (HMII a HM3) byly porovnány za použití T-testu pro nezávislé výběry, Mann-Whitney test nebo Chí-kvadrát test. K zobrazení přežívání bez cévní mozkové příhody a rozdílů mezi jednotlivými

skupinami byl použit Kaplan-Meierův graf. Pro vymezení faktorů souvisejících s přežíváním bez cévních mozkových příhod bylo využito Coxovy regresní analýzy. Proporcionální předpoklad rizika byl testován a splněn u všech regresních modelů. Všechny statistické testy a intervaly spolehlivosti byly oboustranné s použitím hladiny významnosti 0,05.

5. VÝSLEDKY

A

Tucanova Z, Ivak P, Wohlfahrt P, Pol M, Hlavacek D, Konarik M, Szarszoi O, Netuka I, Pitha J. Increased pulsatility index is associated with adverse outcomes in left ventricular assist device recipients. ESC Heart Fail. 2021 Oct;8(5):4288-4295. doi: **IF_{2021}= 3.612**

B

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E

Tucanova Z, Ivak P, Pitha J. The effect of mechanical circulatory supports on the vascular system, AtheroRev 2022; 7(1): 31-34

PRÁCE A je monocentrickou prospektivní studií sledující vliv mechanických srdečních podpor na cévní systém. Hlavní náplní této studie bylo posoudit vliv nepulsatilního toku krve na cévní stěnu pomocí sonografického vyšetření karotických tepen a posouzení tuhosti cévní stěny. V práci byla testována hypotéza o vztahu mezi pulsatilním indexem měřeným v karotických tepnách před implantací mechanické srdeční podpory a 3 a 6 měsíců poté a rizikem vzniku závažných nežádoucích událostí v průběhu terapie. V době vzniku této práce nebyla publikována žádná práce věnující se stejné problematice. Cílem této studie bylo posoudit možnost prediktivního posouzení rizika cévní mozkové příhody nebo úmrtí pacienta na základě neinvazivního sonografického vyšetření karotických tepen. Zároveň práce měla za cíl pomoci objasnit důsledky nepulsatilního toku krve na cévní systém.

Do studie bylo zařazeno 83 pacientů (12 žen, průměrný věk 54 ± 15 let) v terminální fázi srdečního selhání indikovaných k implantaci levostranné dlouhodobé mechanické srdeční podpory s kontinuálním tokem krve jak v indikaci most k transplantaci (BTT, 73.5%), tak destinační terapie (DT, 26.5%). V závislosti na období implantace byla pacientům implantována buď starší pumpa axiálního typu HeartMate II (34 pacientů), nebo novější (později implantovaná) centrifugální pumpa s plně magnetickým levitujícím rotorem HeartMate 3 (49 pacientů). Pacienti s novějším typem HM3 byli typicky starší, měli vyšší prevalenci hypertenze a fibrilace síní a vyšší hodnoty BNP před implantací. Pacienti byli implantování na Klinice kardiovaskulární chirurgie IKEM mezi lety 2014 a 2018 dle standartních postupů našeho pracoviště. Všichni pacienti byli vstupně antikoagulováni heparinem do doby dosažení cílového INR mezi 2.0 a 3.0, pacienti s implantovaným HM3 navíc dostávali denně 100mg kyseliny acetylsalicylové. Patnáct pacientů bylo zařazeno současně do studie s redukovaným antikoagulačním profilem na cílové INR 1.5-1.9 (Netuka et al, 2018). Ischemická etiologie srdečního selhání se vyskytovala u 38 (45.8%) z nich. Pacientům bylo před implantací srdeční podpory a v pravidelných tříměsíčních intervalech po ní prováděno sonografické vyšetření karotických tepen pomocí přístroje Toshiba APLIO 50bXV (Tochibi, Japan). Přítomnost aterosklerózy byla posuzována dle Belcaro score (Belcaro et al, 1996). Posouzení tuhosti cévní stěny bylo provedeno za použití systému Endo-PAT 2000 (Itamar Medical, Israel). Tato technika obnáší použití prstní sondy k digitálnímu měření objemových změn doprovázejících pulsní vlnu.

Výsledky studie ukázaly, že u pacientů, u nichž byl pulsatilní index měřený v karotických tepnách 3 měsíce po implantaci srdeční podpory vyšší než medián, mají vyšší riziko cévní mozkové příhody nebo úmrtí. Pacienti se studijní skupiny, kteří utrpěli cévní mozkovou

příhodu, měli vyšší pulsatilní index ve 3 měsících něž pacienti bez příhody. Za použití Coxovy regrese a přidání věku, pohlaví, fibrilace síní a typu MSP do analýzy byl pulsatilní index označen za ještě silnější prediktor cévní mozkové příhody, zatímco HeartMate 3 byl spojen s protektivním efektem. Po přidání tuhosti cévní stěny do analýzy byl popsán ještě robustnější efekt vyššího pulsatilního indexu na riziko úmrtí a cévní mozkové příhody.

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ORIGINAL RESEARCH ARTICLE

Increased pulsatility index is associated with adverse outcomes in left ventricular assist device recipients

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Abstract

Aims Recipients of left ventricular assist devices (LVAD) are exposed to increased risk of adverse clinical events. One of the potential contributing factors is non-pulsatile flow generated by LVAD. We evaluated the association of flow patterns in carotid arteries and of increased arterial stiffness with death and cerebrovascular events in LVAD recipients.

Methods and results We analysed data from 83 patients [mean age 54 ± 15 years; 12 women; HeartMate II (HMII), *n* = 34; HeartMate 3 (HM3), *n* = 49]. Pulsatile and resistive indexes, atherosclerotic changes in carotid arteries (measured by duplex ultrasound), and arterial stiffness [measured by Endo-PAT 2000 as the augmentation index standardized for heart rate (AI@75)] were evaluated 3 and 6 months after LVAD implantation. Sixteen patients died during follow-up (27.3 months; interquartile range 15.7–44.3). After adjusting for the main variables examined, the pulsatility index measured at 3 months was positively associated with increased hazard ratios (HR) for death and cerebrovascular events [HR 9.8, 95% confidence interval (CI) 1.62–59.42], with HR increasing after adding AI@75 to the model (HR 18.8, 95% CI 2.44–145.50). In HM3 recipients, HR was significantly lower than in HMII recipients (HR 0.31, 95% CI 0.11–0.91), but the significance disappeared after adding AI@75 to the model (HR 0.33, 95% CI 0.09–1.18).

Conclusions The risk of death and cerebrovascular events in LVAD recipients is associated with increased pulsatility index in carotid arteries and potentiated by increased arterial stiffness. The same risk is attenuated by HM3 LVAD implantation, but this effect is weakened by increased arterial stiffness.

Keywords Mechanical circulatory support; Pulsatility index; Clinical events

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Introduction

Use of the left ventricular assist device (LVAD) in patients with end-stage heart failure is considered a standard treatment in routine care.[1] Although it undoubtedly results in improved survival and life quality, LVAD recipients are nonetheless exposed to particular adverse clinical events.[2–4]

Therefore, early identification of patients at high risk of serious clinical events is of great importance. The pathophysiology of vascular changes in LVAD recipients has been the subject of intensive research[5–8] but is still not fully understood. The data are surprisingly sparse on the role of structural changes and blood flow patterns detectable in the peripherally located arteries of LVAD recipients. One potential method for assessing risk of future vascular and other complications in LVAD recipients is simple, non-invasive duplex ultrasound examination of the carotid arteries. According to several studies, LVAD has definitive impacts on carotid arterial structure and blood flow.[9,10] However, impacts on clinical outcomes have yet to be evaluated and, as one parallel study has revealed, factors closely associated

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with flow pulsatility such as arterial stiffness may in fact contribute to increased risk.[11]

On the basis of these assumptions, we conducted a prospective single-centre study to examine the association of morphological changes and flow patterns in carotid arteries with death and cerebrovascular events after LVAD implantation. In the present study, we analysed potential association of pulsatile and resistive indexes in carotid arteries with stroke-free survival. In addition to that, we analysed if these associations are modified by atherosclerotic changes and arterial stiffness.

Methods

This single-centre prospective observational study was conducted in compliance with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practices, and the International Organization for Standardization (ISO 14155:2020, Clinical Investigation of Medical Devices for Human Subjects – Good Clinical Practice). The study protocol was approved by the regulatory boards and ethics committees of the participating institutions. All patients were required to provide their written informed consent prior to inclusion in the study.

From 2014 to 2018, a total of 83 patients were enrolled in the study (mean age 54 ± 15 years; 12 women). All patients were diagnosed with end-stage heart failure and met the institutional criteria for LVAD implantation performed using the HeartMate II (HMII) axial continuous-flow LVAD (Abbott, Abbott Park, Illinois, USA & St. Jude Medical, Pleasanton, California, USA) (n = 34) or the HeartMate 3 (HM3) fully magnetically levitated centrifugal-flow LVAD (Abbott, Abbott Park, Illinois, USA) (n = 49) for an indicated bridge to heart transplantation or destination therapy. HMII was implanted via the subcostal approach in 7 patients and via sternotomy in 27 patients. HM3 was implanted via left anterolateral mini-thoracotomy and via upper J mini-sternotomy in 12 patients and via full median sternotomy in 37 patients. A short-term mechanical circulatory support was administered in seven patients (six HMII and one HM3) preceding implantation of the durable LVAD.

Heparin was continuously and intravenously applied as a bridge until reaching the international normalized ratio (INR) target anticoagulation range for warfarin. The INR anticoagulation therapy target post-implantation was 2–2.5 for HMII and 2.0–2.7 for HM3. Aspirin (100 mg per day) was administered only in HM3 recipients. In 15 HM3 recipients who had taken part in a previous study, the INR anticoagulation therapy target was adjusted to 1.5–1.9.[12]

Baseline characteristics, medical history, laboratory measurements, and medications were collected. Ultrasound and arterial stiffness measurements were performed at pre-specified time-points 3 and 6 months after implantation (±15 days). The median follow-up time was 27.3 months [interquartile range 15.7–44.3]. Examiners (JP and PW) were blinded to the clinical and laboratory data, including the type of LVAD used.

Carotid parameters including pulsatility and resistive indexes

Carotid arteries were examined using the Toshiba APLIO 50 XV (Tochigi, Japan) ultrasound system with a 7.5–10 MHz linear array transducer. Patients were examined in the supine position. With the neck rotated 45° in the direction opposite to the site being examined, a transducer was placed just above the right clavicle.

The presence of atherosclerosis was classified using the Belcaro score,[13] which evaluates the degree of pre-clinical atherosclerosis based on ultrasound criteria, graded from the normal appearance of intima-media thickness (Class I) to plaque with stenosis >50% (Class IV). The mean Belcaro score for the sites of the left and right carotid arteries was used for subsequent analysis. More detailed description of the carotid examination procedure is described at supporting information.

Ultrasound examinations of the right carotid artery were completed in 83 individuals, with a Belcaro score established for both sites in all participants. To assess potential differences between the right and left carotid arteries, in 39 (at 3 months) and 21 (at 6 months) individuals, flow patterns were established on both sides.

Establishment of arterial stiffness

Arterial stiffness was measured using Endo-PAT 2000 software (Endo-PAT 2000®, Itamar Medical, Israel) as the augmentation index (AI).[14,15] This technique involves the use of a finger probe to assess digital volume changes accompanying pulse waves. AI was calculated using a computerized automated algorithm (software version 3.1.2) from peripheral arterial tone pulses recorded during the baseline period. Lower AI values (including negative values) reflect better arterial elasticity. The AI result is used to indicate sex-matched, non-selective populations. For subsequent analysis, we used AI values normalized to a heart rate of 75 bpm (AI@75). Detailed description of the entire procedure is described at supporting information.

Diagnosis of clinical events and stroke

Causes of death and cerebrovascular events were established by clinical assessment and/or autopsies

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according to standard procedures, with the exception of two cases of sudden death. Presence and type of stroke were confirmed by clinical assessment and positive computed tomography (CT) scans. In two patients, discrepancies between positive clinical signs and negative CT scans were detected, with data on these patients added to the clinically assessed analysis. In addition, one fatal haemorrhagic stroke was established post-mortem during autopsy.

Statistical methods

Data are expressed as mean ± standard deviation, median (interquartile range), or frequency (percentage). Longitudinal changes in carotid haemodynamics and the lumen were analysed using a paired *t*-test. Differences between HMII and HM3 patients were compared using the independent-samples *t*-test, Mann–Whitney U-test, or χ^2 test as appropriate. The Kaplan–Meier plot was used to visualize stroke-free survival, with differences between groups analysed using the log-rank test. Cox regression was used to determine factors associated with stroke-free survival. The proportional hazard assumption was tested and fulfilled for all regression models. All statistical tests and confidence intervals (CI) were two-sided using a significance level of 0.05. The Kolmogorov–Smirnov test and the Shapiro–Wilk test were used to test the normality of the data.

Results

Study parameters and changes during follow-up

All 83 patients were Caucasian (mean age 54 ± 15 years; 12 female patients) and indicated for implantation of HMII (n = 34) or HM3 (n = 49). Ischaemic aetiology of heart failure was present in 38 patients (45.8%), with a bridge to transplant the predominant indication for implantation (73.5%). In the majority (78.3%) of patients, INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support) profiles were 2–4 (*Table 1*). HM3 recipients were typically older, displayed increased prevalence of hypertension and atrial fibrillation, and had higher brain natriuretic peptide concentrations compared with patients implanted with HMII (*Table 1*). Three months after implantation when duplex ultrasound of the carotid arteries and AI measurements were performed, patients with HM3 had more advanced carotid atherosclerosis, a moderately higher diameter of the lumen of the carotid arteries, moderately higher PI and RI and significantly lower AI@75 (*Table 2*).

Between 3 and 6 months, no significant changes in PI, RI, or lumen diameter (mean changes) were observed. None of the other study parameters changed significantly during this period. No differences between the HMII and HM3 groups were observed for the above parameters. We observed no differences between the right and left carotid arteries (measured in 39 patients at 3 months) regarding main parameters under study (PI and RI), and no haemodynamically significant

Table 1 Characteristics of patients prior to implantation according to the type of left ventricular assist device used

Variable	All patients, n = 83	HeartMate II, n = 34	HeartMate 3, n = 49	HMII vs. HM3, P
Women, n (%)	12 (14.5)	5 (14.7)	7 (14.3)	0.99
Age (years)	54.4 ± 14.9	48.3 ± 15.6	58.6 ± 12.9	0.002
Body mass index (kg/m^2)	26.5 ± 4.8	26.3 ± 4.9	26.6 ± 4.7	0.78
Arterial hypertension, n (%)	37 (44.6)	12 (35.3)	25 (51.0)	0.08
Ischaemic aetiology of heart failure, n (%)	38 (45.8)	14 (41.2)	24 (50.0)	0.43
History of thromboembolic disease (%)	6 (7.2)	2 (5.9)	4 (8.2)	0.99
History of atrial fibrillation (%)	42 (50.6)	12 (35.3)	30 (61.2)	0.02
Diabetes mellitus, n (%)	18 (21.7)	7 (20.6)	11 (22.4)	0.84
Active smoking, n (%)	10 (12.0)	6 (17.6)	4 (8.3)	0.30
INTERMACS 1/2/3/4/5, n (%)	4/15/35/15/14 (5/18/42/18/17)	3/10/13/4/4 (9/29/38/12/12)	1/5/22/11/10 (2/10/45/22/20)	0.11
Systolic blood pressure (mmHg)	106.1 ± 13.4	106.5 ± 11.7	105.8 ± 14.5	0.81
Ejection fraction of left ventricle assessed by echocardiography (%)	18.7 ± 5.9	19.2 ± 8.1	18.4 ± 3.7	0.53
Brain natriuretic peptide factors (BNP) (ng/L)	1610 (791–2845)	2080 (1003–2964)	1436 (682–2291)	0.06
Lactate dehydrogenase (LDH) units	4.0 (3.3–5.9)	4.6 (3.7–7.8)	3.7 (3.2–5.1)	0.78
Glycaemia (mmol/L)	5.8 ± 1.7	5.8 ± 1.8	5.9 ± 1.7	0.69
LDL cholesterol (mmol/L)	2.09 ± 0.76	2.06 ± 0.86	2.11 ± 0.68	0.81

INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support.
Data expressed as mean ± SD.

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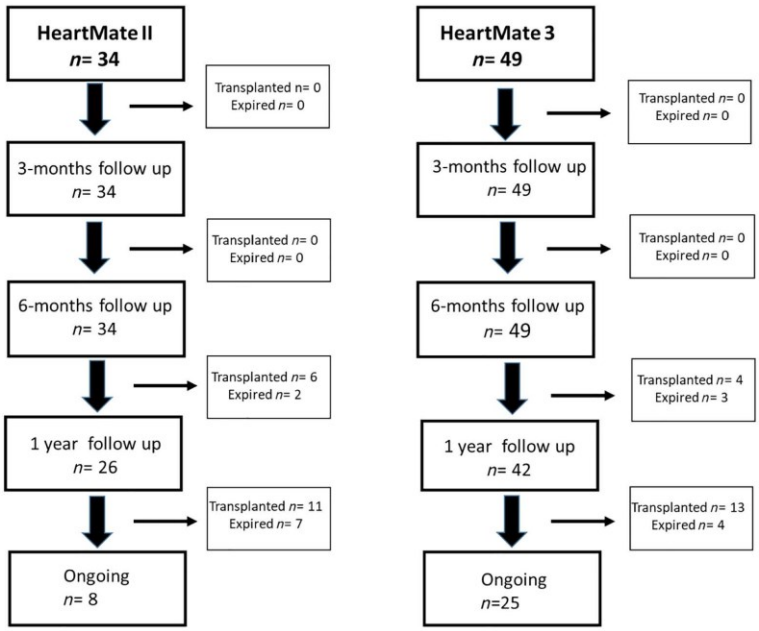
Table 2 Characteristics of patients 3 months after implantation according to the type of left ventricular assist device used

Variable	All patients, *n* = 83	HeartMate II, *n* = 34	HeartMate 3, *n* = 49	HMII vs. HM3, *P*
Aortic valve: open/partly closed/closed (%)	60/12/11 (72.3/14.5/13.3)	26/6/2 (76.5/17.6/5.9)	34/6/9 (69.4/12.2/18.4)	0.23
Belcaro score	2.5 (1.5–3.0)	2 (1–3)	2.5 (2–3)	0.04
Lumen of carotid arteries	6.02 ± 0.78	5.81 ± 0.74	6.11 ± 0.80	0.09
Pulsatility index of carotid arteries	0.50 ± 0.24	0.44 ± 0.18	0.54 ± 0.26	0.05
Resistive index of carotid arteries	0.36 ± 0.12	0.33 ± 0.09	0.38 ± 0.13	0.04
Augmentation index (AI@75)	22.5 (2–39.0)	31 (22–43)	11 (−12–29)	0.001

Data expressed as mean ± SD

Figure 1 Flow chart of patients under study according to the type of left ventricular assist device.



carotid stenosis was detected over the whole course of the study.

Incidence and characteristics of clinical events

Out of the 83 patients enrolled in the study, 16 died (HMII *n* = 9, HM3 *n* = 7). Regarding cause of death, 4 patients died due to stroke: 1 ischaemic and 3 haemorrhagic (HMII *n* = 1/haemorrhagic, HM3 *n* = 3/1 ischaemic, 2 haemorrhagic). Five patients died due to sepsis (HMII *n* = 3, HM3 *n* = 2), 3 died due to multiorgan failure (HMII *n* = 2, HM3 *n* = 1), 2 died due to right heart failure (HMII *n* = 1, HM3 *n* = 1), and 2 due to sudden death of unknown cause (all implanted with HMII). The first fatal event occurred 233 days after implantation of LVAD (HM3). In total, 34 patients were transplanted (HMII *n* = 17, HM3 *n* = 17) (*Figure 1*).

Four patients (HMII *n* = 2, HM3 *n* = 2) suffered from non-fatal ischaemic strokes (Modified Rankin Scores at the time of stroke: 2, 4, 4, and 5). Fifteen patients experienced adverse events related to non-surgical bleeding: seven in the gastrointestinal tract (HMII *n* = 1, HM3 *n* = 6), three in the urinary tract (HMII *n* = 2, HM3 *n* = 1), two in the respiratory tract (all HM3), and three in other locations (all implanted with HM3). In four patients, LVAD was replaced due to thrombosis (HMII *n* = 3, HM3 *n* = 1). One patient with HMII and six

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patients with HM3 were transiently treated by RVAD (mean duration of 30.6 days) shortly after implantation of LVAD, which was successfully removed in all patients. In two patients, HMII was replaced by HM3: in one patient due to a technical fault with pump stop alarms, and in the other patient due to pump thrombosis.

Association between carotid artery haemodynamic/atherosclerotic parameters and death and strokes

In patients with PI above the median at 3 months, the Kaplan–Meier curve indicated lower stroke-free survival. However, using the log-rank test, this difference was not statistically significant (log-rank $P = 0.19$, *Figure 2*). Additionally, in patients who developed stroke, the mean PI at 3 months was higher than in those free of stroke (0.82 ± 0.45 vs. 0.46 ± 0.18), albeit of borderline statistical significance ($P = 0.06$). In patients with HMII, stroke-free survival was lower than in patients with HM3 as indicated by the Kaplan–Meier curve, but again the difference was not statistically significant (log-rank $P = 0.14$, *Figure 3*).

Nevertheless, when using Cox regression and considering age, sex, atrial fibrillation, and HM type, PI was a strong predictor of stroke [hazard ratio (HR) 9.81, 95% CI 1.62–59.42] and HM3 implantation was associated with a protective effect (HR 0.31, 95% CI 0.11–0.91). When arterial stiffness (AI@75) was added to the statistical model, we detected a more robust effect of higher PI on the risk of death and cerebrovascular events (HR 18.80, 95% CI 2.44–145.50) and a weakening of the protective effect of HM3 (HR 0.33, 95% CI 0.09–1.18) (*Table 3*).

Figure 2 Impact of pulsatile index measured in the right carotid artery on stroke-free survival in patients treated by left ventricular assist device (Kaplan Meyer).

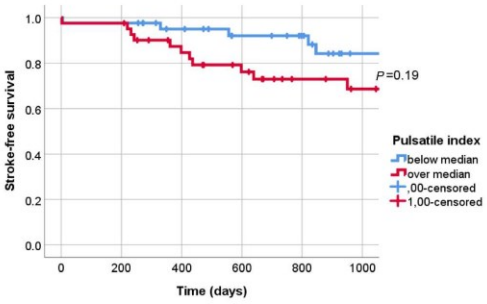
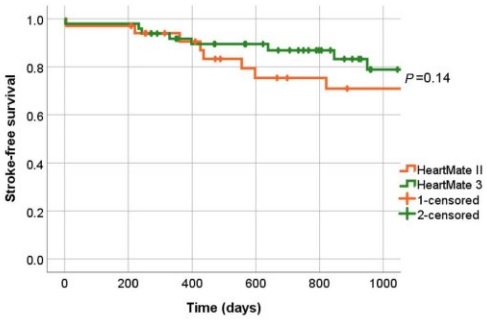


Figure 3 Impact of left ventricular assist device type (HeartMate II vs. HeartMate 3) on stroke-free survival (Kaplan Meyer).



Discussion

Our main finding is that a higher pulsatility index measured in carotid arteries 3 months after LVAD implantation was independently and strongly associated with increased incidence of death and cerebrovascular events. This association became even stronger when arterial stiffness was taken into account. Moreover, we found no association between morphological atherosclerotic changes in the carotid arteries and incidence of clinical events or the pulsatility index, the latter parameter being mutually independent of arterial stiffness.

The effect of particular blood flow patterns in carotid arteries on clinical events including strokes in LVAD recipients were to our best knowledge not studied or published. For this reason, we discuss data from studies of vasculature including carotid arteries after LVAD implantation and from experimental studies of LVAD.

Few studies have focused on vascular changes, including the carotid arteries, after LVAD implantation. In a cross-sectional study of 16 chronic LVAD patients,

Table 3 Multivariate Cox regression of factors associated with stroke-free survival including (Model 1) and excluding (Model 2) arterial stiffness

Variable	HR	95% CI	*P*
Model 1			
Age	0.99	0.96–1.04	0.97
Sex (female)	0.50	0.09–2.70	0.42
Pulsatility index	9.81	1.62–59.42	0.01
HeartMate 3 vs. HeartMate II	0.31	0.11–0.91	0.03
Atrial fibrillation	2.03	0.66–6.25	0.22
Model 2			
Age	0.99	0.96–1.03	0.78
Sex (female)	0.43	0.08–2.38	0.33
Pulsatility index	18.8	2.44–145.50	0.005
HeartMate 3 vs. HeartMate II	0.33	0.09–1.18	0.09
Atrial fibrillation	1.96	0.59–6.55	0.27
Augmentation index (AI@75)	1.02	0.98–1.05	0.25

CI, confidence interval; HR, hazard ratio.

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continuous-flow LVAD support was associated with lower carotid artery compliance, distensibility, and incremental elastic modulus.[16] Another study focused on carotid arteries in 13 patients revealed that while peak systolic velocity was diminished after LVAD placement in both the internal and common carotid arteries, mean flow velocities in the same arteries remained stable.[17] Moreover, further piece of evidence of vascular changes after LVAD implantation stems from our study describing changes in circulating endothelial progenitor cells and stem cells, which are both considered markers of vascular impairment in LVAD recipients.[5–7] In these studies, changes in these markers indicated that improvements in haemodynamic parameters may have negated the deleterious effects of non-pulsatile flow during the first 3 months, but pathological activation of the vasculature and endothelium was detected 6 months. These findings are consistent with another study on 83 LVAD patients, where patients with optimized haemodynamics had greater freedom from haemocompatibility-related adverse events.[18] Several other human studies have described the unfavourable effects of LVAD on the aortic wall[10,17,19] and peripheral vasculature.[20] In addition, an experimental study of 23 calves, comprising a detailed analysis of vascular changes caused by a novel partial-support circulation pump, demonstrated arterial remodelling with subsequent altered haemodynamics in peripheral vessels.[21]

All of the above findings strongly indicate that vascular impairment after LVAD implantation makes LVAD recipients sensitive to clinical events and this risk could be strongly influenced also by pulsatility patterns, even in the presence of non-pulsatile or low-pulsatile flow.

Alternative explanation for our findings is that increased PI only reflected LVAD function and/or just pre-existing pathological changes in the vasculature. Another potential cause is that LVAD with non-pulsatile flow may have triggered further changes in a pre-existing imbalance between the microcirculation of the heart and peripheral and cerebral circulation.[22] Consequently, PI values may have solely reflected these processes responsible for subsequent clinical events.

Additional interesting finding was that HM3 was independently associated with a significantly decreased risk of death and cerebrovascular events compared with HMII. In agreement with the results of the Final Report of the MOMENTUM 3 Study (Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3),[23] this protective effect was moderately attenuated after accounting for arterial stiffness. One possible explanation is that the novel features of HM3, especially enhanced haemocompatibility,[24] serve to suppress the deleterious effect of relative increased pulsatility index despite being attenuated by higher arterial stiffness (*Table 3*). In our study, therefore, the potential advantages of HM3 implantation may have been counterbalanced by the presence of increased arterial stiffness.

Our study is limited by its observational character (reverse causation cannot be excluded) and the relatively low incidence of fatal events. The incidence of stroke, in particular, was lower than that reported in other publications, including MOMENTUM 3, the largest of these studies.[25] Stroke incidence was 6% during the first year after implantation and 9.6% across the whole study, a reduction perhaps attributable to the younger age profile and lower representation of women compared with previous larger studies.[3,23] In our group, the mean age was 54.4 ± 14.9 years compared with 60.0 ± 12.0 years in the HMII group and 59.0 ± 12.0 years in the HM3 group of the MOMENTUM study.[23] In addition, the representation of women was only 14.5% compared with 19.2% in the MOMENTUM study[23] and even to 21.5% in the INTERMACS study.[3] On the other hand, we observed no differences in the main clinical risk factors such as primary cardiac diagnosis, heparin-induced thrombocytopenia, nutritional status, severe diabetes mellitus, dialysis, and anaemia.

Another important possible explanation for some different findings compared with other studies is the specific anticoagulation and antiplatelet therapy strategy, especially in patients with HMII. Compared with the final report of the MOMENTUM study,[23] we found lower incidence of stroke in the HMII group (11.7% compared with 19%), which is perhaps attributable to differences in antithrombotic treatment strategies. While in the MOMENTUM study all HMII patients received warfarin with an INR target of 2.0–3.0 together with a daily dose of aspirin (81–325 mg), our HMII patients only received warfarin with a target range of 2.0–2.5 without aspirin. It should also be noted that 15 of our HM3 recipients were on a reduced anticoagulant regimen due to involvement in studies focused on this kind of therapy.[12]

It should be also noted that the absolute PI value was lower in patients with LVAD than in individuals with physiological pulsatile flow.[26] However, even relatively small change of low PI might be sufficient to trigger clinical events in pre-existing anatomical and/or functional impairment of (micro) vasculature including cerebral vessels. In addition, AI@75 can be modified by a different pattern of pulsatility in LVAD recipients. However, despite AI@75 values are not fully comparable between LVAD recipients and patients presenting with physiological pulsatility, we can reasonably assume that analyses comparing differences between groups of LVAD recipients are quite reliable.

Despite the above mentioned limitations, this study is, to our knowledge, one of the first to describe the impact of the pulsatility index on deaths and cerebrovascular events in a relatively high number of LVAD recipients combined with a parallel study of arterial stiffness. Our data show the potential of an available and easily applicable imaging method for assessing the risk of death and cerebrovascular events in LVAD recipients.

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Conclusions

According to our observations, carotid pulsatility measured by duplex ultrasound may be a strong predictor of death and cerebrovascular events in LVAD recipients.

Conflict of interest

Zuzana Tucanova is a recipient of grants and non-financial support from Abbott, Inc. Peter Ivak is a recipient of grants, personal fees, and non-financial support from Abbott, Inc. and serves as a consultant and overall PI for CARMAT, S.A. Ivan Netuka is a recipient of grants, personal fees, and non-financial support from Abbott, Inc.; serves as a consultant and overall PI for CARMAT, S.A.; and is a board member, stockholder, consultant, overall PI, and recipient of grants, personal fees, and non-financial support from Leviticus Cardio, Ltd.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supporting Information.

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PRÁCE B je zaměřena na posouzení vlivu pulsatility krevního toku na periferní vaskulární systém. Jedná se o monocentrickou prospektivní observační studii, která testovala hypotézu, že arteficiální pulsatilita produkovaná mechanickou srdeční podporou HeartMate3 by mohla mít protektivní efekt na vaskulární systém.

Do studie bylo zařazeno 32 pacientů (5 žen, průměrný věk 55 let) s implantovanou mechanickou srdeční podporou HeartMate3. Kontrolní skupinu tvořilo 30 pacientů simplantovanou MSP HeartMate II (bez arteficiální pulzatility), 25 pacientů s pokročilým srdečním selháním bez implantované mechanické srdeční podpory (NYHA III-IV) a 13 zdravých kontrol. Všem pacientům byla měřena periferní endoteliální funkce pomocí systému EndoPAT 2000. Tento systém umožňuje jednak měření endoteliální responzivity pomocí stanovení reaktivního hyperemického indexu (RHI) a dále stanovení tuhosti cévní stěny pomocí augmentačního indexu (AI). Tato měření byla prováděna u všech pacientů po zařazení do studie před implantací mechanické srdeční podpory a dále 3 a 6 měsíců po implantaci.

Před implantací srdeční podpory byl reaktivní hyperemický index (RHI) u skupiny HM3 nižší, než je průměrem ve zdravé populaci a taktéž nižší než u zdravých studijních kontrol. Po implantaci MSP RHI nadále klesal – mezi nultým a třetím měsícem je pokles statisticky významný, mezi třetím a šestým měsícem již nikoli. Zároveň byl RHI nižší u mladších pacientů pod 60 let. V případě augmentačního indexu (AI) byl trend opačný – po implantaci MSP byl patrný nárůst augmentačního indexu jak ve 3. tak v 6. měsíci. AI byl signifikantně vyšší u kuřáků a pacientů s ischemickou etiologií srdečního selhání. U skupiny pacientů s HM II byla dynamika RHI i AI v čase podobná jako u HM 3, celkové hodnoty však byly vyšší.

Práce přináší další evidenci o zhoršení vaskulární funkce po implantaci jakékoliv mechanické podpory s kontinuálním průtokem, stejně tak jako o přítomnosti endoteliální dysfunkce u pacientů se srdečním selháním. Zjištění popisují pravděpodobné zhoršení endoteliální funkce u pacientů po implantaci LVAD HM3, což je v rozporu s primární hypotézou, že arteficiální pulsatilita této mechanické srdeční podpory má na cévní systém protektivní vliv.

Tato zjištění mohou do budoucna podporovat vývoj srdečních podpor kompatibilních s vlastní srdeční pulsatilitou namísto čerpadel generujících kontinuální tok krve bez ohledu na srdeční revoluci. Nicméně arteficiální pulzatilita zůstává důležitou součásti systému

HM3, která umožňuje pravidelné promytí čerpadla a tím zabraňuje stáze krve a snižuje riziko trombózy. S tímto cílem byla pulzatilita do systému aplikována.

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Clinical Research

The Effect of Artificial Pulsatility on the Peripheral Vasculature in Patients With Continuous-Flow Ventricular Assist Devices

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ABSTRACT

Background: Implantation of left-ventricular assist systems (LVASs) has become the standard of care for advanced heart failure (HF). The absence of pulsatility in previous devices contributes to vascular and endothelial dysfunction related to atherosclerotic or vascular complications. We hypothesized that the artificial pulsatility provided by the HeartMate 3 (HM3) (Abbott, Chicago, IL) LVAS would exert a favourable effect on the vasculature.

Methods: In 32 patients implanted with HM3 (5 female patients, mean age 55 ± 13.6 years), the reactive hyperemia index (RHI) and peripheral augmentation index (AI), markers of endothelial function and arterial stiffness, were measured with an EndoPAT2000 before and in the third and sixth month after implantation. RHI and AI data from 30 HeartMate II (HM II) (Abbott) recipients in the third and sixth month after implantation, from 15 patients with advanced HF without LVASs and from 13 healthy volunteers were also analyzed.

Results: In HM3 recipients, the mean RHI significantly decreased at 3 and 6 months after implantation. The RHI was substantially lower at

RÉSUMÉ

Contexte : L'implantation de dispositifs d'assistance ventriculaire gauche (DAVG) est devenue un standard des soins pour une insuffisance cardiaque (IC) avancée. L'absence de pulsatilité dans les dispositifs précédents contribue à la dysfonction vasculaire et endothéliale liée aux complications athérosclérotiques ou vasculaires. Nous avons émis l'hypothèse que la pulsatilité artificielle fournie par le DAVG HeartMate 3 (HM3) (Abbott, Chicago, IL) entrainerait un effet bénéfique sur le système vasculaire.

Méthodes : Chez 32 patients implantés avec le HM3 (5 femmes, âge moyen 55 ± 13,6 ans), l'indice d'hyperémie réactive (IHR) et l'indice d'amplification périphérique (IAP), marqueurs de la fonction endothéliale et de la rigidité artérielle, ont été mesurés par un EndoPAT2000 avant puis trois et six mois après implantation. Les données IHR et IAP de 30 patients porteurs de HeartMate II (HM II) (Abbott) au troisième et sixième mois post-implantation, de 15 patients atteints d'une IC avancée sans DAVG et de 13 volontaires sains ont également été analysées.

Implantation of left-ventricular assist systems (LVASs) has become a part of the standard of care for advanced heart failure.[1,2] The nearly complete transition to continuous-flow LVASs has enabled both reduced size and improved durability of the devices but at the cost of attenuated pulsatility, with a potential for negative impact on end-organ function and vasculature. The nonpulsatile flow produced by these systems could contribute to the compromise of vascular functional properties.[3-5] Mechanistically, unfavourable vascular effects could occur through dysfunction of the endothelium exposed to a diminished pulsatile pattern. The endothelial dysfunction aggravates thrombotic, proinflammatory, and proliferative mechanisms, resulting in vasospasm, thrombosis, and atherosclerosis, in general.[6,7] Therefore, endothelial dysfunction potentiated by continuous flow could contribute to LVAS-associated clinical complications such as gastrointestinal bleeding, hypertension and stroke.[3,7-9] To mitigate these unfavourable effects, rotor-speed modulation to provide more normal pulsatile perfusion has been introduced.[10-13]

The HeartMate 3 (HM3) (Abbott, Chicago, IL) LVAS is a recent compact, fully magnetically levitated centrifugal-flow

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baseline than that of healthy or the HF reference group. Increasing AI values, indicating worsening arterial stiffness, were also observed. Similar trends were observed in HM II recipients between the third and sixth months but with higher absolute values of RHI and AI.

Conclusions: We detected impaired vascular function in HM3 patients and provided additional evidence on the negative effect of low pulsatility on vascular function after LVAS implantation. The results suggest that the artificial pulsatility of the HM3 does not avert the progression of endothelial dysfunction.

Résultats : Chez les receveurs du HM3, l'IHR moyen a significativement diminué trois et six mois après implantation. L'IHR basal était sensiblement plus faible que celui des personnes en bonne santé ou du groupe IC de référence. Des valeurs croissantes de l'IAP, indiquant une exacerbation de la rigidité artérielle, ont également été observées. Des tendances similaires ont été constatées chez les receveurs du HM II entre le troisième et le sixième mois, mais avec des valeurs absolues de l'IHR et de l'IAP plus élevées.

Conclusions : Nous avons décelé une fonction vasculaire altérée chez les patients porteurs du HM3 et fourni des éléments supplémentaires de l'effet négatif d'une faible pulsatilité sur la fonction vasculaire après l'implantation d'un DAVG. Les résultats suggèrent que la pulsatilité artificielle du HM3 n'empêche pas la progression de la dysfonction endothéliale.

continuous ventricular-assist device. The key novel features of the device—including a magnetically levitated rotor, wide blood-flow path gaps, and artificial pulsatility that allows periodic washout of the device—may lead to decreased rates of major adverse events. Indeed, in a large clinical study, a favourable clinical effect of HM3 was observed compared with a completely nonpulsatile device.[14] The pathophysiological mechanisms responsible for these findings are not fully understood. Hence, the programmed fixed artificial pulsatility in HM3 generated by rapid rotor-speed modulation has attracted additional interest as a potential modifier of the pump flow−microvasculature interaction. Therefore, we hypothesized that the detrimental impact of deficiency of pulsatility on the vasculature might potentially be mitigated by the artificial pulsatility provided by the HM3. To analyze endothelial function, peripheral arterial tonometry was performed with an operator-independent proprietary analyzer EndoPAT2000 (Itamar Medical Ltd, Caesarea, Israel) to evaluate pulsatile arterial volume changes.[15,16]

Materials and Methods

The study was a single-centre prospective observational study. Institutional ethics committee approval of the protocol was obtained before initiation of the study. All patients provided written informed consent before enrollment in the study.

Study group

The study was designed to assess peripheral vascular function in the HeartMate 3 LVAS recipients before implantation and in the third and sixth month after the procedure. Consecutive patients eligible for long-term LVAS support between April 2016 and January 2018 were assessed for participation in the study. The exclusion criteria included age < 18 or > 75 years and acute hemodynamic instability requiring high doses of inotropes or short-term mechanical circulatory support before LVAS implantation.

Baseline characteristics, medical history, laboratory assessments, and medications were collected before and at prespecified time points after implantation. Laboratory parameters were assessed by standard certified institutional laboratory methods.

After implantation, heparin was continuously administered intravenously as a bridge until the target anticoagulation range was reached with warfarin. The aim of anticoagulation therapy was to reach an international normalized ratio (INR) of 2 to 2.5 for the HeartMate II (HM II) (Abbott) and 2.0 to 2.7 for the HM3. Acetylsalicylic acid was administered at a dose of 100 mg per day as a part of an antithrombotic regimen in patients implanted with HM3.

Reference groups

For reference groups, we included patients implanted with the HeartMate II and examined at the third and sixth month after implantation (HM II reference group); patients with advanced HF (New York Heart Association [NYHA] III-IV) who were not (yet) indicated for implantation of LVAS (HF reference group) and group of healthy subjects without any clinically manifested disease (healthy reference group). All participants were examined by an identical protocol, described as follows; advanced HF and healthy reference groups were examined once each.

Examination of vascular function

For the assessment of vascular function, peripheral arterial tonometry was performed with a proprietary analyzer-peripheral arterial tonometry (EndoPAT2000) that evaluated pulsatile arterial volume changes.[15,16] This is an operator-independent, FDA-approved device designed to assess endothelial function by examining the reactive hyperemia index (RHI, a measure of endothelial responsiveness) and peripheral augmentation index (AI, a measure of arterial stiffness).[17-19] RHI and AI were assessed before LVAS implantation and in the third and sixth month after the procedure (± 15 days). The third month for the first follow-up visit was chosen to avoid the effect of complex hemodynamic changes present immediately after implantation.[20] The method has been described in detail previously.[15,16] Briefly, the system uses a finger probe to assess digital volume changes accompanying pulse waves. The examination was performed in all patients in the morning hours in a quiet, temperature-controlled room. All subjects were examined after 5 minutes of rest in the supine position. The baseline pulse amplitude was recorded over a period of 5 minutes before the induction of ischemia. Ischemia was induced by placing a blood-pressure cuff on the upper arm, whereas the opposite arm served as a control. The peripheral arterial tone probes were placed on 1 finger of each hand. After 5 minutes, the blood pressure cuff was inflated to

200 mm Hg for 5 minutes and then deflated to induce reactive hyperemia. RHI and AI were calculated using a computerized automated algorithm (software version 3.1.2) provided with the device. RHI is the ratio of postocclusion to preocclusion PAT signals on the occluded side, normalized to the control side and further corrected for baseline vascular tone. AI is calculated from PAT pulses recorded during the baseline period. Lower AI values (including values below zero) reflect better arterial elasticity.[19]

Statistical analysis

Continuous data with a normal distribution are presented as the mean ± standard deviation, and non-normally distributed variables are presented as the median (interquartile range [IQR]). Categorical data are presented as frequencies (percent). The Spearman correlation coefficient was used to evaluate the association among variables. Differences among groups were tested using the Mann-Whitney test. To account for correlation in the same patients, we used generalized linear mixed models to study the longitudinal trajectories of study variables. In these models, time, age group, and the interaction between time and age group were analyzed as fixed effects, whereas the intercept was treated as a random effect. Gamma regression was used for right-skewed data. The model-derived estimated marginal means with 95% confidence intervals (CIs) are reported, whereas the estimated marginal means and standard error of the mean are shown in graphs. The Bonferroni correction was applied for multiple comparisons. A 2-sided P value < 0.05 was considered statistically significant. Calculations were performed using SPSS version 21 (IBM SPSS Statistics, IBM Corporation, Armonk, NY).

Results

Patients

Fifty-one LVAD candidates were assessed for study enrollment and underwent screening examination. A total of 32 patients (all white, 5 women), aged 19 to 71 years (mean age 55 ± 13.6 years), were enrolled and completed the study (Fig. 1). Patients were implanted with the HM3 between April 2016 and January 2018, via either sternotomy (n = 27; 84%) or left- lateral thoracotomy, combined with upper partial hemisternotomy (n = 5; 16%), both using cardiopulmonary bypass. In the HM3 group, the subjects were predominantly male (n = 27, 84 %) and had a high prevalence of smoking before implantation; ischemic etiology of heart failure was present in less than one-half of the patients (44%); the predominant indication for implantation was bridge to transplant (n = 21; 66%), and **Inter**agency **R**egistry for **M**echanically **A**ssisted **C**irculatory **S**upport (INTERMACS)

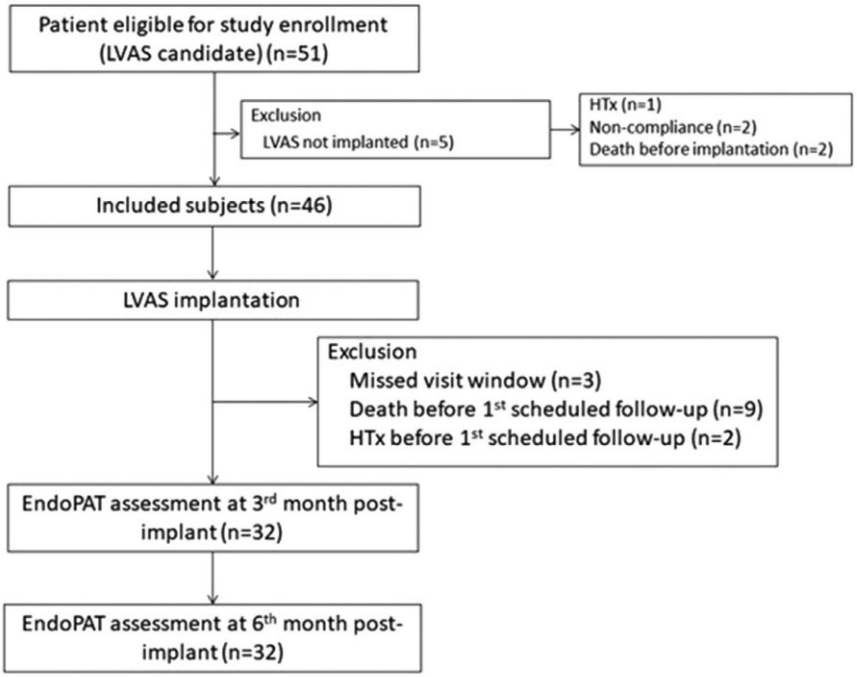

Figure 1. **Con**solidated **S**tandards **o**f **R**eporting **T**rials (CONSORT) diagram of the study. HTx, heart transplantation; LVAS, left-ventricular assist system.

Table 1. Demographics and baseline characteristics of the HM3 group

Age	55 ± 13.6
Male	27 (84)
BSA, m^2	2 ± 0.2
BMI, kg/m^2	26.5 ± 4.6
Ischemic etiology	14 (44)
Indication	
Bridge to transplant	21
Destination therapy	11
INTERMACS profile	
Profile 2	5 (16)
Profile 3	16 (50)
Profile 4	8 (25)
Profile 5	3 (9)
Cardiac index, $L/min/m^2$	1.7 ± 0.6
Left ventricular ejection fraction, %	22 ± 4
Medications	
ACE inhibitor	12 (38)
Angiotensin II antagonist	3 (9)
β blocker	22 (69)
Anticoagulant/antiplatelet drug	27 (84)
Antiarrhythmic drug	12 (38)
Statins	18 (56)
Diuretics	32 (100)
Inotropes	
1	15 (47)
2	5 (16)
Hypertension	16 (50)
Diabetes	7 (22)
Previous sternotomy	3 (9)
Minimally invasive approach	5 (16)
Cardiomyopathy for more than 2 years	26 (81)
Severe COPD	2 (6)
TIA	2 (6)
Stroke	2 (6)
Renal dysfunction	7 (22)
Atrial fibrillation	15 (47)
Pacemaker/defibrillator	24 (75)
Valve disease	16 (50)
Peripheral vascular disease	1 (3)
Smoking within the past 3 months	6 (19)

Values are expressed as the number (%) or mean ± standard deviation.

ACE, angiotensin-converting enzyme; BMI, body mass index; BSA, body surface area; COPD, chronic obstructive pulmonary disease; INR, international normalized ratio; INTERMACS, **Inter**agency **R**egistry for **M**echanically **A**ssisted **C**irculatory **S**upport; TIA, transient ischemic attack.

profiles were indicative of advanced heart failure, with the majority in profiles 2 to 4 (91%) (Table 1).

Routine biomarkers, such as brain natriuretic peptide, plasma creatinine, urea, total bilirubin, and ALT or AST, decreased significantly after LVAS implantation; mean pump flow and speed, pulse index and pump power were stable throughout the study (Table 2).

HM3 patients were older than HM II reference but similar in age to the HF reference group. No intergroup differences were found in the proportion of women or in smoking history, diabetes mellitus status, or renal function. In the healthy reference group, less smoking and no diabetes mellitus were reported (Table 3).

Peripheral vascular function and stiffness

At baseline, the mean RHI of HM3 group was below normal values for a healthy population (RHI = 1.36; 95% CI, 1.21-1.52; cutoff ≤ 1.67 indicative of endothelial dysfunction) and lower than that of healthy reference group (Table 3); RHI further decreased in the third month (RHI = 0.89; 95% CI, 0.80-1.00) as well as in the sixth month after implantation (RHI = 0.94; 95% CI, 0.84-1.06) compared with baseline (both $P < 0.0001$) (Fig. 2, Table 3). The difference between the third and sixth month after implantation was not significant ($P = 0.44$). Significant interaction between age and RHI ($P = 0.023$) in the HM3 group was observed. RHI was higher in subjects ≤ 60 years of age (group median) than in those > 60 years both at baseline ($P = 0.030$) and in the third month ($P = 0.034$). The difference became nonsignificant in the sixth month after implantation ($P = 0.60$) (Fig. 3). In the sixth month, higher pump flow was associated with lower RHI in a cross-sectional analysis ($P = 0.021$). Higher baseline body mass index (BMI) was predictive of a greater RHI decrease in the sixth month ($P = 0.043$). No association was found among the etiology of cardiomyopathy, history of smoking, and endothelial dysfunction ($P > 0.05$ for all interactions). In the HM II reference group, a similar pattern of changes in RHI was observed between the third and sixth month, with RHI values higher than those of the HM3 group. No associations between pump parameters and RHI/AI were observed in the HM II reference group.

In HM3 group, AI significantly increased relative to the baseline value (−32.28; 95% CI, −42.49 to −22.07) at the third (−3.56; 95% CI, −15.34 to 8.23) and the sixth month (−0.62; 95% CI, −11.94 to 10.70) (both $P < 0.001$) (Fig. 4). Differences between the third and sixth month were not significant ($P = 0.72$). At baseline, AI was significantly higher in patients with histories of smoking and ischemic etiology of heart failure ($P = 0.012$ and $P = 0.046$, respectively). AI had no interactions with age or pump parameters.

No significant association between aortic valve opening and RHI (in the third and sixth month) or AI (in the third month) was observed. In the sixth month, aortic-valve opening was associated with a lower (improved) AI in patients with aortic-valve opening within each cardiac cycle than in patients without aortic-valve opening ($P = 0.03$).

In the third month, patients in HM3 group had significantly lower RHI values than in HM II, HF, or the healthy reference groups. The AI in HM3 group was significantly lower than that of HM II or healthy reference groups but higher than that of the HF reference group (Fig. 3). In the HM II reference group, similar longitudinal changes in AI were found, but AI was significantly higher than in the HM3 group. The HF reference group had a higher AI than HM3 group at baseline but a lower AI after 3 and 6 months. The AI of the healthy reference group was higher than that of the HF reference group or the HM3 group but lower than that of the HM II reference group.

Clinical events

In the 6 months after implantation, the HM3 group experienced no fatal clinical events (Fig. 1). Nonfatal events (hemocompatibility adverse events including hemorrhage, thrombosis, ischemic stroke, and hemorrhagic stroke) were observed as follows: 2 ischemic strokes and 1 gastrointestinal bleeding. The low incidence of clinical adverse events precluded analysis relating to RHI and AI.

Table 2. Changes in laboratory and pump characteristics in HM3 group during the course of the study

	Baseline	Third month	Sixth month	*P*1	*P*2	*P*3
Reactive hyperemia index	1.36 (1.21-1.52)	0.89 (0.80-1.00)	0.94 (0.84-1.06)	< 0.0001	0.44	< 0.0001
Augmentation index	−32.28 (−42.49 to −22.07)	−3.56 (−15.34 to 8.23)	−0.62 (−11.94 to 10.70)	< 0.001	0.72	< 0.001
Brain natriuretic peptide (ng/L)	1655 (1232-2223)	428 (318-574)	378 (286-500)	< 0.0001	0.38	< 0.0001
Lactate dehydrogenase (μkat/L)	4.24 (3.68-4.90)	3.69 (3.22-4.23)	3.66 (3.19-4.19)	0.04	0.86	0.04
Hemoglobin (g/L)	117.7 (110.9-124.5)	116.3 (109.5-123.0)	123.7 (116.9-130.5)	0.74	0.28	
Creatinine (μmol/l)	107.9 (96.4-120.8)	84.3 (75.3-94.4)	94.5 (84.4-105.8)	< 0.0001	0.03	0.03
Urea (mmol/L)	10.0 (8.4-12.0)	7.1 (6.0-8.4)	7.5 (6.3-8.9)	0.001	0.54	0.005
Total bilirubin (μmol/L)	31.1 (24.4-37.8)	13.8 (10.3-17.3)	15.7 (12-19.5)	< 0001	0.16	< 0001
Alanine aminotransferase (μkat/L)	1.63 (1.09-2.45)	0.50 (0.34-0.75)	0.56 (0.37-0.83)	0.003	0.71	0.004
Aspartate aminotransferase (μkat/L)	1.03 (0.75-1.40)	0.50 (0.37-0.68)	0.52 (0.38-0.71)	0.003	0.84	0.003
Total cholesterol (mmol/L)	3.4 (3.0-3.8)	4.6 (3.8-5.3)	4.4 (3.8-5.1)	0.03	0.77	0.03
C-reactive protein (mg/L)	32.7 (14.7-50.7)	20.2 (6.9-33.4)	15.88.1-23.6)	0.96	0.51	0.12
White blood cell counts (× 10^9/L)	8.2 (7.3-9.4)	8.3 (7.3-9.4)	7.9 (6.9-8.9)	1.00	1.00	1.00
Pump parameters						
Pump speed (RPM)	NA	5284 (5220-5349)	5291 (5224-5357)	NA	0.79	NA
Pump flow (LPM)	NA	4.1 (3.9-4.3)	4.1 (3.9-4.3)	NA	0.43	NA
Pulse index	NA	4.2 (3.7-4.6)	4.4 (3.9-4.9)	NA	0.27	NA
Pump power (W)	NA	3.8 (3.7-4.0)	3.8 (3.7-4.0)	NA	0.75	NA

Estimated marginal means and 95% confidence intervals are presented. *P*1 difference between baseline and third month; *P*2 difference between third and sixth month; *P*3 difference between baseline and sixth month.
NA, not applicable.

Table 3. Clinical characteristics of the HM3 group and reference groups

	HM3 (n = 32)(Third month)	HM II reference group(n = 30) (Third month)	HF reference group(n = 15)	Healthy reference group (n = 13)
Age, years	55.1 ± 13.8	48.4 ± 15.38	51.2 ± 9.17	50.1 ± 13.52
Sex (male), n (%)	27 (84)	25 (83)	12 (80)	8 (61.5)
(Past) smokers, n (%)	24 (75)	22 (73)	9 (60)	3 (23)*
Diabetes mellitus n (%)	7 (22)	8 (27)	5 (33)	0‡
Ischemic cardiomyopathy n (%)	14 (44)	12 (40)	8 (53)	0
Creatinine, μmol/L	84.3 (75.3-94.4)	97.4 (71.9-123.0)‡	103.6 (89.2-118.1)‡	NA
Urea, mmol/L	7.1 (6.0-8.4)	7.7 (5.4-10.0)	8.7 (7.0-10.4)	NA
Hemoglobin, g/L	116.3 (109.5-123.0)	125 (118.1-131.9)‡	139.8 (129.0-150.6)‡	143.1 (138.6−147.6)‡
Reactive hyperemia index	0.89 ± 0.34	1.50 ± 0.68‡	1.6 ± 0.30‡	1.77 ± 0.54‡
Augmentation index	−3.56 ± 27.12	23.5 ± 22.48‡	−16.53 ± 26.77	6.9 ± 11.45

HF, heart failure; HM3, HeartMate 3 (Abbott, Chicago, IL); HM II, HeartMate II (Abbott); NA, not available.
*$P < 0.05$.
†$P < 0.005$.
‡$P < 0.0001$ vs HM3.

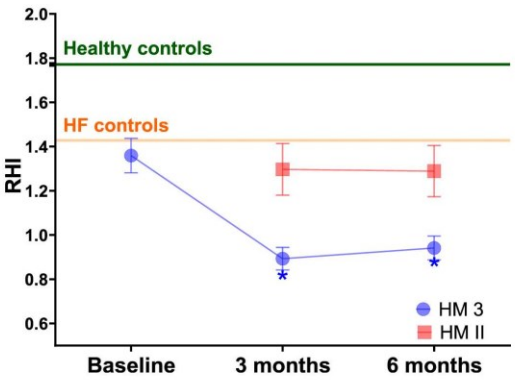

Figure 2. Longitudinal changes in the reactive hyperemia index (RHI) during the study period in HeartMate 3 (HM3) (Abbott, Chicago, IL) group (**blue line**). Longitudinal changes in RHI in HeartMate II (HM II) (Abbott) reference group between the third and sixth months (**red**). Data are presented as estimated marginal means and standard error. *$P < 0.05$ vs baseline. **Solid orange line**, estimated marginal mean for heart failure (HF) reference group; **solid green line**, estimated marginal mean for healthy reference group. **Dashed lines** represent 95% confidence intervals.

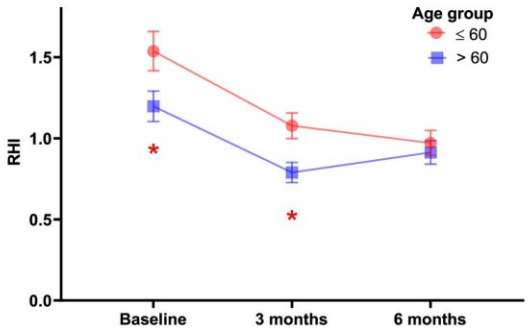

Figure 3. Longitudinal changes in the reactive hyperemia index (RHI) by age group: ≤ 60 vs > 60 years of age. Data are presented as estimated marginal means and standard errors. Statistically significant differences in RHI at baseline and 3 months at the $P < 0.05$ level are marked by **asterisks**.

Discussion

We describe worsened vascular function in patients after HM3 implantation during the 6- month follow-up. The observations do not support our primary hypothesis about the potentially favourable effect of the artificial pulsatility of the HM3 on vascular function measured as the RHI. RHI significantly decreased after HM3 implantation and remained low in the sixth month. RHI was below physiological values at baseline, reflecting the endothelial dysfunction associated with advanced heart failure.[7] Increased arterial stiffness (decreased AI) in the HM3 group after implantation further supports presence of unfavourable vascular changes. Previous reports indicated that continuous-flow LVAS implantation could impair vascular function.[3,20-23] An association between impaired vascular function and occurrence of cardiovascular adverse events was also observed.[3,22] Mechanistic explanations come from experimental studies demonstrating that endothelial function is dependent on mechanical stimulation.[24-26] In this context, our results do not support the hypothesis that the artificial pulsatility produced by the HM3 attenuates unfavourable vascular processes. Instead, these findings confirm that the favourable clinical outcomes of HM3 are mediated by original purpose of artificial pulsatility: to avert blood stasis and enable pump washout.

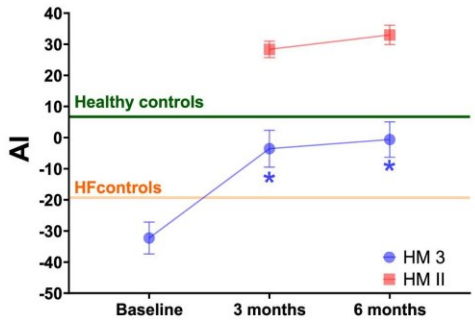

Figure 4. Longitudinal changes in the augmentation index (AI) in HeartMate 3 (HM3) (Abbott, Chicago, IL) group (**blue**). Longitudinal changes in AI in HeartMate II (HM II) (Abbott) reference group between the third and sixth months (**red**). Data are presented as estimated marginal means and standard error. *$P < 0.05$ vs baseline. **Solid orange line**, estimated marginal mean for heart failure (HF) reference group; **solid green line**, estimated marginal mean for healthy reference group. **Dashed lines** represent 95% confidence intervals.

In the HM II reference group, identical trends for RHI and AI were observed, but with higher values of RHI, indicating less severe vascular dysfunction. This finding could be explained by the younger age of HM II recipients (Table 3). As expected, the RHI in HF reference group (measured once) was similar to the baseline values of HM3 group. In the healthy reference group, the RHI was in the physiological range and thus substantially higher than that of any of the other groups. On the other hand, we detected increased arterial stiffness in the HM II reference group compared with the HM3 group. AI was even higher in the healthy reference group than in the HM3 group or the HF reference group, indicating higher arterial stiffness. We suppose that these results were modified by technical limits reflecting the combined effect of abnormal pulsatility and abnormal cardiac output, the latter supported by AI increase in the HM3 group early after implantation. Indirect evidence for potential modification of measurements of arterial stiffness comes from a study of cardiac patients and healthy subjects, in which the AI was determined by chronotropic rather than inotropic effects, thus by factors other than wave reflection.[27]

Therefore, interpretation of the AI data needs to be made with caution. Nevertheless, in our study, the baseline values of the HM3 group were similar to that of the HF reference group. Increase of arterial stiffness in the HM3 group during study period reached higher values than that observed in HF reference group. This finding supports the concept, that HM3 pulsatility does not modify the vascular properties favourably.

Further subanalysis showed that the alterations in RHI were negatively affected by age, BMI, and pump flow in the sixth month. These findings may indicate role of these parameters in the progression of peripheral vascular dysfunction after LVAS implantation. Through the course of the study, the RHI values at baseline and in the third month were influenced by the median age of the patients. The differences were not present in the sixth month after implantation, probably caused by the prolonged influence of the low pulsatility, possibly linked to accelerated processes associated with vascular

aging, even in younger patients.[28] A subanalysis of factors related to pulsatility showed that aortic-valve opening with each heartbeat favourably influenced arterial stiffness in the sixth month. This observation may indicate a favourable effect of pulsatility, in general; this is further supported by a study by Patel et al., describing the difference in aortic strain, distensibility, and stiffness between pulsatile and continuous flow LVASs, favouring the LVASs with pulse.[29]

Limitations

Limitations of this single-centre prospective observational study include moderate number of patients and use of EndoPAT 2000 to measure arterial stiffness. The measurement of arterial stiffness using the EndoPAT2000 is currently used mostly for research purposes. Nevertheless, although AI could be altered also by nonvascular factors, making this parameter less reliable for arterial stiffness assessment, especially in LVAS recipients, RHI was a reliable method to measure vascular function in a broad range of subjects in the study. Also, the data from early postoperative period were not collected and analyzed to avoid the effect of complex hemodynamic changes present immediately after implantation.[20] In addition, only the devices from 1 manufacturer were analyzed, as the devices form other manufacturers (eg, Medtronic HeartWare HVAD) are rarely implanted at our site.

We are well aware of some limitations of our study discussed here. Nevertheless, to the best of our knowledge, only sparse data are available from the longitudinal assessment of endothelial function in LVAS patients. This is the first study in a relatively large population of LVAS recipients that analyzed effects of artificial pulsatility of HM3 on vascular properties. Therefore, this study provides additional evidence that suppressed pulsatility exerts unfavourable effects on peripheral vascular function. Further research focused on a pulse amplitude augmentation and synchronization with native cardiac cycle, and its potential implementation in future devices could provide positive effect on vascular function and thus positively influence the clinical outcomes.

Conclusions

Despite the restoration of central hemodynamics, peripheral vascular function was further compromised 6 months after HM3 implantation. The feature of artificial pulsatility, which enhances blood flow washout, may contribute to a significant improvement in clinical outcomes in HM3 recipients than in other clinically available LVASs.[14] Nonetheless, our observations suggest that the intensity of the artificial pulse wave may not represent a physiologically relevant stimulus that averts endothelial dysregulation in continuous-flow LVAD circulation.

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PRÁCE C je věnována posouzení vlivu kontinuálního toku krve generovaného současnými levostrannými mechanickými srdečními podporami na hladiny mikro RNA (miRNA) v krevní plasmě.

Ztráta pulsatility v důsledku terapie LVAD má potencionálně negativní vliv na vaskulární systém a konkrétně na funkci endotelu, což může vést k endoteliální dysregulaci a dysfunkci. Tyto změny mohou vést ke změnám hladin cirkulujících biomarkerů s poškozením, nebo reparací endotelu. MiRNA jsou krátké nekódující úseky RNA zahrnuté do post-transkripční regulace genové exprese. Změny v expresi miRNA mohou potencionálně ovlivňovat funkci endoteliálních buněk a hladkých svalových buněk cév, což může následně vést k zánětu a progresi aterosklerotických změn. Přítomnost miRNA v krevní plasmě naznačuje, že miRNA může plnit funkci biomarkerů vaskulárního poškození.

Do této monocentrické prospektivní studie bylo zařazeno 33 pacientů (5 žen, průměrný věk 55.7 let) s implantovanou levostrannou mechanickou srdeční podporou v indikaci jak most k transplantaci, tak destinační terapie. 14 pacientů mělo implantovanou starší srdeční podporu axiálního typu HeartMateII a 19 pacientů novější centrifugální pumpu HeartMate3 (oba typy Abbott, Abbott Park, Illionois, USA). Všichni pacienti byli implantováni mezi lety 2015 a 2018 v pražském Institutu klinické a experimentální medicíny. Pacientům byly odebírány vzorky krevní plasmy před implantací a dále 3, 6, 9 a 12 měsíců po implantaci MSP. RNA byla následně extrahována ze vzorků krevní plasmy pomocí miRKURY^{TM} RNA isolačního kitu (Exiqon, Vedbaek, Denmark), následné kvantitativní PCR bylo provedeno za použití systému ABI 7300.

Výsledky měření ukázaly, že hladiny miRNA v krevní plasmě postupně vzrůstají mezi jednotlivými kontrolami. Dále byla pozorována positivní asociace mezi specifickým typem miRNA (miR-126) a Belcaro score (skore určující stupeň aterosklerózy) a naopak inverzní korelace mezi podtypem miR-126 a endoteliální funkcí. Pozorování tedy naznačují, že nepulsatilní tok krve v cévním řečišti je provázen zvýšením hladin miRNA v krvi ve spojitosti s endoteliální dysfunkcí a remodelací cév. Další fyziologický nebo klinický dopad bude možné posoudit v navazujících studiích.

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The effect of long-term left ventricular assist device support on flow-sensitive plasma microRNA levels

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ABSTRACT

Background: Implantation of current generation left ventricular assist devices (LVADs) in the treatment of end-stage heart failure (HF), not only improves HF symptoms and end-organ perfusion, but also leads to cellular and molecular responses, presumably in response to the continuous flow generated by these devices. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression in multiple biological processes, including the pathogenesis of HF. In our study, we examined the influence of long-term LVAD support on changes in flow-sensitive miRNAs in plasma.

Materials and methods: Blood samples from patients with end-stage heart failure ($N = 33$; age $= 55.7 \pm 11.6$ years) were collected before LVAD implantation and 3, 6, 9, and 12 months after implantation. Plasma levels of the flow-sensitive miRNAs; miR-10a, miR-10b, miR-146a, miR-146b, miR-663a, miR-663b, miR-21, miR-155, and miR-126 were measured using quantitative PCR.

Results: Increasing quantities of miR-126 ($P < 0.03$) and miR-146a ($P < 0.02$) was observed at each follow-up visit after LVAD implantation. A positive association between miR-155 and Belcaro score ($P < 0.04$) and an inverse correlation between miR-126 and endothelial function, measured as the reactive hyperemia index ($P < 0.05$), was observed.

Conclusions: Our observations suggest that after LVAD implantation, low pulsatile flow up-regulates plasma levels of circulating flow-sensitive miRNAs, contributing to endothelial dysfunction and vascular remodeling.

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1. Introduction

Left ventricular assist devices (LVADs) have become routine in the treatment of end-stage heart failure [1]. Technical developments introduced in the past decades have led to an almost complete transition towards continuous flow (CF) devices, with improved durability and smaller size. The cost of these improvements lies in the reduction of pulsatility and its potential negative impact on end-organ function, vasculature, and in particular endothelial cells (ECs). Attenuation of physiological pulsatile flow in CF-LVAD patients was previously revealed as an additive factor in the progression of endothelial dysregulation and dysfunction. [2,3]. Additionally, increased stress on the vascular wall may lead to changes in the levels of circulating endothelial progenitor cells and particles, which reflects vascular damage [4,5]. Moreover, as vascular ECs are exposed to non-physiological hemodynamic forces, their function may become modulated and could lead to damage of

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; AST, aspartate aminotransferase; BMI, body mass index; BNP, brain natriuretic peptide; cDNA, complementary deoxyribonucleic acid; CF, continuous flow; CI, cardiac index; CRP, C-reactive protein; DBP, diastolic blood pressure; ECs, endothelial cells; ERK, extracellular signal-regulated kinases; FDA, Food and Drug Administration; FU, follow-up; HF, heart failure; HUVECs, Human umbilical vein endothelial cells; INR, international normalized ratio; INTERMACS, interagency registry for mechanically assisted circulatory support; IQR, interquartile range; LDH, lactate dehydrogenase; LVAD, left ventricular assist device; MAPK, mitogen-activated protein kinases; miRNA, microRNA; NYHA, New York Heart Association functional class; OMT, optimal medical treatment; PCR, polymerase chain reaction; RHI, reactive hyperemia index; RNA, ribonucleic acid; SBP, systolic blood pressure; SD, standard deviation,; SMCs, vascular smooth muscle cells; SPRY1, Sprouty RTK Signaling Antagonist 1; ST, storage time.

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the arterial wall which could be critical if, for example, is present in the coronary arteries [6].

MicroRNAs (miRNAs) are short non-coding RNAs (18–24 nt) involved in sequence-specific post-transcriptional regulation of gene expression. MiRNAs have emerged as key players in a wide array of biological processes [7–11]. Changes in tissue miRNA expression levels due to blood flow have the potential to affect networks of genes regulating endothelial and vascular smooth muscle cell (SMC) function, inflammation, and atherosclerosis [12].

The presence of miRNAs in blood suggests that circulating miRNAs may function as important biomarkers of various disease development and progression. MiRNAs are released from cells either through active transport via extracellular vesicles or as part of protein-miRNA complexes. Moreover, passive release from damaged cells, e.g., after injury or during inflammatory, apoptotic or necrotic processes, may occur. Circulating miRNAs predominantly serve as signaling molecules [13].

The exact pathophysiological mechanisms and impact of flow generated by LVADs on blood and vasculature are not clear but should be better understood in order to manage or even prevent potential future clinical adverse events. The use of LVADs offers a great opportunity for a complex understanding of vascular changes in patients with different blood flow patterns. The key signaling pathways that are targeted by mechano-miRNAs include the endothelial cell cycle, inflammation, apoptosis, and nitric oxide signaling. Emerging evidence indicates that alteration of flow conditions regulates the expression of miRNAs in endothelial cells both in vitro and in vivo [12].

This study aimed to investigate the influence of long-term left ventricular assist devices on circulating flow-sensitive miRNAs. We selected nine flow-sensitive miRNAs believed to participate in vascular remodeling (miR-146a, miR-146b, miR-155, miR-21) or endothelial dysfunction (miR-126, miR-10a, miR-10b, miR-663a, miR-663b). Additionally, to detect possible alterations in endothelial function and/or atherogenic changes and correlate them with miRNA changes, the subjects also underwent both carotid artery ultrasonography and pulse amplitude tonometry in the measurement of endothelial dysfunction.

2. Materials and methods

The protocol of this study was carried out according to the principles of the Declaration of Helsinki. The study was designed as a single-center, prospective observational study. Institutional ethics committee approval of the protocol was obtained. All patients provided written informed consent before enrollment in the study.

2.1. Design and study population

A total of 33 patients (females = 5; age = 55.7 ± 11.6 years) implanted with a LVAD, irrespective of the intended goal of treatment (bridge to transplantation or destination therapy), from May 2015 to July 2018 at the Institute for Clinical and Experimental Medicine in Prague were enrolled in the study. All patients were diagnosed with end-stage heart failure, met institutional criteria for LVAD implantation, and were implanted with the continuous flow axial ventricular assist device HeartMate II ($n = 14$) or with the centrifugal, fully magnetically levitated ventricular assist device HeartMate 3 ($n = 19$) (both designed by Abbott, Abbott Park, Illinois, USA or St. Jude Medical, Pleasanton, California).

Blood samples were collected before LVAD implantation and at follow-up (FU) time points 3, 6, 9, and 12 months after the implantation. Baseline characteristics, medical history, laboratory assessments, and medications were collected before LVAD implantation. Laboratory assessments, medications, device parameters, and clinical outcomes were captured over the course of the study at the defined FU times. Control subjects comprised healthy volunteers ($N = 13$; females = 38.5%; age = 50.1 ± 13.5 years) and NYHA III heart failure patients ($N = 12$; age = 52.3 ± 7.2 years) who were on optimal medical treatment (OMT). Blood samples were collected and analyzed only once from control subjects. Morphological and functional properties of the vascular system were also assessed (see below).

2.2. Plasma samples and miRNA measurements

Blood samples (10 ml) were collected in EDTA tubes and centrifuged at 1500 *xg* for 15 min at room temperature. Plasma samples were then processed within 30 min of blood collection, aliquoted into RNase-free tubes, and stored at −80 °C before RNA extraction.

Total RNA was extracted from 200 μl of plasma using the miRCURY™ RNA isolation kit for biofluids (Exiqon, Vedbaek, Denmark) as previously reported [14,15]. SYBR green-based real-time quantitative PCR was performed using an ABI 7300 system. Passive Reference Dye (ROX™ 500 nm) was included for all PCRs. Interplate calibrators and spike-in controls were included in each analysis to ensure the quality of RNA isolation, the cDNA synthesis reaction, and the PCR.

2.3. Endothelial function and atherosclerotic changes assessment

Peripheral vascular function and arterial stiffness were assessed using the EndoPAT 2000 (Itamar Medical Ltd., Caesarea, Israel), which is an operator-independent, FDA-approved proprietary analyzer designed to assess endothelial function by examining the reactive hyperemia index (RHI, as a measure of endothelial responsiveness). The method of digital measurement of peripheral vascular function using reactive hyperemia and peripheral arterial tonometry has been described in detail previously [16]. Briefly, the system comprises of a finger probe to assess digital volume changes accompanied by pulse waves, with the reactive hyperemia index (RHI) being automatically calculated.

Assessment of atherosclerotic changes was performed by bilateral ultrasound assessment of the carotid arteries by a Toshiba APLIO 50 XV (Tochigi, Japan) ultrasound system with a 7.5–10 MHz linear array transducer. The presence of pre-clinical atherosclerosis was defined by a semiquantitative classification, the Belcaro score [17], which evaluates the degree of pre-clinical atherosclerosis based on ultrasound criteria in four classes, I-IV, where Class I contains normal findings, Class IV contains findings with plaques-causing >50% stenosis, and classes II-III containing intermediate findings. The mean value of the Belcaro score found in the carotid arteries bilaterally was used for further analyses.

2.4. Statistical analysis

Normally distributed data are presented as mean ± standard deviation. Data which were non-normally distributed are presented as median (IQR). The Shapiro-Wilk normality test was used. Mean and standard error is shown is shown in all graphs. Wilcoxon matched-paired signed-rank tests were used for biochemical and clinical parameter comparisons, with the Bonferroni correction further being applied on significance levels. Partial correlation and linear regression were used for the comparison of RHI and Belcaro scores with miRNAs concentration. GenEx SW (Multid Analysis AB, Göteborg, Sweden) was used for miRNA expression analysis. Data was converted to relative quantities and to the log2 transformations of values. To analyze longitudinal changes in miRNA levels after LVAD implantation, we utilized the generalized linear mixed model with random intercept with adjustment for storage time. The One-way analysis of variance was used to compare LVAD miRNAs levels from levels seen in healthy controls and patients with heart failure. Bonferroni adjustment was used to account for multiple comparisons. Calculations were performed using SPSS 21 software (Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

3. Results

3.1. Main characteristics

The demographics of patients are presented in Table 1. In summary, men were represented more than women, patients were on average overweight. As expected, patients were on average hypotensive, with a low ejection fraction of the left ventricle, low cardiac index, and corresponding NYHA classification. Also, INTERMACS profiles were indicative of advanced heart failure, with the majority of patients in profiles 2–4 (64%). Ischemic etiology of heart failure was present in approximately half of the patients. Moreover, a relatively high proportion (15%) of patients suffered from stroke before the beginning of the study. Further, hypertension, diabetes mellitus, dyslipidemia, and smoking were highly prevalent and extensive medical treatment covering the conditions was recorded.

Cardiac damage biomarkers, inflammation, and renal function markers were steadily improving during the FU (Table 2). Hemodynamic and LVAD parameters were captured during follow up visits (Supplementary material, Table 3). No significant interactions between miRNA levels and hemodynamic parameters were observed.

Major post-implant adverse events related to hemocompatibility occurred in 12 patients, of whom 5 suffered a stroke (one hemorrhagic, four ischemic), 3 had pump thrombosis, and one had a thromboembolic event. Bleeding events were observed in 6 patients. Individual clinical events had no reliable associations to miRNA levels.

Table 1
Baseline characteristics of LVAD patients.

	LVAD
Gender Male/Female (N)	28/5
Age (years)	55.7 ± 11.6
BMI (kg/m^2)	27.7 ± 4.9
Current smokers and ex-smokers/ non-smokers (%)	20/13 (60/40)
Hypertension (%)	17 (51.5)
Diabetes Mellitus (%)	8 (24.2)
Dyslipidemia (%)	12 (34.4)
Ischemic heart disease (%)	16 (48.5)
History of Stroke (%)	5 (15)
Type of LVAD	
Heart Mate II	14
Heart Mate 3	19
Atrial fibrillation (%)	6 (18.2)
SBP, mmHg	107.8 ± 11.6
DBP, mmHg	70.3 ± 9
Heart rate, b.p.m.	85 ± 15
Ejection fraction, %	18.9 ± 3.2
ACE-i/ARB (%)	18 (54.5)
Beta-blocker (%)	23 (69.7)
Anticoagulants (%)	28 (84.8)
Diuretics (%)	33 (100)
Antithrombotic drugs (%)	2 (6)
Calcium channel blocker (%)	3 (9)
INTERMACS profiles, *n* (%) (2/3/4/5)	7/14/7/5 (21/43/21/15)
NYHA III/ NYHA IV (%)	9/ 24 (27/73)
CI	1.58 ± 0.4

Data are expressed as mean ± SD or factor proportion.
SD-standard deviation, BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure, ACEi - angiotensin-converting enzyme inhibitor, ARB- angiotensin II. receptor blocker, INTERMACS-interagency registry for mechanically assisted circulatory support, NYHA-New York Heart Association functional class, CI-cardiac index.

3.2. Changes in miRNA quantity with left ventricular assist device support

All selected miRNAs were successfully measured. We identified a correlation between the plasma levels of miRNAs and ST (Suppl. material). After ST adjustment, the levels of miR-126 (between baseline and 6th, and 9th months FU; $P < 0.03$) and the levels of miR-146a (between baseline and 9th, and 12th months FU; $P < 0.02$) were significantly increased in plasma after LVAD implantation (Fig. 1). However, miR-10a, miR-10b, miR-663a, miR663b, miR-155, miR-146b, and miR-21 remained stable or oscillated at the FU time points.

Regarding other findings, at baseline, higher quantities of miR-663a/b ($P < 0.02$), miR-155 ($P < 0.02$), miR-21($P < 0.001$) and miR-146b ($P < 0.04$) were found in LVAD patients compared to healthy controls (Suppl. material). At 6th and 9th month FU, we identified increased levels of miR-126 ($P < 0.003$) in LVAD patients compared to both control groups. Similarly, at the 3rd month FU, we detected higher plasma quantities of miR-146a ($P < 0.02$) compared to healthy and HF subjects. During the FU time points, we found significant decrease of miR-10a and miR-10b in LVAD patients compared to HF patients on OMT ($P < 0.02$).

Finally, we detected differences in particular miRNA concentrations in patients with ischemic vs non-ischemic HF etiology during FU (Suppl. material). Briefly, miR-155 was seen in higher levels in patients with ischemic heart failure at the 3rd month ($P < 0.05$), as did miR-10b, miR-146a, miR-146b, and miR-21 at the 12th month post-implantation follow-up (all $P < 0.04$).

3.3. MiRNAs, endothelial function, and atherosclerotic changes

The Belcaro score was measured in 29 patients at FU time points. Partial correlation analysis revealed a positive association between miR-155 and Belcaro score ($P < 0.04$; Fig. 2A).

The RHI was successfully measured in 30 LVAD patients. The mean RHI was below normal values for healthy populations already during the whole follow-up (cutoff ≤ 1.67 indicative of endothelial dysfunction). The RHI did not significantly decrease within 12-months after LVAD implantation. Moreover, the concentration of miR-126 was inversely associated with RHI ($P < 0.05$; Fig. 2B).

4. Discussion

This prospective observational study was focused on dynamic changes in plasma circulating flow-sensitive miRNAs in patients with end-stage heart failure treated by a durable left ventricular assist device. The main finding was that the concentrations of miR-126 and miR-146a increased during CF-LVAD support with attenuated pulsatility. A potential explanation, also based on the results from our previous study, is that the main affected tissue is most likely ECs [18]. This could explain the increase of miR-126, which is highly abundant in ECs and regulates vascular integrity, angiogenesis [19], and inflammation [20]. Moreover, Zhou et al. [21] reported that the secretion of miR-126 into conditioned media, but not its intracellular expression, was decreased by laminar shear stress and increased by oscillatory shear stress in HUVECs. Endothelial-derived miR-126 regulated SMC turnover in an EC-SMC coculture system. Genetic knockout of miR-126 inhibited neointimal formation in a complete carotid ligation model, whereas local reintroduction of miR-126 in the knockout mice enhanced neointimal formation [22]. MiR-126 is also abundantly expressed in platelets, suggesting a role for miR-126 in the interplay between circulating blood and the vascular wall. It was also proved, that the usage of platelet inhibitors, such as aspirin, could influence the quantity of circulating miR-126 [23]. Other pieces of evidence come from the findings that CF- LVADs significantly elevate shear stress [24] and even overexpression of miR-126 induced by long-term shear stress demonstrated in another study

Table 2
Biomarkers of LVAD patients during FU.

	Baseline	3 months	6 months	9 months	12 months	*P* value
CRP (mg/L)	22.5(40.6)	13.4(21.4)	7.6(9.3)	7.9(13.5)	7.2(13.3)	0.01
Glycemia (mmol/L)	6.5(1.7)	5.1(1.4)	5.6(1.0)	5.5(1.1)	5.4(1.2)	0.09
Total protein (g/L)	66.0 ± 7.7	72.7 ± 5.4	73.6 ± 6.7	72.6 (6.5)	71.6 ± 5.3	0.0006
BNP (ng/L)	1992 ± 1189	229(265)	279(342)	253(207)	220(238)	<0.0001
LDH (μkat/L)	4.0(2.0)	3.6(1.6)	3.8(1.3)	3.8(1.5)	3.9 ± 1.1	0.60
Urea (mmol/L)	10.6(8.4)	7.3(3.7)	7.6(4.7)	7.4(4.9)	7.1(3.3)	0.05
Creatinine (μmol/L)	114.2(58.4)	90.8(24.9)	103.1 ± 29.2	102.9 (39.7)	100.7 (45.6)	0.14
Bilirubin (μmol/L)	25.7(21)	11.6(6.4)	12.1(11.8)	11.6(8.8)	11.8(11.2)	<0.0001
ALT (μkat/L)	0.6(2.6)	0.4(0.3)	0.5(0.2)	0.5(0.2)	0.5 ± (0.2)	0.03
AST (μkat/L)	0.5(0.7)	0.4 ± 0.1	0.5 (0.1)	0.4 ± 0.1	0.5 ± 0.1	0.04
INR (s)	1.2(0.3)	2.6(1.1)	2.5 0.8)	2.5(0.9)	2.5 ± 0.6	<0.0001
APTT (s)	41.9(8.5)	49.8 ± 9.1	47.0 ± 7.0	47.3(10.4)	47.4 ± 7.8	0.005

Data are shown as mean ± SD or as median and (IQR). P-value depicts differences between baseline (0) and 12th months FU time. CRP-C-reactive protein, BNP-brain natriuretic peptide, LDH-lactate dehydrogenase, ALT-alanine aminotransferase, AST-aspartate aminotransferase, INR-international normalized ratio, APTT-activated partial thromboplastin time.

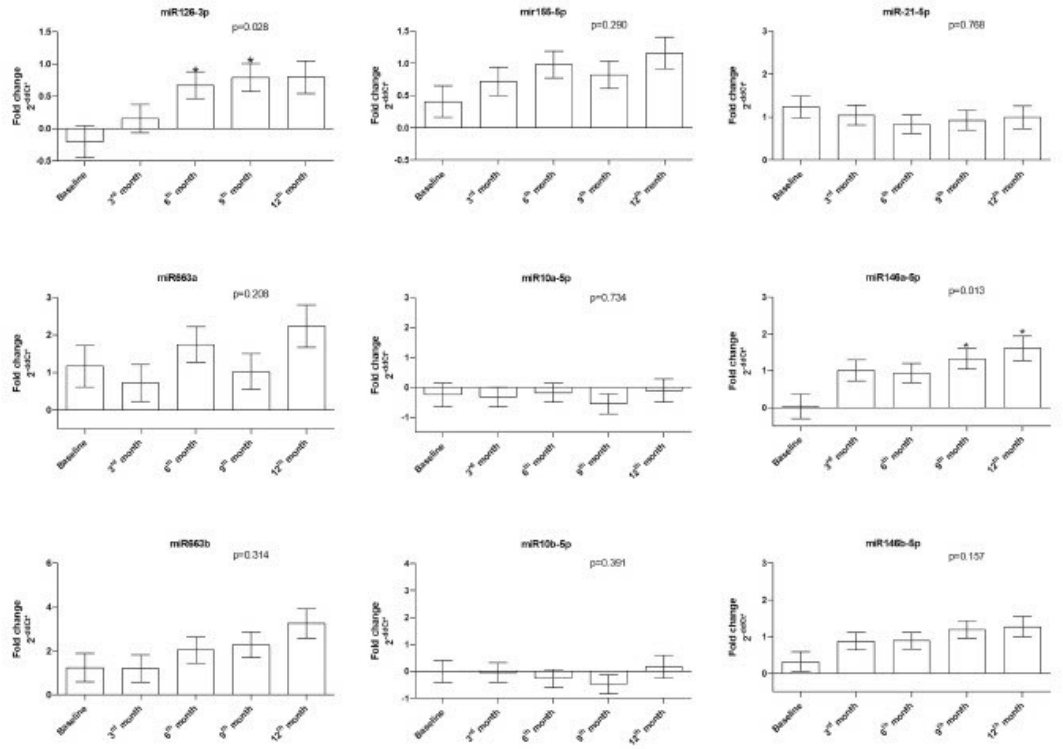

Fig. 1. Dynamics of circulating miRNAs during 12 months of LVAD support. Bar graphs and lines demonstrate mean ± SE. * Indicates significance at $P < 0.05$. SE-standard error.

[25]. Moreover, we observed decreasing trend in the RHI as a measure of endothelial function in LVAD patients after implantation. A lower RHI indicates more severe endothelial dysfunction [26,27]. Our finding of an inverse association between RHI and miR-126 could reflect the vascular dysfunction caused by LVAD implantation.

Our second main finding, the increase of plasma miR-146a, could be caused by its potential role in the regulation of inflammation and immunological responses; this premise is supported by findings, that miR-146a is up-regulated by inflammatory factors such as interleukin 1 and tumor necrosis factor-alpha (TNF alpha) [28]. Mechanistically, miR-146a represses the nuclear factor kappa B and mitogen-activated protein kinase pathways through down-regulation of human antigen R, which promotes endothelial activation in the vessel wall [29]. It was previously shown that inflammatory markers such as TNF alpha are elevated after LVAD implantation [30,31]. Therefore, increased miR-146a levels in our study population could be a marker of the pro-inflammatory post-implant status and this is in concordance with previously published studies [32].

Regarding other interesting findings, we observed higher baseline levels of plasmatic miR-663a, miR-663b, miR-155, miR-21, and miR146b in LVAD patients compared to healthy controls. These findings could be explained by the severity of heart failure with low cardiac output and therefore the overall low flow status, reflected by endothelial dysfunction, which was previously shown to be associated with HF [33]. In agreement with detected miRNAs dynamics during FU, we found higher levels of miR-126 compared to both control groups, which could reflect the endothelial changes seen in LVAD patients. Similarly, miR-146a as an inflammatory marker was detected higher in LVAD during FU, but not before the LVAD implantation. On the contrary, a decreasing trend in miR-21 compared to healthy controls could demonstrate a ventricular regenerative response to MCS. A cardiac enriched miR-21 is essential for cardiac homeostasis and has been demonstrated to act as a cell to cell messenger with diverse functions. MiR-21 activates the ERK-MAPK pathway via the inhibition of SPRY1 and mediates the structural and functional deterioration of cardiac function [34]. The administration of antagomir, which acts against miR-21 in mice with left ventricular pressure overload attenuated the endothelial-to-mesenchymal transition in ECs in vivo [35].

The trend towards stable, lower levels of plasma miR-10a and miR-10b in LVAD patients over 12 months could be interpreted as a response to higher shear stress in order to modulate and potentially regulate protective anti-inflammatory and anti-atherogenic processes.

Our findings of higher concentrations of plasma miR-21, miR-10b, miR-146a, and miR-146b at the 12th FU time point in ischemic versus non-ischemic LVAD patients could reflect worsening of endothelial dysfunction, and the severity of atherosclerosis influenced by LVAD support.

MiR-21 resolves inflammation and down-regulates pro-inflammatory responses. MiR-21 is the most abundant miRNA in macrophages, and its absence could result in accelerated atherosclerosis, plaque necrosis, and vascular inflammation [36]. It was also previously reported that circulating miR-21 was strongly associated with significant coronary stenosis [37].

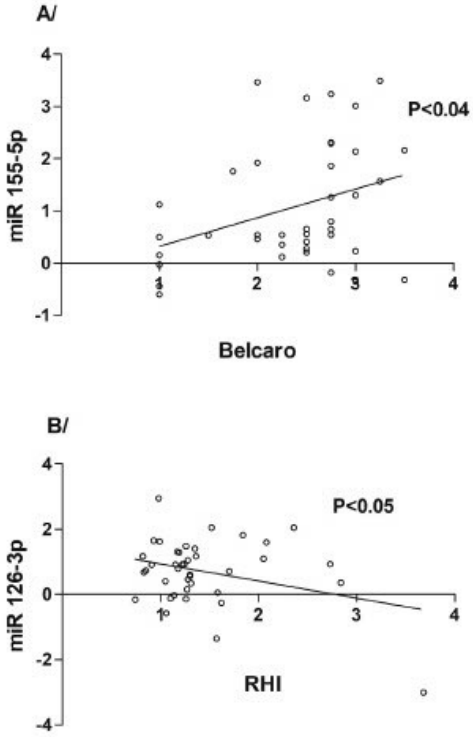

Fig. 2. Association between (A) miRNA levels and Belcaro score; (B) miRNA levels and endothelial function (measured as relative hyperemia index - RHI).

MiR-10b is the crucial downstream mediator of the quality degradation of ECs. It was reported that miR-10b expression in aortic blood plasma was elevated in patients with coronary artery disease with low collateral capacity. A lack of miR-10b expression effectively attenuates the development of atherosclerosis even in the presence of hypercholesterolemia [38].

Overexpression of miR-146a and miR146b negatively regulates inflammation and blunts endothelial activation [29]. Based on the findings that miR-146a is highly up-regulated in atherosclerotic plaques [39] and because it was previously reported that circulating miR-146a positively correlate with the Gensini score/severity of coronary artery disease, [40] we could assume that increased levels of miR-146a could reflect a compensatory response to advanced atherosclerotic changes.

Regarding miR-155, Li et al. [41] reported elevated expression of miR-155 in the plasma and plaques of patients with atherosclerosis. The flow-sensitive miR-155 has been associated with the occurrence and development of atherosclerosis by regulating the functions of CD4 + T lymphocytes, monocytes/macrophages, endothelial cells, and vascular smooth muscle [42,43]. We found a nonsignificant increase in the miR-155 plasma level in LVAD patients after implantation. Therefore, it may be hypothesized that these changes mirror the dysregulation of angiogenesis and inflammation, which may be observed to be aberrant in LVAD patients [44,45]. In this respect, miR-155 has been recently shown to play a fundamental role in the axis between inflammation and the formation of collateral arteries [46], but also miR-155 expression was found to be abundantly expressed in the intima of the thoracic aorta, which is naturally exposed to stable flow in vivo [42]. Furthermore, we observed a positive association with a semiquantitative classification the presence of preclinical atherosclerosis - Belcaro score, further underlining the possible effect of continuous flow on vascular changes.

The limitations of this single-center prospective observational study include the relatively small number of patients, low plasma concentration of some miRNAs, and the need for storage time correction. We identified an influence of long storage time on plasma samples, although constant conditions (−80 °C) and uniform protocols were in place. This factor should be taken into an account in clinical trials using long-term-stored samples for miRNA analysis. Furthermore, miRNAs could be markers of vascular disease and not a causative factor, but despite this, the reflection of endothelial and/or vessel changes is also of importance in understanding physiological and clinical processes.

Despite these limitations, this study could provide valuable insight into the pathophysiology of vascular and inflammatory changes in a specific group of CF-LVAD patients during a long-term follow-up period. The research of epigenetic changes in the circulatory system as well as in vascular biology could clarify the molecular pathways influenced by LVAD support. The occurrence of clinical adverse events and their prediction has become a new challenge in long-term therapy in patients with mechanical circulatory support. Accumulating evidence has suggested that multiple miRNAs may serve as novel biomarkers and new therapeutic targets through their important roles in many biological processes. Relatively non-invasive measurement of circulating miRNAs could be beneficial in the care of patients with LVAD.

In conclusion, the results of the current study suggest that after LVAD implantation, low pulsatile flow upregulates plasma levels of circulating flow-sensitive miRNAs, contributing to endothelial dysfunction and vascular remodeling. Future investigations focused on the explanation of dynamic changes in miRNAs as potential markers or active substances of/in adverse events or even as tools for therapeutic interventions to prevent vascular changes in pathological flow conditions are warranted.

Author contribution

I.N. – Supervision, Conceptualization, Funding acquisition, Investigation, Writing - review & editing. P.I. Project administration, Conceptualization, Data curation, Investigation, Writing - original draft, Writing - review & editing. D.D. Formal analysis, Data curation, Writing - original draft, Writing - review & editing. S.N., M.K., Z.T., and D.H. Data curation and Validation. J.A.H and J.P. Writing - review & editing. P.W. and V.L. Software; Validation.

Declaration of Competing Interest

P. I. is a consultant for Abbott and CARMAT SA. I. N. is a consultant who received grant funds and is on advisory boards for Abbott, CARMAT SA, LeviticusCadioLtd and EvaHeart Inc. M.K. is a consultant for CARMAT SA. P.I., I.N., M.K., Z.T., D.H. have received institutional grant support from Abbott.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.06.050.

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PRÁCE D je zaměřena na vliv kontinuálního, nefyziologického toku krve na cévní systém z pohledu procesů detekovaných přímo ve stěně aorty pomocí určení změn v expresi mRNA a miRNA.

Vlivem implantace mechanické srdeční podpory s kontinuálním tokem krve dochází k zásadním histologickým změnám ve stěně aorty, zejména k degeneraci hladkých svalových buněk a elastických vláken. Nepulsatilní tok krve může vést ke zhoršování funkčních parametrů stěny aorty (jako tuhost cévní stěny), k morfologickým změnám nebo dynamické remodelaci aorty. Jak již bylo uvedeno výše, miRNA jsou krátké nekódující úseky RNA, které regulují expresi cílových genů a ovlivňují množství buněčných procesů a biologických jevů. MiRNA jsou za normálních podmínek přítomny pouze v malém množství, které se ale zvyšuje při mnohých patologických stavech. Hladiny cirkulujících miRNA mohou potencionálně ovlivnit geny regulující endoteliální a hladké svalové buňky, zánět nebo sterosklerózu.

Do této monocentrické, prospektivní studie bylo zařazeno 16 pacientů s implantovanou mechanickou srdeční podporou s kontinuálním tokem krve (4 HMII a 12HM3). Průměrný věk ve studijní skupině byl 57let (18-65). Pacienti byli implantováni v Institutu klinické a experimentální medicíny v Praze mezi lety 2015 a 2018. Etiologie srdečního selhání byla převážně neischemická a průměrná doba trvání byla 382 dní (162-887). Studovány byly párované vzorky stěny aorty odebrané při implantaci LVAD a následně při transplantaci srdce. Detailní popis odběru vzorků a následné analýzy je uveden v přiloženém článku.

Výsledky studie naznačují, že vlivem implantace MSP dochází k významným změnám mRNA zavzatých do extracelulární matrix a organizace kolagenních vláken. Tato skutečnost může způsobovat poruchu homeostázy extracelulární matrix vedoucí ke změně morfologie cévní stěny a její tuhosti. Zároveň bylo nalezeno množství deregulovaných miRNA souvisejících s remodelací cévní stěny a progresí endoteliální dysfunkce. Tato studie jasně ukázala, že změna charakteru krevního toku ovlivňuje cévy a cévní systém již na genetické úrovni.

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An Integrative Study of Aortic mRNA/miRNA Longitudinal Changes in Long-Term LVAD Support

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Abstract: Studying the long-term impact of continuous-flow left ventricular assist device (CF-LVAD) offers an opportunity for a complex understanding of the pathophysiology of vascular changes in aortic tissue in response to a nonphysiological blood flow pattern. Our study aimed to analyze aortic mRNA/miRNA expression changes in response to long-term LVAD support. Paired aortic samples obtained at the time of LVAD implantation and at the time of heart transplantation were examined for mRNA/miRNA profiling. The number of differentially expressed genes (Pcorr < 0.05) shared between samples before and after LVAD support was 277. The whole miRNome profile revealed 69 differentially expressed miRNAs (Pcorr < 0.05). Gene ontology (GO) analysis identified that LVAD predominantly influenced genes involved in the extracellular matrix and collagen fibril organization. Integrated mRNA/miRNA analysis revealed that potential targets of miRNAs dysregulated in explanted samples are mainly involved in GO biological process terms related to dendritic spine organization, neuron projection organization, and cell junction assembly and organization. We found differentially expressed genes participating in vascular tissue engineering as a consequence of LVAD duration. Changes in aortic miRNA levels demonstrated an effect on molecular processes involved in angiogenesis.

Keywords: mRNA; microRNA; aorta; mechanical circulatory support; left ventricular assist device

1. Introduction

The use of continuous-flow left ventricular assist devices (CF-LVADs) in patients with end-stage heart failure has become a widely used and sustainable treatment strategy, both as a bridge to transplant (BTT) and as destination therapy (DT). CF-LVAD may induce pathological changes to the aortic wall and aortic valve [1]. Important histologic changes in the aortic wall, before and after CF-LVAD implantation, with degeneration of smooth muscle cells and elastic fibers, were previously reported [2]. CF-LVADs may contribute to the deterioration of aortic functional parameters (e.g., aortic stiffness) or structural changes (e.g., increase of wall thickness or collagen content) through adverse effects of the nonphysiological flow [3]. The nonpulsatile flow of CF-LVAD generates dynamic remodeling within the aorta. Remodeling within the aortic root and proximal ascending aorta may also contribute to the pathophysiology of aortic regurgitation with CF-LVAD.

Studies have demonstrated that the proximal aorta dilates after chronic CF-LVAD support, and that an increasing aortic diameter was associated with the development of aortic regurgitation [4].

MiRNAs are endogenous small noncoding RNAs that regulate mRNA translation of target genes through the RNA interference pathway, strongly influencing a wide range of cellular processes and biological pathways [5]. As such, miRNAs are fine-tuners of gene expression patterns in response to pathophysiological stimuli. Most miRNAs are more ubiquitously expressed and are not cell-type specific. Thus, many miRNAs are expressed at relatively low levels under basal conditions, but during pathological stress, are strongly upregulated [6]. Changes in tissue miRNA expression levels due to blood flow can potentially affect networks of genes regulating endothelial and vascular smooth muscle cell (SMC) function, inflammation, and atherosclerosis [7].

Alteration in gene expression reflects changes in cellular function and behavior, in development, and disease states. The major cardiovascular diseases, including coronary artery disease, myocardial infarction, congestive heart failure and common congenital heart disease, are caused by multiple genetic and environmental factors, as well as the interactions between them. The underlying molecular pathogenic mechanisms for these disorders are still largely unknown, but gene expression may play a central role in the development and progression of cardiovascular disease [8]. Gene expression analysis can also contribute to understanding and discovering novel and sensitive biomarkers of cardiovascular disease. Over the past two decades, methods of measuring gene expression have improved dramatically with a plethora of hybridization arrays available, followed by RNA-Seq, the sequencing of short or long RNA reads using massively parallel sequencing technology [9].

Studying the long-term use of LVADs offers an opportunity for a complex understanding of the pathophysiology of vascular changes in patients with mechanical circulatory support, which produce nonphysiological blood flow patterns. The aim of the present study was therefore to detect accurate mRNA/miRNA associations in the aorta in response to long-term LVAD.

2. Results

The principal component analysis of expression profiles segregated samples before and after LVAD support (Supplementary Figure S1). The mRNAs profile (Figure 1A) demonstrated differentially expressed genes (DEGs). We identified a total of 277 DEGs (Pcorr < 0.05) after long-term LVAD support. 141 DEGs were upregulated in aortic tissue after LVAD support. Between twenty DEGs, we identified Collagen Type I Alpha 2 Chain (*COL1A2*); Chemokine-like receptor 1 (*CMKLR1*); S100 calcium-binding protein A4 (*S100A4*); Elastin (*ELN*); and Collagen Type III Alpha 1 Chain (*COL3A1*) to be upregulated (all Pcorr < 0.0001). Solute Carrier Family 12 Member 2 (*SLC12A2*); Pyruvate Dehydrogenase Kinase 4 (*PDK4*); Inhibitor of DNA Binding 1 (*ID1*); Arginine and Glutamate Rich 1 (*ARGLU1*) and noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) were downregulated (all Pcorr < 0.0001, Supplementary Figures S2 and S3). The Wald test performed on samples after support to assess the effect of HeartMate II (HM II), and HeartMate 3 (HM 3) devices did not show any significantly differentially expressed genes (Supplementary Data S1).

Gene ontology (GO) analysis identified 31 biological processes in GO terms enriched in explanted tissue (false discovery rate (FDR) q-value < 0.001), predominantly involved in extracellular matrix organization and collagen fibril organization. Moreover, 17 molecular functions of GO terms, and 10 cellular components of GO terms were also enriched (Supplementary Data S1).

The whole miRNome profile revealed 69 DE miRNAs (Pcorr<0.05, after Bonferroni correction (BFC), Figure 1B). A total of 30 miRNAs were upregulated (Supplementary Data S1), and the most increased expression was detected for let-7a/d/e/f; miR-181a/b; miR-29b; miR-149 and miR-99b (all Pcorr < 0.01). The opposite effect of LVAD was found on miR-19a/b; miR-654; miR-664a; miR-885; and -511 (all Pcorr < 0.01).

With the usage of miRWalk and miRDB, 170 DEGs were identified as potential targets of upregulated miRNAs and 159 DEGs as potential targets of downregulated miRNAs. From these, 138 DEGs were possible targets of both group miRNAs (Supplementary Data S1).

(**A**) mRNAs profile

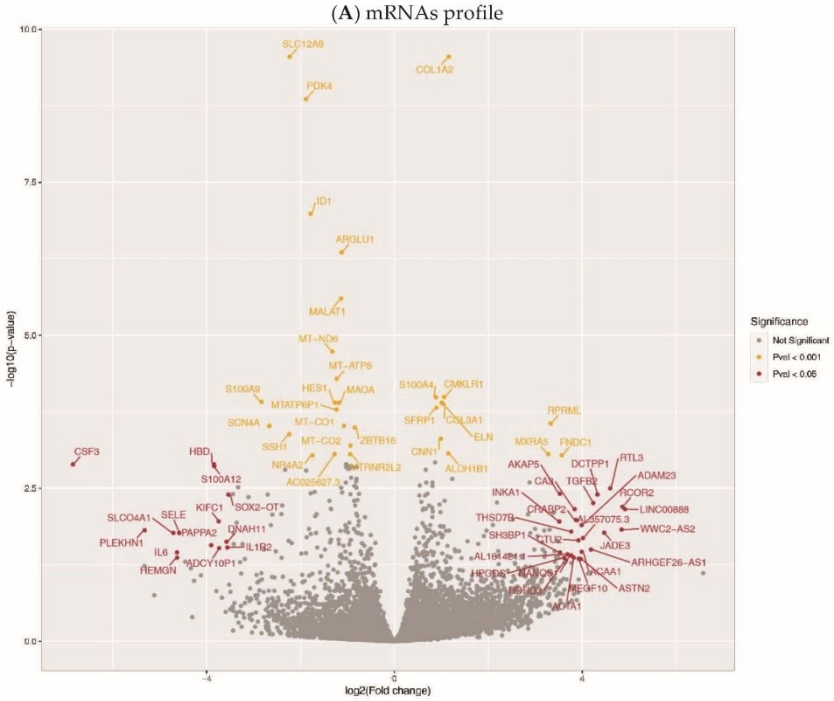

Figure 1. *Cont.*

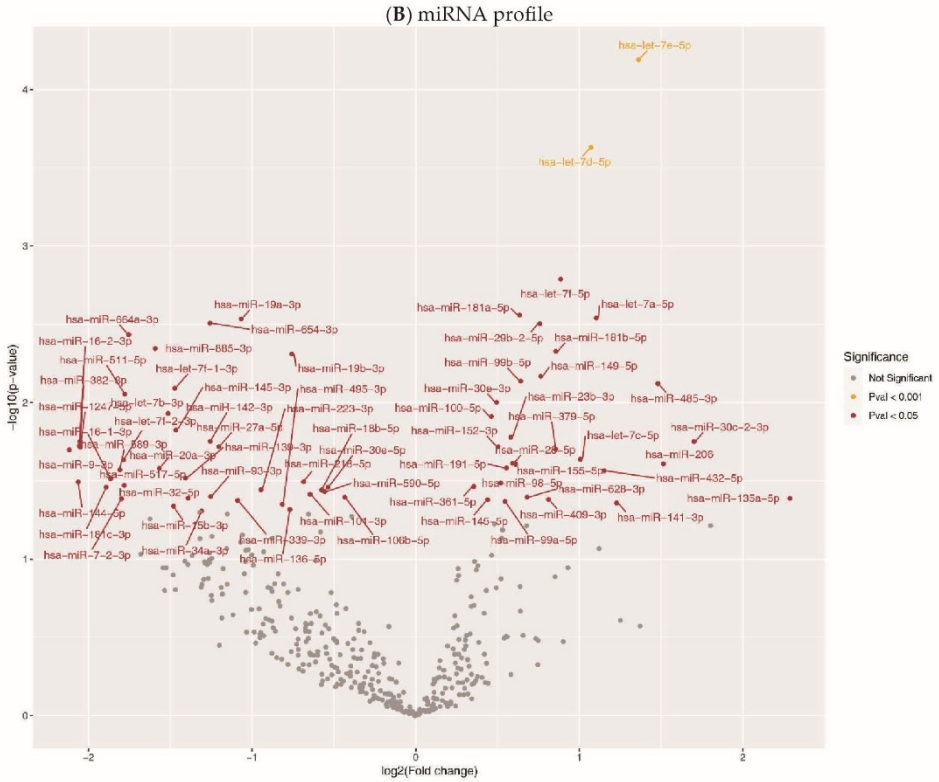

Figure 1. Expression profiles in aortic tissue. Volcano plot of comparative mRNA resp. miRNAs expression profiles in samples before and after LVAD support; x-axis indicates difference in expression level on a log2 scale; y-axis represents corresponding *P*-values on a negative log scale. (**A**) mRNA profile: yellow points indicate mRNAs with Pcorr < 0.001; red points indicate mRNAs with Pcorr < 0.05, & Pcorr > 0.001, & |logfc| > 3.5.; (**B**) miRNA profile: yellow points indicate miRNAs with Pcorr < 0.001; red points indicate miRNAs with Pcorr < 0.05, & Pcorr > 0.001.

In order to discover the relationship between deregulated miRNAs and mRNAs, Pearson correlation between differentially expressed miRNAs and all potential target mRNAs was assessed (Figure 2). This integrated mRNA/miRNA analysis revealed that potential targets of miRNAs upregulated in explanted samples are mainly involved in GO biological process terms related to cell junction assembly and cell junction organization (FDR q-value < 0.05, Supplementary Data S1). Enriched GO terms in targets of downregulated miRNAs were related to dendritic spine organization and neuron projection organization (FDR q-value < 0.05, Supplementary Data S1). Moreover, an enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway related to endocytosis was found (FDR q-value < 0.05, Supplementary Data S1).

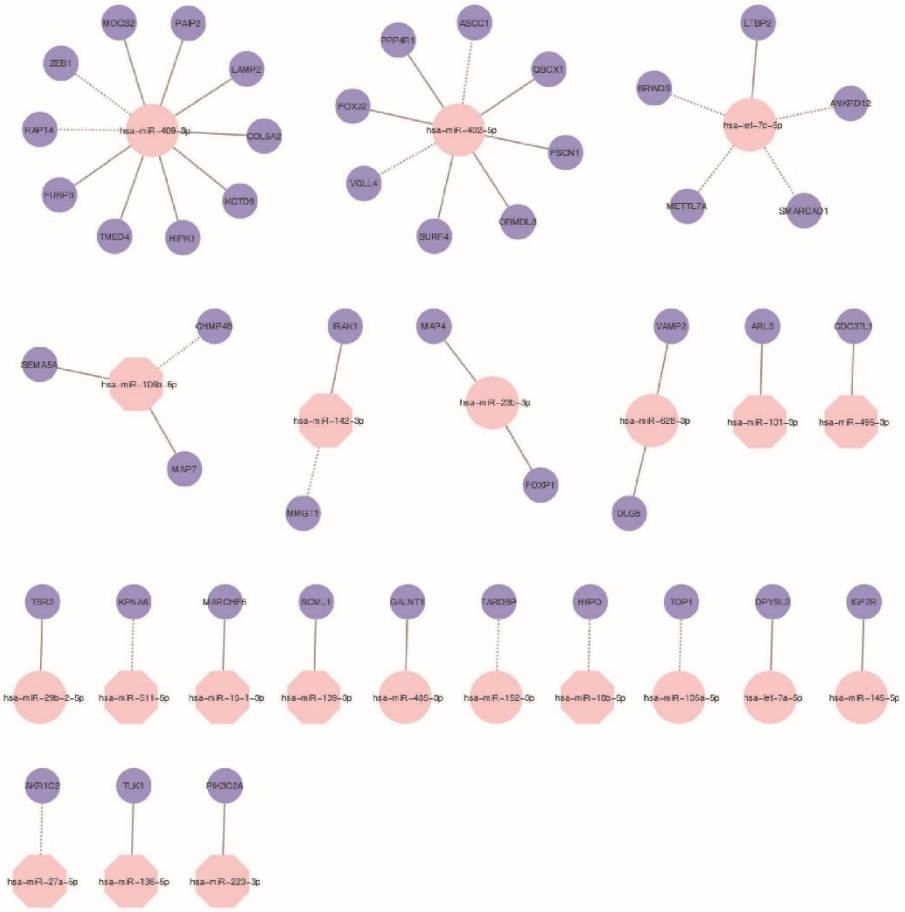

Figure 2. MiRNAs regulatory network. Examples of flexible and selective regulatory networks between miRNAs and mRNAs in samples after LVAD support. Selected overexpressed miRNAs are highlighted in pink circles, and underexpressed miRNAs are highlighted in pink octagons. The targets are highlighted in blue circles. The full line indicates a positive correlation, and the discontinued line indicates a negative correlation with target mRNAs.

3. Discussion

Our multiple regression analysis is, to our best knowledge, the first study focused on changes in mRNA/miRNA expression in paired aortic samples collected before LVAD implantation and at the time of LVAD explantation, during heart transplantation (HTx).

We compared gene expression profiles using a robust and simple mRNA sequencing method. We found the significant DEGs were predominantly involved in extracellular matrix (ECM) and collagen fibril organization in aortic tissue after LVAD explantation. ECM is an active and dynamic structure with a fundamental role in regulating vascular function in normal and pathological conditions. Homeostasis of the vascular ECM may affect intrinsic properties of the arterial wall and arterial stiffness [10]. The ECM is a key component of the local cellular microenvironment. It comprises structural proteins (e.g., elastin and collagen), proteoglycans, and glycosaminoglycans. Among the different cell types, smooth muscle cells (SMCs) and fibroblasts are examples of cells that produce signif-

icant ECM. Interestingly, Coffey et al. [11], using analysis of the integrated miRNA/mRNA network, identified pathways predominantly involved in extracellular matrix function in patients affected by aortic stenosis.

The previously reported histologic analysis showed significant degenerative changes in the aortic wall, SMCs disorientation and depletion, elastic fiber fragmentation and depletion, medial fibrosis, and atherosclerosis changes in ascending aortic tissue at the follow-up than at device implantation [2]. Furthermore, there was evidence of structural remodeling within the aortas of CF-LVAD patients, including an increase in total wall thickness, an increase in collagen content, and reduced elastin content that may explain the increase in vessel stiffness [12]. We identified elevated expression of collagens *COL1A1* and *COL3A1*. Collagen and elastin are the most abundant ECM proteins of the aortic wall, and they are responsible for characteristic mechanical properties—tensile strength and elasticity. Over-accumulated collagen in the aorta may lead to medial fibrosis, hypothetically resulting in decreased arterial distensibility [13]. Surprisingly, we also detected elevated expression of *ELN*, which is usually lowered in processes related to atherosclerosis, such as pathological flow. We may only hypothesize whether this phenomenon can be attributed to compensatory mechanisms reacting on the non-physiological flow pattern or should be a subject for further research, as the reason for this observation is unclear at this stage of research.

We found overexpressed *S100A4*, a member of the large family of S100 proteins, under LVAD support. S100A4 controls different cellular pathways, exerting numerous effects on processes that are cell- and tissue-type dependent. In activated fibroblasts, endothelial, dendritic, and mast cells, as well as in macrophages, monocytes, neutrophils, and T-lymphocytes, S100A4 has a significant role in stimulating invasion and migration, cytoskeletal dynamics and in promoting proinflammatory phenotypes [14]. S100A4 represents a well-known marker that characterizes a complex biological process where endothelial cells assume a mesenchymal phenotype, known as the "endothelial-to mesenchymal transition", changing morphology and functions, acquiring accentuated motility and contractile properties, typical of fibrotic processes [15]. Furthermore, upregulated *CMKLR1* is currently the only chemerin receptor. CMKLR1 receptor, and the proposed pro- and anti-inflammatory properties of chemerin, suggest a role of this adipokine in inflammatory states and possibly atherosclerosis. It was reported that foam cell *CMKLR1* expression strongly and positively correlates with aortic atherosclerosis, but only marginally with coronary atherosclerosis [16]. Our findings of aortic increased expression levels of *COL1A2*; *CMKLR1*; *S100A4*; and *COL3A1* may support previously mentioned morphological changes in the aorta under CF-LVAD.

Changes in aortic wall functional properties as the possible consequence of a pulsatility decrement caused by implantation of CF-LVAD were described [3]. Patel et al. reported that patients with CF-LVADs before heart transplant had an increase in proximal aortic stiffness compared with patients without an LVAD or with pulsatile flow LVADs before transplant [3,17]. DEGs involved in ECM organization could also suggest a potential link with the development of acquired aortic insufficiency (AI), a significant complication that develops following the implantation of CF-LVAD [18,19]. We detected downregulated *MALAT1*, a gene coding stiffness-sensitive long non-coding RNA. This non-coding RNA regulates stiffness-dependent VSMC proliferation and migration [20], which may influence aortic functional properties.

Despite the advantages and improving results of the CF-LVAD therapy, the loss of pulsatility may lead to different complications on the micro and macrovascular levels. Vascular changes may be linked with the occurrence of clinically adverse events related to CF-LVAD therapy, such as non-surgical bleeding, e.g., gastrointestinal bleeding related to arteriovenous malformations [21] or von Willebrand factor (vWF) deficiency [22], or other clinical complications such as cerebrovascular events [23,24], device thrombosis [25,26] or development of aortic insufficiency [27,28].

One of the pathogenetic mechanisms of cardiovascular complications with CF-LVADs may be endothelial dysfunction. Endothelial dysfunction is related to heart failure in general. After the implantation of the device, the endothelial dysfunction does not improve and may even deteriorate [29]. In our miRNAs profile, we identified deregulated multiple miRNAs involved in vascular remodeling (Table 1) which potentially involved endothelial dysfunction progression. Several miRNAs have been shown to control the varying mechanisms which govern SMC plasticity [30]. In response to LVAD, we found upregulated miRNAs that influence SMC dynamics and downregulated miRNAs known to stimulate apoptosis during atherosclerosis plaque development. Leeper et al. reported that chronic SMC apoptosis accelerates vascular disease progression, promotes calcification, and induces features of medial degeneration, like atrophy, elastin fragmentation, and enhanced glycosaminoglycan deposition, thus worsening endothelial dysfunction [30].

Table 1. miRNAs participated in vascular remodeling in aortic tissue.

miRNA	Expression	Target (s)	Function
miR-23b	**up**	*TLP3, FOXO4, CHI3L1, SMAD3*	SMCs proliferation, differentiation, cytokine production
miR-29b	**up**	*COL1A1, COL3A1, COL5A1, ELN, MMP2, MMP9, PTEN, ADAMTS7*	ECM production, SMCs proliferation, arterial calcification, cell apoptosis
miR-155	**up**	*SMAD, BCL6, CTLA4, MMP1, MMP3, SOCS, NF-κB signaling transcription factor*	SMCs differentiation, regulation of inflammation
miR-206	**up**	*ARF6, SLC8A1*	SMCs differentiation
miR-34a	**down**	*SIRT1, NOTCH*	SMCs proliferation, differentiation
miR-145	**up**	*KLF4/5, MYOCD, ELK1, SRF, SOX9*	SMCs differentiation, proliferation Inhibits TGF-β signaling, ECM production, regulation of fibrosis
miR-19a/b	**down**	*FZD4, LRP6, TLR2, TGFBRI/TGFBRII*	ECs proliferation, differentiation, angiogenesis, WNT signaling pathway, regulation of fibrosis
miR-20a	**down**	*MKK3, TLR4*	Reduction of ECs migration and angiogenesis, TXNIP signaling, inflammation
miR-149	**up**	*FGFR1, GPC1*	Regulation of angiogenic functions of ECs
Let-7a/c/e/f	**up**	*TGFBR3, TBX5, ADRB1, EDN1, FGF5, IL6, IκBβ*	Regulation of angiogenesis of ECs and inflammation
miR-100	**up**	*mTOR, NOX4*	Regulation of neovascularization
miR-99b	**up**	*NOX4, TGFβ*	Differentiation of ECs
miR-30c/e	**up**	*CTGF*	Promotion of the synthesis of ECM and collagen, regulation of fibrosis
miR-142-3p	**down**	*ADAM9, HMGB1, AZIN1, JNK1*	Regulation of fibrosis
miR-15b/16	**down**	*TGF-βR1, p38, SMAD3, SMAD7, ENDOGLIN, AKT3*	Regulation of fibrosis, cell apoptosis, and angiogenesis
miR-885	**down**	*ULK2*	Cell autophagic processes
miR-511	**down**	*FOXC1*	Regulation of angiogenesis
miR-664a	**down**	*TGFBR2, AKT*	Inhibits TGF-β signaling, ECM production, regulation of fibrosis
miR-654	**down**	*PTEN, ATM, ADAM10, RAB22A*	Regulation of fibrosis and inflammation

For more details see [30–44].

Several authors, including Morgan et al., observed worsened endothelial function in long-term CF-LVAD patients [31]. In our study, we observed dysregulation in miRNAs participating in the regulation of vascular development, growth, and differentiation [32], which may indicate the role of these miRNAs in the further development of endothelial dysfunction. Interestingly, we also identified upregulated miRNAs that regulate mRNAs encoded by genes in human endothelial cells related to vascular function and blood pressure regulation [33].

Correlation analysis revealed that miR-409-3p (upregulated in explanted tissue), one of the most potent fibrinogen downregulating miRs [45], potentially affects the expression of the highest number of genes (10 genes with a correlation coefficient higher than |71|). However, only two genes correlated negatively, suggesting they may be direct targets (*ZEB1* and *RAP1A*). *ZEB1* gene is associated with the regulation of vasculogenesis [46], whereas *RAP1A* promotes angiogenesis and dynamic regulation of endothelial barrier [47].

It should be mentioned that in our study, patients with two types of LVAD were studied—an axial-flow LVAD HeartMate II and the HeartMate 3, a centrifugal-flow pump with intrinsic artificial pulsatility. Nevertheless, this intrinsic pulsatility was originally designed to enhance pump washout and prevent blood stasis and thrombosis. Our previous work found that this pulsatility does not avert endothelial dysfunction [29,48]. Therefore, the HeartMate 3 should also be considered as CF-LVAD. We are aware that our population of patients is not exceedingly large; nevertheless, we did not observe any differences between the pump types.

The vasculature is one of the most dynamic tissues that encounter numerous mechanical cues derived from pulsatile blood flow, blood pressure, the activity of smooth muscle cells in the vessel wall, and the transmigration of immune cells [49]. Endothelial cell junction assembly and cell junction organization play pivotal roles in tissue integrity, barrier function, and cell–cell communication, respectively [50]. In this study, a multistep approach combining mRNA and miRNA expression profiles and bioinformatics analysis was adopted to identify the mRNA/miRNA regulatory network. Enriched GO terms in targets of upregulated miRNAs were related to cell junction assembly and cell junction organization.

4. Materials and Methods

4.1. Subjects

All examined individuals provided their informed consent, which the institution's ethics committee approved together with the study protocol. The protocol of this study was conducted according to the principles of the Declaration of Helsinki [51].

A total of 16 patients (median age 57 years, range 18–65) who required mechanical circulatory support from HeartMate II (N = 4) and HeartMate 3 (N = 12) as a bridge to transplantation or destination therapy from July 2015 to March 2018 at the Institute for Clinical and Experimental Medicine in Prague, were enrolled in our study. The etiology of heart failure was predominantly nonischemic dilated cardiomyopathy (N = 13). The median LVAD support duration was 382 days (ranging from 162 to 887 days). The basic characteristics of the patients are summarized in Table 2.

Table 2. Demographics of the patients selected for mRNA/miRNA analysis.

N (female %)	16 (18.8%)
Age (years)	49.6 ± 16.8
BMI (kg/m^2)	25.6 ± 5.5
Diabetes mellitus (%)	1 (6.3%)
Hypertension (%)	8 (50%)
Hyperlipidemia (%)	5 (31.3%)
CVA/TIA (%)	0
NYHA classification IV (%)	11 (68.8%)
Etiology of nonischemic DCM (N)	13
Idiopathic	11
Familial	1
Toxic	1
Etiology of hypertrophic cardiomyopathy	1
Etiology of ischemic DCM (N)	1
Etiology of noncompact DCM (N)	1
CRTD/ICD before LVAD implant, %	12 (75%)
Type of LVAD	
Heart Mate II	4
Heart Mate 3	12
Days of LVAD support (days)	382 (325.5)

HF, heart failure; CVA, cerebrovascular accident; TIA, transient ischemic attack; NYHA, New York Heart Association; DCM, dilated cardiomyopathy; CRTD, cardiac resynchronization therapy defibrillator; ICD, implantable cardioverter-defibrillator. Categorical data are presented as number (%), continuous data as mean ± SD or median (IQR), respectively. Familial DCM is only defined if the patient has one or more family members diagnosed with idiopathic DCM or has a first-degree relative who experienced sudden unexplained death under 35 years. Days of LVAD support are based on patients who already underwent HTx.

4.2. Sampling

Paired aortic tissue was obtained at the time of LVAD implantation and at the time of HTx from CF-LVAD patients. Approximately 30 mg of tissue was immediately after exclusion from aorta inserted into RNase/DNase-free tubes pre-filled with All Protect Tissue Reagent (Qiagen GmBH Strasse 1, Hilden, Germany). Samples were stored for 2–4 weeks at 4 °C and then at −80 °C before RNA extraction. The storage time of tissue ranged from 33 to 876 days.

4.3. mRNA and miRNA Analysis

Total RNA, including miRNA, was extracted from 10 mg of aortic tissue according to protocol using the miRCURY^{TM} RNA isolation kit for tissue (Qiagen GmBH Strasse 1, Hilden, Germany). RNA quality and quantity were assessed using a Fragment Analyzer system (Agilent technologies, 301 Stevens Creek Blvd., Santa Clara, CA, USA).

Gene expression was measured in paired samples from 10 patients. QuantSeq 3′ mRNA sequencing for RNA quantification was performed using a high-throughput technique using 3′mRNA-Seq Library Prep Kit FWD and 3′mRNA-Seq Library Prep Kit REV (https://www.lexogen.com/quantseq-3mrna-sequencing/; accessed on 20 March 2020) at Lexogen (Campus Vienna Biocenter 5, 1030 Vienna, Austria). QuantSeq. 3′ mRNA library preparation predominantly produces fragments for sequencing close to the 3′ end of polyadenylated mRNA, generally from the last exon and the 3′ untranslated region (3 UTR) [52]. The total RNA input was 20 ng. There was no prior poly(A) enrichment or rRNA depletion. The QuantSeq Forward kit has an oligo (dT) primer containing the Illumina-specific Read 2 linker (P7), which is annealed to the 3′ end of the mRNA fragment to synthesize the first cDNA strand via reverse transcriptase. The second strand synthesis is commenced by random priming and DNA polymerase extension. The random primer contains the Illumina-specific Read 1 linker sequence (P5). Sequencing commences from the Read 1 sequencing primer and goes toward the poly(A) tail with only one fragment produced per transcript [9].

SYBR green-based real-time quantitative PCR (RT-PCR) for miRNA profiling (in a total of 16 patients) was performed using miRNome Panels (Qiagen GmBH Strasse 1, Hilden, Germany). Passive Reference Dye (ROX^{TM} 30 nm) was included for all PCR reactions. Measurement was performed using the QuantStudio6 Flex instrument (ThermoFisher Scientific, 81 Wyman Street, Waltham, MA, USA). Inter-plate calibrators (IPC) for calibration between PCR plate runs, and spike-in controls to ensure the quality of RNA isolation, cDNA synthesis reaction, and PCR was included in each measurement [53].

4.4. Processing of mRNA Sequencing Data

QuantSeq 3′ mRNA sequencing produced 140 million reads with 35–76 bp length. Raw reads were trimmed of bases with Phred 33 quality lower than 30 using Cutadapt software v2.9 [54]. Reads mapping to rRNA and UniVec (common vector contaminations in RNA sequencing) databases were discarded. Mapping was performed using a bowtie aligner [55], with one mismatch allowed. The remaining reads were mapped with STAR aligner to the human genome (GRCh 38.95), with one mismatch allowed [56]. Only uniquely mapping reads were assigned to individual genes using featureCounts software [57].

4.5. DESeq2 Analysis of mRNA Expression

Genes with less than 10 counts per all samples were removed before further analysis. Raw counts were normalized using the median ratio method built in DESeq2 software [58]. To account for paired samples, parameters included in the DESeq2 model were patient IDs and conditions before (implant) and after (explant) LVAD. Differential expression between implant and explant samples was tested with one parameter Wald test built in DESeq2; p values were adjusted for multiple testing with Bonferroni correction (BFC). A p-value < 0.05 was considered statistically significant.

4.6. Gene Ontology Analysis

Gene ontology was performed using Gene Ontology enRIchment anaLysis and visuaLizAtion tool (Gorilla), a web-based tool [59]. All genes expressed in measured samples (threshold > 10 raw counts per all samples) were ranked using the following formula –log (p-value) * log2FC; the resulting list was used as input for calculating the p-value of the minimum hypergeometric score, as described in detail by [60]. KEGG pathway analysis was performed with clusterProfiler tool using 10,000 permutations and a gene set size between 3–800 genes. As input for KEGG pathway analysis, the same ranked list of genes was used for GO analysis [61,62].

4.7. miRNA Profile Analysis

Gene Expression software (GenEx SW, Multid Analysis AB, Göteborg, Sweden) was used for miRNA expression analysis. Ct values higher than 35 were replaced by 35. A total of 330 miRNAs with a call rate <40% (i.e., more than 60% data are invalid of that miRNA) were removed from further analysis. The missing data, exceedingly low miRNA levels, were replaced by deltaCt + 2 (representing at least 1/4 of the detectable miRNAs amount). Data were normalized with the mean expression of all miRNAs and converted to relative quantities and Log2. P values were corrected for multiple testing with BFC. A p-value < 0.05 was considered statistically significant.

4.8. Integrated mRNA/miRNA Analysis

Differentially expressed miRNAs were used to predict mRNA targets in miRWalk (http://mirwalk.umm.uni-heidelberg.de/search_mirnas/; accessed on 18 October 2020) and miRDB (http://www.mirdb.org/; accessed on 18 October 2020) databases. miRDB target prediction was restricted to gene targets with prediction scores less than 60, and miRNAs with more than 2000 genes in the genome were excluded. For prediction in the miRWalk database, no restrictions were used. All genes expressed in our samples, which appeared in the predicted targets in either of the databases, were used for correlation analysis.

Pearson correlation was used to correlate $\log_{10}$ transformed normalized expression values of differentially expressed miRNAs with $\log_{10}$ transformed normalized expression values of expressed genes. Only pairs with a correlation better than −0.7/0.7 were considered for further GO analysis and KEGG pathway. GO analysis and KEGG pathway were performed using clusterProfiler with the same parameters described in Section 4.6.

5. Conclusions

The study provides additional insight into the pathophysiology of vascular changes observed in patients after LVAD implantation. Significant regulation of mRNAs involved in ECM and collagen fiber organization in response to the implantation of LVAD was observed, which may suggest infliction of ECM homeostasis resulting in changes of intrinsic properties of the vascular wall and arterial stiffness.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22147414/s1.

Author Contributions: P.I. and I.N. designed and directed the study, performed the clinical aspects of the project, contributed to the interpretation of the results, wrote and edited the article. D.D. designed and performed the experiments, analyzed the data, and wrote the article. Data curation was performed by Z.T. Data analysis conducted by S.B. Data review was performed by J.A.H. The original draft was written by D.D. and P.I. All authors reviewed the original draft and approved the final version of the manuscript.

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Institutional Review Board Statement: The study was approved on 10 June 2015 with approval number G-15-06-14 by the Human Ethics Review Board, Institute for Clinical and Experimental Medicine and Thomayer Hospital, Prague, Czech Republic, and was performed in accordance with the guidelines in the Declaration of Helsinki (2000) of the World Medical Association.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: P.I. is a consultant for Abbott and CARMAT S.A.; I.N. is a consultant, has received grant funds and is on advisory boards for Abbott, CARMAT S.A., Leviticus Cadio Ltd., and Eva Heart Inc.; P.I., I.N., and Z.T. have received institutional grant support from Abbott.

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PRÁCE E představuje souhrn současných poznatků týkajících se vlivu MSP na cévní systém. V úvodu popisuje jednotlivé typy nynější srdečních podpor a jejich vliv na fyziologii cévního průtoku. Levostranné srdeční podpory během svého vývoje byly nejprve pulsatilní ve snaze co nejvíce napodobit fyziologický tok krve. Množství součástí a jejich kontakt s krví pacienta se však odrazil v nižší spolehlivosti a životnosti čerpadel, zejména v porovnání se současnou generací. Vývoj poté směroval k podporám generujícím kontinuální tok krve. To umožnilo zmenšení velikosti i prodloužení životnosti, avšak za cenu ztráty pulsatility. A právě vliv nepulsatilního toku krve na cévní systém a vznik komplikací je v současné době stále velmi málo prozkoumanou oblastí. Práce dále popisuje nejčastější komplikace spojené s tímto typem terapie terminálního srdečního selhání.

Práce se podrobněji věnuje studiím věnujícím se změnám v karotickém řečišti vlivem MSP. Tyto změny jsou dobře detekovatelné prostým sonografickým vyšetřením. Všechny studie se shodují na tom, že ke změnám v karotických tepnách dochází, a dokonce je možno na základě těchto změn určit možné riziko závažných komplikací pro konkrétního pacienta. Dále se práce zaměřuje na změny cirkulujících biomarkerů vaskulárního poškození, konkrétně cirkulujících mikročástic, endoteliálních progenitorových buněk a kmenových buněk a cirkulujících mikroRNA. U všech těchto markerů dochází ke změně hladin po implantaci MSP, ať už ve srovnání s kontrolami, nebo postupně v čase od implantace. Je tedy zřejmé, že kontinuální tok levostranných mechanických srdečních podpor cévní systém ovlivňuje. Některé výsledky dokonce naznačují, že bude potencionálně možné využít dané poznatky k predikci rizika nežádoucích událostí. Závěr studie potvrzuje jasně zřetelný vliv kontinuálního toku MSP na cévy a cévní systém. Zároveň se jedná o velmi důležité poznatky pro vývoj dalších a stále dokonalejších generací mechanických srdečních podpor.

Vliv mechanických srdečních podpor na cévní systém

The effect of mechanical circulatory supports on the vascular system

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Abstrakt
Implantace mechanických srdečních podpor je spolu s transplantací srdce základní modalitou terapie konzervativně neřešitelného srdečního selhání. Dnes nejčastěji využívané levostranné srdeční podpory s kontinuálním tokem krve svým příjemcům zlepšují přežívání i kvalitu života, zároveň je ale ohrožují množstvím specifických komplikací. Jedním z faktorů, které k tomuto jevu přispívají, může být vliv kontinuálního toku krve na cévní systém. K posouzení tohoto jevu lze použít množství metod, nejčastěji zobrazovacích, reprezentovaných především ultrasonografickým vyšetřením cévního systému, ale i metod laboratorních reprezentovaných například kvantitativním měřením cirkulujících mikročástic, endoteliálních progenitorových buněk, případně mikroRNA. Cílem této práce je shrnout naše dosavadní výsledky a zkušenosti v této oblasti.

Klíčová slova: cévní systém – mechanické srdeční podpory – srdeční selhání

Abstract
The implantation of mechanical circulatory supports is, together with heart transplantation, the basic modality of therapy for the end-stage heart failure. The most frequent left ventricular assist devices with continuous flow improve survival and quality of life of their recipients, but they also put them at risk through a number of specific complications. One of the potential side effects under intensive discussion is unfavorable impact of continuous blood flow on the vascular system. A number of methods are used to study this phenomenon; among the most frequently used are imaging methods, including vascular ultrasound, but also laboratory methods including circulating microparticles, endothelial progenitor cells, microRNA etc. In this article we would like to summarize our previous findings and experiences in this field.

Key words: heart failure – mechanical circulatory support – vascular system

Úvod

Dlouhodobé mechanické srdeční podpory (MSP) jsou v současné době již zlatým standardem terapie terminální fáze srdečního selhání [1]. Jedná se o implantabilní pumpy našité nejčastěji na levou, vzácně pak na pravou komoru srdeční, nahrazující její funkci. Vně těla pacienta je patrný pouze napájecí kabel ústící v oblasti epigastria, který je napojený na řídící jednotku a baterie (obr. 1). Nejčastější indikací implantace MSP celosvětově je tzv. destinační terapie (DT – Destination Therapy) [2], druhou nejčastější je „most k transplantaci" (BTT – Bridge To Transplant,). Doba trvání MSP je u obou skupin pacientů v rámci měsíců a let.

Ačkoliv první mechanické srdeční podpory fungovaly na principu pulzatilního toku krve [3], ale brzy se u nich ukázalo vyšší riziko selhání pumpy pro množství složitých komponent a také vyšší výskyt infekce [4].

Proto se vývoj posunul směrem ke 2. generaci MSP, u nichž je pumpou axiálního typu generován kontinuální, nepulzatilní tok krve (např. HeartMate II – HM II, Abbott, Abbott Park, Illinois, USA). Zatím nejdokonalejší 3. generace MSP

je založena na principu elektromagnetického nebo hydrodynamického závěsného rotoru bez přítomnosti ložisek (obr.). Tím je umožněno zmenšení velikosti čerpadla a omezení kontaktu s krevními elementy, což snižuje riziko trombózy a dalších komplikací a zvyšuje životnost přístroje. Příkladem tohoto nejnovějšího typu MSP je HeartMate3 (HM III, Abbott, Abbott Park, Illinois, USA), u kterého elektromagnetický levitující rotor v pravidelných intervalech snižuje a zvyšuje rychlost pumpy, čímž vytváří arteficiální pulzatilitu sloužící zejména k zamezení stázy krve uvnitř přístroje, čímž je dále sníženo riziko trombózy pumpy. Avšak riziko trombotických komplikací je přítomno i nadále, proto je u obou výše zmíněných typů MSP nutná trvalá antikoagulační terapie, nejčastěji warfarinem, spojená s antitrombotickou terapií kyselinou acetylsalicylovou. Nastavení správné úrovně a síly antikoagulace a vyvážení trombotických a krvácivých nežádoucích událostí u pacientů s MSP je v současné době předmětem několika mezinárodních studií [5,6].

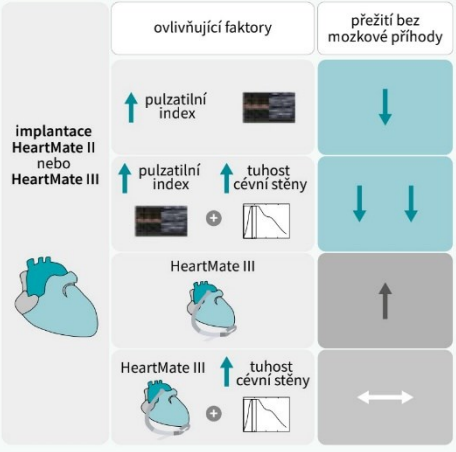
Levostranné mechanické srdeční podpory s kontinuálním tokem krve (CF-LVADs – Continuous Flow Left Ventricuar Assist Devices) výrazně zlepšují kvalitu života a snižují úmrtnost pacientů v terminální fázi srdečního selhání, ale tito pacienti jsou zároveň ohroženi některými závažnými komplikacemi [7,8,9]. Mezi nejzávažnější patří významné krvácení a tromboembolické komplikace postihující i centrální nervovou soustavu [10]. Rizikovým faktorem pro tyto komplikace by mohl mít i nepulzatilní charakter průtoku. I dle našich zkušeností je možné vliv srdeční podpory zjistit ultrasonografickým cévním vyšetřením, kvantitativním stanovením cirkulujících biomarkerů endoteliálního poškození, případně dalšími metodami. Cílem této práce je shrnout naše dosavadní výsledky a zkušenosti v této oblasti.

Změny v karotickém řečišti u pacientů s mechanickou srdeční podporou detekované ultrasonograficky

Patofyziologie cévních změn u pacientů s mechanickou srdeční podporou je předmětem mnoha studií [11–14]. Žádná ze studií však zatím tyto změny plně nevysvětlila ani neposoudila jejich vliv na prognózu pacientů. Jednou z možností, jak predikovat riziko zejména vaskulárních komplikací, může být prosté sonografické vyšetření karotických tepen doplněné posouzením tuhosti cévní stěny a endoteliální (dys)funkce. V našem případě jsme určili index hyperemie a augmentační index systémem EndoPAT2000 a v předchozí práci jsme zjistili, že po implantaci MSP došlo k poklesu indexu hyperemie mezi 3. a 6. měsícem po implantaci a zároveň ke zvýšení augmentačního indexu, a tedy ke zhoršení tuhosti cévní stěny [14]. Kombinaci ultrasonografického vyšetření karotických tepen doplněnou o měření arteriální tuhosti jsme využili v monocentrické, prospektivní studii k určení vztahu morfologických a hemodynamických změn v karotickém řečišti u pacientů s CF-LVAD k riziku úmrtí nebo cerebrovaskulárních příhod [15]. Do studie bylo zařazeno 83 pacientů indikovaných k implantaci (provedené mezi lety 2014 a 2018). Bylo provedeno neinvazivní vyšetření karotických tepen pomocí sonografie a byly posuzovány jak morfologické, tak hemodynamické parametry (Belcaro score, pulzatilní index, rezistivní index). Zároveň byla pomocí systému Endopat 2000 měřena tuhost cévní stěny jako augmentační index. Vyšetření

Obr | Mechanická srdeční podpora HeartMate III (použito se svolením polečnosti Abbott U.S.)

Schéma | Vliv implantace mechanické srdeční podpory (Heart Mate II nebo III) a cévních parametrů (pulzatilního indexu v karotických tepnách a augmentačního indexu) na klinické příhody (median sledování 27 měsíců)



probíhalo před implantací a 3 a 6 měsíců po implantaci, medián doby sledování byl 27 měsíců. Během sledování 16 pacientů zemřelo a 4 pacienti prodělali nefatální ischemickou cévní mozkovou příhodu. Hlavním nálezem bylo, že zvýšený pulzatilní index v karotických tepnách měřený 3 měsíce po implantaci MSP byl silným prediktorem rizika úmrtí a cévní mozkové příhody; tato asociace byla ještě silnější, pokud byla do analýzy přidána tuhost cévní stěny vyjádřená jako augmentační index. Dalším nálezem bylo, že implantace HM III (Heart Mate) měla protektivní efekt (schéma). Význam stanovení parametrů průtoků v karotických tepnách podporují i menší studie. Například studie u 13 pacientů ukázala, že vrcholová systolická rychlost se snížila po implantaci levostranné MSP ve vnitřních i společných karotických tepnách, zatímco střední rychlosti v těchto tepnách zůstaly stabilní [16]. Další průřezová studie se 16 pacienty s CF-LVAD poukazuje na nižší poddajnost a roztažnost karotických tepen po implantaci [17].

Je však třeba zmínit, že vzhledem ke kontinuálnímu charakteru toku krve je absolutní hodnota pulzatilního indexu v karotických tepnách nižší než u pacientů bez MSP. Na ultrasonografii detekovatelná pulzní vlna může být důsledkem otevírání aortální chlopně při neúplném unloadingu levé komory při zachovalé kontraktilitě obou komor nebo i rozdílu tlaku mezi levou komorou a aortou z důvodu senzitivity čerpadla k preloadu. MSP 3. generace HM III má arteficiání pulzatilitu způsobenou periodickým zvyšováním a snižováním rychlosti pumpy. Ta však nemá za cíl nahradit fyziologický pulz, nýbrž zajistit adekvátní proplach pumpy.

Cirkulující biomarkery vaskulárního poškození

Další ukazatele stavu cévního systému u pacientů s MSP jsou cirkulující biomarkery, zahrnující i cirkulující mikročástice, cirkulující endoteliální progenitorové a kmenové buňky a vaskulární mikroRNA (MiRNA).

Cirkulující mikročástice

Cirkulující mikročástice jsou drobné částice vznikající v oběhovém systému a v buňkách cévní stěny při jejich aktivaci nebo poškození [18]. Mikročástice jsou jedním z konečných produktů apoptózy a jejich množství bývá zvýšeno u řady chorobných stavů včetně srdečního selhání a cévních chorob. Nejčastější místo produkce mikročástic jsou endoteliální buňky a trombocyty. Nepulzatilní tok krve generovaný MSP způsobuje zvýšené zatížení a následně poškození buněk cévního systému. Studie provedená na 30 pacientech s LVAD HM II prokázala signifikantní pokles cirkulujících mikročástic 3 měsíce po implantaci srdeční podpory [12]. Tyto výsledky mohou naznačovat, že minimálně první 3 měsíce od implantace převládá pozitivní efekt MSP na kardiovaskulární systém, tedy včetně cévních struktur.

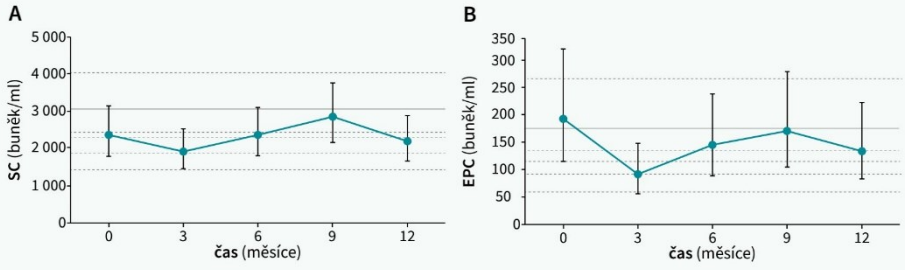
Endoteliální progenitorové buňky a kmenové buňky

Cirkulující endoteliální progenitorové (EPC – Endothelial Progenitor Cells) a kmenové buňky (SC – Stem Cells) jsou zejména ukazatelem reparace buněk, jsou tvořeny v kostní dřeni při akutním vaskulárním poškození a nutnosti obnovení endotelu. Studie čítající 14 pacientů s LVAD posuzovala množství EPC a SC před implantací podpory a dále ve 3., 6., 9. a 12. měsíci po implantaci a srovnávala tato data s daty pacientů se srdečním selháním bez MSP a se zdravými kontrolami. Po implantaci MSP došlo ke snížení hladiny SC po 3 měsících, jejich navýšení 9 měsíců po implantaci a jejich mírný pokles ve 12 měsíci (graf). Množství EPC významně kleslo 3 měsíce po implantaci a nevýznamně se zvýšilo mezi 3. a 9. měsícem [14]. Tyto výsledky mohou poukazovat na časné zlepšení stavu cévního systému korekcí důsledků těžkého srdečního selhání implantací MSP, ale i na následnou endoteliální dysfunkci spojenou s tímto postupem.

Cirkulující mikro RNA

MiRNA jsou krátké, nekódující částice RNA zapojené do sekvenčně specifické posttranskripční regulace genové exprese. Některé MiRNA jsou uvolňovány z poškozených endoteliálních buněk a aktivně se podílejí na dysregulaci cévního systému mechanizmem podobným hormonálnímu působení [19]. Hladiny „cévně aktivních“ MiRNA u pacientů již před implantací

Graf | Vývoj počtu kmenových buněk po implantaci srdeční podpory (Heart Mate II)



kontinuální křivka- jednorázově stanovené hodnoty u zdravých dobrovolníků

EPC – Endothelial Progenitor Cells/cirkulující endoteliální progenitorové buňky **SC** – Stem Cells/kmenové buňky

LVAD a dále 3, 6, 9 a 12 měsíců po implantaci narůstaly [20]. Z tohoto výsledku lze usoudit, že po implantaci LVAD s kontinuální tokem krve skutečně dochází k endoteliální dysfunkci a přestavbě cévní stěny.

Shrnutí

MSP jsou zásadním milníkem v terapii dříve neřešitelných stadií srdečního selhání. Nicméně, kromě jasně pozitivního krátkodobého i střednědobého přínosu je vzhledem k vzrůstajícímu počtu pacientů na destinační terapii nutné podrobněji zkoumat i jejich dlouhodobý vliv na cévní systém. Jednou z hlavních otázek v této oblasti je vliv (absence/snížení) pulzatility na cévní systém po implantaci MSP. Naše studie posuzující změny v karotických tepnách prokázala, že relativně vyšší pulzatilní index i při jeho absolutně nižších hodnotách může být prediktorem úmrtí a mozkových příhod u pacientů po implantaci MSP. Tento efekt je navíc zřetelný i při porovnání HM II s novější HM III: novější pumpa má výrazně nižší incidenci trombózy [21]. Výše uvedené poznatky poukazují na významný vliv mechanických srdečních podpor na cévní systém a tento faktor by měl být brán v úvahu při vývoji dalších generací MSP.

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6. DISKUSE

Mechanické srdeční podpory jsou v dnešní době standardem v terapii terminálních fází srdečního selhání. Dlouhodobé výsledky této terapie se při použití nejnovějších čerpadel a při pečlivém výběru optimálního pacienta můžou přibližovat výsledkům přežívání po transplantaci srdce. Významným problémem však zůstávají specifické komplikace této terapeutické modality, zejména ty související s hemokompatibilitou (cévní mozkové příhody ischemické i hemoragické, krvácení, trombotické komplikace). V současnosti implantovaná čerpadla generují převážně kontinuální, nepulsatilní tok krve. Důsledky této nefyziologické cirkulace na cévní systém nejsou stále podrobně objasněné a jsou přetrvávajícím předmětem výzkumu. Jejich posouzení může být zásadní jak pro správně nastavení terapie již implantovaných pacientů, tak pro směřování vývoje nových typů mechanických srdečních podpor.

Hlavním tématem předkládané práce byl vliv pulsatility krevního toku na cévní stěnu a cévní systém. Byla testována hypotéza, že vliv kontinuálního krevního proudu lze monitorovat v cévním řečišti neinvazivně jak pomocí sonografického vyšetření, tak sledováním cirkulujících biomarkerů vaskulárního poškození. Dále byl posuzován možný vztah mezi změnami v cévním řečišti a rizikem úmrtí a vzniku nežádoucích příhod.

Výsledky studie ukázaly, že u pacientů, u nichž byl pulsatilní index (PI) měřený v karotických tepnách 3 měsíce po implantaci srdeční podpory vyšší než medián, měli vyšší riziko cévní mozkové příhody nebo úmrtí. Ve shodě s tímto pacienti, kteří v době sledování utrpěli cévní mozkovou příhodu, měli vyšší pulsatilní index. Tato asociace se ukázala ještě silnější, když se do analýzy přidala tuhost cévní stěny. Zároveň však nebyl nalezen vztah mezi morfologickými aterosklerotickými změnami v karotických tepnách a rizikem nežádoucích příhod. Recentně publikovaná práce čítající 141 pacientů s HMII, kterým byly vyšetřovány karotické tepny před a po implantaci MSP, naopak prokázala vztah mezi střední až významnou stenosou karorických tepen na podkladě aterosklerózy před implantací srdeční podpory a následným vznikem nežádoucích událostí (Kiyatkin et al, 2021). Zde se však jednalo pouze o pacienty se starším typem MSP axiálního typu (HMII), zatímco v naší studijní skupině jsou zastoupeny jak HMII, tak HM3.

Implantace HM3 měla v naší studii protektivní vliv oproti pacientům s HMII. Recentně publikovaná práce zahrnující celkem 148 pacientů (41 HM3, 32 HMII, 43 srdeční selhání a 32 zdravých kontrol) prokázala, že arteficiální pulsatilita generovaná HM3 (pravidelné

zrychlování a zpomalování pumpy sloužící zejména k jejímu lepšímu promývání a prevenci trombózy) je patrná až po úroveň mikrocirkulace, avšak neovlivňuje hodnotu pulsatilního indexu (Stohr et al, 2023). Protektivní vliv HM3 může být tedy spíše vysvětlen lepší hemokompatibilitou této srdeční podpory.

Další část studie se věnovala posouzení vlivu pulsatility krevního toku na periferní vaskulární systém za pomoci měření periferní endoteliální funkce systémem EndoPAT 2000. Výsledné měření ukázalo, že již před implantací srdeční podpory HM3 byl relativní hyperemický index (RHI) nižší, než u zdravé populace (v rámci endoteliální dysregulace ve fázi pokročilého srdečního selhání), a po implantaci systému nadále klesal. V případě augmentačního indexu (AI) byl trend opačný, což svědčí o nárůstu tuhosti cévní stěny. Je však patrné, že u pacientů po implantaci HM3 nedochází k takovému zlepšení vaskulární funkce, jaké při arteficiální pulzatilitě a zejména ve srovnání s předchozí generací bylo očekáváno. Zajímavým zjištěním bylo, že pokud se u pacientů otevírala aortální chlopeň každý stah a byla tedy zachována alespoň částečná pulsatilita krevního toku, byl patrný positivní vliv na tuhost cévní stěny. Tento výsledek potvrzuje i studie provedená Patelem a kolektivem (Patel et al, 2017, Wever-Pinzon et al, 2013) srovnávající změny stěny aorty mezi pulsatilními a nepulsatilními srdečními podporami s jasným positivním efektem u pulsatilní varianty.

Další sledovanou veličinou ve vztahu k vlivu MSP na cévní stěnu byly miRNA v krevní plasmě. Tyto krátké nekódující úseky RNA jsou považovány za biomarkery poškození a dysfunkce endotelu. V souladu s naším očekáváním hladiny miRNA v krevní plasmě postupně vzrůstaly s časem od implantace MSP, což opět naznačuje postupující endoteliální dysfunkci a změny v cévní stěně spojené s nepulsalitou krevního toku. Vyšší hladiny miRNA byly patrny ve skupině s MSP již před implantací ve srovnání se zdravými kontrolami, což podporuje hypotézu endoteliální dysfunkce přítomné v pokročilých fázích srdečního selhání ve shodě s dříve publikovanou literaturou (Giannitsi et al., 2019).

Návazným krokem bylo studium miRNA přímo ve stěně aorty v párovaných vzorcích odebraných před implantací MSP a poté při explantaci systému během transplantace srdce. Z nich je jasně patrné, že dochází k významným změnám miRNA ovlivňujících expresi extracelulární matrix a organizaci kolagenních vláken vedoucí ke změně morfologie cévní stěny a její tuhosti. Tato zjištění jasně dokumentují, že nefyziologický charakter krevního toku ovlivňuje cévy a cévní systém již na genetické úrovni.

Všechny výše zmiňované nálezy potvrzují silný vliv mechanických srdečních podpor na vaskulární systém a s tím spojenou náchylnost ke komplikacím. Tato asociace se zdá být silně ovlivněna parametry pulsatility, a to i přesto, že se u těchto pacientů jedná o nepulsatilní nebo minimálně pulsatilní krevní tok.

7. ZÁVĚRY

I. Vyšší pulsatilní index v karotických tepnách měřený 3 měsíce po implantaci LVAD je asociován s vyšším rizikem vzniku závažných cerebrovaskulárních komplikací nebo úmrtí. Zároveň nebyla potvrzena asociace mezi aterosklerotickými směnami karotid a výskytem nežádoucích událostí. Implantace MSP HM3 měla protektivní vliv.

II. Implantace MSP, nezávisle na jejím typu, souvisí s prohloubením endoteliální dysfunkce, která je přítomna již u pacientů se srdečním selháním před implantací MSP. Progrese endoteliální dysfunkce není zmírněná ve spojitosti s arteficiální pulzatilitou u HeartMate 3.

III. Výrazně vyšší výskyt některých podtypů cirkulujících miRNA (miR-126 a miR-146a) u pacientů s LVAD ve srovnání se zdravými kontrolami velmi pravděpodobně potvrzuje změny cévního systému při zavedení terapie pomocí MSP, které se odráží i na úrovni genové exprese. Změny v těchto tzv. flow-senzitivních miRNA jsou odrazem endoteliální dysfunkce a vaskulární remodelace.

IV. Vlivem implantace levostranné mechanické srdeční podpory dochází k významným změnám mRNA podílejících se na tvorbě extracelulární matrix a organizaci kolagenních vláken. Tím může nastat porucha homeostázy extracelulární matrix vedoucí ke změně morfologie cévní stěny a její tuhosti. Další detekované deregulované miRNA souvisí s s remodelací cévní stěny a endoteliální dysfunkcí. Změna charakteru krevního toku tedy ovlivňuje cévy a cévní systém již na úrovni genové exprese a proteinogeneze.

V. Dizertační práce poskytuje souhrn současných poznatků o jednotlivých typech MSP a o jejich vlivu na endotel a cévní systém a to od přístrojového vyšetření velkých cév až po vyšetření na úrovni genové exprese. Výsledky ukazují vliv MSP na cévní systém na všech vyšetřovaných úrovních, dokonce s potenciálem predikce některých komplikací. Bližší studium jednotlivých procesů může být nápomocné při včasné detekci komplikací, při selekci vhodných kandidátů k implantaci LVAD a také při vývoji nových generací mechanických srdečních podpor.

8. SOUHRN

Stoupající prevalence srdečního selhání v současné populaci a zvětšující se počet pacientů vyžadujících terapeutickou intervenci vede ke značnému rozvoji nových metod v tomto odvětví. Své zásadní místo mají vedle farmakologické léčby a transplantace srdce i mechanické srdeční podpory. Ty se postupně vyvinuly od velkých strojů neumožňujících propuštění pacienta z nemocnice po dnešní nové koncepty plně implantabilních zařízení. Cílem této terapeutické modality je zlepšení přežívání pacientů a kvality jejich života, ať už čekají na transplantaci srdce, nebo mají srdeční podporu v režimu destinační terapie do konce života. K tomuto je nutné snažit se maximálně omezit nežádoucí události související s implantací a následným provozem pumpy a ideálně se jim snažit předejít. Většina současných mechanických srdečních podpor generuje nepulsatilní, kontinuální tok krve, který je však pro člověka nefyziologický. A právě jeho vlivu na cévní systém a následný vnik nežádoucích komplikací se věnovala tato disertační práce. Závěr všech pozorování jasně ukazuje, že důsledky ztráty pulsatility jsou v krevním řečišti patrny jak na funkční, tak na makro- a mikroskopické a dokonce i na genetické úrovni. Cévy ztrácí poddajnost a dochází ke změnám ve stavbě jejich stěny, endotel je patologicky aktivován. Důsledkem mohou být jak mnohé krvácivé i trombotické komplikace během terapie mechanickou srdeční podporou, tak třeba i těžká a leckdy fatální vasoplegie po následné transplantaci srdce. K objasnění dalších mechanismů budou potřebné další studie, ale již teď tyto výsledky pomáhají určovat další směřování vývoje mechanických srdečních podpor směrem k pumpám pracujícím v souladu se srdeční revolucí, u kterých bude fyziologická pulsatilita zachována.

9. SUMMARY

The rising prevalence of heart failure in the current population and the increasing number of patients requiring therapeutic intervention has led to a significant development of new methods in this field. Mechanical circulatory support has its essential place alongside pharmacological treatment and heart transplantation. These have gradually evolved from large machines that do not allow the patient to be discharged from hospital to today's new concepts of fully implantable devices. The goal of this therapeutic modality is to improve patient survival and quality of life, whether they are waiting for a heart transplant or have cardiac support in destination therapy mode for the rest of their lives. To achieve this, efforts must be made to minimize and ideally prevent adverse events related to the MCS. Most current mechanical cardiac supports generate a non-pulsatile, continuous blood flow, but this is unphysiological for humans. The effect of this non-pulsatile flow on the vascular system and the subsequent onset of adverse events is the focus of this study. In conclusion, all observations clearly show that the consequences of loss of pulsatility are evident in the bloodstream at the functional, macro- and microscopic, and even genetic levels. Blood vessels lose elasticity and their wall structure changes, the endothelium is pathologically activated. The consequence may be many bleeding and thrombotic complications during MCS therapy, as well as severe and often fatal vasoplegia after subsequent heart transplantation. Further studies will be needed to elucidate other mechanisms, but these results already help to guide the future direction of mechanical circulatory supports towards pumps that work in accordance with the cardiac revolution and in which physiological pulsatility is preserved.

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Původní vědecké práce, které nejsou podkladem této disertační práce:

Netuka I, **Tucanova Z**, Ivak P, Gregor S, Kolesar DM, Marek T, Melenovsky V, Binova J, Dorazilova Z, Hegarova M, Podolec M, Riha H, Connors JM, Mehra MR. A Prospective Randomized Trial of Direct Oral Anticoagulant Therapy with A Fully Magnetically Levitated LVAD: The DOT-HM3 Study. Circulation. 2024. **IF2024 = 10.1161**

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12. PŘÍLOHY

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A Prospective Randomized Trial of Direct Oral Anticoagulant Therapy with A Fully Magnetically Levitated LVAD: The DOT-HM3 Study

Running title: *Netuka et al.; Apixaban versus Warfarin with the HeartMate 3 LVAD*

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Non-standard Abbreviations and Acronyms

LVAD = Left Ventricular Assist Device
HM 3 = HeartMate 3
VKA = Vitamin K Antagonist
DOAC = Direct Oral Anticoagulants
DOT-HM3 = Direct Oral Anticoagulant Therapy with the HeartMate 3 LVAD
LDH = Lactate Dehydrogenase
TTR = Time in Therapeutic Range
HTx = Heart Transplant

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The fully magnetically levitated HeartMate 3 (HM3) LVAD (Abbott) is superior in reducing pump thrombosis and stroke but use of anti-thrombotic therapy with a Vitamin-K Antagonist (VKA) remains suboptimal and resource intensive.[1] Whether direct oral anticoagulants (DOAC) that inhibit Factor Xa are safe and preserve hemocompatibility-related adverse event outcomes with the HM3 pump remains unknown. Importantly, a small study with Dabigatran in patients with the HeartWare HVAD (Medtronic) demonstrated an increase in thrombotic complications.[2]

In the Direct Oral Anticoagulant Therapy With the HeartMate 3 LVAD (DOT-HM3) study we enrolled patients receiving the HM3 LVAD > 3 months post-implant, free of a bleeding episode or stroke, who tolerated a VKA targeted to an INR of 2-3 (protocol details available at https://clinicaltrials.gov/study/NCT04974684). Participants were randomized by a non-study scientist (using a computer derived scheme with study team blinded to allocation) to either apixaban 5mg twice daily or continued therapy with VKA (2:1), with those allocated to apixaban further randomized (1:1) to either continued use of 100 mg aspirin once daily or withdrawal. All patients provided informed consent and the single-center study was approved by the institutional Ethics Review Board. All data and materials will be made available for any reasonable request to the first author once the study is fully concluded. At trial initiation, the ARIES-HM3 study results (which showed reduced bleeding rates with elimination of aspirin) were unknown.[1] Pump thrombosis surveillance was mandated by assessment of Lactate dehydrogenase (LDH) once weekly for 4-weeks, then once every 2-weeks for the next month and monthly thereafter; Echocardiography was performed at baseline, 1, 3 and 6-months.

The primary safety endpoint was survival-free of pump thrombosis, disabling stroke, or major bleeding at 3 months post-randomization, with clinical outcomes ascertained at completion of 6-month follow-up (if no safety concerns). Heart transplantation was considered success and other withdrawals, a failure. No study power was assigned in this exploratory study and intent to treat principles were used in describing outcomes.

We enrolled 45 patients at a median of 630 (range 90-2291) days after LVAD implantation (warfarin plus aspirin n=14, apixaban plus aspirin n=15 and apixaban alone n=16).(Figure 1) The enrolled population included 40 males (100% Caucasian), mean age 62 years (range 24-77), 51% ischemic etiology, 67% NYHA IV (69% on inotropic therapy), 75% atrial fibrillation, 33% diabetes and 55% bridge to transplant goal.

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Characteristics were balanced with no significant differences among groups. All patients reached 6 months unless withdrawn or transplanted since safety at 3 months was determined. No thromboembolism (pump thrombosis, stroke, or arterial thromboembolism) occurred in either arm at 6-months of follow-up. In the VKA group, 2 uterine bleeds (day 21 and 54) and 1 epistaxis (day 2) occurred (Time in Therapeutic Range (TTR) achieved was 74.4%). 1 patient in the apixaban plus aspirin arm experienced a gastrointestinal bleed (day 20). Six heart transplants were performed in apixaban treated patients and 1 among the warfarin group, without excessive post-operative bleeding. Apixaban doses were held for at least 24 hours prior to transplant surgery, prothrombin complex concentrates were used in all patients and a cytokine adsorption system employed on cardiopulmonary bypass. Reversal antidotes to apixaban were not required. Four patients were withdrawn for reasons other than a transplant procedure (1 in the VKA arm for palliative care on day 85; 3 patients in the apixaban groups for a gastrointestinal bleed, mediastinitis, and medical non-adherence).(Figure1)

The DOT-HM3 study has demonstrated a) feasibility of using a DOAC (with or without aspirin) in chronically supported HM3 LVAD patients for a duration of 6-months b) No thromboembolism was noted in the DOAC groups, c) heart transplantation while on apixaban was not accompanied by excessive bleeding post-surgery. In contrast to adverse outcomes with prior LVADs that used dabigatran, the fully magnetically levitated rotor and wider blood flow pathways of the HM3 pump may confer hemocompatibility with improved risk-benefit ratio of the balance between thrombosis and bleeding to support continued assessment of DOACs in this situation. Whether mechanism of dabigatran (direct thrombin inhibition) compared with factor Xa inhibition (proximal in the contact activation pathway) contributes to observed differences remains uncertain. A retrospective study evaluated apixaban to warfarin and suggested no adverse thromboembolic complications with the HM3 pump at 6-months.[3] Another single-center study that intended to enroll 40 patients but only randomized 30 patients (15 each to Apixaban or Warfarin) with HM3 pumps demonstrated similar findings.[4] That study randomized more black persons to warfarin than apixaban (79% versus 20%), a group known to experience more hemocompatibility related adverse events and achieved a TTR of 46% with warfarin.[5] Our study is limited by its single-center design, small numbers and short follow-up but includes a larger DOAC treated cohort and optimal warfarin management.

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We conclude that in carefully selected stable patients after HM3 LVAD implantation, a strategy of switching a VKA to apixaban is feasible for either bridge to transplantation or destination therapy patients. These findings require study in large-scale and long-term trials before adoption in clinical practice.

Article Information

ClinicalTrials.gov ID NCT04974684

Disclosures

Ivan Netuka discloses relationships with CARMAT SA (Consultant and Investigator, Advisory Board Member), Fineheart SA (Consultant and Investigator, Advisory Board Member), LeviticusCardio (Advisory Board Member, Board Member, Stock holder), Abbott (Consultant, Institutional support); Zuzana Tucanova discloses relationships with Abbott (Consultant, Institutional support) and Fineheart SA (Institutional support); Peter Ivak discloses relationships with CARMAT SA (Consultant), Abbott (Consultant, institutional support), Fineheart SA (Institutional support);Hynek Riha discloses relationships with Abbott (Consultant, Institutional support), Carmat SA (Institutional support), Fineheart SA (Institutional support); Jean M. Connors reports receiving personal consulting fees from Abbott and Alnylam, receiving personal fees from Werfen, Bayer, Pfizer, BMS Scientific, Sanofi, Roche and Anthos for serving as a speaker or advisor outside the submitted work; Mandeep R. Mehra reports consulting fees derived from Abbott (paid to his institution), Natera, Paragonix, Moderna, NupulseCV, Fineheart, Transmedics, Leviticus, Cadrenal and Second Heart Assist; The remaining authors have no relevant disclosures other than institutional support for the study from Abbott.

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The study was designed by Dr. Mehra with lead PI Dr. Netuka and conducted solely at Institute for Clinical and Experimental Medicine, Prague, Czech Republic as a single center study. These details are provided on the Clinical Trials registration website where the complete study protocol is available at https://clinicaltrials.gov/study/NCT04974684).)

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Figure 1. Study Flow and Clinical Outcomes Depicting the Enrolled Patients, Withdrawals For Reasons of Either Heart Transplantation (considered success for the clinical outcome endpoint) or Another Cause (considered failure of the clinical outcome endpoint).

The 3-month time point was a safety end point achieving which 6 months of follow-up was mandated. Note that 2 uterine bleed events occurred in the same patient in the warfarin arm (accounted for as a single patient event in the primary endpoint). HTx = Heart Transplant

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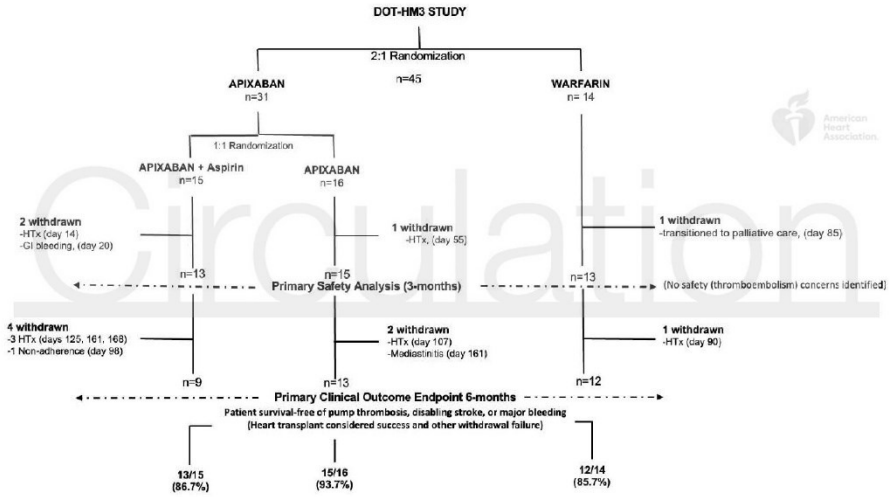
Figure 1: Study Flow and Clinical Outcomes



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FEATURED PAPERS

Evaluation of low-intensity anti-coagulation with a fully magnetically levitated centrifugal-flow circulatory pump—the MAGENTUM 1 study

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KEYWORDS:
HeartMate 3;
INR management;
left ventricular assist device;
LVAD;
reduced intensity anti-coagulation;
Rosendaal method;
TTR;
time in therapeutic range

BACKGROUND: The HeartMate 3 left ventricular assist system is engineered to avoid pump thrombosis, yet bleeding complications persist. We investigated the safety of low-intensity anti-coagulation in patients with the HeartMate 3.

METHODS: The Minimal AnticoaGulation EvaluatioN To aUgment heMocompatibility (MAGENTUM 1) pilot study is a prospective, single-arm study of low-intensity warfarin anti-coagulation in patients implanted with the HeartMate 3 pump. After standard warfarin anti-coagulation (international normalized ratio [INR] 2.0 to 3.0) and aspirin for 6 weeks post-implant, patients were transitioned to a lower INR target range of 1.5 to 1.9. The primary end-point was a composite of survival free of pump thrombosis, disabling stroke (modified Rankin score [MRS] >3), or major bleeding (excluding peri-operative bleeding) with at least 6-month post-implant follow-up. Time in therapeutic range (TTR) was measured to assess anti-coagulation target efficacy using the Rosendaal method. A safety algorithm to monitor for signs of pump thrombosis was developed and implemented.

RESULTS: We enrolled 15 patients (mean age 57.3 ± 13.3 years), 13 men with advanced heart failure (67% with INTERMACS Profiles 2 or 3), irrespective of therapeutic goal of bridge-to-transplant or destination therapy. The primary end-point was met in 14 of 15 (93 ± 6%) patients; 1 patient developed recurrent gastrointestinal bleeding. The TTR during the reduced anti-coagulation phase (6 weeks to 6 months) was 75.3 ± 8.6%. No thrombotic events occurred.

CONCLUSIONS: This pilot study suggests low-intensity anti-coagulation targeting an INR between 1.5 and 1.9 is achievable and safe with the HeartMate 3 cardiac pump in the short-term phase, 6-months post-implant. A large-scale trial is now warranted.

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The use of anti-platelet and anti-thrombotic therapy is a mainstay in left ventricular assist systems (LVAS) to mitigate complications such as pump thrombosis or systemic thromboembolism.[1–3] Typically, an anti-platelet agent, such as acetylsalicylic acid, and a vitamin K antagonist are used in therapeutic doses with the international normalized ratio (INR) targeted to 2.0 to 3.0. This approach, although effective, tilts the adverse effect profile toward surgical and non-surgical bleeding-related complications. As elderly patients are implanted with such devices for destination therapy with increasing frequency, bleeding complications have risen, largely due to coexisting morbidity.[4] Any attempt at reduction in anti-coagulation intensity is usually met with clinical concern for an increased risk of pump thrombosis and stroke with current devices, although this has not been systematically investigated.

The HeartMate 3 (HM3) LVAS (Abbott, Chicago, IL) is a continuous centrifugal–flow device with a fully magnetically levitated rotor, engineered with wide blood-flow paths and intrinsic pulsatility facilitated by speed changes of the rotor at fixed programmed intervals. In a series of experiences from Europe and the United States, this LVAS has shown absence of pump thrombosis (de novo; occurring within the pump) in the short term at 6 months.[2,3] However, these benefits have been observed in the setting of therapeutic use of aspirin and standard vitamin K antagonist anti-coagulation targeting an INR of 2.0 to 3.0. Encouraged by this early experience, we hypothesized that a lower intensity anti-coagulation range than that used currently may be employed with the HM3 LVAS, and this may reduce bleeding-related adverse events, without increasing thromboembolic complications. Thus, the Minimal Anti-coaGulation EvaluatioN To aUgment heMocompatibility (MAGENTUM 1) study was designed as a pilot trial to study feasibility and safety of a strategy to reduce anti-coagulation goals (INR 1.5 to 1.9) in stable patients supported with the HM3 LVAS, with closely monitored clinical surveillance and a structured anti-coagulation management protocol.

Methods

Study design

MAGENTUM 1 is a prospective, single-center, single-arm trial to evaluate safety and feasibility of a low-intensity anti-coagulation regimen in patients implanted with the HM3 LVAS. Low-intensity anti-coagulation was defined as a target INR of 1.5 to 1.9 (reduced from the standard target of 2.0 to 3.0 for HM3) starting at 6 weeks post-implant. The primary end-point of the study was survival free of pump thrombosis, disabling stroke (modified Rankin score [MRS] >3), and major bleeding with at least 6 months of post-implant follow-up, measured during the low-intensity anti-coagulation phase. All adverse events, principally those in the hemocompatibility (thrombosis and bleeding) domain, were collected as secondary end-points. Adequacy of anti-coagulation during the low-intensity phase was ascertained by calculating the time in therapeutic range (TTR) using the Rosendaal method. The trial is registered on ClinicalTrials.gov number NCT03078374.

Patients receiving the HM3 LVAS, irrespective of intended goal of therapy (either bridge to transplantation or destination therapy), were enrolled. The institutional ethics committee approved the protocol for a 6-month follow-up. Once patients reached the 6-month pre-specified goal of follow-up, the steering committee extended the follow-up to 12 months, with a conditional extension within the cohort for the entire duration of support on the HM3 (institutional ethics committee approval was also obtained). This strategy facilitated a safety measure in case futility of the approach was demonstrated during the initial phase of low-intensity anti-coagulation.

The trial was conducted at the Institute for Clinical and Experimental Medicine (IKEM), Prague, after design input from collaborators at Brigham and Women's Hospital/Harvard Medical School and Abbott. All adverse events were reviewed by the steering committee (IKEM and Brigham and Women's Hospital/Harvard Medical School) of the trial during weekly review of the trial. Data were collected and maintained by the study team at IKEM; the Brigham and Women's team reviewed and analyzed the data to calculate anti-coagulation efficacy, per protocol. The authors had access to the data and vouch for the completeness and accuracy of the data and the analyses.

Study conduct

Consecutive patients surgically implanted with the HM3 were managed based on institutional standard-of-care procedures and screened for study enrollment. Individuals who met study criteria and provided informed consent were enrolled. The reduced anti-coagulation regimen was commenced 6 weeks post-implant (on post-operative day [POD] 43). For details see the CONSORT diagram (Figure 1).

Study enrollment

The observation period of 6-week post-HM3 implantation was chosen to ensure clinical stability with anticipated discharge to the ambulatory setting. In addition, anti-coagulation management compliance was evaluated to ensure that patients could adhere to the rigorous follow-up, as judged by the principal investigator.

Exclusion criteria were a pre-implant history of major thrombotic event (e.g., deep vein thrombosis, pulmonary embolism); presence of any artificial valve prosthesis, except biological aortic valve; persistent atrial fibrillation or atrial flutter not amenable to left atrial appendage resection/exclusion; and hemodynamically significant or symptomatic carotid artery stenosis. All patients were tested before enrollment for such major hypercoagulable disorders by assessing Factor V Leiden, Prothrombin G20210A, anti-phospholipid syndrome, and lupus anti-

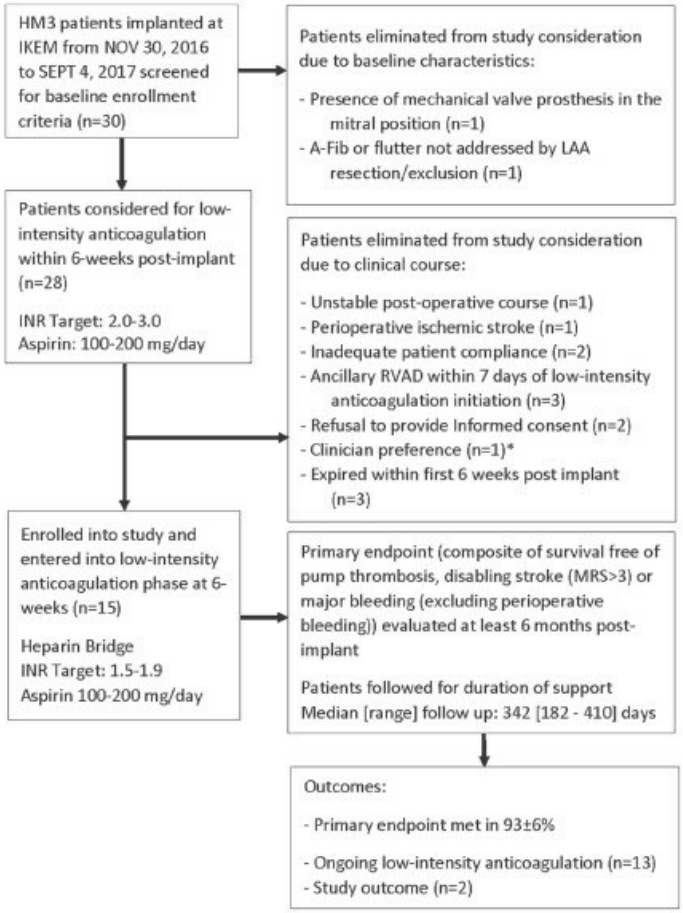

Figure 1 Study CONSORT diagram. *One BTT not enrolled due to clinician preference.

coagulant. Of note, presence of known hypercoagulable disorder did not affect enrollment nor the therapeutic strategy (unless a previous thromboembolic event history was identified).

Anti-coagulation management

Post-implant, all patients were bridged with unfractionated heparin until target anti-coagulation with warfarin was reached. Based on the standard anti-thrombotic regimen, all patients were maintained with a target INR of 2.0 to 3.0 for the first 6 weeks. Each patient received anti-platelet therapy (acetylsalicylic acid 100 to 200 mg) beginning on POD 3. If INR dropped below the therapeutic range, low-molecular-weight heparin was used until therapeutic range was restored.

Standardized warfarin management was based on a protocol established at Brigham and Women's Hospital/Harvard Medical School (refer to sections S1 to S3 in Supplementary Material available online at www.jhltonline.org). A dedicated inter-institu tional expert team, which included the study site's clinical pharmacist responsible for anti-coagulant dosing, was established to provide protocol-based INR management. The TTR was calculated using the Rosendaal method[5] as a measure of the efficacy of this anti-coagulation management strategy. INR testing was performed using venipuncture samples in a certified laboratory facility at least once per week throughout follow-up. Home INR point-of-care testing was not allowed in this study.

Safety measures

A safety algorithm to detect and manage pump thrombosis based on the patient's individual lactate dehydrogenase (LDH) profile and device log files analysis was developed and implemented as depicted in section S4 (refer to Supplementary Material online).

Adverse event definitions

Standard Interagency Registry for Mechanically Assisted Circu-latory Support (INTERMACS) definitions (version 3.0, www.intermacs.org) for adverse events were utilized. Pump thrombosis and stroke events were subject to immediate reporting and adjudication by the steering committee to determine safety of continuation of the study.

Statistical analysis

Categorical data are presented as both number and percent (%), whereas continuous variables are presented as mean ± standard deviation (SD). Time in therapeutic range was calculated using the Rosendaal interpolation method, presented as a percentage of days within range for each patient and as mean ± standard deviation (SD) for individual patients. The center TTR was calculated as the TTR for all values. TTR was calculated in the early 6-week post-implant phase for the target range 2.0 to 3.0, and then during the low-intensity anti-coagulation period. We excluded INRs from the TTR calculation during initial heparin bridging and during transition to the lower INR target per convention to allow clear distinction and separation of the switch in target INR ranges.[6]

The second INR measurement that was within the INR range after discontinuing heparin and after transitioning to the lower target range were used as the starting point for TTR calculations for the different INR target ranges. No other INR values were excluded from the individual or center TTR calculations, including INR values when patients required outpatient low-molecular-weight heparin (LMWH) bridging for sub-therapeutic INR. Uncensored TTR results are also calculated and reported in Table S5 (refer to Supplementary Material online). Actuarial analysis was conducted using the Kaplan–Meier plot estimate method to evaluate clinical outcomes after reduction in anti-coagulation. All outcomes and adverse events were evaluated through at least 6 months post-implant or last available follow-up.

Results

Patients' characteristics

Fifteen (n = 15) patients were implanted between November 30, 2016 and September 4, 2017, which included 13 men, all Caucasian, with a mean age of 57.3 ± 13.3 (range 18 to 72) years. Patients were distributed equally among bridge to transplantation, bridge to candidacy, and destination therapy (5 patients each); had INTERMACS Profiles of 2 or 3 (67%); and a mean cardiac index 1.6 ± 0.4 liters/min/m^2. Heterozygous Factor V Leiden mutation was found in 1 patient without a history of thromboembolic events. Patients were implanted via the sternotomy approach in 12 of 15 (80%) cases and via left thoracotomy in 3 of 15 (20%) cases. Left atrial appendage exclusion was performed in 5 patients (33%) using the Atriclip device (Atricure, Mason, OH). Detailed baseline characteristics and pre-operative risk factors are summarized in Table 1. All patients provided informed consent and completed a minimum of 6 months of post-implant follow-up.

Table 1 Demographics and Baseline Characteristics

Age [median (range)], years	61 (18 to 72)
Male sex	13 (87)
BMI (kg/m^2)	26.6 ± 2.7
Ischemic etiology	8 (53)
Indication	
Bridge to transplant	5 (33)
Bridge to candidacy	5 (33)
Destination therapy	5 (33)
INTERMACS profile	
Profile 2	3 (20)
Profile 3	7 (47)
Profile 4	3 (20)
Profile 5	2 (13)
Cardiac index (liters/min/m^2)	1.6 ± 0.4
LVEF (%)	22 ± 4
Atrial fibrillation	5 (33)
TIA or stroke	2 (13)
Pre-existing aortic bioprosthesis	1 (7)
Prior sternotomy	3 (20)

Values expressed as number (%) or mean ± standard deviation, unless otherwise stated. BMI, body mass index; INTERMACS, Inter-agency Registry for Mechanically Assisted Circulatory Support; LVEF, left ventricular assist device; TIA, transient ischemic attack.

Trial experience

The median primary hospitalization was 29 (range 11 to 62) days. Rehospitalizations, for any reason, occurred in 7 of the 15 (47%) patients for a median of 20 (range 6 to 54) days within the first 6 months of follow-up. The median follow-up duration for all patients was 342 (range 182 to 410) days. Patients spent a median of 133 (range 83 to 162) days out of the hospital within the first 6 months post-implant. For additional details see Table S6 (refer to Supplementary Material online).

Bridging for a sub-therapeutic INR was performed on 14 of 15 (93%) patients within the low-intensity anti-coagulation phase of the trial. Further details regarding LMWH bridging are presented in Table 2. Supra-therapeutic INR readings were found in all patients and accounted for 130 of the 930 (14%) INR readings.

Two patients had high baseline LDH: 1 patient (M1-04), with a baseline LDH 707 U/liter, had a partially dehisced mitral valvuloplasty ring resulting in hemolysis, and the second patient (M1-08), with an LDH 701 U/liter, had a confirmed history of Danon disease, which results in elevated LDH. LDH profiles excluding these 2 outliers are shown in Figure S7 (refer to Supplementary Material online).

The primary end-point of survival free of pump thrombosis, disabling stroke, and major bleeding was reached in 93 ± 6% of the patients (Figure 2A). No patients died, underwent transplant, or were explanted due to myocardial recovery within 6 months of implant. No (0%) neurologic complications (transient ischemic attack, seizure, or disabling stroke) were observed within 6 months of implant. Both hemocompatibility-related[7] and overall adverse events are presented in Table 3.

Hemocompatibility-related adverse events

No (0%) episodes of clinically relevant hemolysis were noted. In 1 patient (M1-09), an episode of suspected gastrointestinal bleeding with a drop in hemoglobin was encountered on POD 119 with a recurrent event on POD 210. In the first event, the patient presented with anemia (hemoglobin 4.9 g/dl) and an elevated INR of 3.34. The patient was transfused with 3 units of packed red blood

Table 2 Details of Anticoagulation Management: TTR and Bridging for Low INR

				INR Range: 1.5 – 1.9		
				6 weeks - 6 months		Bridging with LMWH for Low INR
Magentum ID	Sex	Age	Indication	TTR	% Time Above 1.8	Instances of Bridging (total days)
M1-01	M	60	BTC	56.4%	58.7%	2 (11)
M1-02	M	60	BTT	80.5%	18.5%	3 (4)
M1-03	M	50	BTT	80.6%	8.8%	4 (15)
M1-04	M	61	BTC	86.5%	26.9%	1 (2)
M1-05	F	51	BTT	67.2%	30.8%	3 (5)
M1-06	M	69	DT	81.9%	19.0%	1 (3)
M1-07	M	67	DT	77.2%	20.7%	3 (11)
M1-08	M	18	BTT	76.4%	23.1%	1 (3)
M1-09	M	64	BTT	65.4%	42.3%	2 (10)
M1-10	F	41	BTC	74.2%	22.2%	1 (4)
M1-11	M	64	DT	74.0%	21.6%	1 (5)
M1-12	M	72	DT	80.1%	12.1%	2 (14)
M1-13	M	50	BTC	77.8%	8.0%	3 (15)
M1-14	M	65	BTC	88.4%	20.3%	0 (0)
M1-15	M	67	DT	62.0%	45.6%	2 (8)
Mean				75.3%	25.2%	

cells, aspirin therapy was reduced from 200 to 100 mg/day, and the INR target reduced to a target close to 1.5. No source of active bleeding was found on gastroscopy, colonoscopy, or capsule enteroscopy, although internal hemorrhoids were observed. Upon re-admission for recurrent bleeding, the patient was treated with further reduction in anti-coagulation and use of octreotide, and subsequently underwent a successful transplant (refer to section S8 in the Supplementary Material online).

Anti-coagulation management

The center TTR in the reduced anti-coagulation phase from 6 weeks post-implant through Month 6 was 75.3 ± 9.0% (56.4% to 88.4%); individual patient TTR results are shown in Figure 2B and Table 2. Ability to achieve and maintain the INR target range of 1.5 to 1.9 throughout the full follow-up is depicted in Figure 3. Over 67% of all INR values in the low-intensity INR range fell between 1.5 and 1.9 for full patients follow-up. The uncensored center TTR value and uncensored individual TTR results are presented in Table S5 in the Supplementary Material online.

Safety monitoring—pump thrombosis and LDH algorithm

The safety algorithm was activated a total of 3 times in 2 patients, both presenting with elevated baseline LDH within the first 6 months. Beyond 6 months, safety monitoring was performed in 2 patients (once each). The first patient (M1-08) was among the 2 patients with elevated baseline LDH. The second patient (M1-02) presented at

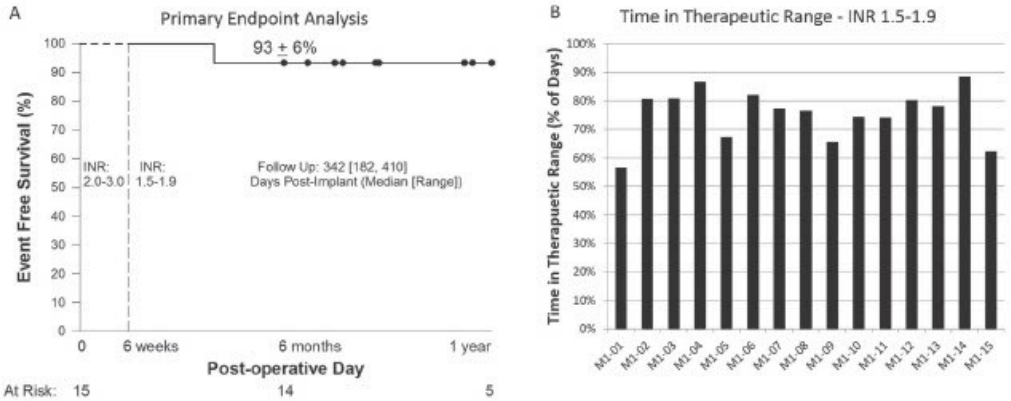

Figure 2 Primary end-point: event-free survival and patient TTRs.

Table 3 Adverse Events

Patient	Sex	Age	Indication	Hemocompatibility Related Adverse Events	All Other Adverse Events
1	M	60	BTC	none	none
2	M	60	BTT	none	Ventricular Arrhythmia (66, 283 POD)
3	M	50	BTT	none	Driveline Infection (28, 126 POD)
4	M	61	BTC	none	Urinary Tract Infection (49 POD)
5	F	51	BTT	none	none
6	M	69	DT	none	Hematoma in Urinary Bladder (17 POD)
7	M	67	DT	none	Revision for Cardiac Tamponade (13 POD) Infection – Sternotomy (102, 147, 239, 341 POD)
8	M	18	BTT	none	none
9	M	64	BTT	Suspected GI Bleeding (119, 210 POD)	none
10	F	41	BTC	none	none
11	M	64	DT	none	Ventricular Tachycardia, Cardioversion (2 POD)
12	M	72	DT	none	Respiratory Failure (8 POD) Urinary Tract Infection (29, 106 POD)
13	M	50	BTC	none	none
14	M	65	BTC	none	Urinary Tract Infection (3 POD) Gout (37 POD) Enlarged prostate resulting in TURP (49 POD)
15	M	67	DT	none	Driveline Infection (135 POD)

POD 229 with INR of 1.49 and LDH 341 U/liter, which was elevated relative to the post-clinical course (between 174 and 252 U/liter), but not relative to baseline (341 U/liter). Transthoracic echocardiography was normal and log-file interrogation was negative. The patient was bridged with LMWH according to the study protocol. Control LDH level at 2 days after was 294 U/liter. No additional complications were noted through POD 413. Safety monitoring detected no thrombosis-related adverse events.

Discussion

The MAGENTUM 1 pilot trial with the HM3 LVAS suggests that low-intensity anti-coagulation is feasible within a narrow INR range (1.5 to 1.9), and is not associated with an increase in thromboembolic complications, especially pump thrombosis, in a group of closely monitored and managed patients.

Reported rates of pump thrombosis at 12 months for patients implanted with commonly used LVASs are 6% to 12% for axial-flow pumps[8,9] (HeartMate II LVAS) and 8% with hydrodynamic centrifugal-flow devices[10,11] (HeartWare HVAD, Medtronic, Minneapolis, MN). In contrast, recent observations from both a single-arm study at 2 years[12] and a randomized trial at 6 months[3] with the HM3 LVAS, a magnetically levitated centrifugal-flow assist device, reported absence of de novo pump thrombosis. Similarly, an observational study showed greater preservation of von Willebrand high-molecular-weight multimers with the HM3 LVAS as compared with the HeartMate II device.[13] However, these attributes have not translated into decreased incidence of non-surgical bleeding during LVAD support, and the incidence of gastrointestinal bleeding at 6 months was similar and unchanged in a randomized trial experience comparing the centrifugal-flow HM3 and axial-flow HeartMate II LVAS (15.9% for both devices). Thus, a clinical rationale to reduce anti-coagulation targets exists for the HM3 LVAS; however, there is concern that the observed benefit of a low rate of pump thrombosis may be attenuated if such an approach were adopted.

Anti-platelet agents are used to provide not only rheologic but also anti-inflammatory effects.[14] Controversy

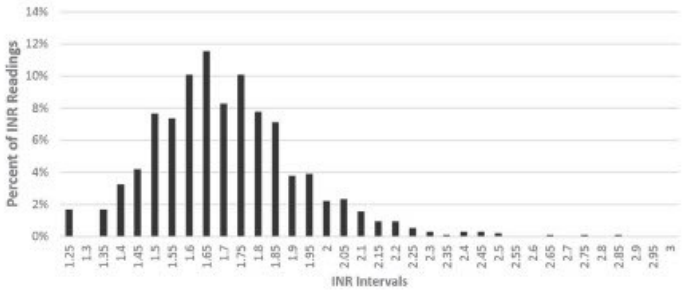

Figure 3 Proportion of INR values at various levels, with target INR 1.50 to 1.90 (INR measurements > 3.0 were excluded in 4 instances, representing 0.42% of measurements).

reigns with their use and whether they represent a critical component of therapy.[15] However, knowledge of their mechanistic utility remains incomplete in the context of LVAS and recent studies have suggested that platelets activate differentially in various clinical contexts and such activation, as assessed by the platelet activity assay, may correlate with thrombotic outcomes.[16] Furthermore, in the centrifugal-flow HeartWare HVAD pump, correlations with reduced anti-platelet therapy and heightened risk of ischemic stroke have emerged.[17] Thus, we chose to maintain anti-platelet therapy so that we could reliably investigate one component of the anti-thrombotic regimen, decreasing the INR target range.

Management of anti-coagulation and maintenance of the INR in a therapeutic range remains challenging. Poor INR control (TTR <60%) is typically the norm in patients with LVAS and there is emerging evidence that better control (as adjudicated by TTR >60%) is associated with a reduced risk of thromboembolic and bleeding complications.[18] A multidisciplinary initiative to optimize anti-coagulation practice, by establishing a standardized anti-coagulation management protocol, standardizing INR target ranges, and integrating a clinical pharmacist as a consultant into the care team, improves the TTR achieved in patients implanted with an LVAS.[19] In MAGENTUM 1, we adopted protocol-based, centralized management, developed at Brigham and Women's Hospital/Harvard Medical School. This INR management protocol was implemented at the study center in Prague, with patient warfarin management by a dedicated anti-coagulation–trained clinical pharmacist, to ensure a strictly controlled multidisciplinary initiative. In this study, we measured INR weekly exclusively with venipuncture samples at a certified laboratory and adjusted the warfarin dose according to the INR management protocol. To ensure safety, we did not allow use of point-of-care testing at home due to the reported inaccuracy of such systems, where a difference of 0.4 to 0.8 in INR for home monitoring is commonly reported.[20] We addressed 2 other potential safety concerns within the study. First, in patients with baseline atrial fibrillation, we uniformly performed left atrial appendage exclusion and patent foramen ovale closure, if detected. Second, we established a structured algorithm for biomarker surveillance of LDH and an early alert system using clinical and advanced LVAS interrogation parameters, as we reported in a previous study.[21]

Limitations to this single-center, prospective feasibility study include the small number of patients and the tightly monitored and controlled anti-coagulation management algorithm that achieved a high center TTR. We encountered a greater frequency of supra-therapeutic INRs that required adjustment as compared with sub-therapeutic range INRs. This may reflect: (a) the lower limit INR being closer to what would be considered a "normal" INR; (b) the inherent variability in pharmacokinetics and pharmacodynamics of warfarin dosing; (c) the impact of lifestyle changes such as exercise and dietary patterns; and (d) interceding illnesses (such as an infection) or drug–drug interactions. We were unable to discern a clear pattern in the time-curves for supra-therapeutic INRs; however, we did find that this occurred only 14% of time. A center TTR of ≥66% is considered excellent and has served as a benchmark for warfarin management in trials of atrial fibrillation with direct thrombin inhibitors or direct factor Xa inhibitors compared with vitamin K antagonists. A meta-analysis of all such trials[22] demonstrated that, once TTR increases, the advantages of direct thrombin inhibitors or direct factor Xa inhibitors compared with vitamin K antagonists decreases significantly. Furthermore, there are concerns about generalizability outside the controlled trial environment and it would be unwise to translate these preliminary pilot findings into routine clinical practice. Reproducibility and safety within larger patient cohorts will be required before these early findings can be adopted.

In conclusion, the results of this pilot study demonstrate feasibility and short-term safety at 6 months for use of closely monitored low-intensity anti-coagulation with a target INR range of 1.5 to 1.9 in patients implanted with the HM3 LVAS. We believe that this investigation has established the rationale for a large-scale, randomized, controlled clinical trial to test the clinical utility of low-intensity anti-coagulation with the HM3 LVAS on hemocompatibility-related adverse effects.

Disclosure statement

I.N. is a consultant, has received grant funds, and is on the advisory boards for Abbott and Carmat. SA. P.I., Z.T., S.G., and O.S. have received grants from Abbott. P.S. and D.C. are employees of Abbott. J.R. has no disclosures. J.M.C. is a consultant for Abbott. M.R.M. is a consultant for Abbott, Medtronic, Janssen, Portola, Mesoblast, and Bayer, and is on the advisory board for NupulseCV, Inc.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at www.jhltonline.org.

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Association of thrombophilia prospective detection with hemocompatibility related outcomes in left ventricular assist device patients

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Abstract

Introduction: Inherited thrombophilias represent a concerning risk factor due to a proclivity to an aberrant clot formation. However, in patients with left ventricular assist device (LVAD), their impact on bleeding and thrombotic complications remains still poorly understood. The aim of the present study was to evaluate the effect of thrombophilic mutation directed anticoagulation therapy on adverse clinical outcomes in LVAD patients.

Materials and methods: About 138 consecutive patients indicated for LVAD implant (HeartMate II, Abbott, Plymouth, USA) were prospectively screened for three major thrombophilic mutations: factor II (prothrombin), factor V Leiden, and homozygous methylenetetrahydrofolate reductase (MTHFR). Subsequently, discordant individualized anticoagulation targets of INR 2.5–3.0 in thrombophilia positive and INR 1.8–2.2 in negative patients were established; notably without anti-platelet agents given the center standard of care.

Results: Mean age was 50 ± 12.7 years, 83% male. Mean duration of support was 464.5 days (SD 482.9; SEM 41.1) and median of 310 days (IQR 162; 546). Full thrombophilia positive cohort analysis has not revealed any significant impact on event free survival. In contrast, detailed analysis of specific thrombophilias subsets has revealed Factor II prothrombin mutation as a significant predisposition for the pump thrombosis risk (SHR 10.48; $p = 0.001$) despite more aggressive prespecified anticoagulation target. Moreover, the incidence of bleeding events in prothrombin group was also significantly increased (SHR 6.0; $p = 0.03$).

Conclusions: Our observations suggest that specific thrombophilias in LVAD patients may pose different intensity predisposition for thrombotic complications. Factor II (prothrombin) positive mutation was identified as significant risk factor associated with the pump thrombosis.

Keywords

Thrombophilias, individualized anticoagulation, ventricular assist device, hemocompatibility, pump thrombosis

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Introduction

Left ventricular assist devices (LVADs) have emerged as a mainstay lifesaving treatment option for end-stage heart failure patients. Current actuarial survival rates at 1- and 2-years are reported as high as 81% and 70%, respectively.[1] In selected patient cohorts implanted with a fully magnetically levitated left ventricular assist device, the 2-year survival might reach up to 77%[2] and 83%,[3] respectively. Notwithstanding this undebatable progress continuous-flow LVADs are not free of caveats. Significant numbers of adverse events have been reported, in particular bleeding complications, cerebrovascular accidents and in most devices also the pump thrombosis.[4–7]

Previous reports of unexpected uptick in the incidence of pump thrombosis with axial flow pumps,[8] albeit highly variable among the centers,[9] shed interest in this topic, and has stimulated a detailed root cause assessment.[10,11] Much of this effort aims at developing patient and device-specific predictors of thrombus formation. Besides surgical implant technique and pump migration aspects, a huge effort has been devoted to analysis of bioreactive pump surfaces properties,[12] aberrant pump flow shear stress-induced platelet activation, hemolysis, and stasis. Likewise, relentless effort has been aimed at optimization of unloading algorithms and its use in early pump thrombus detection.[13] Consequently, a need for pump speed adjustments, an intensity and a frequency of aortic valve opening might play important role in a prevention of thromboembolism.[13]

Results of such clinical initiatives may indeed contribute to further outcomes improvement. However, other important factors, such as comprehensive analyses and understanding of systemic, in fact, inherent predispositions for thrombotic events known as thrombophilia still remain largely overlooked. LVADs related literature covering this topic is sparse. Carboni[14] and subsequently our group (Szarszoi et al.)[15] described a high-risk prothrombotic nature of these abnormalities and suggested their prospective identification. These observations were further supported by a retrospective analysis of the association of LVAD outcomes and pre-existing hematological conditions.[16] Current European expert consensus (2019) based on this limited evidence recommends "Evaluation of all long-term mechanical circulatory support candidates for coagulopathies and hypercoagulable states (e.g., thrombophilia; class I, level of evidence C)."[17]

Notably, prospective thrombophilias detection approach reflected by individualized anticoagulation regimen has not yet been published. In brief, consecutive axial-flow LVAD patients were screened prior to the implant on a presence of prespecified inherited thrombophilias. Positively screened patients were considered as potentially at higher risk of thrombotic complications and as such were subject to higher target range anticoagulation strategy. Conversely, those at presumably lower risk were maintained on considerably less intense anticoagulation.

Materials and methods

The study conducted between May 2009 and February 2015 encompassed all consecutive patients receiving axial continuous-flow device LVAD HeartMate II (Abbott, Plymouth, USA) uniformly as a bridge to heart transplantation. Procedure was performed via median sternotomy in standard fashion on cardiopulmonary bypass. Heparin bridge has been applied in all individuals until target anticoagulation with warfarin has been reached. Based on institutional expert consensus, three types of hereditary thrombophilias were identified as potentially high risk in respect to thrombotic events (homo/heterozygotic mutation in factor V Leiden, factor II prothrombin G20210A, and homozygous MTHFR gene variants C677T and A1298C). All the patients enrolled in the study were screened for the gene mutations mentioned above and subdivided into two arms based on thrombophilia presence. Method of Real-Time PCR was used to detect thrombophilia mutations in the study population (utilizing the GeneProof Factor II Prohtrombin PCR Kit, Factor V Leiden PCR Kit, MTHFR C677T PCR Kit and MTHFR A1298C PCR Kit; Gene Proof Plc., Brno, Czech Republic). An informed consent was a prerequisite for the mutation detection, in the other words, for participation in the study.

Comprehensive counsel of our expert hematologists proposed two differential target ranges with international normalized ratio (INR) of 1.8–2.2 in thrombophilia negative patients whereas 2.5–3.0 in those exhibiting positive screening.

Prior to procedure, patients with ischemic heart disease were treated with acetylsalicylic acid and statins in contrast to patients with non-ischemic etiology of heart failure. Notably, antiplatelet therapy has been discontinued prior to surgery and has not been further resumed through a course of LVAD support reflective of the institutional standard of care regarding antithrombotic regimen.

Patient follow-up ended in January 2019, was 100% complete and totaled 175.6 patient-years.

Collected data included patient characteristics, medical history, laboratory assessments, anticoagulation/antiplatelet medications, re-hospitalizations, and patient outcome (as and when they occur) over the course of LVAD support.

Post-discharge adverse events were collected (as and when they occur) and INTERMACS adverse event definitions (Version 2.2) were used for stroke, transient ischemic attack (TIA), pump thrombosis, and bleeding.

Baseline data are presented as percentage of the sample or mean with standard deviation. Chi-square test was used for comparison of categorical variables. Multiple groups analysis of continuous variables was performed with Kruskal–Wallis one-way analysis of variance test.

Table 1. Baseline characteristics in breakdown for each individual thrombophilia positive and thrombophilia negative subgroups.

	Factor V Leiden $n=16$	Prothrombin $n=8$	MTHFR homozygote $n=14$	Thrombophilia negative $n=100$	p-Value
Age (years)	53 ± 10.5	55 ± 12.3	45 ± 14.0	50 ± 12.6	0.830
Male (*n*/%)	14 (87.5)	5 (62.5)	10 (71.4)	85 (85)	0.229
INTERMACS profile	2.94 ± 1.09	2.75 ± 1.09	2.93 ± 0.96	2.56 ± 0.94	0.05
1 (*n*/%)	2 (12.5)	2 (25)	1 (7.1)	12 (12)	N/A
2 (*n*/%)	3 (18.8)	0	4 (28.6)	36 (36)	
3 (*n*/%)	6 (37.5)	4 (50)	4 (28.6)	39 (39)	
4–6 (*n*/%)	5 (31.3)	2 (25)	5 (35.7)	13 (13)	
Hypertension (*n*/%)	1 (6.3)	1 (12.5)	0 (0)	6 (6)	0.869
DM (*n*/%)	5 (31.3)	3 (37.5)	4 (28.6)	18 (18)	0.084
Smoking (*n*/%)	6 (37.5)	2 (25)	4 (28.6)	34 (34)	0.788
Stroke or TIA (*n*/%)	0 (0)	0 (0)	0 (0)	4 (4)	0.211
COPD (*n*/%)	2 (12.5)	2 (25)	1 (7.1)	10 (10)	0.595

COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; INTERMACS: interagency registry for mechanically assisted circulatory support; MTHFR: methylenetetrahydrofolate reductase; N/A: not available; TIA: transient ischemic attack.

The linearized rate for each adverse event was calculated as total number of observed events divided by total patient-months of follow-up and expressed as episodes per one patient—year (EPPY). Kaplan-Meier analysis was used to visualize the differences in outcomes by prothrombotic mutation presence. Because Cox regression is not able to correctly estimate marginal probability of an event in the presence of a competing event, competing risk factors model was used to analyze freedom from from pump thrombosis and freedom from bleeding, with death analysed as a competing event. Sub-hazard ratios with the level of significance are shown in the figures, with the group without a thrombophilic mutation considered as the reference. Calculations were performed using SPSS 21 software (Chicago, IL, USA). Two-tailed tests were used in analyses. A p value of <0.05 was considered significant.

Results

The cohort comprised 138 patients; 114 were males (82.6%). Mean age of the group was 50 ± 12.7 years, with a total time of follow-up 64,098 days. All of the patients received the LVAD in a bridge to transplant designation. INTERMACS profiles were reflective of severe advanced heart failure with 82% of patients being in profiles 1, 2, or 3. Detailed baseline characteristics including cardiovascular risk factors for individual subsets are described in Table 1. Strikingly, the prevalence of pre-defined thrombophilias was 27.5%. Of a note, no interaction was revealed between thrombophilias positivity and actuarial survival, censored at the time of transplant, explant, and at 1 year after device implantation (Figure 1). Mean duration of support was 464.5 days (SD 482.9; SEM 41.1) and median of 310 days (IQR 162; 546). For the patients still being on device, mean duration of support was 1677.5 days (SD 373.8; SEM 112.7) and median of 1546 days (IQR 1452; 1779).

About 96 (70%) of the patients were transplanted, 28 (20%) expired, 3 (2%) had the device explanted due to sufficient degree of myocardial recovery while 11 (8%) patients were still ongoing on device support at the time of this analysis.

Overall thrombotic and bleeding events are depicted in Figure 2 as an incidence of adverse events per patient years divided between thrombophilias positive and negative subgroups. Among all the patients, 5.8% (0.046 EPPY) of the patients experienced a bleeding event, 5.8% (0.046 EPPY) experienced a hemorrhagic stroke, 5.8% (0.046 EPPY) experienced an ischemic stroke, and 8.7% (0.068 EPPY) had pump thrombosis. Pump thrombosis subgroup encountered for 12 patients in total. All but one patient (deceased shortly before surgery) of the thrombosis-affected patients underwent successful pump exchange.

Statistical comparison has not revealed any significant impact of thrombophilias positivity on event free survival as documented at Figure 1. However, more detailed analysis for particular thrombophilias subsets has revealed striking increase of pump thrombosis in patients positively screened for factor II mutation (SHR 10.48; $p=0.001$) as shown at Figure 3 albeit increased anticoagulation target has been adopted. Surprisingly, incidence of bleeding events in prothrombin group was also significantly increased (SHR 6.0; $p=0.03$) as demonstrated by Figure 4. None of thrombophilia positive subgroups have been tending to higher incidence of hemorrhagic or ischemic stroke.

Discussion

In our prospective study we have observed that patients with different thrombophilias do not have the same susceptibility to thromboembolic complications. We have seen that positive mutation for factor II represents serious risk factor for pump thrombosis despite increased

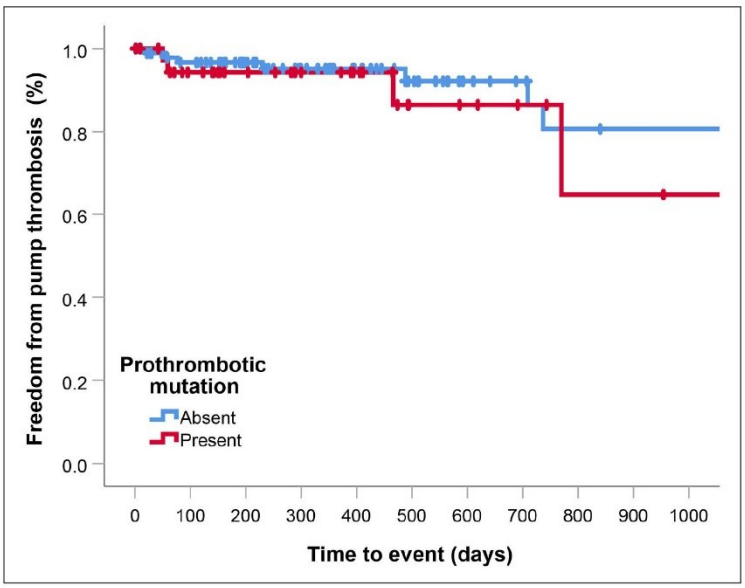

Figure 1. Impact of thrombophilias positivity on event free survival—no interaction was revealed between thrombophilias positivity and actuarial survival, censored at the time of transplant, explant, and at 1 year after device implantation.

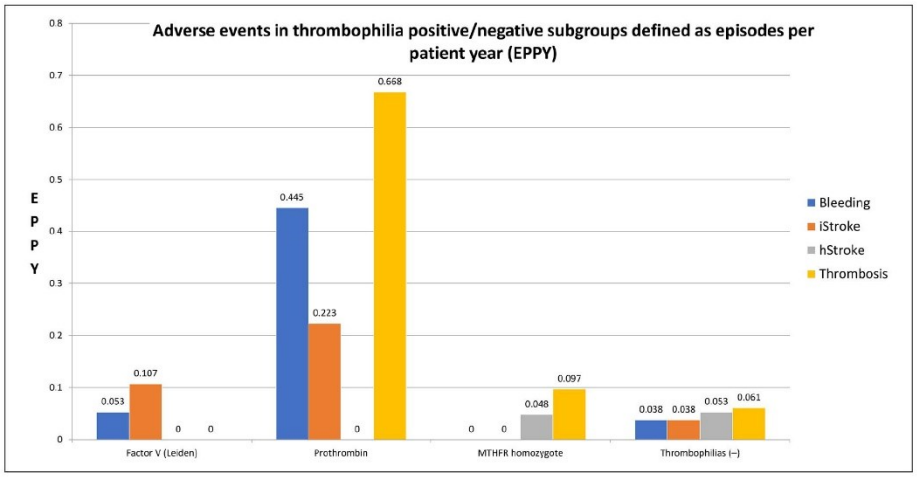

Figure 2. Adverse events in thrombophilia positive/negative subgroups defined as episode per patient year (EPPY).

anticoagulation target and moreover, these patients experienced increased hemorrhagic events.

Thrombophilia can be defined as a predisposition to excessive clot formation. Likewise, in other areas of medicine, in cardiac surgical patients, it can cause severe complications. Previous studies reported a high incidence of perioperative and postoperative thromboembolic events in patients with heterozygous Factor V (Leiden) mutation

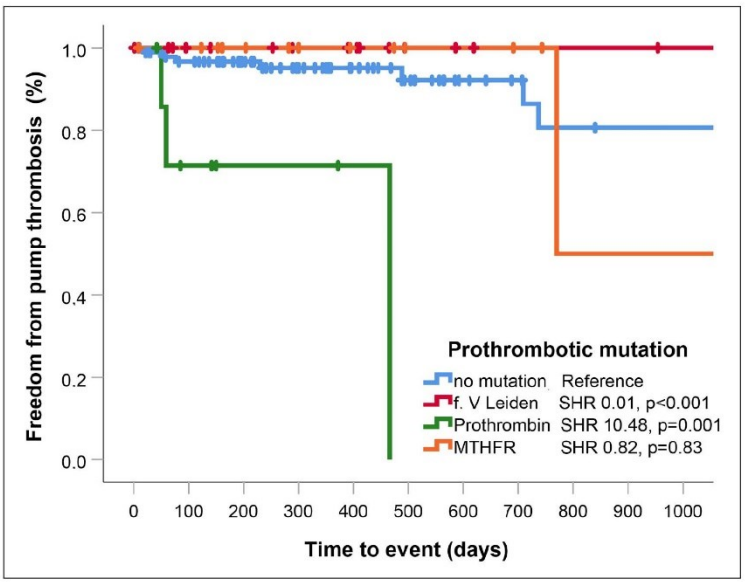

Figure 3. Freedom from pump thrombosis in individual study subsets—striking increase of pump thrombosis in patients positively screened for factor II mutation (SHR 10.48; $p = 0.001$).

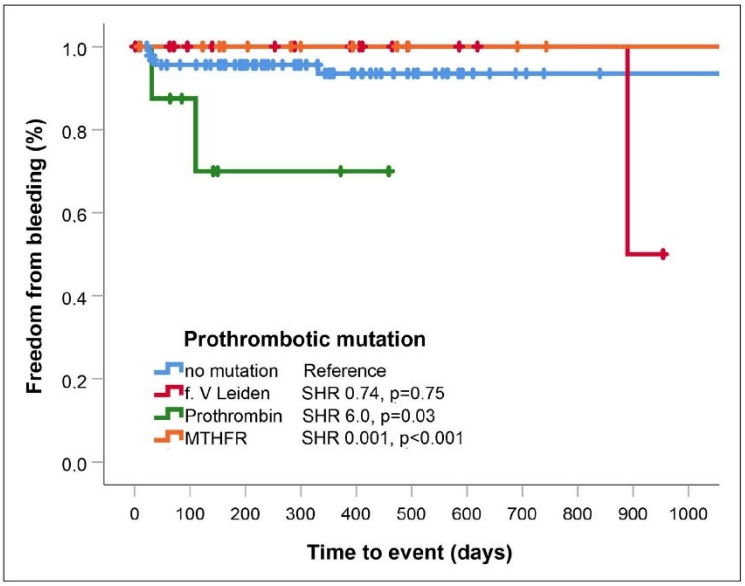

Figure 4. Freedom from bleeding events in individual study subsets—significantly increased incidence of bleeding events in patients positively screened for factor II mutation (SHR 6.0; $p = 0.03$).

who had undergone coronary artery bypass grafting.[18,19] Moreover, the presence of artificial materials in the circulatory system may render prothrombotic disease even more dangerous. Compelling data assessing this putative risk factor in patients implanted with mechanical circulatory support devices are missing.

A prevalence of thrombophilias, for example, Factor V (Leiden) mutation, exhibit clear dependence based on ethnical origin with highest rates in Caucasians—2%–15% in general population. To demonstrate a magnitude of an issue, recent study of random sample of over 1500 individuals at our referral metropolitan area confirmed relatively high prevalence of two abnormalities tested in our study alike—factor V Leiden and factor II of 4.5% and 1.3% respectively.[20] Importantly, in our cohort these abnormalities together accounted for up to 17%, thus suggesting potential clustering of these hereditary prothrombotic milieu individuals in end-stage heart failure population. However, as there are no strong data in current literature supporting this thesis, we do talk about an assumption. Randomized multicentric trials would be warranted to clarify this topic.

Based on results from the HM II clinical trial, the antithrombotic recommendation for the HM II requires aspirin (81–325 mg) daily associated with Warfarin aiming at an International Normalized Ratio (INR) of 1.5–2.5.[21] However, given the aforementioned facts, it seems intuitive, that more individualized approach may additionally contribute to optimizing outcomes.

In the setting of a non-randomized study, it is very challenging to identify an appropriate comparator to a single-arm cohort. In comparison to Boyle et al.,[7] who summarizes post-discharge patients' outcomes of the same type of device, our overall cohort analysis (Figure 2) revealed favorable bleeding rates and the similar rate of ischemic stroke, while pump thrombosis was twice higher but still in acceptable levels. Compared to recent report of Starling et al.,[4] adverse events probability of our study group was clearly lower.

Comparison of thrombophilias positive and negative subsets outcomes have not shown any statistical significance. That is in contrast to recent retrospective analysis which clearly demonstrated prominent increase in thrombotic complications in patients with prior hematological disorders (0.57 thrombotic EPPY).[16] A potential explanation might be that those patients have been treated with more relaxed antithrombotic protocol. The authors conclude, that based on the results of these high-risk patients, more aggressive protocol, apparently similar to ours, has been introduced.

By reviewing in a detailed breakout of each individual thrombophilia subset, in prothrombin group we have identified highly significant increase in pump thrombosis accounting for 37.5% of cases (0.668 EPPY) (Figure 2), which closely corresponds to the rates reported by Uriel et al.[11] It suggests high risk nature of this inherited abnormality. Conversely, Factor V Leiden and MTHFR appear relatively benign while managing patients with increased anticoagulation protocol (Figure 3). Moreover, homozygotic MTHFR mutation exhibits its potential thrombogenicity rather indirectly by hyperhomocysteinemia, which if detected, can be effectively normalized by folic acid administration. Thus, it may not be necessarily considered as a risk factor in LVAD patients.

On the other side, there have been other abnormalities which were not encountered in hematological workup, in particular antibody-mediated anti-phospholipid syndrome or vitamin K dependent proteins C and S deficiency.[14] However, hypercoagulable potential of latter two over the initial phase of vitamin-K antagonist therapy can be mitigated by our routine use of Heparin bridge.

A deleterious effect of bleedings on LVAD patients is mirrored at elevated blood requirements and the subsequent risk of infection and immunosensitization. Importantly, it has been documented, that alterations of anti-thrombotic therapy in response to bleeding, either reversal or cessation of anticoagulation, are associated with an increased risk of subsequent thromboembolic events.[22] The authors concluded that prevention of bleeding may ultimately contribute also to lower thromboembolic events rate, particularly in an elderly LVAD population.

In our observations, we have demonstrated evenly low rates of bleeding events compared to previously published multicentric series.[7] Interestingly, rates were not elevated by increased anticoagulation target in the overall thrombophilias positive subsets. However, the aforementioned paper identified a positive correlation of DT strategy, older age (>65 years) and female sex with an increased risk of bleeding post discharge. Thus, it is possible that reduced incidence of bleeding in our cohort is at least in part due to the characteristics of our study cohort, considered relatively lower risk in view of these findings.

Supplementary factor to explain more favorable freedom from bleeding may be our routine use of anti-platelet free protocol. Data from single center reports indicate that many HM II patients have been maintained on reduced anti-coagulation. Experience on the cost-benefit associated with anti-platelet free antithrombotic therapy in patients with CF-LVADs remains limited. Typically, these modified regimens are in response to a bleeding event, albeit some European centers, including ours, have instituted a reduced anti-thrombotic monotherapy as standard of care with encouraging results.[23]

A rational behind this strategy posits that the impact of LVAD implantation on platelet activation is at least somewhat offset by the presence of a von Willebrand's syndrome due to the deficiency of high-molecular von Willebrand multimers, one of key players in platelet adhesion and aggregation.[24–26] Reflexively, in the setting of von

Willebrand's syndrome, antiplatelet therapy may additionally suppress platelet function and thus lead to an incremental risk of bleeding complications. Thus, avoiding antiplatelet agents may reduce bleeding complications, while appropriately targeted anti-vitamin K agents' therapy might still protect against thrombotic events.

Furthermore, LVAD bleeding events are not confined to hematological aspects only. Interestingly, as reported by Wever-Pinzon et al.,[27] there is a clear correlation between a decrease of residual pulsatility indicated by HeartMate II pulsatility index and a rise of non-surgical bleeding.

Limitations

There are obvious limitations to this single-center prospective observational study. There was no randomization or blinding, and therefore, caution should be used in interpreting the results. There was not any head-to-head comparator for solely arbitrary set anticoagulation target for presumably high and low risk subgroups based on intra-institutional experts' consensus and only a subset of thrombophilias have been screened.

Our data were limited to a sample size of 138 patients with uneven subgroup size distribution and relatively low occurrence of adverse events, which limits statistical power of our findings. Notably, the study population may not be fully representative of the overall LVAD landscape while encompassing a cohort of younger and exclusively bridge-to-transplant population in contrast to a majority of recently published papers including elderly DT therapy individuals. Further, one major limitation in the follow-up may have been deviations from targeted INR levels and a limited evidence of INR levels course due to standard twice a week check-up. Additionally, it is possible that unmeasured confounders may have influenced outcomes. Lastly, it is of critical importance to note that bleeding and thrombotic complications in LVAD patients are truly multifactorial phenomenon and that contributors such as residual pulsatility and mean blood pressure have not been systematically analyzed.

Conclusions

To the best of our knowledge, we believe that our study is quite unique. It is the first prospective thrombophilias detection study in patients prior to continuous-flow LVAD implantation. We observed that various thrombophilias exhibit differential impact on thrombotic complications. Positive screening for factor II mutation represents serious risk factor of pump thrombosis whereas factor V Leiden and homozygous mutation for MTHFR remain insignificant while maintained on proposed increased INR target. Nevertheless, overall thrombophilia positive group patients, considered as high-risk population in regard to thrombosis, did not tend to bleed more despite a higher level of therapeutic anticoagulation. However, the factor II mutation subgroup showed a higher bleeding rate compared to the other groups.

This momentous observation urges for further clarification. If confirmed on broader based observations, it may eventually arise a question of changing recommendation on eligibility of this subgroup for device therapy and provocatively, even prioritization on waiting list for heart transplantation. However, it should also be taken into consideration that recent generations of centrifugal pumps may have a different proportion of adverse events.

Notwithstanding aforementioned limitations, it is clear that these findings warrant further data collection of this predisposing factors in LVAD patients as a prospective registry. Such an approach would ensure definition of risk profiles to define more stringent recommendation on the suitability of these, assumingly high-risk patients, for device therapy and subsequently broader based individualized anticoagulation strategy alike. Consequently, prospective randomized studies are warranted to determine whether a targeted anticoagulation reflective of inherent ambient proclivity to thrombosis might further optimize the balance between bleeding and thrombosis.

Author contributions

Miroslav Konarik: Data collection, data analysis/interpretation, drafting article. Ivan Netuka: Concept/design, critical revision of article. Peter Ivak: Data collection, data analysis/interpretation. Hynek Riha: Critical revision of article. Zuzana Tucanova: Data collection. Peter Wohlfahrt: Statistics, data analysis/interpretation. Jiri Maly: Critical revision of article. Ondrej Szarszoi: Concept/design, critical revision of article.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Ivan Netuka is a consultant, has received grant funds (outside of the submitted work) and is on advisory boards for Abbott, CARMAT SA and LeviticusCadio Ltd. P. Ivak is a consultant for Abbott. J. Maly is a consultant for Abbott. The other authors declare no conflict of interest.

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A multicenter evaluation of external outflow graft obstruction with a fully magnetically levitated left ventricular assist device

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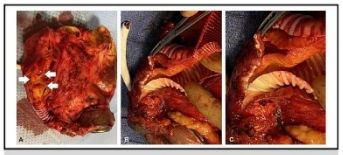
ABSTRACT

Background: The HeartMate 3 (HM 3; Abbott) left ventricular assist device (LVAD) has improved hemocompatibility-related adverse outcomes. In sporadic cases, external compression of the outflow graft causing obstruction (eOGO) can result from substance accumulation between the outflow graft and its bend relief. We sought to evaluate the prevalence, course, and clinical implications of eOGO in an international study.

Methods: A multicenter retrospective analysis of HM 3 LVADs implanted between November 2014 and April 2021 (n = 2108) was conducted across 17 cardiac centers in 8 countries. We defined eOGO as obstruction >25% in the cross-sectional area in imaging (percutaneous angiography, computed tomography, or intravascular ultrasound). The prevalence and annual incidence were calculated. Serious adverse events and outcomes (death, transplantation, or device exchange) were analyzed for eOGO cases.

Results: Of 2108 patients, 62 were diagnosed with eOGO at a median LVAD support duration of 953 (interquartile range, 600-1267) days. The prevalence of eOGO was 3.0% and the incidence at 1, 2, 3, 4, and 5 years of support was 0.6%, 2.8%, 4.0%, 5.2%, and 9.1%, respectively. Of 62 patients, 9 were observed, 27 underwent surgical revision, 15 underwent percutaneous stent implantation, 8 received a heart transplant, and 2 died before intervention. One patient underwent surgical revision and later stent implantation. The mortality with therapeutic intervention was 9/53 (17.0%).

Conclusions: Although uncommon, HM 3 LVAD-supported patients might develop eOGO with an increasing incidence after 1 year of support. Although engineering efforts to reduce this complication are under way, clinicians must maintain a focus on early detection and remain vigilant. (J Thorac Cardiovasc Surg 2022;■:1-9)



Split bend relief with gelatinous substance. Gelatinous substance is marked with arrows.

CENTRAL MESSAGE

External outflow graft obstruction is an uncommon postoperative adverse event in patients with a HeartMate 3 (Abbott). Our analysis of 2108 patients revealed an increasing incidence after 1 year of support.

PERSPECTIVE

Our findings suggest that centers should develop a standardized strategy of surveillance to screen patients with HM 3 LVADs for eOGO and that imaging should be performed without delay when this complication is suspected.

See Commentary on page XXX.

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Abbreviations and Acronyms
CT = computed tomography
eOGO = external outflow graft obstruction
HM 3 = HeartMate 3
LVAD = left ventricular assist device
PA = percutaneous angiography

Scanning this QR code will take you to the table of contents to access supplementary information.

The incidence of pump thrombosis for the HeartMate 3 (HM 3; Abbott) left ventricular assist device (LVAD) is lower than for the HeartMate II (Abbott) or the HeartWare HVAD (Medtronic) LVADs.[1-4] However, obstruction of the outflow graft due to twisting,[5] intraluminal thrombus formation,[6] and extraluminal compression might occur with the HM 3 system.[7-9] The twist problem was solved by improved outflow graft attachment designs. Kinking or anastomotic stenosis is caused by suboptimal surgical techniques. External outflow graft obstruction (eOGO) is specific to the pump design and it remains unclear whether this can be avoided by modifying the bend relief during implantation. Therefore, our study focused on extraluminal compression by a gelatinous substance between the outflow graft and the bend relief, which might result in severe, life-threatening hemodynamic impairment. In this study we study aimed to determine the prevalence, incidence, clinical implications, treatment, and outcomes of eOGO.

METHODS

Study Design

This was a multicenter, retrospective study for which we collected data from patients supported with a fully magnetically levitated centrifugal-flow HM 3 LVAD. The study was approved by the institutional review board of each of the 17 participating centers in Austria, the Czech Republic, Denmark, Germany, Italy, Saudi Arabia, the Netherlands, and the United States. The authors vouch for the complete and accurate acquisition of all the data, the validity of the results, and the conclusion. The institutional review board or equivalent ethics committee of the authors' institutions approved the study protocol and publication of data. The study was approved by the institutional review board at the Charité—Universitätsmedizin Berlin (approval number: EA1/120/21) on May 20, 2021. The patients provided informed written consent for the publication of the study data.

Study Population

We analyzed data of patients with a reliable diagnosis of eOGO, confirmed using imaging, and defined as outflow graft obstruction by external compression due to an accumulation of gelatinous substance between the outflow graft and the bend relief. Patients with a thrombus inside the outflow graft, a twisted or kinked outflow graft, stenosis of the distal part of the outflow graft or aortic anastomosis, and patients supported with two HM 3 pumps for biventricular support or with a total artificial heart configuration were excluded from the analysis.

During the course of the study, 2 modifications were made to the outflow graft system of the HM 3. This resulted in 3 different outflow graft attachment designs, which eliminated the twist: no clip/no nut lock, clip, and nut lock. These changes do not affect the build-up of gelatinous substance between the bend relief and the outflow graft and therefore they were not recorded and evaluated in the present study.

Data Acquisition

We included all patients with eOGO who underwent HM 3 LVAD implantation between November 2014 and March 2021. The outcomes of interest were pump replacement or removal for heart transplantation, weaning, or death. Data were collected by means of a review of electronic medical records. Microsoft Excel was used for data collection.

Statistical Analysis

Categorical and continuous variables are summarized as frequencies, percentages, and mean/median with interquartile range, respectively. A Kaplan–Meier analysis and cumulative incidence function is presented for patients diagnosed with eOGO. The analysis was conducted in R version 4.02 (R Foundation for Statistical Computing).

RESULTS

Patients

Seventeen centers participated in this study. From June 2014 through March 2021, a total of 2108 patients received an implanted HM 3 LVAD, and of these, 62 patients (2.9%) were diagnosed with eOGO during the course of the study period. The mean support time until the eOGO diagnosis was 953.4 ± 462.5 days, and baseline characteristics and detailed surgical techniques used are described in Table E1 and Table 1. A full sternotomy was performed in most of the patients (83.87%). In 56 patients the outflow graft was connected to the ascending aorta. In 33 patients the surgeon had chosen a long run of the outflow graft (eg, around the right atrium and the right ventricle). Twenty-two patients (36%) had at least 1 additional concomitant surgical procedure with the HM 3 implantation. In many of these

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TABLE 1. Baseline characteristics of patients (N = 62)

Characteristic	Value
Sex	
Male	54
Female	8
Age, y	59 ± 14
Cardiac pathology	
Dilated cardiomyopathy	26
Ischemic cardiomyopathy	25
Nonischemic cardiomyopathy	6
Valvular cardiomyopathy	2
Combined ischemic/valvular cardiomyopathy	1
Combined ischemic/dilated cardiomyopathy	1
Chemotherapy-induced cardiomyopathy	1
Intended goal of pump support	
Destination therapy	33
Bridge to transplantation	26
Bridge to candidacy for transplantation	2
Bridge to decision	1

Data are presented as mean ± SD or n.

cases (n = 10), the tricuspid valve was repaired concomitantly. Four patients required postoperative surgical revision due to outflow graft kinking, bend relief dislocation, or cardiac tamponade. Five patients underwent a secondary transcatheter aortic valve replacement procedure and 1 patient subsequently underwent coronary artery bypass graft surgery (Table E1, Table 1).

Clinical Presentation

One-third of the patients (32.79%) presented with a low-flow alert when admitted to the hospital. Six other patients (9.84%) had a combination of low-flow alert and associated symptoms (eg, dyspnea, anemia, or cardiac decompensation). Thirteen patients (20.97%) reported dyspnea as the main symptom and 9 other patients (14.75%) had dyspnea and another symptom like arrhythmia or angina pectoris. Three patients (4.92%) reported nonspecific symptoms like abdominal pain, fatigue, or epistaxis. In 6 patients (9.84%), eOGO was asymptomatic and was diagnosed incidentally (Table E2). Anticoagulation at admission, LVAD parameters, and laboratory parameters at admission and discharge are provided in Tables E3 through E5.

Computed tomography (CT) or percutaneous angiography (PA) was performed for the eOGO diagnosis in 50 and 19 patients, respectively. In most cases the imaging results contained only a qualitative statement, for example, "(severe) external stenosis," "filling defect," "compression," or simply "(proof of) obstruction." In case of a qualitative statement, the eOGO was defined as being equivalent to a degree of obstruction of 25%. Seventeen of 50 patients underwent PA and CT imaging. For 20 CT studies a quantitative estimation of the degree of eOGO was reported. Two patients showed obstruction ≤50%, 14 patients had obstruction with 51% to 74% stenosis, and 4 patients showed an eOGO ≥75%. For 30 CT reports a qualitative diagnosis of eOGO was made. Two PA reports described an eOGO ≤50%; 1 of these patients underwent intravascular ultrasound. Three PA reports described an eOGO ≥75%. The 2 patients whose CT scan showed <50% stenosis were symptomatic: 1 exhibited dyspnea and the other a low-flow alert. The first patient died before treatment and the second underwent successful transplantation. The 2 patients with <50% stenosis in the PA were symptomatic and successfully stented. One of these patients exhibited severe stenosis (with a residual diameter of 7 mm) in the CT scan (Table E6). We did not find a correlation between the degree of stenosis and presenting symptoms at admission (Table E7). Echocardiography was performed in 51 patients at admission. The data are provided in Table E8.

The annual incidence of developing eOGO was 0.6% at 1 year, 2.8% at 2 years, 4.0% at 3 years, 5.2% at 4 years, and 9.1% at 5 years of HM 3 support. The incidence range across participating centers was 0% to 1.7% at 1 year, 0% to 7.7% at 2 years, 0% to 25% at 3 years, 0% to 20% at 4 years, and 0% to 100% at 5 years. The 100% result is because in this particular center only 1 patient was supported for 5 years, and stenting of eOGO was successful in this patient (Figure 1).

Treatment

Twenty-four patients underwent re-thoracotomy with removal of gelatinous substance and consequent relief of eOGO. As a consequence of eOGO, 8 patients underwent urgent heart transplantation. Three patients underwent pump exchange, whereas 15 patients had a percutaneous intervention with dilatation and/or stenting of the outflow graft. One additional patient underwent re-thoracotomy with removal of gelatinous substance; this patient later needed a percutaneous intervention with stenting of the

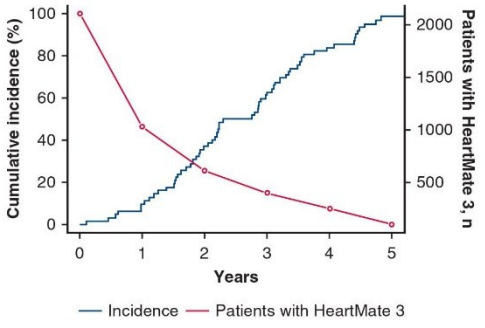

FIGURE 1. Cumulative sum.

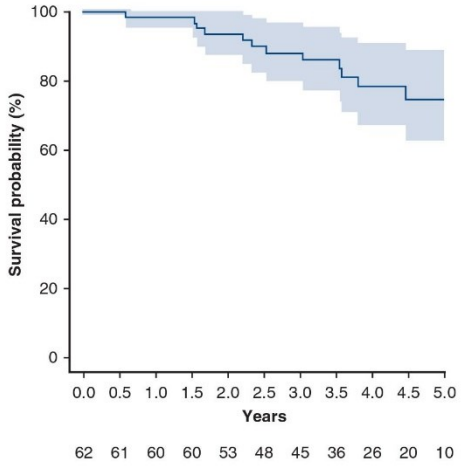

FIGURE 2. Survival probability after left ventricular assist device implantation with 95% CI indicated by *blue shading*.

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outflow graft. For 9 patients a watchful waiting strategy without invasive treatment was chosen (Table E9).

Overall, 14 patients died after the eOGO diagnosis. Nine patients died in the hospital where the eOGO was first diagnosed on admission. Two patients died in-hospital after stenting. Five patients died in-hospital after surgery. Two patients died before percutaneous intervention or surgical intervention could be performed. There was no in-hospital death after heart transplantation. Five patients died during the follow-up due to noncardiac reasons (Table E10). The Kaplan–Meier survival curve of our 62 patients after their LVAD implantation is shown in Figure 2.

DISCUSSION

The principal findings of this international collaborative analysis suggest that eOGO occurs sporadically, varies in prevalence and incidence according to center, and has an overall prevalence of 3.0%. Importantly, this complication has an increasing incidence as time of LVAD support grows. We confirm that the overall incidence is comparable with that previously reported.[10] Recently published data from large registries show that the overall survival of eOGO patients is similar to that of all patients supported with the HM 3. Patients (n = 516) enrolled in the Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3 (MOMENTUM 3) trial were followed-up for 2 years or until death. The 2-year survival was 0.75.[2] The third report of the European Registry for Patients with Mechanical Circulatory Support (EUROMACS) showed similar results for 1254 patients. The cumulative incidence of mortality after 5 years of follow-up was 0.35.[11]

We note that the emergence of low-flow alarms, especially when coupled with de novo symptoms suggestive of heart failure, should raise immediate suspicion for this potential complication, particularly in the later duration of LVAD support. After excluding other diagnoses, imaging should be undertaken via intravascular ultrasound, PA, or contrast CT with 3D volume rendering (Figures 3 and 4). The finding of eOGO appears to commonly require intervention, and the initial approach in most centers was rethoracotomy with opening of the bend relief and removal of the compressing gelatinous substance (Figure 5).

Because of the small number of eOGO patients we were unable to determine a statistical difference between the centers regarding the surgical technique at initial LVAD implantation (Table E1). We had 3 patients who underwent a lateral approach with splitting of the bend relief and

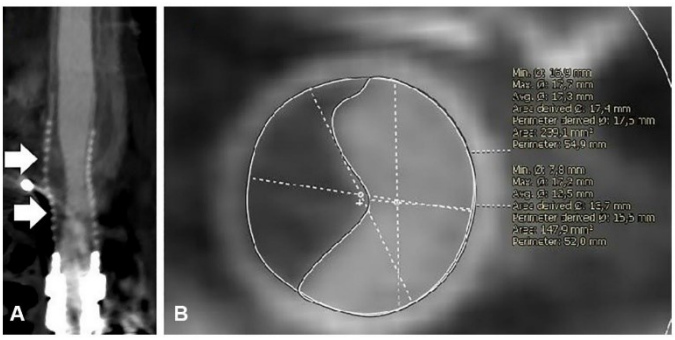


FIGURE 3. Computed tomography images of external outflow graft obstruction in longitudinal (A) and axial (B) views. *Arrows* point directly at external outflow graft stenosis (A).

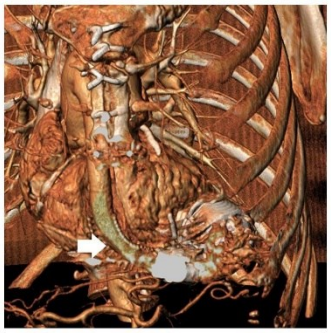


FIGURE 4. Three-dimensional volume rendering of computed tomography image showing external outflow graft stenosis. The *arrow* points directly at the external outflow graft stenosis.

removal of gelatinous substance. In these patients, an outflow graft connection to the descending aorta was performed during the initial LVAD implantation. The lateral approach might be preferable to re-sternotomy.

Patients with internal outflow graft obstruction due to thrombus formation visible on the CT scan or PA were excluded from our analysis. Internal or external obstruction is analyzed using different imaging modalities and can be recognized and differentiated with CT imaging or PA.[12]

A twisted outflow graft is suspected of being a cause of eOGO. It is conceivable that eOGO can be provoked by enlargement of the free space between the bend relief and the outflow graft and the increased pressure before the twist. These 2 changes inside the bend relief might lead to more oozing and accumulation of the gelatinous substance, but this is an epiphenomenon of the twist. In our study all cases with a twisted outflow graft were excluded from the analysis. In October 2018 the manufacturer Abbott modified the technical design of the system by adding an outflow graft clip.[13] Since this modification there have been no reported cases of a twisted outflow graft, but in our study 8 patients with eOGO underwent implantation after October 2018. In this study no patient with eOGO had a twisted outflow graft after the initial LVAD implantation. One patient with eOGO postoperatively underwent revision because of a kinked outflow graft. This patient developed eOGO with dyspnea after 1143 days of LVAD support (Table E1).

Over time, minimally invasive percutaneous stenting of the outflow graft was performed at some centers with good results.[14,15] In this regard, successful stenting refers only to the elimination of stenosis. In our study 2 patients died after successful intervention with stenting due to multiple organ failure. Urgent heart transplantation might be a desirable option in selected cases at centers with a short waiting time for an organ donor. However, in an unstable hemodynamic situation, surgical or percutaneous intervention is the best immediately available option.

Not all eOGO cases require immediate intervention. In our series, clinicians observed patients in whom no overt device alarms or symptoms prompted a diagnosis, and eOGO was mostly diagnosed coincidentally during imaging for other reasons. Although it is less common (15% in our series), asymptomatic eOGO obstructing <50% of the cross-sectional area might be closely observed with a serial CT imaging follow-up strategy. However, in symptomatic patients, if an improvement in filling pressures cannot be achieved without markedly increasing the pump speed, and in patients who are asymptomatic but with obstruction >50% of the cross-sectional area, caution is advised because rapid progression of heart failure is a threat. Naturally, such a recommendation requires further investigation to determine the rate of progression, and a strategy of serial surveillance imaging (ideally with CT scans) of all patients supported with the HM 3 might be an option to detect eOGO at an early stage and to study the natural history of early lesions. However, this kind of strategy is resource-intensive, associated with radiation exposure, and might not be feasible if clinical contraindications (such as kidney disease) exist. In the course of this study 1 participating center began to screen all supported patients using serial CT scans once and discovered that 3 of 29 patients exhibited eOGO with 60% to 70% stenosis; these patients might have been otherwise overlooked clinically. However, such a strategy cannot be advocated at this time and must be considered anecdotal.

Interestingly, we noted marked intercenter variability in the development of this complication. One of our largest centers (according to implantation volume) reported no cases of eOGO (Table 2). The shorter duration of support because of the high frequency of heart transplantation might explain this phenomenon. At another center with a high incidence of eOGO, we noted a longer run of the outflow graft requiring gelatinous substance removal and shortening of the outflow graft during reoperation with cardiopulmonary bypass.

The outflow graft of the HM 3 is made of gelatin-impregnated, woven polyester. The bend relief is made of polytetrafluoroethylene and is impermeable (Figure E1). In all patients in our study, the gelatinous substance accumulated in the space between the outflow graft and the bend relief. However, some cases have been reported in which surgeons covered the distal part of the outflow graft with a Gore-Tex membrane to prevent adhesions. The Gore-Tex membrane is also made of polytetrafluoroethylene. This resulted in development of eOGO in the distal part. In our study there was no case of eOGO when a Gore-Tex membrane was used. The gelatinous substance is completely different from a thrombus and similar to an "organized seroma."[16] Gelatinous substance accumulation

TABLE 2. Participating centers

Center	Total implanted HM 3	eOGO	Mean support time eOGO, d	Prevalence,%
1	290	0	–	–
2	236	12	1240.6 ± 433.3	5.01
3	32	1	1238	3.13
4	90	2	677.5 ± 161.9	2.22
5	55	1	1802	1.82
6	147	10	919 ± 490.9	6.8
7	170	4	1236 ± 832.4	2.36
8	110	2	1855 ± 108.9	1.82
9	218	5	908 ± 461.1	2.29
10	41	5	909 ± 243.8	12.12
11	41	1	809	2.44
12	105	6	908.8 ± 438.5	5.71
13	202	1	1299	0.5
14	60	3	701 ± 736.4	5
15	94	1	1143	0.01
16	77	4	796 ± 493.1	5.20
17	140	4	642 ± 378.4	2.86
Total	2108	62	953.4 ± 462.5	3.0

Data are presented as mean ± SD or n. *HM 3*, HeartMate 3; *eOGO*, external outflow graft obstruction.

is not a new phenomenon. It has been reported with the HeartWare HVAD[17] and with HeartMate II.[18] The more pronounced clinical presentation and low-flow alert in patients with HM 3 might be on the basis of the different behavior of the pressure flow curves of the HM 3 compared with the HeartMate II.[19] The etiology of perigraft seroma through a vascular polyester graft is not well understood.[20] Some centers have begun to modify the bend relief by longitudinal splitting or by adding multiple perforations to prevent accumulation of this gelatinous material.[10,17,21] However, 1 center in our series reported some degree of outflow graft obstruction (<50%) in 2 patients even after such a modification of the bend relief. Furthermore, modifications of the bend relief have only been made in a few cases thus far. If we eliminate the outflow bend relief graft, the eOGO problem might be avoided, but it might create other problems. Splitting the outflow bend relief might solve this; however, the currently available insights do not allow us to draw any conclusions.

The last European Association for Cardio-Thoracic Surgery expert consensus mentions the possibility of stenting outflow graft stenosis. There is no recommendation for early detection of eOGO.[22] In our opinion, one way to recognize an impending eOGO early on would be to decrease the threshold for a low-flow alarm (so that it emerges even when a mild reduction in flow is consistently noted). Subtle changes to power consumption over time could also aid in early detection. This would require a comprehensive collection of all log files. We do not currently have sufficient information to make a recommendation to modify the bend relief.

Because of the retrospective design of this study we cannot conclude a correlation between early development of eOGO and mortality. However, we note that mortality among patients who required therapeutic intervention was

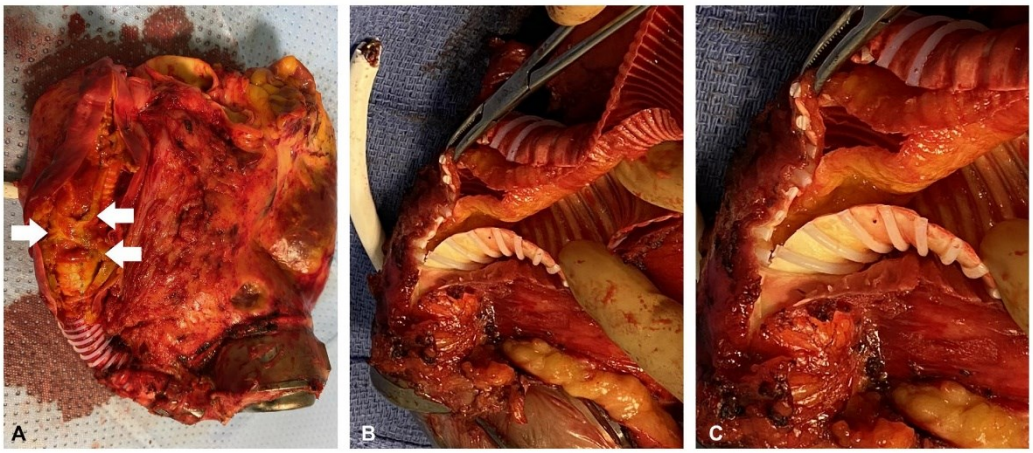
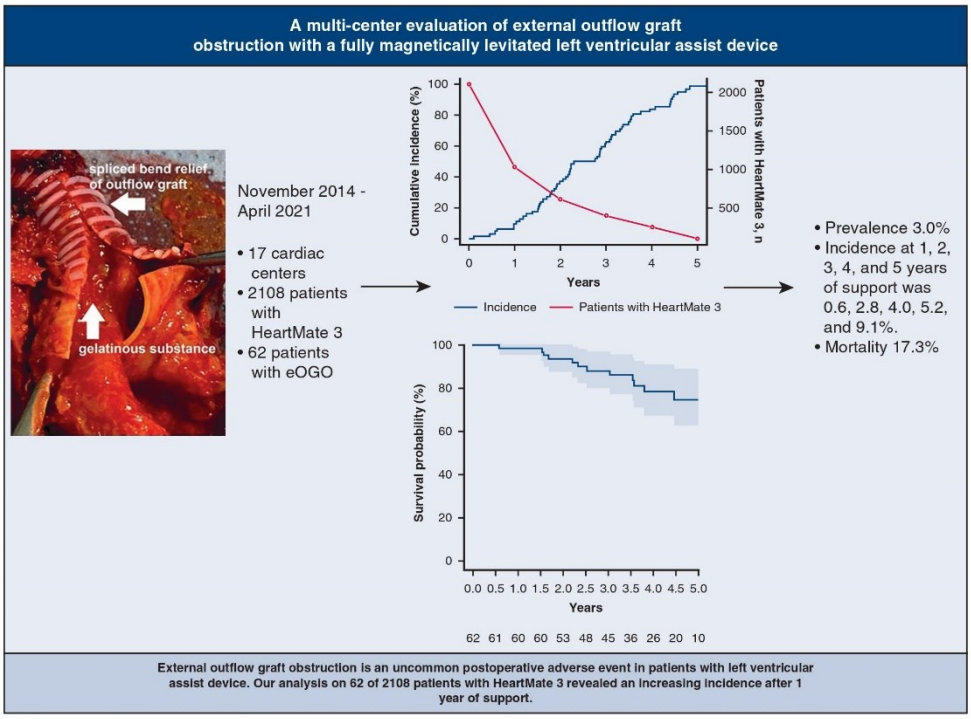


FIGURE 5. Split bend relief with gelatinous substance. *Arrows* point directly at the gelatinous substance (A). B and C, Exposition of the gelatinous substance.



eOGO: external outflow graft obstruction; LVAD: left ventricular assist device; HM3: HeartMate 3

FIGURE 6. Graphical abstract. *eOGO*, External outflow graft obstruction.

high despite the experience of high-volume centers specialized in mechanical circulatory support. This underlines the need for early recognition and to monitor such patients closely before their symptoms or condition deteriorates and decreases their eligibility as a surgical candidate.

This study has several limitations. The detection of eOGO and the variable imaging modalities by which eOGO is identified was not standardized across centers so we cannot be certain whether the prevalence of this complication is underestimated. In addition, we present only the reported prevalence. Our study shows only the cumulative incidence and not the incidence rate. We calculated the proportion of HM 3 patients who developed eOGO during a specific period. There are no analyses of survival data or other detailed data of the patients without diagnosed eOGO. More granular data can be collected in large registries or in a prospective manner. Observer bias due to recognition and diagnostic challenges might also underestimate the actual detection of this complication. We do not believe that the surgical technique—full sternotomy, left lateral anterior thoracotomy, or left anterior thoracotomy combined with partial sternotomy—would change the development of the gelatinous substance between the woven polyester graft and the polytetrafluoroethylene bend relief. Therefore, we kept the nonsternotomy patients in the analysis. However, an upcoming follow-up study with more eOGO patients will include a subgroup analysis. We are also limited in determining the predictors of eOGO and only structured studies that are prospective in design with close attention to surgical techniques and medical management would answer such a question. We also cannot be certain on advocating the best approach for an intervention, which we believe will require structured evaluations over time and in a standardized manner across centers.

CONCLUSIONS

eOGO caused by compression of gelatinous substance between the outflow graft and the bend relief is an uncommon but potentially life-threatening complication in HM 3 LVAD patients who require prolonged support (Figure 6). These findings suggest that centers should develop a standardized strategy of surveillance to screen HM 3 LVAD

patients for eOGO and that imaging should be performed without delay when this complication is suspected. Although engineering efforts to reduce this complication are under way, clinicians must maintain a focus on early detection and remain vigilant. A low-flow alarm should be interpreted as an early warning sign that something is progressing in the background. We noted that mortality among patients requiring (surgical) therapy is high. This shows the need to diagnose the complications early on. It is our duty as a group of experts to find an adequate screening protocol.

Conflict of Interest Statement

G.C.S.: Participation on a Data Safety Monitoring Board or Advisory Board: Procyrion Inc (chair of clinical trial safety committee [Aortix]), Abbott Laboratories (evidence base advisory board [HeartMate 3]). M.R.M.: Consulting fees: Abbot, Janssen, Paragonix, Natera, Moderna, Baim Institute for Clinical Research, and Briadview Ventures. Participation on a Data Safety Monitoring Board or Advisory Board: Mesoblast and Fineheart. Stock or stock options: Leviticus, NupulseCV, and Fineheart. A.M.: Grants or contracts from any entity: Abbott (Fellowship grant made to institution). U.P.J.: Grants or contracts from any entity: Research grant Abbott. Consulting fees: Abbott and Edwards. Support for attending meetings and/or travel: Abbott and AncoraHeart. Leadership or fiduciary role in other board, society, committee, or advocacy group, paid or unpaid: ISHLT board. Stock or stock options: Revamp Medical. 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Key Words: left ventricular assist device, HeartMate 3, outflow graft obstruction

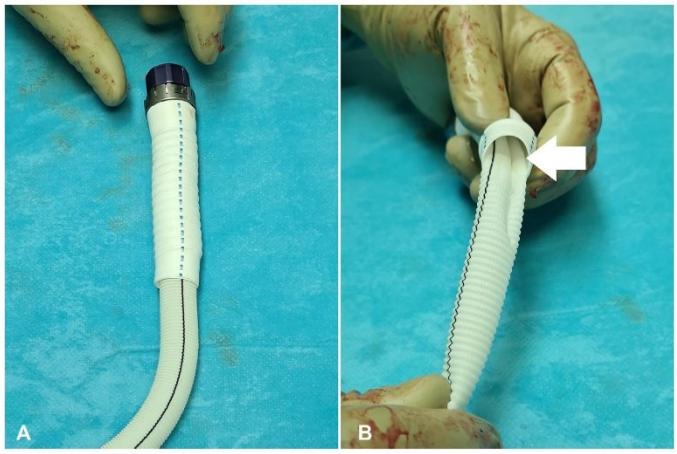

FIGURE E1. Outflow graft with bend relief before implantation (A). The *arrow* points at space between outflow graft and bend relief (B).

TABLE E1. Technical baseline characteristics

Characteristic	n
Surgical access for VAD implantation	
Full sternotomy	52
Lateral thoracotomy	8
Partial sternotomy with lateral thoracotomy	2
Aortic outflow graft connection	
Outflow graft connection to ascending aorta	56
Outflow graft connection to descending aorta	6
Anatomical course of outflow graft*	
Short run of graft to descending aorta	6
Short run of graft (eg, from apex to ascending aorta under sternum)	18
Long run of graft (eg, around right atrium and right ventricle)	33
Very long run of graft (eg, s-shape or kinked)	2
Modification of bend relief at initial LVAD implantation	
No modification	60
Perforation of bend relief	2
Concomitant cardiac surgery or intervention	
Aortic valve replacement and PFO/ASD closure	1
Prosthetic aortic valve replacement	1
Aortic valve replacement and tricuspid valve reconstruction	2
Tricuspid valve repair	5
Tricuspid valve repair and temporary RVAD	1
Tricuspid valve repair and PFO/ASD closure	3
Tricuspid valve repair, temporary RVAD, PFO/ASD closure	1
Left ventricular reduction	1
Temporary RVAD	4
PFO/ASD closure	3
Later cardiac surgeries or interventions	
Revision, outflow graft	1
Revision, bend relief	1
Revision, cardiac tamponade, open chest	1
Revision, cardiac tamponade and temporary RVAD, open chest	1
Transposition of driveline and reposition of greater omentum	1
TAVR	5
Temporary RVAD and TAVR	1
CABG	1

VAD, Ventricular assist device; *LVAD*, left ventricular assist device; *PFO/ASD*, patent foramen ovale/atrial septal defect; *RVAD*, right ventricular assist device; *TAVR*, transcatheter aortic valve replacement; *CABG*, coronary artery bypass grafting. *Imaging was not available for 3 patients.

TABLE E2. Patients' main clinical symptoms at admission

Symptom	n
Heart failure symptoms	
Low-flow alert	20
Low-flow alert and dyspnea and hydropic decompensation	2
Low-flow alert and cardiogenic shock	2
Low-flow alert and dyspnea and cardiac decompensation	1
Low-flow alert and anemia	1
Hydropic decompensation	2
Dyspnea	13
Dyspnea and low-flow alert	5
Dyspnea and right heart failure	2
Dyspnea and cardiac arrhythmia	1
Dyspnea and angina pectoris	1
Pulmonary congestion and peripheral edema and dyspepsia	1
Pulmonary edema and low cardiac output	1
Other	
ARDS	1
Abdominal pain and nausea and vomiting	1
Reduced vigilance and hyperglycemia	1
Epistaxis	1
Asymptomatic or incidental finding	6

ARDS, Acute respiratory distress syndrome.

MCS

TABLE E3. VAD parameters at admission and discharge

VAD parameter	Admission	Discharge
Speed, rpm	5556.9 ± 898.4*	5455.8 ± 683.5†
Flow, L/min	3.8 ± 1.1‡	4.6 ± 0.6§
Motor power, W	4.0 ± 0.7‖	4.2 ± 0.6¶

Data are presented as mean ± SD. *VAD*, Ventricular assist device. *Data collected from 52 patients. †Data collected from 43 patients. ‡Data collected from 52 patients. §Data collected from 44 patients. ‖Data collected from 51 patients. ¶Data collected from 42 patients.

TABLE E5. Anticoagulation at admission

Medication	n
Warfarin	22
Warfarin and aspirin	37
Warfarin and clopidogrel	3

MCS

TABLE E4. Laboratory parameters at admission and discharge

Laboratory parameter	Admission	Discharge
Serum lactate dehydrogenase, U/L	287.9 ± 117.7*	268.8 ± 74.0†
Plasma-free hemoglobin, mg/L	119.6 ± 313.0‡	103.2 ± 318.4§
INR	2.4 ± 0.6‖	2.1 ± 0.6¶
aPTT, s	46.7 ± 10.6#	52.4 ± 20.3**

Data are presented as mean ± standard deviation (SD). *INR*, International normalized ratio; *aPTT*, activated partial thromboplastin time. *Data collected from 55 patients. †Data collected from 44 patients. ‡Data collected from 38 patients. §Data collected from 28 patients. ‖Data collected from 60 patients. ¶Data collected from 48 patients. #Data collected from 52 patients. **Data collected from 46 patients.

TABLE E6. Judgement of the degree of stenosis at admission on the basis of CT and PA

	n
CT	
Total	50*
Quantitative statement	
≤49%	2
50%-74%	14
≥75%	4
Qualitative statement	30
CT not performed or not defined	12
PA	
Total	19*
Quantitative statement	
≤49%	2†
50%-74%	0
≥75%	3
Qualitative statement	14
PA not performed or not defined	42

CT, Computed tomography; *PA*, percutaneous angiography. *17 Patients received CT and PA imaging. †1 Patient received intravascular ultrasound.

TABLE E7. Patients' symptoms, degree of stenosis at admission, therapy, and outcome

Patient	Degree of stenosis at admission	Presenting symptoms at time of diagnosis	Survival after eOGO diagnosis, d	Therapy	Survival after therapy, d	Outcome
1	Qualitative evaluation	Asymptomatic	669	Intervention, stenting	494	Ongoing
2	Qualitative evaluation	Asymptomatic	2	Surgery, decompression	2	Deceased
3	Qualitative evaluation	Asymptomatic	32	Surgery, decompression	30	Ongoing
4	50%	Asymptomatic	1047	Watchful waiting	–	Ongoing
5	Qualitative evaluation	Asymptomatic	679	Watchful waiting	–	Ongoing
6	50%	Asymptomatic	402	Watchful waiting	–	Ongoing
7	50%	Low-flow alert	200	Surgery, decompression	200	Ongoing
8	Qualitative evaluation	Low-flow alert	232	Surgery, decompression	229	Deceased
9	70%	Low-flow alert	42	Intervention, stenting	42	Ongoing
10	Qualitative evaluation	Low-flow alert	78	Conservative	64	Ongoing
11	Qualitative evaluation	Low-flow alert	551	Surgery, pump exchange	551	Deceased
12	Qualitative evaluation	Low-flow alert	1075	Surgery, decompression	1069	Ongoing
13	Qualitative evaluation	Low-flow alert	848	Surgery, decompression	847	Ongoing
14	70%	Low-flow alert	239	Surgery, transplantation	135	Ongoing
15	30%	Low-flow alert	229	Surgery, transplantation	30	Ongoing
16	50%	Low-flow alert	665	Surgery, transplantation	3	Ongoing
17	Qualitative evaluation	Low-flow alert	0	Not possible	0	Deceased
18	Qualitative evaluation	Low-flow alert	488	Surgery, pump exchange	488	Ongoing
19	Qualitative evaluation	Low-flow alert	362	Surgery, decompression	358	Ongoing
20	Qualitative evaluation	Low-flow alert	132	Intervention, stenting	131	Ongoing
21	50%	Low-flow alert	962	Surgery, transplantation	923	Ongoing
22	Qualitative evaluation	Low-flow alert	3	Surgery, decompression	2	Deceased
23	Qualitative evaluation	Low-flow alert	5	Surgery, decompression	5	Deceased
24	50%	Low-flow alert	612	Intervention, stenting	609	Ongoing
25	Qualitative evaluation	Low-flow alert	1018	Surgery, decompression	1011	Ongoing
26	Qualitative evaluation	Low-flow alert	2	Surgery, decompression	2	Deceased
27	Qualitative evaluation	Low-flow alert and dyspnea and hydropic decompensation	36	Surgery, decompression, intervention, stenting	18	Ongoing
28	Qualitative evaluation	Low-flow alert and dyspnea and hydropic decompensation	375	Intervention, stenting	368	Ongoing
29	Qualitative evaluation	Low-flow alert and cardiogenic shock	14	Surgery, pump exchange	14	Deceased
30	Qualitative evaluation	Low-flow alert and cardiogenic shock	755	Surgery, decompression	755	Deceased
31	Qualitative evaluation	Low-flow alert and dyspnea and cardiac decompensation	770	Surgery, decompression	747	Ongoing
32	70%	Low-flow alert and anemia	85	Intervention, stenting	85	Deceased
33	75%	Hydropic decompensation	72	Intervention, stenting	66	Ongoing
34	Qualitative evaluation	Hydropic decompensation	427	Watchful waiting	–	Ongoing
35	50%	Dyspnea	494	Surgery, transplantation	14	Ongoing
36	Qualitative evaluation	Dyspnea	841	Conservative	838	Ongoing

(Continued)

TABLE E7. Continued

Patient	Degree of stenosis at admission	Presenting symptoms at time of diagnosis	Survival after eOGO diagnosis, d	Therapy	Survival after therapy, d	Outcome
37	Qualitative evaluation	Dyspnea	589	Surgery, decompression	568	Ongoing
38	Qualitative evaluation	Dyspnea	39	Surgery, transplantation	39	Ongoing
39	Qualitative evaluation	Dyspnea	177	Surgery, transplantation	175	Ongoing
40	25%	Dyspnea	219	Not possible	0	Deceased
41	50%	Dyspnea	301	Surgery, transplantation	250	Ongoing
42	50%	Dyspnea	134	Intervention, stenting	130	Ongoing
43	Qualitative evaluation	Dyspnea	18	Intervention, stenting	18	Deceased
44	Qualitative evaluation	Dyspnea	342	Surgery, decompression	320	Ongoing
45	Qualitative evaluation	Dyspnea	319	Intervention, stenting	317	Ongoing
46	Qualitative evaluation	Dyspnea	593	Surgery, decompression	592	Ongoing
47	Qualitative evaluation	Dyspnea	173	Intervention, stenting	173	Ongoing
48	Qualitative evaluation	Dyspnea and low-flow alert	575	Surgery, decompression	573	Ongoing
49	Qualitative evaluation	Dyspnea and low-flow alert	42	Surgery, decompression	26	Ongoing
50	85%	Dyspnea and low-flow alert	14	Surgery, decompression	13	Ongoing
51	80%	Dyspnea and low-flow alert	978	Surgery, decompression	978	Ongoing
52	Qualitative evaluation	Dyspnea and low-flow alert	282	Surgery, decompression	282	Ongoing
53	60%	Dyspnea and right heart failure	48	Watchful waiting	–	Ongoing
54	70%	Dyspnea and right heart failure	124	Surgery, transplantation	6	Ongoing
55	Qualitative evaluation	Dyspnea and cardiac arrhythmia	677	Surgery, decompression	677	Ongoing
56	75%	Dyspnea and angina pectoris	1100	Intervention, stenting	744	Ongoing
57	60%	Pulmonary edema and low cardiac output	317	Intervention, stenting	210	Ongoing
58	Qualitative evaluation	Pulmonary congestion and peripheral edema and dyspepsia	109	Watchful waiting	–	Ongoing
59	Qualitative evaluation	Abdominal pain and nausea and vomiting	145	Watchful waiting	145	Ongoing
60	Qualitative evaluation	ARDS	630	Surgery, decompression	629	Deceased
61	50%	Reduced vigilance and hyperglycemia	149	Intervention, stenting	127	Ongoing
62	80%	Epistaxis	750	Intervention, stenting	733	Ongoing

eOGO, External compression of the outflow graft causing obstruction; *ARDS*, acute respiratory distress syndrome.

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TABLE E8. Echocardiography parameters at admission

Parameter	n
Left ventricular ejection fraction	
≥50%	1
40%-49%	2
<40%	10
No data	49
Left ventricular end-diastolic diameter	
4.2-5.8 cm, men	12
3.8-5.2 cm, women	1
≥5.9 cm, men	23
≥5.3 cm, women	5
No data	21
Right ventricular ejection fraction	
≥32%	3
Normal, measured quantitatively	14
25%-31%	2
Mild, measured quantitatively	7
18%-24%	0
Moderate, measured quantitatively	13
≤17%	0
Severe, measured quantitatively	3
No data	20
Right ventricular end diastolic diameter	
2.7-3.3 cm	3
Normal, measured quantitatively	7
≥3.4 cm	11
≤2.6 cm	0
Dilated, measured quantitatively	10
No data	31

TABLE E9. Consequence of outflow graft obstruction diagnosis

Consequence	n
Surgery	
Re-thoracotomy with removal of gelatinous substance	24
Pump exchange	3
Heart transplantation	8
Intervention	
Dilatation and/or stenting	15
Re-thoracotomy with removal of gelatinous substance and secondary intervention with stenting	1
Conservative therapy/watchful waiting	9
Death before possibility of therapy	2

TABLE E10. Outcomes

Outcome	Value
Death, overall	14
Cause of death, overall	
Cardiac	3
Noncardiac	11
In-hospital death	9
Survival after eOGO diagnosis, overall, d	382 ± 347
Survival after eOGO therapy, overall, d	330 ± 323
Stenting	
Stenting, OGO therapy	16
Survival, stenting, after OGO therapy, d	269 ± 251
In-hospital death, after stenting	2
Surgery	
Surgery, OGO therapy	28
Survival, surgery, after OGO therapy, d	399 ± 346
In-hospital death, after surgery	5
Heart transplantation	
Heart transplantation, OGO therapy	8
Survival, heart transplantation, after OGO therapy, d	255 ± 313.2
In-hospital death, after heart transplantation	0

Data are presented as mean ± SD or n. *eOGO*, External compression of the outflow graft causing obstruction; *OGO*, obstruction of the outflow graft.

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