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IMAGES IN CARDIOVASCULAR MEDICINE

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ISAR-REACT 5 — What have we learned?

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Paris, France 2019 — several days ago, the results of the ISAR-REACT 5 were finally presented during the European Society of Cardiology ESC Congress 2019 in Paris. The Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment (ISAR-REACT) 5 trial was an investigator-initiated, phase 4, multicenter, randomized, open-label trial [1]. The study was designed to demonstrate the superiority of ticagrelor *vs.* prasugrel; in contrast, the main finding of this trial was the reported superiority of prasugrel over ticagrelor with respect to the composite endpoint of death, myocardial infarction, or stroke 1 year after randomization, in patients with acute coronary syndromes. Reduced incidence of the composite endpoint in the prasugrel arm was not associated with an increased risk of bleeding.

However, quite the opposite results were commonly expected based on the existing body of evidence from the previously published trials.

The ISAR-REACT 5 trial did not directly compare two antiplatelet agents, but rather different antiplatelet strategies mimicking the ESC guidelines, but not quite so [2–4]. Thus, the results do not reflect current clinical practice.

In our opinion, the study design and its organization lead to a bias that is difficult to estimate due to several reasons. Both the open-label design of the trial and the fact that it was performed only in two countries with a disproportion of enroll-ing centers (21 centers in Germany and 2 centers in Italy) could have had some, however probably minor, impact on the outcomes. On the other hand, the adherence to the trial medication could have had much more influence on the results. The stated non-adherence to the treatment of 0.9% in prasugrel arm and 0.4% in ticagrelor arm seem to be unequivocally underestimated as patients were followed up mainly by telephone only (83% of contacts), at hospital or by outpatient visit (10%), or by using structured follow-up letter (7%). Moreover, after the in-hospital phase of the trial commercially available ticagrelor or prasugrel were prescribed by the physician and had to be purchased by the patients themselves. No specific method for adherence evaluation, i.e. medication event monitoring systems, pill count, or drug availability according to the purchase of prescribed drugs etc., was reported. As the patient-reported, drug intake was previously shown to be misleading, the true adherence to trial medication was probably much lower than that reported by the authors [5, 6].

The intention-to-treat analysis, i.e. with the inclusion of all patients according to the randomly assigned trial group, irrespective of the actual treatment received, is a widely accepted method for such kind of clinical studies. In this particular trial, however, such design might have led to serious distortion of the results, as only 1602 of 2012 and 1596 of 2006 subjects in ticagrelor and

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prasugrel groups, respectively, were discharged from the hospital on the allocated medication. Moreover, additional 243 and 199 patients in each arm, respectively, discontinued study medication after discharge, and finally, 19 and 18 patients were lost to follow-up in each group. The intention-to--treat principle applied in the ISAR-REACT 5 has led to the inclusion of at least 1262 patients in final analysis, who were not treated with the assigned medication (653 of 2012 subjects (32.5%) and 609 of 2006 subjects (30.4%) in ticagrelor and prasugrel arms, respectively).

A primary endpoint (the composite endpoint of all-cause death, myocardial infarction, or stroke) at 1 year after randomization, occurred in 184 of 2012 patients (9.3%) in the ticagrelor group and in 137 of 2006 patients (6.9%) in the prasugrel group (HR, 1.36; 95% CI, 1.09 to 1.70; P = 0.006). Taking into account that the analysis of 4018 patients included 1262 (31.4%) who were supposed to be on study medication, whereas they were not treated according to the study protocol, the absolute difference in primary endpoint incidence of 47 events can hardly be considered relevant.

In the analysis of the primary endpoint occurrence among subjects discharged on the study medication (1602 patients on ticagrelor and 1596 patients on prasugrel) in the period from discharge to the time of discontinuation of treatment or end of follow-up ("on treatment" analysis), the difference between the arms has been shown not to be significant (92 events in the ticagrelor group and 71 in the prasugrel group; HR, 1.34; 95% CI, 0.98–1.82).

The observed bleeding rates are also the subject of questions. In the modified intention-to-treat analysis the safety endpoint (major bleeding BARC type 3 through 5) was observed in 95 patients (5.4%) in ticagrelor arm and in 80 patients (4.8%) in the prasugrel arm (HR, 1.12; 95% CI, 0.83 to 1.51; P = 0.46). The absolute difference of 15 events between the groups, while as many as 233 of 2006 patients (11.6%) from prasugrel group and only 23 of 2012 patients (1.1%) from the ticagrelor group were excluded from this analysis without any justification, is difficult to interpret.

Thus, the result of both efficacy and safety needs to be carefully validated.

In conclusion, the ISAR-REACT 5 trial, indeed instead of providing answers, rather rises questions. Trying to answer the title question: "ISAR-REACT 5 — What have we learned?" we found ourselves in trouble, as we can not provide any definitive statement.

Conflict of interest: Lecture honoraria

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VIA MEDICA

REVIEW ARTICLE

Calcific lesion preparation for coronary bifurcation stenting

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Abstract

Bifurcating coronary lesions are a very common challenge in interventional cardiology because of the technical complexity in their treatment, the risk of side branch occlusion and an overall worse outcome when compared to non-bifurcating lesions.

The presence of calcifications represents further complexity due to the difficulty in device delivery and stent expansion as well as enhanced risk of side branch occlusion.

Rotational and orbital atherectomy, scoring and cutting balloons, coronary lithoplasty are available tools which have been introduced over the last three decades to overcome such issue. Nevertheless, their application in different contexts of bifurcations presents specific caveats and the studies directed at comparing such techniques have never been expressly oriented in the subset of the bifurcating lesion.

In this paper, we review these devices and their usefulness in bifurcations by analyzing consistent data from clinical trials, and we propose a practical algorithm for the treatment of severely calcified bifurcating lesions according to their anatomical features. (Cardiol J 2019; 26, 5: 429–437)

Key words: bifurcation, calcified lesion, plaque modification, rotational atherectomy, coronary lithoplasty

Introduction

Bifurcating coronary lesions are a very common challenge for interventional cardiologists because of the technical complexity in their treatment, a higher risk of procedural complications and an overall worse outcome compared to non-bifurcating lesions [1]. The inherent difficulty of bifurcation percutaneous coronary intervention (PCI) stems from the risk that main vessel (MV) stenting may hamper flow in the side branch (SB).

The European Bifurcation Club (EBC) consensus document recommends a single "provisional" stenting technique, although acknowledging that bifurcations with diffuse SB involvement often requires double stenting [2]. Several stent techniques have been developed in order to tailor double stent deployment to the complex anatomy

of the bifurcation. Hence, significantly higher risk of myocardial infarction and stent thrombosis have been associated with double when compared with single stenting [3].

In this scenario, the main issues to effectively guarantee optimal vessel patency are prevention of plaque shifting and careful carena reconstruction; further complexity may be due to calcification that increases the risk of SB occlusion or hampers crossability or adequate lesion dilatation [4].

Various strategies have been tested in order to prepare the plaque in the bifurcation and to reduce or displace the amount of calcium before stenting, and the inherent scientific literature presents a consistent controversy. The aim of this paper is to review current strategies adopted to modify calcific plaque before stent deployment in coronary bifurcations.

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Figure 1. Coronary calcified bifurcating lesion. Diffuse calcifications of left anterior descending artery (LAD); **A.** Fluoroscopy view (arrows indicate calcifications) of LAD; **B.** Angiographic view; **C.** Fluoroscopy view of incomplete balloon expansion.

Table 1. Calcium quantification by imaging modality.

Angiography	Radio-opacities before contrast injection noted with cardiac motion (moderate), or without cardiac motion (severe)
Optical coherence tomography	Signal-poor with sharply delineated leading, quantified by thickness (mild < 0.5 mm; severe > 0.5–1 mm), area, and volume
Intravascular ultrasound	Hyperechoic leading edge with acoustic shadowing, quantified mainly by angle (concentric if > 270°) and length

Calcification at bifurcating lesions

When dealing with a bifurcation lesion, the first issue should be an assessment of the relevance of the SB; in this view, Kim et al. [5] clearly documented that a length ≥ 73 mm, not the diameter of the SB, identifies a vessel supplying a "significant" portion of myocardium, notably a fractional myocardial mass ≥ 10%.

Hence, following a widely accepted classification by Medina et al. [6], SB plaque involvement should be checked. The so called "true bifurcations" define lesions where plaque enters the SB, namely Medina 1,1,1–1,0,1–0,1,1, although there is growing evidence that not the sole SB involvement, but SB lesion length > 9 mm is an independent predictor of adverse events [7].

The use of intravascular imaging is of great value to ascertain the distribution of the plaque, the true vessel size and the extent of calcium in bifurcating lesions, even beyond the bifurcation of the left main (LM) [8], that is currently the only site where guidelines recommend the use of intravascular ultrasound (IVUS) [9].

Extensive calcification is the main determinant of balloon and stent underexpansion during PCI (Fig. 1) [10]. The sensitivity of intravascular imaging is far higher than angiography in detecting coronary calcifications, as angiography is able to detect a calcified plaque only for calcium angles almost > 100°, by either IVUS or optical coherence tomography (OCT). Nevertheless, all disagreement between the angiography and intravascular imaging is related to thin calcifications that have not been shown to affect stent expansion (Table 1) [4]. Even with the availability of newer drug-eluting stents (DES), patients with severely calcified lesions still have worse clinical outcomes compared with those without [11]. Clinical relevance of coronary calcification cannot be neglected, as treating stent underexpansion in a heavily calcified lesion is more difficult than preventing it [12]. However, although there is general agreement that the greater the arc length, or thickness of calcium, the greater the likelihood of stent underexpansion, there are no published cutoffs that can be used for recommending lesion modification prior to stent implantation or the need for high-pressure adjunctive balloon inflations afterward.

The presence of extensive calcification at the site of bifurcation evaluated by OCT portends a higher risk of occlusion of the side branch, as documented by Fujino et al. [4]. In multivariate analysis, the presence of a calcified plaque in

the bifurcation segment of MV (odds ratio [OR]: 12.32; 95% confidence interval [CI]: 2.58–58.83; p = 0.002) as assessed by OCT was the most relevant feature associated with SB deterioration, being bifurcation angle > 70° (OR: 11.83; 95% CI: 2.00–70.02; p = 0.007) and baseline % diameter of stenosis (DS) of SB (OR 1.07; 95% CI: 1.02–1.13; p = 0.012) other independent predictors of SB deterioration, as assessed by angiography.

The mechanism of SB closure due to calcium has not been fully elucidated, but a higher risk of carina shift due to reduced compliance of the wall opposing the SB and a lower resistence encountered by the inflated balloon at the SB ostium could be a potential hypothesis. Carina is typically spared from atherosclerosis distribution owing to high local shear stress [13], but is the potential source of subsequent stent failure. Careful lesion preparation might therefore "soften" the lesion and reduce the risk of plaque shift (Table 2).

Modifying balloons

The **cutting balloon** has been available for almost 30 years. It is a semi-compliant balloon with three thin sharp blades mounted on its body, designed to cut the continuity of fibrocalcific plaque once the pressure of the balloon forces them against the vessel wall, creating fissures on the plaque.

The main drawback of these tools is their high rigidity that hinders system advancement and lesion negotiation through tortuous and calcified vessels [14]. Moreover, cutting balloon angioplasty showed a higher rate of coronary perforation (0.8% vs. 0%, p = 0.03) and had no advantages in terms of restenosis compared to balloon angioplasty (31% vs. 30%, p = NS) [15]. Therefore, after the first optimistic feasibility reports [16], scoring the plaque to facilitate stent deployment has been reported only in occasional cases for treatment of calcified bifurcations. This technology has recently undergone some important revisions: in the newer generation of cutting balloons (Wolverine™ Cutting Balloon, Boston Scientific, USA), the atherotome's support thickness has been reduced, without affecting the functional height of the blade, resulting in an overall smaller crossing profile and improved crossability.

The principle of using a "buddy wire" to fracture calcified plaque [17] promoted the development of the **scoring balloon**: wires apposed externally to the body of the balloon increase local punctual pressure, achieving plaque fissuration [18].

Otsuka et al. [19] recently proposed that prolonged inflation might improve the success rate of these devices with a "creep phenomenon": a sustained tensile load produces microcrack formation and propagation leading to a phasic tissue elongation.

Scoring balloons are available in two different families, the first one is engineered as a traditional balloon with three segments of wire apposed spirally or linearly on the outer surface of the balloon; the second kind has only one external wire and is engineered as a rapid exchange balloon with a very short monorail involving only the tip of the balloon, thus allowing the guidewire to course along the balloon and to serve, together with the other, as a scoring wire. Scoring balloon, especially the latter has a better crossing profile than old generation cutting balloons but still has less deliverability than a standard balloon. Several reports suggest a very good success rate but many authors still consider these devices limited to a less-than-severely calcified lesion.

Using a provisional approach with a scoring balloon for the SB and a DES for the MV in "true" bifurcation lesions yielded promising results in a single arm prospective study, with a rate of crossover to stent deployment in the SB as low as 11%, and a target lesion revascularization rate of 3.3% [20].
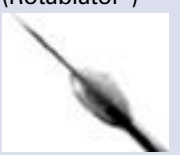
Atherectomy

Rotational atherectomy (RA) (Rotablator, Boston Scientific-Scimed Corporation, Natick, Massachusetts), firstly introduced more than 30 years ago [21], is a plaque modification method achieved by a high-speed diamond chips-coated rotating burr that allows selective abrasion of calcified hard tissues. In the pre-stent era RA was conceived as a stand-alone approach to obtain plaque debulking in order to gain lumen diameter in a severely calcified lesion. It subsequently became evident that RA may offer its best contribution as a major "plaque modifier" for subsequent balloon angioplasty and stent implantation. Calcified ostial and bifurcating lesions were effectively treated by RA in the pre-stent era [22]. Its main effect is to restore adequate lumen by breaking the continuity of calcium plaque, increasing lesion "crossability" and making the artery more compliant to balloon dilatation.

Rotational atherectomy relies on two principles [23]:
— "Differential cutting" defines the ability to selectively ablate hard plaque components while

Table 2. Features of the main plaque modifying devices.

Device	Material	Technical features	Ref.
Focused force dilatation balloon (Scoreflex™)	Semi-compliant or non-compliant balloon A nitinol integral wire (~0.011") and the "conventional" guide wire act as two opposite scoring elements	Balloon size 2.0–4 mm Working range 6–16 atm Crossing profile 0.032" F 5F Guiding catheter compatible Guide wire 0.014"	[17]
Scoring balloon (Angiosculpt®)	Semi-compliant balloon Nitinol-enhanced balloon deflation Electropolished, rectangular, spiral scoring element (~0.005")	Balloon size 2.0–3.5 mm Working range 2–20 atm Crossing profile 0.047" F 6F Guiding catheter Guide wire 0.014"	[17, 20]
Cutting balloon (Wolverine™)	Nylon non-compliant balloon Microsurgical blade, called: "Atherotome" (functional height: ~0.005")	Balloon size 2.0–4.0 mm Working range 8–16 atm Reduced crossing profile then Flexotome® 5F Guiding catheter compatible	[16]
Rotational atherectomy (Rotablator®)	Diamond-coated elliptical burr rotating up to 190,000 rpm	Multiple burrs size (1.25–> 2.5 mm) RotaWire™ (330 cm, 0.014", extra support or floppy) Catheter 6–10 F (according to the size burr)	[23]
Orbital atherectomy system (Diamondback 360®)	Eccentrically mounted diamond-coated crown (1.25 mm) rotating up to 200,000 rpm	6F Guiding catheter ViperWire Advance® (0.014")	[27, 28, 29]
Intravascular lithotripsy (Shockwave®)	Semi-compliant balloon containing a series of unfocused, electrohydraulic lithotripsy emitters	Balloon size 2.5–4 mm Inflated to 4 atm and administered 4 cycles of 10 s Crossing profile 0.044" 6F Guiding catheter Guide wire 0.014"	[36]

displacing and sparing soft tissues, that are deflected away. At rotational speeds > 60,000 rpm the friction, which occurs when sliding surfaces are in contact, is virtually eliminated. The "differential cutting" should theoretically allow RA to accomplish a selective abrasion of plaque in the proximity of a branch, thus increasing the procedural success rate and reducing the need for side-branch intervention.

— "Orthogonal displacement of friction" refers to a change in effective longitudinal friction, which is almost eliminated, resulting in reduced surface drag and unimpeded advancement of the burr in tortuous and diseased segments of the coronary tree.

Rotablator® advances over a guidewire (Rotawire), is a 325 cm long and 0.009" thin shaft, with a 0.014" 2.2 cm long floppy spring tip, which has a lower performance in crossing lesions and tortuosity than a traditional guidewire. Rotawire can therefore be positioned through a microcatheter after an exchange with a "workhorse" guidewire.

The burr is mounted on a spiral drive shaft that is connected to an advancer and covered by a sheath.

The burr's passage through the lesion creates fragments with a diameter theoretically smaller than blood cells; such microparticles should easily cross microcirculation and then be eliminated by the reticuloendothelial system. Nevertheless, the no-reflow phenomenon has frequently been reported after RA-assisted PCI, and therefore the use of a flushing solution containing vasodilators such as calcium antagonists, nitrates or adenosine alone or in combination has been suggested [24]. A widely used solution to cool the Rotablator® turbine and to flush the coronary circulation from debris generated during the ablation is a saline solution with equal proportions of verapamil, nitrates, and heparin (5 mg/5 mg/5,000 U in 500 mL of saline). The use of the Rotablator® is technically demanding and is associated with a significant incidence of adverse events. Among the procedural complications burr's lodging, coronary perforation, large dissection, acute thrombosis with abrupt coronary occlusion and atrioventricular-block have been reported. In order to minimize the complication rate, the EAPCI consensus document [25] recommends distal positioning of the Rotawire in order to have adequate support and stability and the use a rotational speed in the range of 135,000–180,000 rpm to obtain effective plaque modification, with a gentle picking to-and-fro movement of the burr, avoiding brisk deceleration (> 5,000 rpm) and to stop rotation when the burr is over the lesion to minimize the risk of lodgment. During RA procedures the increased quantity of injected contrast medium may cause deterioration of renal function. Therefore, all strategies useful to contain acute kidney injury should be applied [26], although there are no randomized trials exploring this issue.

Currently, as the role of RA has changed from debulking to plaque preparation, a burr-to-artery diameter ratio of 0.5–0.6 (smaller than previously recommended 0.7) should be targeted in order to balance efficacy in lesion ablation and risk of coronary wall damage.

After several failures to cross the lesion, downsizing of the burr is recommended. In case the smallest burr does not pass, a change to a more supportive, or even a larger guiding catheter must be considered.

More recently, the Diamondback 360®, a coronary **orbital atherectomy** system (OAS) has become available. Mechanism of OAS is a differential sanding to reduce plaque burden with a carbon-coated crown (1.25 mm). Theoretically, softer tissue flexes away from the crown while fibrotic tissue or arterial calcium is engaged. A drive shaft with an eccentrically mounted diamond-coated crown provides proximal and distal sanding; the crown's orbital diameter expands radially via centrifugal force [27]. Operators can control the speed of rotation, with a higher speed creating a larger sanding diameter by increasing lateral pressure. Pivotal trials have documented safety and feasibility of OAS in preparation of severely calcified plaques before stent deployment [28, 29], although clinical evidence is still limited.

The interest in calcified lesion preparation was recently revived in the Comparison of Strategies to Prepare Severely Calcified Coronary Lesions (PREPARE-CALC) trial, where modifying balloons (cutting or scoring) were compared with RA, in the setting of stable coronary artery disease. Bifurcating lesion were present in 42% of cases. The trial showed a clear superiority in terms of procedural success in the RA group (98% vs. 81%, p < 0.001), driven by a 20% of cross-over to RA, while, at 9 months, mean in-stent late lumen loss was similar in the two groups, as well as stent thrombosis (0% in both groups) [30].

Plaque debulking in coronary bifurcation with any atherectomy device (both RA and OAS) may pose some technical challenges because of the need of single wire use, impeding the protection of side branches (Fig. 2). Reportedly, a tricky approach with a child-in-mother guiding catheter allowed RA with the use of multiple guidewires in order to protect side branches proximal to the target lesion and to gain more support [31].

As for calcified bifurcating lesions, there are several observational studies highlighting the safety and effectiveness of RA, that achieved a high (> 90%) success rate with a low rate of major adverse events (MACE < 5%) and the need for bailout side-branch stenting (< 20%) [32, 33]. Recently, Chambers et al. [34], in a series of patients undergoing atherectomy with either OAS or RA for severely calcified plaques, documented similar low 30-day MACE rates among patients with bifurcation as compared with non-bifurcation lesions. OAS was associated with significantly shorter procedure and fluoroscopy time, as compared with RA.

Lithoplasty

Intravascular lithotripsy (IvL) is the most recent tool for the treatment of calcified lesion after being introduced in peripheral vascular angioplasty. IvL consists of a balloon catheter that uses sonic
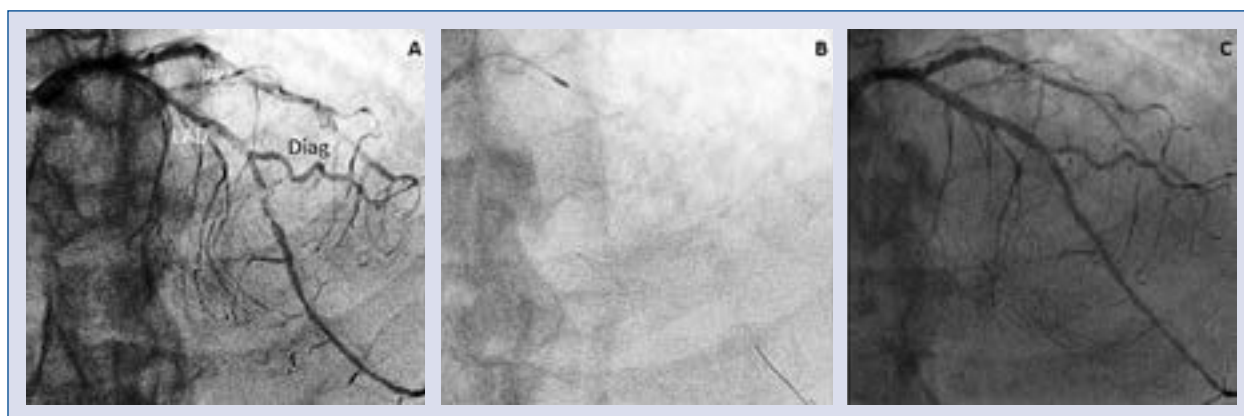
Figure 2. Coronary calcified bifurcating lesion treated by rotablator. Diffuse calcified atherosclerosis of left anterior descending artery (LAD) resulting in severe stenosis involving the bifurcation site of a large diagonal branch (Diag); **A.** Angiographic view; **B.** Rotawire positioned distally in the main branch with Rotablator burr approaching the lesion; **C.** final result after provisional stenting.
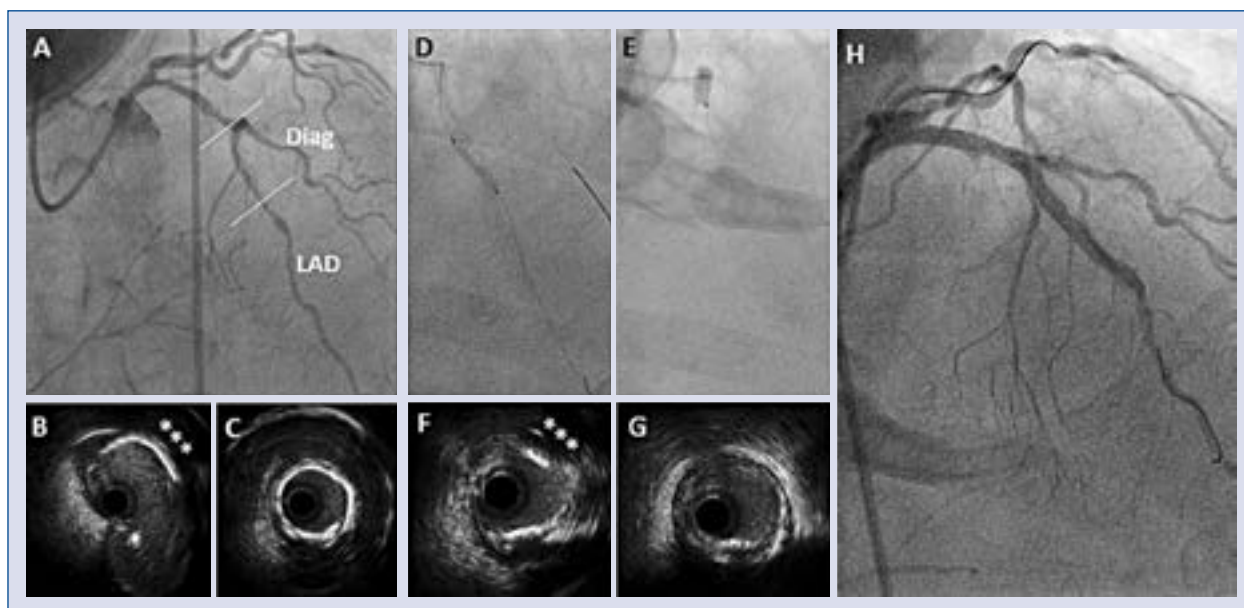
Figure 3. Coronary calcified bifurcating lesion treated by coronary lithotripsy. Long left anterior descending artery (LAD) lesion involving the bifurcation site of a large diagonal branch (Diag); **A.** Angiographic view; **B.** Intravascular ultrasound (IVUS) section at bifurcation site, with an eccentric calcific plaque (asterisks); **C.** IVUS section at mid LAD, with a concentric calcific plaque ("annular ring"); **D, E.** Intravascular lithotripsy (IvL) balloon inside of the lesions; **F, G.** IVUS section after IvL, showing effective disruption of the calcific plaques; **H.** Final angiographic view after LAD stenting.

pulses to fracture calcified tissues with virtually no debris production. The concept of acoustic waves to selectively crack hard bodies has been employed in urology for decades. This approach aims to avoid the trauma produced by blades and burrs and their related risk, still offering an effective disruptive action on calcium (Fig. 3).

IvL device is composed of a 12 mm long balloon catheter with three emitters inside the balloon between two radiopaque markers; it has a profile smaller than other modifying balloons, quite comparable to a non-compliant balloon and is available in 7 sizes ranging from 2.5 to 4.0 mm. An electrical discharge at the emitters vaporizes the
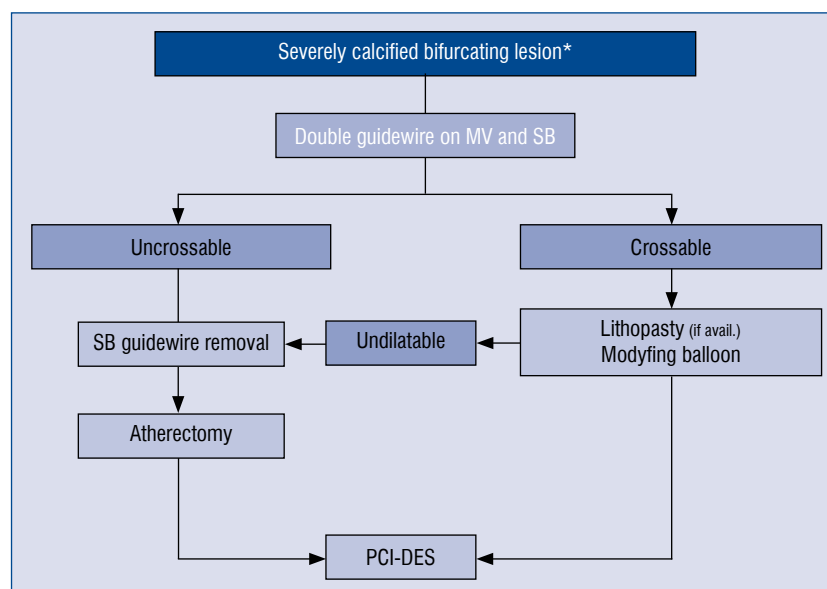
Figure 4. Proposed algorithm for the treatment of severely calcified bifurcating lesions; *Side branch longer than 73 mm, with a diameter ≥ 2 mm; GW — guidewire; MV — main vessel; SB — side branch; PCI-DES — percutaneous coronary intervention–drug-eluting stent.

fluid generating a series of sonic pulses that propagate and selectively interact with calcified plaques even in their inner adventitial layer. In the absence of any system mounted onto the balloon itself, the profile of IvL should guarantee better crossability than other modifying balloons, although in severely calcified lesions uncrossable by any balloon, atherectomy seems to stand as a last resource. In another way, unlike RA, IvL can be used with more than a guidewire to protect side branches; its effect might theoretically extend to calcified side branches ostia and, reportedly, it can be used with the kissing balloon technique because of the presumed ability of IvL to propagate across a second balloon. Moreover, IvL has been employed to fracture a calcified plaque outside an underexpanded stent, allowing appropriate stent expansion at subsequent high-pressure dilatation [35]. An OCT study [36] demonstrated that vessel preparation with IvL led to an increase in minimum lumen area and a reduction in area stenosis, allowing stents to be delivered into all target lesions, with an efficacy proportional with calcification severity, with a very low complication rate (no perforation, nor slow/no flow phenomenon). The prospective, multicenter, single arm Study "Disrupt CAD II", recently documented the safety and the effectiveness of IvL [37].

Conclusions

Percutaneous coronary intervention of a severely calcified bifurcating stenosis is a challenge due to the inherent risks related to inadequate stent expansion and side branch compromise. Therefore, adequate lesion preparation with dedicated tools is often required. Modifying balloons are effective in lesion preparation, but their use is undermined by low crossabilty. Atherectomy, has been available for 30 years only as rotational, more recently it has evolved as orbital, allowing a high success rate, with an increased complication rate as a trade-off. IvL are now promising devices that obtain calcific plaque fragmentation with sonic pulses locally delivered, but the clinical translation of such benefit is still to be determined.

Here we propose a practical algorithm for the treatment of severely calcified bifurcating lesions (Fig. 4); in crossable lesions, modifying balloons, scoring better than cutting, can be the first choice and now IvL is extremely promising. In case of crossing failure, atherectomy still stands as the last resource [38], but it requires SB removal.

Conflict of interest: None declared

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REVIEW ARTICLE

VIA MEDICA

Established and potential echocardiographic markers of embolism and their therapeutic implications in patients with ischemic stroke

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Abstract

Cardiogenic strokes comprised 11% of all strokes and 25% of ischemic strokes. An accurate identification of the cause of stroke is necessary in order to prepare an adequate preventive strategy. In this review the confirmed and potential causes of embolic strokes are presented, which can be detected in echocardiography in the context of present treatment guidelines and gaps in evidence. There remains a need for further studies assessing the meaning of potential cardiac sources of embolism and establishment of rules for optimal medical prevention (antiplatelet therapy [APT] vs. oral anticoagulation [OAC]) and interventional procedures to reduce the incidence of ischemic strokes. Currently available data does not provide definitive evidence on the comparative benefits of OAC vs. APT in patients with cryptogenic stroke or embolic stroke of undetermined source. There is a lack of antithrombotic treatment scheme in the time between stroke and the completed diagnosis of potential sources of thromboembolism. (Cardiol J 2019; 26, 5: 438–450)

Key words: cardioembolic stroke, anticoagulant therapy, cardiac sources of embolism, ischemic stroke, cryptogenic stroke

Introduction

Approximately 1.1 million inhabitants of Europe suffera stroke each year, and ischemic stroke accounted for approximately 80% of cases [1, 2]. Although global stroke incidence is declining, rates observed in young adults are on the rise, thus suggesting a need for strategies to improve prevention [2]. In addition, because of an ageing population, the absolute number of strokes is expected to dramatically increase in coming years: by 2025, 1.5 million European people will suffer a stroke each year. Stroke is associated with increased long-term mortality. Beyond vital prognosis, stroke patients are also at increased risk of poor outcome within the first year of the event including re-hospitalization (33%), recurrent event (7% to 13%), dementia (7% to 23%), mild cognitive disorder (35% to 47%), depression (30% to 50%), and fatigue (35% to 92%), which all contribute to, and affect health related quality of life. Ischemic stroke is a heterogeneous disease with different mechanisms and etiologies and their specific treatments. An accurate identification of the cause is essential in order to prepare an adequate preventive strategy. A substantial proportion of stroke risk remains unexplained [3].

Using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, ischemic strokes may be further subdivided into following types:
1. Thrombosis or embolism associated with large vessel atherosclerosis;
2. Embolism of cardiac origin (cardioembolic stroke);
3. Small blood vessel occlusion (lacunar stroke);
4. Other determined cause;
5. Undetermined (cryptogenic) cause (no cause identified, more than one cause, or incomplete investigation) [4].

The incidence of each cause is variable and depends on patient age, sex, race, geographic location, risk factors, clinical history, physical findings, and results of various tests. Embolism of cardiac origin accounts for around 15–30% of ischemic strokes. It is worth noting that stroke from a cardiac source carries a poorer outcome compared with other sources, having a 50% mortality at 3 years. The diagnosis of a cardioembolic source of stroke is frequently uncertain and relies on the identification of a potential cardiac source of embolism in the absence of significant autochthone cerebrovascular occlusive disease [5]. While a stuttering course has usually been attributed to atherothrombotic stroke, cardioembolic strokes can have a progressive course in at least one-fifth of cases given that emboli can recanalize, move and fragment after initial impaction. Rapid regression of symptoms (the spectacular shrinking syndrome) reflecting early recanalization has also been related to cardioembolic stroke [6].

In patients who are at risk for or have already had potentially embolic strokes, the primary role of echocardiography is to establish the existence of the source of embolism, determine the likelihood that such a source is a plausible cause of stroke or systemic embolism, and guide therapy in an individual patient. Ultimately stroke is classified as cryptogenic or undetermined when no cause is identified after a thorough study [7]. Embolic stroke of undetermined source (ESUS) is one-fourth among cerebral infarction, but most of them could be ascribed to embolic stroke. This review presents the confirmed and potential causes of embolic strokes which can be detected in echocardiography in the context of present treatment guidelines and gaps in evidence. There is still a need for further studies assessing the meaning of potential cardiac sources of embolism and establishment of the rules of the optimal medical prevention (antiplatelet therapy [APT] vs. oral

anticoagulation [OAC]) and interventional procedures to reduce the incidence of ischemic strokes.

Cryptogenic stroke and ESUS

The term cryptogenic stroke has been extensively used in the literature to describe ischemic strokes of undetermined etiology. However, this does not take into account the extent and quality of the investigation performed or classification system used [8]. In the TOAST classification system, stroke of undetermined cause may refer to a stroke with incomplete workup, more than one potential cause, or indeed no determined etiology after investigations are complete [9]. In 2014, the term ESUS, was coined by the cryptogenic stroke /ESUS international working group, ESUS refers to non-lacunar infarct (subcortical infarct ≤ 1.5 cm on computed tomography [CT] or ≤ 2.0 cm on magnetic resonance imaging [MRI]) in the absence the following: extracranial or intracranial atherosclerosis causing > 50% luminal stenosis in the artery supplying the ischemic region, major cardioembolic sources permanent or paroxysmal atrial fibrillation (AF), sustained atrial flutter, intracardiac thrombus, prosthetic cardiac valve, atrial myxoma or other cardiac tumors, mitral stenosis (MS), myocardial infarction (MI) within the past 4 weeks, left ventricular (LV) ejection fraction (LVEF) < 30%, valvular vegetation's or infective endocarditis (IE), and no other specific cause of stroke (e.g., dissection, arteritis, migraine/ vasospasm, drug misuse) [7]. Extensive evaluation including transesophageal echocardiography (TEE) and cardiac monitoring over an extended period of time could identify the etiology of ESUS patients. Although an antiplatelet drug is recommended in ESUS is the current guideline, clinical trials are ongoing to determine the efficacy of non-vitamin K antagonist oral anticoagulant in ESUS patients [9].

The established and potential cardiac reasons of ischemic stroke

Echocardiography (both, transthoracic echocardiography [TTE] and TEE) is a widely used and versatile technique that can provide comprehensive information of thromboembolic risk in patients with stroke [8, 9]. In many conditions more than one embolic source may be present (coexistence of embolic sources) or one cardioembolic condition may lead to another (interdependent of embolic sources). For instance, MS is associated with spon-

taneous echocardiographic contrast, AF, clot, and even endocarditis. Conditions that are known to lead to systemic embolization are subdivided into high-risk and low-risk groups on the basis of their embolic potential and are presented in Tables 1 and 2 [6, 8].

The established cardiac sources of ischemic stroke

Atrial fibrillation

Atrial fibrillation is the most common cardiac arrhythmias, and a major cause of morbidity and mortality due to cardioembolic stroke. Prevalence of AF increases with age, up to 15% in octogenerians, and continues to grow rapidly due to the increasing proportion of aging in the population. Moreover, the elderly population of today has a higher prevalence of predisposing conditions for AF, such as diabetes, heart failure, hypertension, and coronary heart disease [10]. Newly detected AF is identified in 10% of the patients who experience a stroke or a transient ischemic attack (TIA), and an additional 11% cases of AF are newly detected when patients undergo 30 days of continuous electrocardiographic monitoring [11]. The left atrial appendage (LAA) is the major site of thrombus formation in non-valvular AF. TEE is considered to be the gold-standard technique to detect LAA thrombi, with values of sensitivity and specificity approaching 99% [11].

In the Stroke Prevention in Atrial Fibrillation (SPAF) III and other trials, LAA thrombi, dense spontaneous echo contrast, LAA peak flow velocities 20 cm/s, and complex aortic plaques were independently associated with increased thromboembolic risk in AF patients [12].

Atrial fibrillation detection is important in stroke prevention, because anticoagulation is known to decrease stroke risk in the presence of this arhythmia. A meta-analysis of 50 studies involving more than 10,000 patients with a recent stroke found that 7.7% had AF on their admitting electrocardiogram. Within 3 weeks during and after hospitalization, another 16.9% were diagnosed. A total of 23.7% of these stroke patients had silent AF; that is AF diagnosed after hospital admission [13]. Olsen et al. [14] aimed to evaluate whether speckle tracking echocardiography improves risk stratification for AF. After 5.5 years patients who presented a composite of new-onset AF and ischemic stroke had significantly reduced systolic function by LVEF and global longitudinal strain, however, only global

Table 1. The conditions with a high embolic potential.

The sources of intracardiac thrombi
Atrial arrhythmias:
— Valvular atrial fibrillation
— Non-valvular atrial fibrillation
— Atrial flutter
Akinetic segments and diffuse ventricular hypokinesia:
— Recent myocardial infarction
— Ventricular aneurysm
— Non-ischemic cardiomyopathies
Prosthetic valves and devices
Intracardiac vegetations
Myxoma
Papillary fibroelastoma
Other tumors
Intracardiac vegetations
Infective endocarditis
Marantic endocarditis
Aortic atheroma
Thromboembolism
Cholesterol crystal emboli

Table 2. The conditions with a low embolic potential.

Septal defects and anomalies
Patent foramen ovale
Atrial septal defect
Atrial septal aneurysm
Intracardiac calcifications
Mitral annular calcifications, mitral stenosis (mainly in the course of rheumatic heart disease)
Calcific aortic stenosis
Substenotic atherosclerotic plaques
Potential precursors of intracardiac thrombi
Mitral valve prolapse
Pouches of atrial septum
Enlargement of left atrium
Structures in dextral atrium like Eustachian valve or Chiari's network

longitudinal strain remained a significantly independent predictor (hazard ratio [HR] 1.12, 95% confidence interval [CI] 1.00–1.25, p = 0.042, per 1% decrease) after multivariable adjustment for baseline predictors (age, sex, diabetes, hypertension, diastolic dysfunction, and LVEF) [14].

Myocardial infraction and cardiomyopathies

Myocardial infarction and dilated cardiomyopathy (DCM) are most frequently associated with LV thrombus formation attributable to stasis caused by regional or global myocardial dysfunction. From 1% to 2.5% of patients with acute MI suffer a stroke within 4 weeks, one-half in the first 5 days. LV thrombus was more likely in patients with EF of < 40%, anterior wall MI and LV aneurysm [11]. Embolic events are estimated to occur in 4% of patients with DCM who have a LVEF ≤ 35%. Further, the incidence of LV thrombus in patients with DCM and sinus rhythm is 13%, and DCM may also have a high incidence of clinically asymptomatic silent cerebral infarction [15]. Isolated LV noncompaction (LVNC) is a genetic cardiomyopathy characterized by prominent ventricular trabeculations and deep intertrabecular recesses, or sinusoids, in communication with the LV cavity. The clinical sequelae of these deformities are the syndrome of heart failure and the risk for arrhythmias and stroke. The diagnostic studies for evaluation of LVNC are echocardiography, cardiac magnetic resonance (CMR) and cardiac CT. The Stöllberger et al. [16] revealed that stroke//embolism in LVNC is not always cardioembolic, but may also have an atherosclerotic cause. The CHADS$_2$ score may be useful for clinical decision-making about OAC for the prevention of stroke//embolism in LVNC patients [17].

Rheumatic heart disease

Rheumatic heart disease is a common cause of stroke in developing countries. Among patients with rheumatic heart disease, MS is the valve lesion most strongly associated with stroke and systemic embolism [18, 19]. Studies from the middle part of the last century found an annual incidence of systemic embolism among patients with rheumatic mitral valve disease of 1.5% to 4.7% [20]. Reports on the association of embolic stroke with mitral valve prolapse have been inconsistent [19–21]. A population-based study of patients from Olmsted County, Minnesota, found an increased risk ratio (RR) of stroke or TIA among patients with mitral valve prolapse who were initially in sinus rhythm (RR 2.2; 95% CI 1.5–3.2) [21]. The presence of left atrium enlargement and AF increase the incidence of silent brain infarction in patients with MS, whereas the presence of moderate to severe mitral regurgitation decreases the incidence [21].

Endocarditis

Stroke often complicates IE, affecting 16–25% of patients with IE [21].

Most diagnoses of stroke and IE are made close together in time, but a period of heightened stroke risk becomes apparent several months before the diagnosis of IE and lasts for several months afterward [22, 23]. In the study of Merkler et al. [24] among 17,926 patients with IE, 2,275 strokes occurred within the 12-month period surrounding the diagnosis of IE.

The risk of stroke was highest in the month after diagnosis of IE (1,640 vs. 17 strokes in the corresponding month 2 years prior) [24]. A transthoracic echocardiography must be performed in every case of suspected IE. Vegetations (infected tissue mass) appear as irregular echogenic masses usually attached to the atrial side of the atrioventricular valves or the ventricular side of the semilunar valves along the line of leaflet coaptation. The vegetations are seen to move independently to the underlying valve motion [15]. IE requires administration of systemic antibiotics and, if indicated, surgery. Non-bacterial thrombotic endocarditis (NBTE), ormarantic endocarditis, is composed of small, sterileplatelet — fibrin vegetations on the cardiac valves. There is little inflammatory reaction at the foot of the growth so the thrombi detach easily, causing multiorgan infarcts. The pathogenesis of NBTE is not completely understood. It is particularly associated with adenocarcinomas of the gastrointestinal tract and lungs and mechanisms are considered to be similar to the ones underlying cancer hypercoagulability [21, 23]. A clinical triad of a known disease process associated with NBTE, heart murmur and signs of multiple systemic emboli should alert the physician to NBTE. NBTE could be treated with tumor-suppressive, antiretroviral, or immunosuppressive therapy with systemic anticoagulation, preferably heparin-based however treatment options are limited because of Disseminated Intravascular Coagulation which is often present.

Fibrous and fibrinous lesions of the heart valves and endocardium traditionally occur in patients with systemic lupus erythematosus (Libman-Sacks endocarditis), antiphospholipid antibody syndrome. Fibrin-platelet aggregates may attach to these fibrous and fibrinous lesions [23, 24].

Cardiac tumors

Primary tumors of the heart are rare with the incidence of 0.02–0.05% [25].

The majority of them are myxomas, accounting for only 0.02% of primary tumors, and 75% of them are benign. Embolic manifestations occur in 20–45% of patients with a cardiac myxoma, sometimes as the onset symptom [26]. From 75% to 80% of myxomas are located in the left atrium. Myxomas arising from the right ventricle are extremely rare. Most cases (90%) of atrial myxoma are sporadic with no known cause. In the remaining (10%), a familial pattern occurs having an autosomal dominant pattern known as Carney complex which is characterized by multiple tumors, including atrial and extracardiac myxomas, cutaneous spotty pigmentation, non-myxomatous extracardiac tumors, schwannomas and various endocrine tumors, through a causative mutation of the PRKAR1α gene located on the long arm of chromosome 17 [25]. Cardiac myxomas produce interleukin (IL)-6, leading to constitutional manifestations such as recurrent fever, anemia, arthralgia, and weight loss which disappear after removal of the tumor [26, 27]. Myxomas are frequently found particularly between the third and sixth decades of life. There are 2 types of cardiac myxomas: friable polypoid type and smooth-surface rounded type. The polypoidal type tends to result in embolism because of its friable consistency and intracavitary location [28]. Furthermore, the LV has a higher risk of embolization resulting from high mobility and pressure in it [29, 30]. The second most common benign primary tumor of the heart is papillary fibroelastoma. Its prevalence is 0.002% to 0.28% among the general population. The average age at diagnosis is 56, with a primarily male preponderance (58%). These kinds of tumors are often found on cardiac valvular surfaces. The clinical presentation of fibroelastoma varies widely, ranging from clinically asymptomatic to severe thromboembolic events. It is important to differentiate fibroelastomas from cardiac myxomas and thrombi because of differences in their medical treatment. A clear surgical margin is necessary for excision of a myxoma due to its high rate of recurrence. On the other hand, fibroelastomas rarely recur after resection, thus it is recommended to preserve valvular function by shaving off the tumor [25]. Diagnosis at present is established most appropriately with two dimensional echocardiography. CMR can help out in demarcating tumor size, attachment and its mobility and this information may be further used during surgical resection. Positron emission tomography scan has been useful in identifying cardiac involvement in patients with metastatic tumors and atrial myxoma [29]. Although, since tumor fragments may embolise, early anticoagulation may not be protective in reducing disability and mortality [30]. Prompt excision using cardiopulmonary bypass, first carried out by Crafoord in 1954, has been established as the only acceptable mode of treatment for these tumors. The results of surgical resection are generally very good, with most series reporting an operative death rate of < 5%.

Potential sources of thromboembolism

Patent foramen ovale and atrial septal defect

Patent foramen ovale (PFO) is the most common congenital cardiac abnormality present in approximately 25% of the population and is responsible for up to 95% of right-to-left shunts (Fig. 1). PFO is a communication across the interatrial septum between a nonadherent septum primum and septum secundum and is considered to be a risk factor for serious clinical syndromes including paradoxical systemic embolism such as embolic strokes, MI, decompression sickness in divers, and complications of pulmonary embolism [27]. PFO prevalence among patients with cryptogenic stroke are typically high with median prevalence of approximately 40% and more commonly present in young people. Saline contrast injection or agitated saline mixed with air (also referred to as a "bubble study") during TTE or TEE can detect a PFO if microbubbles which are seen within the left atrial chamber within three cardiac cycles after right atrial opacification [8]. In situ thrombus formation and propensity for cardiac arrhythmias in patients with PFO have been suggested stroke mechanisms [1]. No association has been documented between shunt amount and stroke recurrence. Until now, whether PFO is a risk factor for stroke has been unsettled. In the SISIFO study — a multicenter, prospective, single-wave, cross-sectional survey conducted on 1,130 consecutive patients with acute ischemic stroke admitted to selected clinical centres underline PFO alone must not be considered a significant independent predictor for stroke; so the presence of PFO alone doesn't permit rushed causal correlations or 'therapeutic aggressiveness' [31]. By far, paradoxical embolization is the most commonly proposed mechanism for stroke in PFO patients. Therefore, finding a proximal source such as a deep venous thrombus is warranted, otherwise the association is rendered theoretical. Besides PFO closure did not prevent stroke. Clinical conditions such as deep vein thrombosis, a prolonged immobility/postopera-
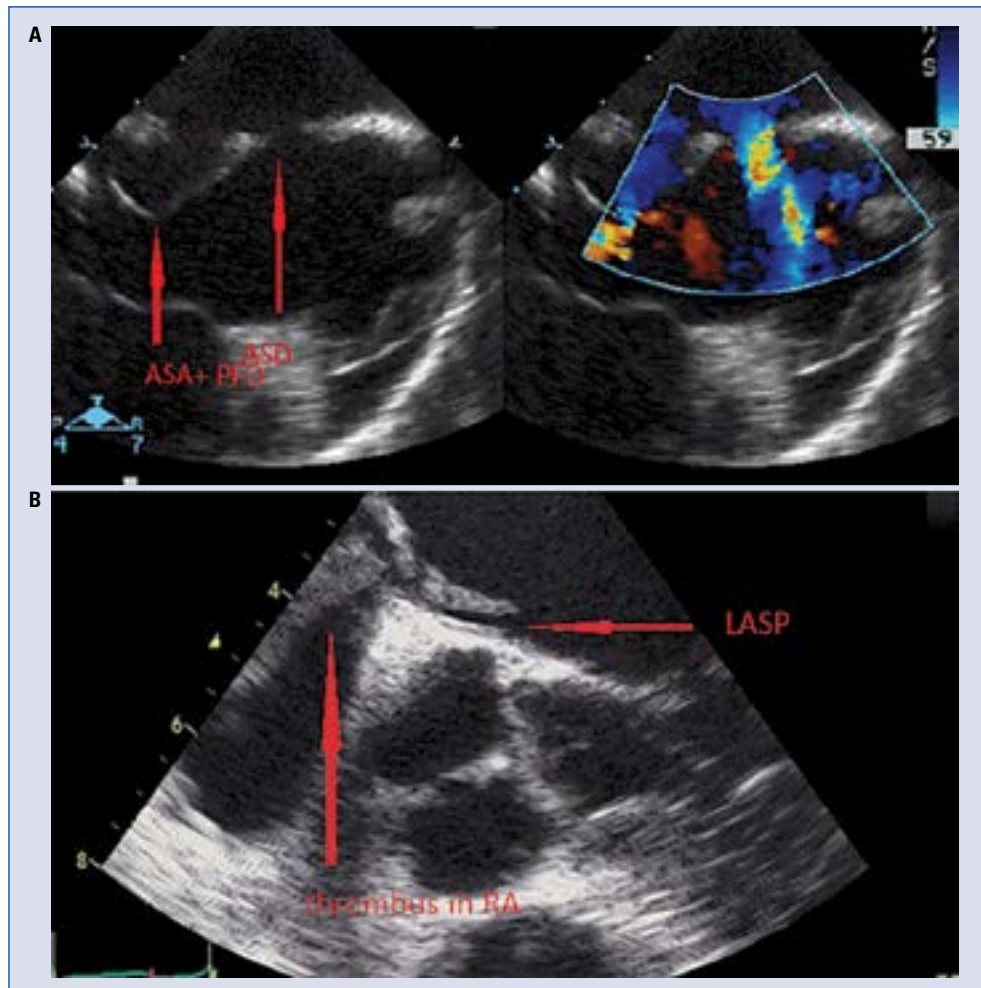
Figure 1. A. Transesophageal echocardiography (TEE), atrial septal aneurysm (ASA), patent foramen ovale (PFO) and atrial septal defect (ASD) in patient after ischemic stroke; **B.** TEE. The left atrial septal pouch (LASP) and mobile thrombus in right atrium (RA).

tive period, and the Valsalva maneuver were also not associated with embolism recurrence. Taken together, a mechanism other than paradoxical embolism may contribute to the development of embolic stroke in patients with PFO [32–34]. The probability of having a PFO as a possible cause of stroke in cryptogenic stroke patients may vary according to patient characteristics [35, 36]. Several factors possibly associated with increased risk of stroke recurrence in patients with PFO include a right-to-left shunt detectable in resting conditions, amount of right-to-left shunt under Valsalva, and a combination of PFO with either atrial septal aneurysm (ASA) or increased interatrial septal mobility (Fig. 1) [37–39]. Several clinical and brain imaging features, including the 10-point Risk of Paradoxical Embolism (RoPE) score, have been suggested to determine a high-risk PFO (Table 3) [39].

Substenotic atherosclerotic plaques

Substenotic atherosclerotic plaques can possibly cause ischemic stroke by plaque rupture and artery-to-artery embolization. In particular, complicated atherosclerotic plaques with evidence of intraplaque hemorrhage on imaging have been suggested to be a potential mechanism in ESUS [7].

There are more potential causes of cardiac strokes, which are not entirely examined as:
— pouches of atrial septum;
— structures in dextral atrium like Eustachian valve (EV) or Chiari's network (CN);
— enlargement of left atrium;
— ASA.

Pouches of left atrial septum

The left atrial septal pouch (LASP) was described in 2010 by Krishnan and Salazar as

Table 3. Ten-point risk of paradoxical embolism (RopE) score to determine a high-risk patent foramen ovale (PFO).

Characteristic	Points
No history of hypertension	+1
No history of diabetes	+1
No history of stroke or TIA	+1
Nonsmoker	+1
Cortical infarct on imaging	+1
Age [years]:	
18–29	+5
30–39	+4
40–49	+3
50–59	+2
60–69	+1
≥ 70	0

SCORE INTERPRETATION

RoPE score	PFO-attributable fraction (95% CI)	Estimated stroke/ /TIA recurrence at 2 years
0–3	0% (0–4)	20% (12–28)
4	38% (25–48)	12% (6–18)
5	34% (21–45)	7% (3–11)
6	62% (54–68)	8% (4–12)
7	72% (66–76)	6% (2–10)
8	84% (79–87)	6% (2–10)
9–10	88% (83–91)	2% (0–4)

CI — confidence interval; TIA — transient ischemic attack

a potential source of embolism. Some data suggest that when a foramen ovale closes spontaneously, the septum primum and the septum secundum fuse initially at the caudal limit of the zone of overlap of the two structures (Fig. 2). This incomplete fusion results in a pouch that, in the majority of instances, communicates with the left atrium cavity [40]. The presence of LASP with access to the systemic circulation also raises the possibility that, similar to the LAA, during low-flow states, this pouch might serve as a site for thrombus formation and embolization. But the results of clinical studies are inconclusive [30].

In a case-control study of relatively older subjects (mean age 69 years), Tugcu et al. [33] found that LASP was not associated with ischemic or cryptogenic strokes. Negative results between LASP and cryptogenic stroke were also reported in a cohort study of 566 consecutive patients undergoing TEE by Wayangankar et al. [34]. The

same author also carried out a retrospective case-control study in patients with a high prevalence of cardiovascular risk factors. The prevalence of LASP was 18%, irrespective of age or pathology. Their study did not show any association between LASP and ischemic stroke or cryptogenic stroke [35]. On the other hand, Wong et al. [35] studied 212 consecutive TEE patients with mean age 57 years and found an increased prevalence of LASP among cryptogenic stroke patients compared to ischemic stroke patients of other subtypes. Sun et al. [31] demonstrated among 324 patients, evidence of an association between LASP and ischemic stroke in either univariable analysis and after adjustment for other stroke risk factors using multiple logistic regression analysis. The risk of ischemic stroke in this study was two-fold among patients with LASP than cases without LASP. Additional investigation should take place to determine the clinical significance of LASP and what interventions are required to prevent ischemic stroke in at-risk individuals [31].

The Eustachian valve

The EV is a remnant of the embryonic right valve of the sinus venosus. Embryologically, the EV directs oxygenated blood from the inferior vena cava across the PFO into the systemic circulation. By directing the blood from the inferior cava to the interatrial septum, a persisting EV may prevent spontaneous closure of PFO after birth and may, therefore, indirectly predispose one to paradoxical embolism. However, there have been relatively few studies performed looking at stroke risk in this population and not all authors agree that prominent EV predisposes to additional risk. In a study by Schuchlenz et al. [36] the mean EV size was 1.0 ± 0.4 cm; 70% of patients with an EV had a PFO (p < 0.001). In contrast the prevalence of PFO in the control group was 30% and 61% for those with presumed paradoxical embolism (p < 0.001). Thus, an EV was significantly more common for patients with presumed paradoxical embolism than in control patients (143 of 211 — 68% vs. 31 of 95 — 33%, respectively, p < 0.001) [36].

The Vale et al. [37] investigated the relationship between EV length and atrial septal movement in 72 consecutive patients referred to their centre for PFO closure following presumed cryptogenic stroke. The authors proposed that while a large degree of atrial septal movement significantly increases propensity to cerebral embolism in patients with PFO, its absence does not negate this risk. They have shown that long EV may function

All patients with ESUS:

— History + physical examination + neurological assessment

— MRI or CT scanning of the brain

— ECG + chest ray + laboratory test

— Ultrasound or MRI/CT angiography of extracranial carotid arteries

— Transcranial Doppler USG

— ECG cardiac monitoring for at least 72 h

— TTE

No cardiac sources suspected	Cardiac sources suspected
Other etiologies: — Lacunar stroke — Atherothrombotic stroke — Hemodynamic stroke — Thrombotic stroke Hypercoagulable state test: — Protein C — Protein S — Antiphospholipid antibodies — Leiden mutation — Hyperhomocystein — Antythrombin III	TEE, if negative — TTE Additional ECG monitoring by long-term non-invasive ECG monitors or implanted loop redcorders if high suspicion of paroxysmal AF

Figure 2. Diagnostic algorithm for cardiac workup of ischemic stroke. AF — atrial fibrillation; CT — computed tomography; ECG — electrocardiography; ESUS — stroke of undetermined source; MRI — magnetic resonance imaging; USG — ultrasonography; TEE — transesophageal echocardiography; TTE — transthoracic echocardiography.

independently from atrial septal movement to potentiate paradoxical embolism [37].

Chiari network

The CN, present in approximately 2% of the population, and is a reticulated network of fibers originating from the Eustachian connecting to different parts of the right atrium. Its presence results from incomplete reabsorption of the right valve of the sinus venosus [41]. CN is often clinically insignificant. However, it has been reported to be involved in the pathogenesis of thromboembolic disease, endocarditis, arrhythmias, and entrapment of catheters upon percutaneous intervention [42]. The network is associated with PFO in 80% of cases. The role of a CN in the pathogenesis of stroke is discussed. CN may create turbulent blood flow leading to thrombus formation. The fibers of the network are sometimes torn during life and may break free. The fenestrated types may rarely remove emboli from the circulation, but this is purely by chance, and further emboli are likely to reach the lung [38]. Rigatelli et al. [39] during over a 24-month period, prospectively enrolled 50 consecutive patients (mean age 37 ± 12.5 years,

38 females) with previous stroke and migraine referred for PFO catheter-based closure. Patients with EV and CN had more frequently a curtain pattern on transcranial Doppler, a larger right-to-left shunt, more recurrent cerebral paradoxical embolism before closure, and a higher preoperative MIDAS score. This study suggests that EV and CN have a deep impact on migraine with aura and paradoxical embolism pathophysiology: EV, CN, and migraine with aura should be considered as adjunctive risk factors for paradoxical embolism in the work-up of both symptomatic and asymptomatic PFO patients [39].

The left atrial enlargement

Little is known about the risk of stroke associated with left atrial enlargement in patients in sinus rhythm, and whether such patients may need a thromboprophylaxis. Formal stroke risk stratification among patients with left atrial enlargement may further help to identify patients who stand to gain from preventive antithrombotic therapy [43]. The normal left atrial volume index (LAVI) using echocardiography is 22 ± 6 mL/m²; thus, on the basis of the sensitivity and specificity

for predicting cardiac events [3, 6–8], the American Society of Echocardiography (ASE) considers left atrial enlargement as > 28 mL/m^2 (i.e., 1 SD from the mean). However, for the purpose of identifying LV diastolic dysfunction, an LAVI cut point > 34 mL/m^2 (i.e., 2 SD) was endorsed in both ASE and the European Association of Echocardiography guideline document [26]. Biteker et al. [44] prospectively followed 310 consecutive first-ever acute ischemic stroke patients aged 50 years or older who were admitted to hospital within 24 h of the onset of stroke symptoms. The optimal cut off value, sensitivity, and specificity of LAVI to distinguish cardioembolic stroke from non-cardioembolic stroke were 30 mL/m^2 (81% and 64%, respectively). Kaplan-Meier analysis showed that there was a stepwise increase in risk of mortality with each increment of LAVI category. Overvad et al. [40] identified nine cohort studies and analyzed a total of 67,875 participants and 3,093 stroke outcomes. All studies reported a higher risk of stroke with larger/enlarged left atrium compared to smaller/normal sized left atrium. The underlying etiology explaining this observed higher risk is likely to be multifactorial and not confined to a potential direct effect of left atrial enlargement on thromboembolic risk [44]. In the study of Yaghi et al. [41] in 655 first ischemic stroke patients over a median of 4 years, there were 65 recurrent ischemic strokes (29 were cardioembolic or cryptogenic). In multivariable models adjusted for confounders, including AF and heart failure, moderate-severe left atrial enlargement compared with normal left atrial size was associated with greater risk of recurrent cardioembolic/cryptogenic stroke, but not total ischemic stroke [42].

Left atrial dysfunction has been reported in patients with PFO. It is postulated that left atrial dysfunction could be involved in the development of an arterial embolism in patients with PFO [43]. One hypothesis is that a pathological PFO might contribute to atrial enlargement. In contrast, another possibility is that cardiac thrombus that is secondary to left atrial enlargement might be a determinant of stroke and PFO is additive or even incidental.

Atrial septal aneurysm

An ASA consists of redundant atrial septal tissue bulging into the right or the left atrium (Fig. 1). Some studies indicate that existence of ASA may be a potential risk factor of stroke. In patients with ASA and a history of embolic events, ASA may enhance migration of a thrombus constituted in situ or transiting through it. Marked mobility of ASA may also increase the risk of peripheral embolus. Rigatelli et al. [39] revealed that moderate-to-severe ASA might be associated with left atrial dysfunction in patients with PFO. In the majority of cases ASA is associated with other cardiac abnormalities such as PFO and atrial septal defects as well as mitral valve prolapse or atrial arrhythmias. Mattioli et al. [22] have demonstrated a statistical association between PFO and ASA and have shown that both morphological abnormalities were independent predictors of embolic events in a multivariate analysis [44].

Anticoagulation affairs and stroke risk

Acetylsalicylic acid, clopidogrel, or the combination of acetylsalicylic acid and dipyridamole, are all acceptable options for secondary prevention in patients with ischemic stroke or TIA of arterial origin [45]. Currently, OAC therapy with warfarin or one of the non-vitamin K antagonist oral anticoagulants (NOACs), including a direct thrombin inhibitor (dabigatran) and factor Xa inhibitors (rivaroxaban, apixaban, and edoxaban) is the most effective prophylactic approach available to patients with AF at high risk of thromboembolic events. Contemporary guidelines from the European Society of Cardiology, the American College of Cardiology, the American Heart Association (AHA), and the Heart Rhythm Society for managing stroke prophylactic treatment in AF patients advocate for the use of the CHA_2DS_2-VASc score (Congestive heart failure [1 point], Hypertension [1 point], Age 75 years [2 points], Diabetes [1 point], Stroke [2 points], Vascular disease [1 point], Age 65–74 years [1 point], and female sex category [1 point]) for stroke risk stratification [46, 47]. In 'low risk' patients with no additional stroke risk factors, oral anticoagulant treatment is not recommended. The United States guideline offers a Class IIb recommendation on either no treatment, acetylsalicylic acid therapy, or oral anticoagulant treatment in AF patients with a CHA_2DS_2-VASc score of 1. The European guideline recommends that female sex as a single risk factor should be disregarded, and hence, low risk is defined as a CHA_2DS_2-VASc score of 0 in males, 1 in females, where no antithrombotic therapy is recommended [48, 49]. Thus, oral anticoagulant treatment should be considered in AF patients with a one stroke risk factor (i.e. CHA_2DS_2-VASc score of 1 in males, or 2 for females; Class IIa recommendation), or recommended in those with a CHA_2DS_2-VASc score ≥ 2 (Class I recommendation) [50, 51]. Patients with mechanical valve prosthesis

and with AF and moderate or severe MS should be treated with vitamin K antagonist. There is a high risk of stroke and systemic thromboembolism but also a high risk of bleeding if anticoagulants are prescribed. Newer surgical or percutaneous interventions (WATCHMAN, LARIAT, and Amplatzer devices) have been developed for stroke prevention in patients with AF. These devices are typically used in AF patients who have high risk for thromboembolic events and cannot tolerate prolonged anticoagulation therapy. Although these interventions have shown feasibility, their long-term superiority to medical management remains a matter of debate [12]. The cryptogenic strokes are being recognized as sharing many characteristics with cardioembolic strokes [4]. The major challenge in supervising stroke is secondary stroke prevention of cryptogenic strokes, especially in choosing antithrombotic therapy [4]. A relatively frequent complication of ischemic stroke is hemorrhagic transformation. It happens in 2.2% to 44% of clinical cases [51].There has been no definitive study assessing the comparative effectiveness of OAC vs. APT in this population. Currently available data do not provide definitive evidence on the comparative benefits of OAC vs. APT in patients with cryptogenic stroke or ESUS [44]. There are ongoing studies assessing advantages and disadvantages of these two kinds of therapies. The Randomized, double-blind, Evaluation in secondary Stroke Prevention comparing the EfficaCy and safety of the oral Thrombin inhibitor dabigatran etexilate vs. acetylsalicylic acid in patients with Embolic Stroke of Undetermined Source (RE-SPECT ESUS) is a prospective, randomized, double-blind, multicenter trial involving approximately 6000 patients and 550 centers. Subjects are randomized to dabigatran or acetylsalicylic acid and are treated for an expected minimum of 6-months and for up to approximately 3 years. The primary efficacy outcome is a time to first recurrent stroke (ischemic, hemorrhagic, or unspecified). Key secondary outcomes are time to first ischemic stroke and time to first occurrence in the composite outcome of nonfatal stroke, nonfatal MI, and cardiovascular death. The primary safety outcome is major hemorrhage, including symptomatic intracranial hemorrhage [50]. The ATTICUS randomized trial is designed to determine whether the factor Xa inhibitor apixaban administered within 7 days after ESUS, is superior to acetylsalicylic acid for prevention of new ischemic lesions documented by brain MRI within 12 months after index stroke. The primary outcome is the occurrence of at least one new ischemic le-

sion identified by axial T2-weighted FLAIR MRI and/or axial diffusion-weighted imaging MRI at 12 months when compared with the baseline MRI. Key secondary outcomes are the combination of recurrent ischemic strokes, hemorrhagic strokes, and systemic embolism [52].

The New Approach riVaroxaban Inhibition of Factor Xa in a Global trial versus ASA to prevent Embolism in ESUS (NAVIGATE ESUS) is a multinational, randomized, double-blind, superiority trial comparing antithrombotic therapies for secondary stroke prevention in a well-defined cohort of patients with nonlacunar cryptogenic stroke with embolic features. Main results are anticipated in 2018.

There is a possibility that a covert AF is the underlying pathogenesis in ≈40% of ESUS patients. In this context the present antithrombotic strategy might be suboptimal, which in turn could have important consequences on their outcome. Indeed, the reduced risk of ischemic stroke in response to warfarin is offset by a risk of hemorrhage in patients with reduced LVEF [51]. Therefore, it is not clear whether patients with sinus rhythm and reduced LV function should be treated with anticoagulation therapy during or after treatment for heart failure [53]. The ACCF/AHA guidelines for the management of valvular heart disease recommend acetylsalicylic acid therapy for patients with mitral valve prolapse who experience TIAs (Class I; Level of evidence C) and warfarin for these patients with a history of stroke and mitral regurgitation, AF, or left atrial thrombus (Class I; Level of evidence C). There is no evidence that anticoagulant therapy reduces the risk of stroke in patients with mitral annular calcification (MAC) [21]. The decision to use antiplatelet agents vs. anticoagulants in patients with MAC should include the consideration of other potential comorbid factors such as: AF (that can occur 12 times more often in patients with MAC in comparison to those without MAC) or endocarditics [26, 53]. Current evidence-based guidelines do not support routine closure of PFO in the absence of deep venous thrombus or proximal source and recommend antiplatelet therapy. Three major randomized controlled trials did not show a net benefit from closure of PFO in this population. A meta-analysis evaluating these 3 and an additional 11 non-randomized observational studies failed to prove superiority of closure against medical therapy, with an increased incidence of new onset AF in the closure group (RR 3.50). However, it should be noted that previous PFO closure trials differed in study criteria, devices used, and lack of

Table 4. Potential sources of embolism and type of anti-thrombotic therapy in patients after ischemic stroke.

Potential source of embolism	Type of anti-thrombotic therapy	
	Established	Not established
Atrial arrhythmias:		
— Valvular AF	x	
— Non-valvular AF	x	
— Atrial flutter	x (anticoagulation according CHADSVASC score)	
Prosthetic valves and devices	x (prostetic valve — VKA, device according indications)	
Sinus rhythm and reduced LV function		x
Patients with mitral valve prolapse who experience TIAs	x (antiplatelet therapy)	
Patients with mitral valve prolapsed and a history of stroke and mitral regurgitation, AF, or left atrial thrombus	x (anticoagulation)	
Mitral annular calcification		x
PFO/ASD without DVT	x (antiplatelet therapy)	
PFO/ASD with DVT	x (anticoagulation)	
ASA, Chiari Network, Eustachian valve, LAE, LASP		x

AF — atrial fibrillation; ASA — atrial septal aneurysm; ASD — atrial septal defect; DVT — deep venous thrombus; LAE — left atrial enlargement; LASP — left atrial septal pouch; LV — left ventricular; PFO — patent foramen ovale; TIA — transient ischemic attack; VKA — vitamin K antagonist

standardized design. Ongoing trials will hopefully find which patients are ideal for PFO closure. At the present time, patients should be thoroughly evaluated and risk stratified before considering closure of PFO in ESUS [52]. Furthermore, current evidence-based guidelines do not recommend anticoagulation over APT in patients with PFO. A large randomized control trial evaluating outcomes in PFO patients with anticoagulation vs. APT with acetylsalicylic acid reported a 2-year event rates of 9.5% in the warfarin-treated group and 17.9% in the acetylsalicylic acid-treated group, but could not conclude statistical significance (HR 0.5; 95% CI 0.2–1.7). In addition, there was no significant difference between patients with isolated PFO, those associated with an atrial septal defect, or among small or large PFO's. More recently, an individual participant meta-analysis evaluating OAC or APT in 2385 patients found no statistically significant difference in recurrent stroke, TIA, death; or stroke alone. Furthermore, subgroup analysis did not find significant heterogeneity of treatment effects in both groups, supporting the finding [8, 53]. Patients with cryptogenic strokes should be evaluated for the presence of venous thromboembolism. If venous thromboembolism is present, treatment is the same as for pulmonary embolism: anticoagulation. If venous thromboembolism is not present, APT is indicated [54].

Conclusions

About 80% of ischemic strokes occur in persons without AF, and it is therefore important to develop a path to examine the optimal prevention of stroke when there is no obvious AF. The benefits of oral anticoagulation for patients with heart failure in sinus rhythm have yet to be established. Many cryptogenic strokes/ESUS are presumed to have an embolic etiology. There are ongoing studies: Re-SPECT ESUS, ATTICUS and NAVIGATE ESUS assessing the comparative effectiveness of OAC vs. APT in patients after ESUS. Further research is needed to determine whether anticoagulant use may reduce risk of recurrence in ischemic stroke patients with moderate to severe left atrial enlargement and other potential sources of thromboembolism (Table 4). At present there is a lack of antithrombothic treatment scheme in the time between stroke

and finishing the diagnosis of potential sources of thromboembolism in ESUS. It should be discussed if OAC therapy should not be introduced for about 3 months during which patient could have at least 7 days electrocardiogram monitoring, PFO and deep veins assessment, eventually genetic assessment and based on these results a final decision on treatment can be stated.

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ORIGINAL ARTICLE

VIA MEDICA

Rate of acquired pulmonary vein stenosis after ablation of atrial fibrillation referred to electroanatomical mapping systems: Does it matter?

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Abstract

Background: *Thermal injury during radiofrequency ablation (RFA) of atrial fibrillation (AF) can lead to pulmonary vein stenosis (PVS). It is currently unclear if routine screening for PVS by imaging (echocardiography, computed tomography) is clinically meaningful and if there is a correlation between PVS and the electroanatomical mapping system (EAMS) used for the ablation procedure. It was therefore investigated in the current single center experience.*

Methods: *All patients from January 2004 to December 2016 with the diagnosis of PVS after interventional ablation of AF by radiofrequency were retrospectively analyzed. From 2004 to 2007, transesophageal echocardiography was routinely performed as screening for RFA-acquired PVS (group A). Since 2008, diagnostics were only initiated in cases of clinical symptoms suggestive for PVS (group B).*

Results: *The overall PVS rate after interventional RFA for AF of the documented institution is 0.72% (70/9754). The incidence was not influenced by screening: group A had a 0.74% PVS rate and group B a 0.72% rate (NS). Referred to as the EAMS, there were significant differences: 20/4229 (0.5%) using CARTO®, 48/4510 (1.1%) using EnSite®, 1/853 (0.1%) using MediGuide®, and 1/162 (0.6%) using Rhythmia®. Since 2009, no significant difference between technologies was found.*

Conclusions: *The present analysis of 9754 procedures revealed 70 cases of PVS. The incidence of PVS is not related to screening but to the application of different EAMS. Possible explanations are technological backgrounds (magnetic vs. electrical), learning curves, operator experience, and work-flow differences. Furthermore, incorporation of new technologies seems to be associated with higher incidences of PVS before workflows are optimized.* (Cardiol J 2019; 26, 5: 451–458)

Key words: pulmonary vein stenosis, radiofrequency ablation, atrial fibrillation, electroanatomical mapping system

Introduction

Thermal injury during radiofrequency ablation (RFA) of atrial fibrillation (AF) can lead to pulmonary vein stenosis (PVS), a rare but commonly known adverse event [1–4]. Severe symptoms such as dyspnea and hemoptysis may occur. Catheter-based interventional treatment for PVS

still remains a challenging field [3, 5–9]. To improve anatomical understanding and to reduce radiation exposure during ablation procedures, electroanatomical mapping systems (EAMS) were introduced [10]. Because the electroanatomical map is not able to portray the complex anatomy of the left atrium in its entirety, an image integration step of a pre-recorded heart extracted from computed tomography

(CT) or magnetic resonance imaging (MRI) was introduced [11]. The most widely used systems are CARTO® (Biosense Webster, Baldwin Park, CA, USA) and EnSite® (Abbott/St. Jude Medical, St. Paul, MN, USA). The main technological difference between these three-dimensional (3D) mapping systems is the electromagnetic localization of catheters in CARTO® and the impedance-based approach of the EnSite NavX/Velocity® technology. Since the introduction of EnSite Precision® in 2016 providing magnetic catheter localization as well, no fundamental technological difference is present. It is unclear whether there is a relationship between the occurrence of PVS and the used EAMS during the ablation procedure for AF. Herein described, is a present single center experience.

Methods

From January 2004 to December 2016 all patients with the diagnosis of PVS after interventional ablation of AF by radiofrequency were collected. Cases with catheter-based cryoablation or intraoperative RFA for AF were excluded.

The study was approved by the local ethics committee and was done in accordance with the Declaration of Helsinki.

Definition of PVS

Minimal luminal diameter of stenosed pulmonary veins (PVs) was measured on multi-planar reformatted 3D angiographic datasets (by contrast enhanced CT or MRI) of pre- and post-ablation imaging and PVS was expressed as percentage of post- vs. pre-ablation luminal diameter reduction. The degree of PVS is classified as severe with luminal narrowing over 70%, moderate for 50–70% narrowing, and mild for < 50% narrowing [12].

Screening for PVS

From 2004 to 2007, transesophageal echocardiography (TEE) was performed as routine screening for RFA-acquired PVS. The time of screening TEE was between 6 and 12 months after PV isolation or at the time of new symptoms suggestive of PVS. In cases of abnormal echo findings for PVs such as an accelerated peak flow over 1 m/s, a subsequent contrast enhanced CT or MRI timed for opacification of the PVs was performed to confirm and quantify the PVS. Also, in case of insufficient TEE quality, but typical symptoms suggestive for PVS, a subsequent imaging diagnostic was initiated.

Due to the low number of detected PVS and the logistic efforts required, routine screening was stopped. Since 2008, diagnostics were initiated only in cases of clinical symptoms suggestive for PVS. CT, MRI and/or PV angiography were the imaging methods used at this time. During this period, PVS of asymptomatic patients were detected as incidental findings in the context of re-ablation procedures.

Ablation procedure

The present ablation approach has been previously described [13]. Briefly, interventional RFA for AF at the documented institution is performed under analgosedation. After the transseptal approach, the PVs are isolated point-by-point with an irrigated tip catheter using radiofrequency as the energy source. The ablation line is made antral circumferential around the left- and right-sided PVs. Ablation inside the PVs was avoided. Additional ablation lines were performed at the discretion of the operator. Catheter navigation was supported by fluoroscopy and 3D EAMS. The choice of mapping tool and ablation catheter was at operator discretion. Intracardiac ultrasound guidance was not used.

EAMS — CARTO®

At the documented institution, CARTO XP® was introduced in 1998. CARTO® works based on a magnetic field generated by a location pad placed under the patient's chest [14]. Sensors embedded in the catheter tip enable catheter localization. A further sensor on the patient's skin is used as a location reference. With further improvement of the system (CARTO3®) by a combination of magnetic and current-based technology, visualization of multiple catheters has been available since 2009. In contrast to the EnSite NavX® system, the CARTO® allowed from the onset a reconstruction of the PVs as well as the whole atrium in one map (initially as point-by-point with CARTO XP® and later as fast anatomical mapping sampled by roving the catheter supported by CARTO3®). Since 2005, it is possible to merge the surfaces of a pre-recorded CT or MRI with the reconstructed map by the CARTOmerge® software. Further registration steps are not necessary (Fig. 1: 1A–D).

EAMS — EnSite®

EnSite NavX® has been in use since 2005 in the present institution. The system works based on an electric field created by six skin electrodes in three orthogonal planes [15]. Catheter localization is implemented by an impedance gradient in relation to a reference electrode. Later, for com-
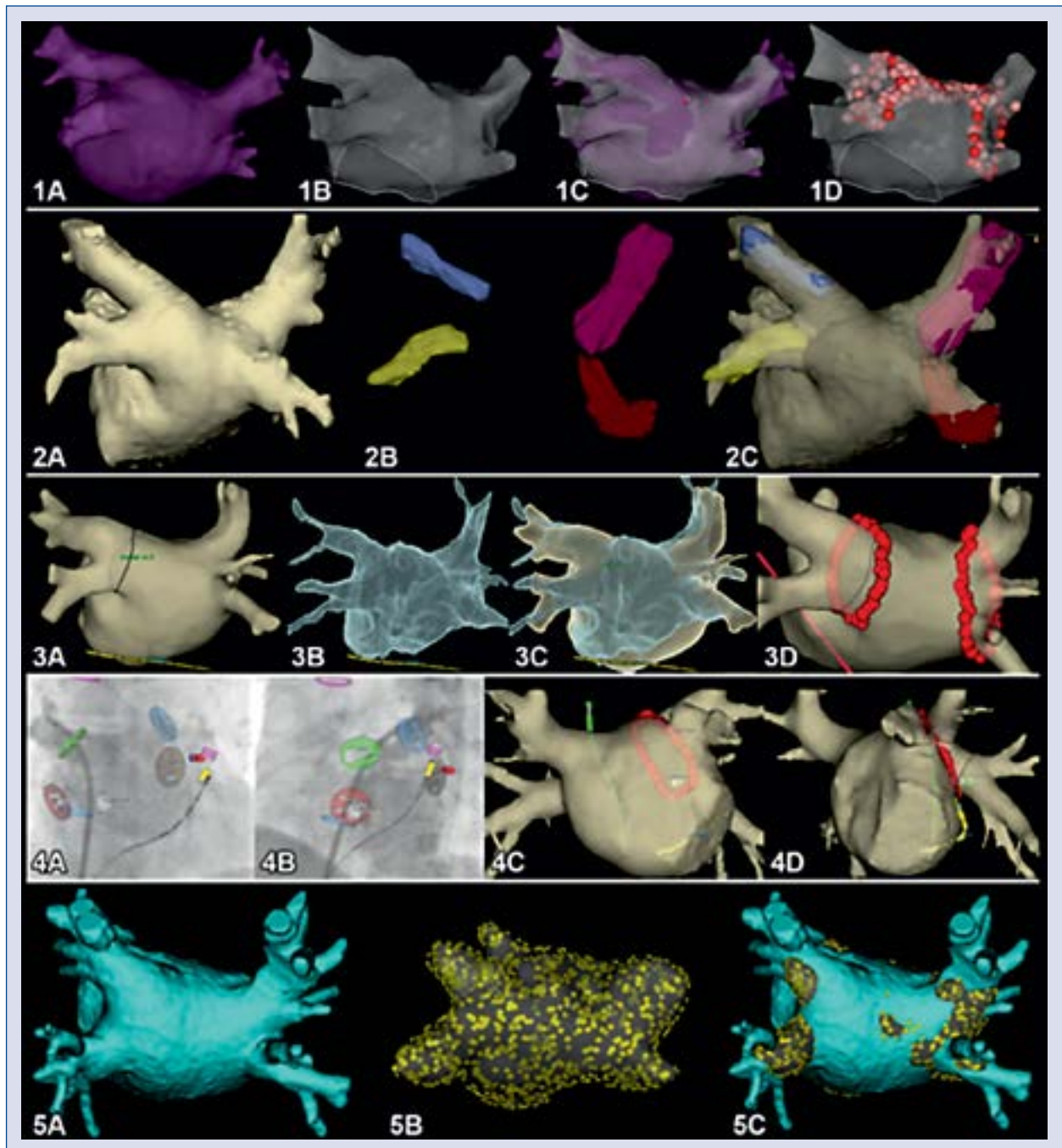
Figure 1. Workflow of the different electroanatomical mapping systems. **Panel 1A–D.** CARTO®, **1A.** Segmented computed tomography (CT) shell of the left atrium; **1B.** Reconstructed map by fast anatomical mapping; **1C.** Fusion of the models by surface merging after setting a landmark at the posterior wall; **1D.** Automatic visualization of abla-tion points by the VISITAG tool without any additional steps of registration. **Panel 2A-C.** EnSite NavX® 2005–2008; **2A.** Segmented CT of the left atrium; **2B.** Reconstruction of each pulmonary vein as separate geometry; **2C.** Regis-tration of the reconstructed three-dimensional shell in the electroanatomical mapping system. **Panel 3A–D.** EnSite Velocity® 2008–2016; **3A.** Segmented magnetic resonance imaging (MRI) of the left atrium; **3B.** Reconstruction of the left atrium and each pulmonary vein in one map; **3C.** Fusion of 3A and 3B by fiducial points; **3D.** Registered MRI shell with ablation points (red) projected on the 3D shell; **Panel 4A–D.** EnSite Velocity® supported by MediGuide®; **4A, B.** Snapshots of simultaneously displayed MediGuide® pre-recorded fluoroscopy loops in a right anterior oblique view (**A**) and left anterior oblique view (**B**) and corresponding location of the catheter ablation tip in the EnSite Velo-city® supported map (**C, D**). The circular markers tag ostia of pulmonary veins after localization by contrast-enhanced angiography (**A, B**); **Panel 5A–C.** Rhythmia®; **5A.** Segmented MRI of left atrium; **5B.** Reconstruction of left atrium and each pulmonary vein; **5C.** Fusion of map 5A and 5B.

Table 1. Baseline characteristics.

Variable	All (n = 9754)	CARTO® (n = 4229)	EnSite® (n = 4510)	MediGuide® (n = 853)	Rhythmia® (n = 162)	P
Age [years]	66.6 ± 10.3	67.1 ± 10.2	66.7 ± 10.2	63.4 ± 10.3	65.3 ± 10.3	< 0.001*
Male sex	6332 (65)	2751 (65)	2893 (64)	597 (70)	94 (58)	0.002+
Hypertension	6741 (69)	2850 (67)	3244 (72)	542 (64)	108 (66)	< 0.001§
Diabetes mellitus	1575 (16)	645 (15)	801 (18)	107 (13)	24 (15)	< 0.001§
CAD	1425 (15)	638 (15)	673 (15)	91 (11)	24 (15)	0.008*
Heart failure	2595 (27)	1100 (26)	1252 (28)	193 (23)	50 (31)	0.007+
Paroxysmal AF	5995 (62)	2734 (65)	2815 (62)	396 (46)	50 (31)	< 0.001+
Non-paroxysmal AF	3759 (38)	1495 (35)	1699 (38)	457 (54)	113 (69)	< 0.001+

Continuous data are represented as mean ± standard deviation and categorical data as number (percentage). P-values are given as overall value within the whole cohort; *Level of significance reached due to the MediGuide® group; +Level of significance reached due to the Medi-Guide® and Rhythmia® group; §Level of significance reached between each group; AF — atrial fibrillation; CAD — coronary artery disease

pensation of cardiac and respiratory motion, an intracardiac reference catheter is usually placed into the coronary sinus.

At the beginning of the EnSite NavX® supported RFA for AF, the workflow was to reconstruct the 4 PVs separately followed by a transmission into a pre-recorded CT or MRI model of the patient. A reconstruction of the atrium as well as PVs in one map was not possible (Fig. 1: 2A–C). Since the introduction of EnSite Velocity® as a next generation in 2008, the system allowed a reconstruction of the PVs as well as the whole atrium in one map followed by a merge with the CT or MRI. Thereafter registration steps of the merged map to confirm its accuracy were necessary (Fig. 1: 3A–D).

Since the introduction of EnSite Precision® in 2016, which provides magnetic catheter information as well, no fundamental technological differences are present between the systems at present.

MediGuide® technology

In 2011, the MediGuide® technology (Abbott/St. Jude Medical, St. Paul, MN, USA) was introduced and became a standard in the present institution. By electromagnetic sensor-embedded catheter tracking in prerecorded fluoroscopy loops, the system allows additional X-ray imaging information as well as integration of a PV angiography [16]. Working in combination with EnSite®, it represents a combination of magnetic and impedance based tools that had been available for years before the introduction of EnSite Precision® in 2016 (Fig. 1: 4A–D).

Rhythmia®

The recently developed EAMS Rhythmia Mapping® (Boston Scientific, Marlborough, Massa-

chusetts, USA) uses both magnetic and impedance information. The most important innovation of this system is an automatic record of high-resolution electroanatomical maps without manual annotation of the mapping points [17]. For RFA of AF, the workflow is to reconstruct the left atrium using a specially-designed mini basket (IntellaMap Orion®) followed by visual alignment with the pre-recorded CT/MRI shell (Fig. 1: 5A–C).

Statistical analysis

All analyses were performed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous data with a normal distribution are reported as a mean and standard deviation. Discrete variables are reported as frequency (percentage). Groups were compared using the χ^2 test for categorical variables and ANOVA for continuous data. Bonferroni adjustment for multiple testing was done for post-hoc tests. All tests were performed two-tailed at significance level $\alpha = 1\%$ (p < 0.01) due to the high number of patients.

Results

Baseline characteristics

From January 2004 to December 2016, 9754 patients underwent interventional RFA for AF in this institution. The most frequently used mapping systems were the CARTO® system with 4229/9754 (43%) cases and the EnSite® system with 4510/9754 (46%). 853/9754 (9%) of the procedures were supported by MediGuide® technology and 162/9754 (2%) by Rhythmia®.

The patient cohort had a mean age of 66.6 ± ± 10.3 years and were 65% male, presenting with paroxysmal AF in 62% of cases (Table 1). As for
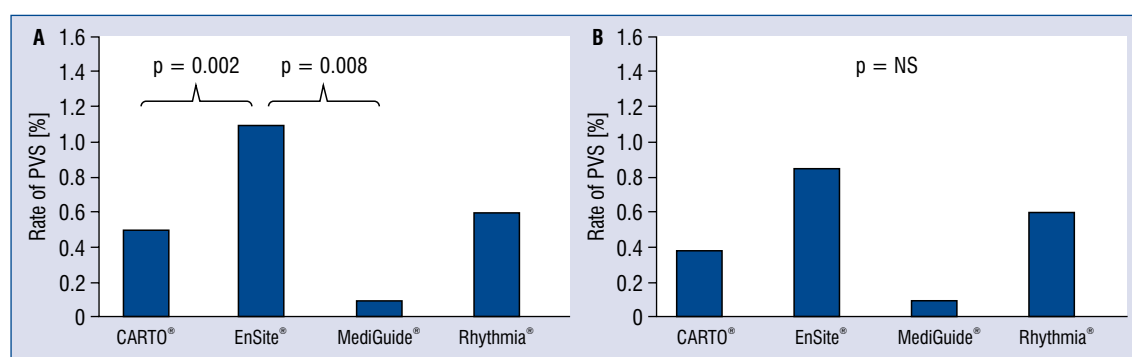
Figure 2. Rates and significance levels of pulmonary vein stenoses referred to the used mapping system in total (**A**) and since 2009 (**B**); PVS — pulmonary vein stenosis. The level of significance was only reached in CARTO® vs. EnSite® and EnSite® vs. MediGuide® in total. Since 2009, no significant difference between technologies was found.

general cardiac risk factors, arterial hypertension was present in 69% of patients, diabetes mellitus in 16%, coronary artery disease in 15%, and heart failure in 27%. The distributions of these risk factors were statistically significant, with the difference being mainly driven by the MediGuide® group. These patients were healthier with regards to their cardiovascular risk factors but with a higher amount of persistent AF. In comparing the baseline characteristic between CARTO® and EnSite®, the level of significance was only reached for arterial hypertension and diabetes mellitus (data not shown).

Overall rate of PVS

Out of 9754 interventional RFA for AF, a total of 70 patients with PVS were identified. The overall PVS rate at the present institution is 0.72%. During the TEE screening period (group A), 9/1223 (0.74%) patients with PVS were identified vs. 61/8531 (0.72%, p = 0.972) without routine screening by TEE and CT/MRI (group B).

Rate of PVS referred to mapping system

The rate of PVS revealed a significant difference referred to the applied mapping system: 20/4229 (0.5%) for CARTO® vs. 48/4510 (1.1%) for EnSite® vs. 1/853 (0.1%) for MediGuide® and 1/162 (0.6%) for Rhythmia® cases (p < 0.001) (Fig. 2).

The significance level was due to the difference between the CARTO® vs. EnSite® cases as well as the EnSite® vs. the MediGuide® cases in the whole cohort (Fig. 2).

The curve of incidence rates related to the year of RFA procedure showed for EnSite® mapping a peak around 2007 whereby in this year the number of EnSite® supported procedures was relatively small. In comparison, the CARTO® group showed

a relatively stable percentage (Fig. 3). Since 2009, there was an alignment of the curves without a statistical difference in the PVS rate (Figs. 2, 3).

The mean degree of stenosis between the EnSite® and CARTO® group was 63 ± 28% narrowing vs. 72 ± 24% (p = 0.184) and the amount of treated PVS with 31% vs. 30% (p = 1.0) was equal.

Discussion

The overall PVS rate for approximately 10,000 RFA procedures of AF is 0.72% in the present institution. Since the change from ablation in or near the PVs to wide circumferential antral lesions, as well as the use of 3D mapping systems, the incidence of PVS has decreased from initially 6.3% (estimated from publications between 1999 and 2004) [18] to 1% [1, 4]. Compared to the reported data, the present single-center PVS rate reflects a comparable level.

Interestingly, routine screening using TEE and CT/MRI did not influence the percentage of identified PVS. Even though some asymptomatic PVS may have remained undiagnosed in group B, it was clinically reasonable to stop routine screening given the fact that no treatment would have been performed in these asymptomatic patients.

Referring to the applied mapping system used during the RFA procedure, the lowest cumulative PVS rate was revealed for MediGuide® cases with 0.1% and CARTO® cases with 0.5%. The overall PVS rate of EnSite® cases was significantly higher with 1.1%, although since 2009 an alignment of PVS rates was recorded. There are several different explanations for this result.

Technical background. Before introducing the new Precision platform in 2016, EnSite techno-
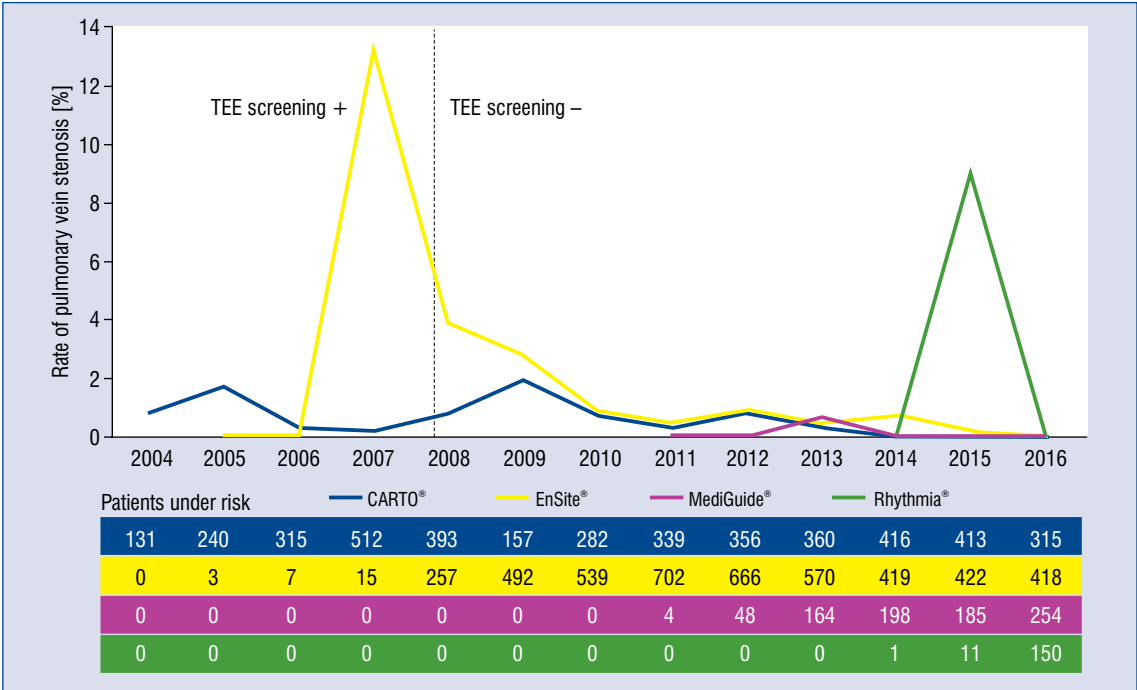
Figure 3. Rates of pulmonary vein stenoses referred to mapping systems and year of occurrence; TEE — transesophageal echocardiography.

logy was based exclusively on electrical information for catheter localization. The potential limitation of this technology is its dependency on the body's non-linear impedance distribution. Starting in 2008, the next generation system EnSite Velocity® was enabled to correct for this using a computer algorithm called "field scaling". Another frequent observation was map-shifting after patient motion or dislocation of the reference electrode. In contrast, CARTO® technology was impedance-based as well as electromagnetic-based. In a phantom experiment a significantly better point localization by CARTO3® in comparison to EnSite Velocity® could be demonstrated [19].

A randomized comparison of CARTOmerge® vs. EnSite NavX® in RFA for AF could demonstrate a significantly higher accuracy in lesion distance to the shell: 2.0 mm vs. 3.4 mm [20].

MediGuide® technology is a non-fluoroscopic catheter tracking system which works in combination with EnSite® [21]. Because of the fact that MediGuide® itself is electromagnetic based, the EnSite® system was functionally upgraded to a combined impedance and electromagnetic mapping tool. Its accuracy was shown to be superior to EnSite® only in a phantom [22].

Since the introduction of EnSite Precision® in 2016, no fundamental technological differences between the competing systems are present to date.

Reconstruction and map fusion. Significant differences existed in the workflow with regards to EAM tools. The first version of EnSite NavX® was not able to reconstruct the whole atrium and PVs in one map. Furthermore, the lack of a field scaling algorithm often resulted in a flattened version of the left atrial anatomy. So it might be conceivable that inclusion of reconstructed PV's with their orifices into pre-recorded CT was imprecise. Since 2008 with the next version EnSite Velocity®, this problem was solved which might be a reason for the decrease in PVS rates since then. To integrate the reconstructed map into the pre-recorded CT/MRI shell, some fiducial points must be chosen in both. Due to the subjective election of its localization, it appears as a source of error.

On the other hand, reconstruction of the whole atrium and PVs in one map and a subsequent operator-independent surface merging with pre-recorded CT/MRI as the workflow of the CARTO® system right from the beginning could contribute to less registration errors and consequently lower PVS rates.

Registration. After map fusion by several fiducials, the EnSite® system required a further

registration step to validate the map. It should be noted that a single incorrect registration point could produce a shifted map with unprecise localization of the PV ostia. There are data showing a higher precision of the surface-merge without a subsequent registration step for the CARTO® system in comparison to the point-by-point technique of EnSite® [19]. The values ranged from 0.73 mm for CARTO3® vs. 2.02 mm for EnSite Velocity®.

In comparison, MediGuide technology unifies additional anatomical information of a PV angiography whereas PV ostia are tagged by setting markers in pre-recorded cine loops.

Operator experience. Knowledge of anatomical orientation as well as know-how in the workflow of the different EAMS and their pitfalls might influence the rate of PVS substantially.

With only a small number of Rhythmia® supported cases performed, a fundamental statement seems to be unreasonable. Prior data did not show a difference in registration accuracy between pre-recorded CT or MRI for AF ablation procedures [23].

The influence of screening for the rate of PVS

In group A (year 2004–2007), over 1000 consecutively screened patients by TEE a PVS rate of 0.74% was evidenced. There was no significant difference in comparison to the period without screening in group B. TEE was shown to have a sensitivity of 84% and specificity of 98% in detecting moderate PVS and appears to be a useful diagnostic tool [24, 25]. Nevertheless, measurements of flow parameters and PV diameters are not accurate, especially of inferior PVs due to an unfavorable Doppler angle. Furthermore, detection of mild PVS remains a challenge [24].

However, there is no doubt that the rate of PVS is underestimated because of asymptomatic PVS and lack of routine screening. Obviously the PVS rate is influenced by factors such as energy titration, registration steps, and operator experience rather than on routine TEE screening. According to the current Consensus Statement on catheter and surgical ablation of AF from 2017, a routine screening for PVS after PV isolation is not recommended [12].

The opinion herein, is that routine screening is not necessary because patients with severe PVS, but no symptoms, would not be treated particularly with regard to the risk-benefit balance. Despite a well-known increase of morbidity due to PVS, there is actually no data showing a higher mortality.

Limitations of the study

The baseline characteristics between the groups of different EAMS are not balanced because of the retrospective nature of this registry analysis. However, there is no data showing an influence of cardiovascular comorbidities on the rate of PVS. There may be selection bias due to preference of the operators to different EAMS and varying levels of experience leading to an influence on PVS rates. Furthermore, when the MediGuide® was introduced, operators were already experienced with the EnSite® EAMS. Additionally, the study was performed in a single high-volume center with extensive experience in ablation of AF. As such, these data may not be easily applicable in cases of less experienced operators. Hence, analysis of experience from other centers would be desirable.

Conclusions

According to available research, this is the first analysis of the rate of PVS in a cohort of almost 10,000 patients. It was demonstrated that equal PVS rates, with and without screening, show significant differences between commonly used EAMS and the incidence of PVS after RFA for AF. Apart from technological differences between the EAMS, variances in workflow as well as operator experience might be influencing factors. Furthermore, the incorporation of new technologies seems to be associated with higher incidences of PVS before workflows are optimized.

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ORIGINAL ARTICLE

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Survival benefit from recent changes in management of men and women with ST-segment elevation myocardial infarction treated with percutaneous coronary interventions

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Abstract

Background: *Nowadays, the majority of patients with myocardial infarction with ST-segment eleva-tion (STEMI) are treated with primary percutaneous coronary interventions (PCI). In recent years, there have been ongoing improvements in PCI techniques, devices and concomitant pharmacotherapy. However, reports on further mortality reduction among PCI-treated STEMI patients remain incon-clusive. The aim of this study was to compare changes in management and mortality in PCI-treated STEMI patients between 2005 and 2011 in a real-life setting.*

Methods: *Data on 79,522 PCI-treated patients with STEMI from Polish Registry of Acute Coronary Syndromes (PL-ACS) admitted to Polish hospitals between 2005 and 2011 were analyzed. First, tem-poral trends of in-hospital management in men and women were presented. In the next step, patients from 2005 and 2011 were nearest neighbor matched on their propensity scores to compare in-hospital, 30-day and 1-year mortality rates and in-hospital management strategies and complications.*

Results: *Some significant changes were noted in hospital management including shortening of median times from admission to PCI, increased use of drug-eluting stents, potent antiplatelet agents but also less frequent use of statin, beta-blockers and angiotensin converting enzyme inhibitors and angiotensin II receptor blockers. There was a strong tendency toward preforming additional PCI of non-infarct related arteries, especially in women. After propensity score adjustment there were significant changes in in-hospital but not in 30-day or 1-year mortality rates between 2005 and 2011. The results were similar in men and women.*

Conclusions: *There were apparent changes in management and significant in-hospital mortality reductions in PCI-treated STEMI patients between 2005 and 2011. However, it did not result in 30-day or 1-year survival benefit at a population level. There may be room for improvement in the use of guideline-recommended pharmacotherapy.* (Cardiol J 2019; 26, 5: 459–468)

Key words: ST-segment elevation myocardial infarction, percutaneous coronary intervention, temporal trends, treatment strategy, in-hospital mortality, 1-year mortality, sex-differences

Introduction

Most of recent studies have confirmed a significant reduction in mortality rates among patients with myocardial infarction with ST-segment elevation (STEMI) during the last 10–20 years [1–3]. The increased use of percutaneous coronary interventions (PCI) has unquestionably been key improvement in STEMI treatment. Nevertheless, there are other important changes evolving in PCI techniques and new evidence-based concomitant pharmacotherapy. Recent advances in angioplasty devices, including manual aspiration catheters and drug-eluting stents (DES), potent antiplatelet and anticoagulant agents, have significantly enhanced outcomes for STEMI patients [4], not to mention shorter door-to-balloon (D2B) times, growing experience of operators performing PCI and efforts put into implementation of evidence-based treatments into real-life clinical practice. However, the scope and reasons for the observed decline in mortality remain inconclusive, especially among PCI-treated patients and in sex-specific analyses.

A large study from Northern Italy presented a weak temporal trend in mortality reduction from 2000 to 2010 in men only, despite increases in the use of an invasive approach in both sexes [5]. In contrast, an American study including patients with STEMI who underwent primary PCI from 2003 to 2008 reported a tendency toward decreased in-hospital mortality only among women but even that was not statistically significant [6]. Some newer studies including mostly patients treated with PCI showed that there was no further improvement regarding in-hospital [7], 30-day [8] or 1-year mortality despite changes in patient characteristics and concomitant treatment [9]. On the other hand, French data demonstrated a decrease in 30-day mortality rates also among patients treated with PCI from 1995 to 2010 [10]. Similarly, British investigators found that 6-month survival improved significantly from 2003 to 2010 for STEMI patients who received reperfusion therapy [11].

Clinical profiles of STEMI patients have been changing over time and it has already been demonstrated in the Polish population [12]. Female STEMI patients differ significantly from males [13] and may undergo independent temporal changes in terms of clinical characteristics and modes of treatment [12, 14], which warrants separate analyses of both sexes. It was reported that women are less likely to undergo proper reperfusion treatment [1, 3, 15] and to receive early drug therapies even after adjustment for baseline characteristics [1].

Recently published registry data have also shown that, despite advances in care, women continue to experience higher mortality rates compared with men in STEMI [16] or after PCI for coronary artery disease [17].

The aims of this study are to compare changes and analyze temporal trends in hospital management of men and women with STEMI treated with PCI from 2005 to 2011 and determine if it resulted in better in-hospital, 30-day and 1-year survival rates.

Methods

The Polish Registry of Acute Coronary Syndromes (PL-ACS) is an ongoing, nationwide, multicenter, prospective, observational study of patients hospitalized with acute coronary syndromes (ACS). The registry is a joint initiative of the Silesian Center for Heart Diseases and the Polish Ministry of Health. Patients admitted with suspected ACS were screened for their eligibility to enter the registry, but they were not enrolled until ACS was confirmed. During the study period, 449 hospitals participated in the registry, 132 of them with PCI facilities and 20 with onsite cardiac surgery. The registry covered around 70% of all hospitals where STEMI patients were treated in Poland including primary, secondary and tertiary-level hospitals as well as academic and university centers.

In the current study all patients enrolled in the PL-ACS Registry hospitalized between 2005 and 2011 with the diagnosis of STEMI were evaluated (111,148). Of them, 79,522 (71.5%) were treated with PCI and were included in further analyses (25,155 women and 54,367 men). STEMI was defined as the presence of ST-segment elevation of ≥ 2 mm in the contiguous chest leads and/or ST-segment elevation of ≥ 1 mm in two or more standard leads or a new left bundle branch block, together with positive cardiac necrosis markers (cardiac troponin or creatine kinase-MB). For the patients who presented more than once during the study period only the first hospitalization was analyzed. All-cause mortality data were obtained from the official mortality records of the National Health Fund. The vital statuses at discharge, 30-day and 1-year were available for all patients included. The study adhered to the Declaration of Helsinki and its revision from 2008 and was approved by the Bioethics Committee at the Swietokrzyska Chamber of Physicians.

Temporal trends for in-hospital PCI-related treatment strategies were presented (D2B times,

PCI type, Thrombolysis in Myocardial Infarction [TIMI] flow 3 after PCI, additional PCI of any non-infarct-related artery (IRA) during index hospitalization). Continuous variables were presented as means ± standard deviation or median ± interquartile range, depending on the normality of the distribution. Categorical variables were presented as counts and percentages. The significance of the time trends was tested with Jonckheere-Terpstra test for continuous variables and Cochran-Armitage test for categorical variables.

To adjust data from 2005 and 2011 available baseline characteristics of PCI-treated patients (Table 1) were incorporated into a regression model to estimate a propensity score (PS) of each individual. In the next step, the patients from 2011 were nearest neighbor matched on their PS to patients from 2005. A total of 15,886 individuals were successfully matched within a pre-defined PS distance. Women and men were analyzed separately. Standardized differences were calculated for assessing balance in baseline characteristics between subjects from 2005 and 2011 (Table 1). The overlap and the region of common support between the groups were checked by visual analysis. In-hospital treatment strategies including pharmacotherapy, in-hospital complications (myocardial reinfarction, ischemic stroke and major bleeding) as well as in-hospital, 30-day and 1-year mortality rates were compared between patients from 2005 and 2011. Significance of differences between the study groups was assessed by the Student t-test or Mann-Whitney U test for continuous variables and χ^2 test for categorical variables. A two-sided p value ≤ 0.05 was considered significant.

The calculations and statistical analyses were performed with STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA), MedCalc (MedCalc Software, Belgium) and SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

The fraction of patients treated with PCI was increasing annually from 53.3% in 2005 to 93.8% in 2011. A majority of patients included in the analysis were treated with primary PCI. Small and declining percentages of all PCI-treated patients had PCI after thrombolysis or PCI followed by emergent coronary artery bypass grafting during index hospitalization. Detailed unadjusted trends are presented in Tables 2 and 3. TIMI flow after PCI was reported in 99% of patients.

When comparing crude data men were more often treated with PCI and more often had TIMI 3

flow after PCI than women — both in 2005 and 2011 (p < 0.001). Bare metal stents (BMS) were more often implanted in men in 2005 (p = 0.001) but not in 2011 (p = 0.53) whereas DES were more often implanted in men in 2011 (p = 0.003) but not in 2005 (p = 0.07). Women more frequently had at least one additional PCI of non-IRA in 2011 (p < 0.001) but not in 2005 (p = 0.55). There was a strong trend (1.6% average absolute change per year) towards increased fractions of women undergoing additional PCI of non-IRA whereas a corresponding trend in male patients was only 0.3% per year. The differences in D2B times were not statistically significant between sexes (p = 0.32 in 2005 and p = 0.1 in 2011). However, the 1–2 min longer D2B times in women were reported relatively constantly throughout the study period.

Following adjustment of 2005 and 2011 populations with PS matching technique many notable differences were observed in treatment strategies including in-hospital pharmacotherapy (Tables 4 and 5).

A substantial increase in additional PCI of non-IRA, particularly in women, was also confirmed in PS-matched subgroups; thus, it proved to be likely unrelated to temporal changes in initial characteristics. There was an increase in hospital colpidogrel and glycoprotein IIb/IIIa inhibitors usage in both sexes. At the same time there was a decrease in acetylsalicylic acid (ASA), beta-blockers, statins and angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARBs) usage in both sexes. The percentage of patients who received ASA at discharge increased in 2011 when compare to in-hospital usage and was not significantly different from the percentage observed in 2005. At the same time some patients who were given in-hospital clopidogrel were discharged without this drug. In-hospital complications were rare; there was a further decline in the number of myocardial reinfarctions and ischemic strokes (only in women) and non-statistically significant increase in major bleeding was reported during hospitalization. In-hospital mortality rates of STEMI patients decreased between 2005 and 2011 in both sexes. However, there was no significant change in 30-day or 1-year mortality rates in neither men nor women (Tables 4 and 5).

Discussion

The main finding of this study is that, despite numerous advances in hospital management and in-hospital mortality reduction, no significant decrease in 30-day or 1-year mortality was observed. In fact, the first 30 days was critical in terms of STEMI patients

Table 1. Clinical characteristics on admission after propensity score matching.

	Women (n = 5253)			Men (n = 10633)		
	2005 N = 2615	2011 N = 2638	S. Diff.	2005 N = 5328	2011 N = 5305	S. Diff.
Age	67.1 ± 11.1	67.5 ± 12.2	**0.05**	60.5 ± 11.2	60.8 ± 11.2	**0.04**
Hypertension	68.9%	66.5%	**–0.05**	58.7%	59.9%	**0.02**
Diabetes	28.6%	25.7%	**–0.07**	16.7%	17.5%	**0.02**
Hypercholesterolemia	43.7%	42.9%	**–0.02**	40.4%	40.8%	**0.01**
Current smoking	31.8%	30.7%	**–0.02**	60.7%	61.7%	**0.02**
Obesity	24.9%	22.8%	**–0.05**	13.9%	15.1%	**0.03**
Prior MI	7.8%	7.9%	**0.00**	9.7%	9.3%	**–0.01**
Prior PCI	1.4%	1.4%	**0.00**	2.2%	2%	**–0.01**
Prior CABG	1%	1%	**0.00**	1.8%	1.7%	**–0.01**
Systolic BP on admission [mmHg]:						
< 100	8.1%	9.3%	**0.04**	7.4%	7%	**–0.02**
100–160	73.6%	72.7%	**–0.02**	75.8%	75.3%	**–0.01**
> 160	18.3%	18%	**–0.01**	16.8%	17.7%	**0.02**
ECG on admission (rhythm):						
Sinus rhythm	92.2%	91.2%	**–0.04**	93.6%	93.9%	**0.01**
Atrial fibrillation	5.8%	5.8%	**0.00**	4%	4%	**0.00**
Pacing	0.2%	0.3%	**0.02**	0.2%	0.2%	**0.00**
Other	1.8%	1.9%	**0.01**	2.2%	1.9%	**–0.02**
HR > 100/min	7.7%	7.8%	**0.00**	6.6%	6.5%	**0.00**
ECG on admission (intraventricular conduction):						
Normal	89.1%	88.9%	**–0.01**	88.6%	88.8%	**0.01**
LBBB	1.8%	1.9%	**0.01**	1.5%	1.4%	**–0.01**
RBBB	2.6%	2.6%	**0.00**	3.5%	3.2%	**–0.02**
Other	6.5%	6.6%	**0.00**	6.5%	6.5%	**0.00**
Infarct location:						
Anterior	39.8%	41.1%	**0.03**	40.2%	40%	**0.00**
Inferior	50.3%	50.2%	**0.00**	51.5%	51.3%	**0.00**
Other	9.9%	8.8%	**–0.04**	8.3%	8.7%	**0.01**
Time from symptom-onset to admission [h]:						
0–2	21.5%	21.7%	**0.00**	25.8%	25.5%	**–0.01**
2–12	63.5%	61.6%	**–0.04**	58.8%	60.1%	**0.03**
> 12	15%	16.7%	**0.05**	15.3%	14.4%	**–0.03**
Prehospital cardiac arrest	2%	2.3%	**0.02**	3.3%	2.8%	**–0.03**
Killip class on admission:						
IV	6.2%	5.8%	**–0.02**	4.7%	4.7%	**0.00**
III	2.1%	1.9%	**–0.01**	1.7%	1.5%	**–0.02**
II	10.7%	10.1%	**–0.02**	9.4%	9.9%	**0.02**
I	81%	82.2%	**0.03**	84.2%	83.8%	**–0.01**
LVEF [%]:						
> 50%	49%	47.1%	**–0.04**	48.7%	49.1%	**0.01**
30–50%	46%	47.8%	**0.04**	46.6%	46%	**–0.01**
< 30%	5%	5.2%	**0.01**	4.7%	4.8%	**0.00**

BP — blood pressure; CABG — coronary artery bypass grafting; ECG — electrocardiogram; HR — heart rate; LBBB — left bundle branch block; LVEF — left ventricular ejection fraction; MI — myocardial infarction; PCI — percutaneous coronary intervention; RBBB — right bundle branch block; S. Diff. — standardized difference

Table 2. Trends in management of percutaneous coronary intervention (PCI)-treated women with ST-segment elevation myocardial infarction (STEMI) from 2005 to 2011.

Year (n = number of women with STEMI)	2005 N = 6422	2006 N = 6790	2007 N = 5434	2008 N = 4396	2009 N = 4365	2010 N = 5206	2011 N = 5047	P for trend	Average absolute change per year
Treated with PCI	3052 (47.5%)	3374 (49.7%)	3138 (57.7%)	2900 (66.0%)	3443 (78.9%)	4605 (88.5%)	4643 (92.0%)	< 0.001	
With thrombolysis	36	38	9	6	15	14	12	< 0.001	
With CABG	34	23	13	19	0	2	0	< 0.001	
Door to balloon time [min]	46 (30–75)	45 (30–71)	45 (30–73)	45 (29–75)	41 (29–65)	44 (30–70)	43 (30–65)	< 0.001	–0.54 min
Additional PCI of non-IRA	8.6%	8.0%	8.7%	11.5%	11.4%	15.0%	17.9%	< 0.001	1.6%
PCI type:									
Balloon angioplasty	9.9%	8.1%	8.5%	7.9%	7.4%	6.5%	7.4%	< 0.001	–0.4%
Bare metal stent	89.1%	90.2%	89.6%	90.0%	88.4%	85.0%	76.4%	< 0.001	–1.8%
Drug eluting stent	1.1%	1.7%	1.9%	2.1%	4.2%	8.4%	16.2%	< 0.001	2.2%
TIMI 3 flow after PCI	88.9%	89.6%	88.4%	89.7%	90.3%	90.0%	89.9%	0.069	NS

CABG — coronary artery bypass grafting; non-IRA — non-infarct related artery; TIMI — Thrombolysis in Myocardial Infarction

Table 3. Trends in management of percutaneous coronary intervention (PCI)-treated men with ST-segment elevation myocardial infarction (STEMI) from 2005 to 2011.

Year (n = number of men with STEMI)	2005 N = 12180	2006 N = 13083	2007 N = 11020	2008 N = 8355	2009 N = 8775	2010 N = 10934	2011 N = 9141	P for trend	Average absolute change per year
Treated with PCI	6866 (56.4%)	7715 (59.0%)	7363 (66.8%)	6129 (73.4%)	7601 (86.6%)	10028 (91.7%)	8665 (94.8%)	< 0.001	
With thrombolysis	104	73	45	29	31	28	10	< 0.001	
With CABG	66	61	22	24	4	2	2	< 0.001	
Door to balloon time [min]	45 (30–72)	44 (29–69)	43 (29–69)	44 (29–70)	40 (28–65)	42 (30–65)	42 (30–63)	< 0.001	–0.6 min
Additional PCI of non-IRA	8.9%	7.7%	8.5%	11.1%	10.6%	9.9%	9.9%	< 0.001	0.3%
PCI type:									
Balloon angioplasty	8.20%	7.30%	6.30%	6.50%	6.30%	6.30%	5.90%	< 0.001	–0.3%
Bare metal stent	91.10%	90.70%	91.50%	91.40%	89.50%	83.70%	75.90%	< 0.001	–2.2%
Drug eluting stent	0.70%	2%	2.20%	2%	4.20%	10%	18.30%	< 0.001	2.5%
TIMI 3 flow after PCI	91.1%	91.4%	91.3%	92.2%	91.6%	91.8%	92.2%	0.032	0.2%

CABG — coronary artery bypass grafting; non-IRA — non-infarct related artery; TIMI — Thrombolysis in Myocardial Infarction

Table 4. Changes in management and 30-day mortality of percutaneous coronary intervention (PCI)-
-treated women with ST-segment elevation myocardial infarction from 2005 and 2011 matched on
propensity scores.

	2005	2011	P
Door to balloon time [min]	47 (30–75)	43 (30–65)	< 0.001
Additional PCI of non-IRA	8.6%	18.4%	< 0.001
PCI type:	9.6%	7.1%	< 0.001
Balloon angioplasty			
Bare metal stent	89.3%	76.3%	< 0.001
Drug eluting stent	1.1%	16.6%	< 0.001
TIMI 3 flow after PCI	88.8%	90.6%	0.032
In-hospital pharmacotherapy:	96.0%	89.2%	< 0.001
ASA			
Clopidogrel	77.4%	98.9%	< 0.001
GP IIb/IIIa inhibitors	24.6%	31.1%	< 0.001
Beta-blockers	79.3%	70.1%	< 0.001
Statins	84.3%	75.4%	< 0.001
ACEIs or ARBs	77.1%	64.6%	< 0.001
In-hospital complications:	3.1%	0.2%	< 0.001
Myocardial reinfarction			
Ischemic stroke	0.8%	0.3%	0.005
Major bleeding	1.3%	1.7%	0.23
Pharmacotherapy at discharge:			
ASA	95.9%	95.9%	0.93
Clopidogrel	41.4%	92.5%	< 0.001
In-hospital mortality	7.5%	5.7%	0.011
30-day mortality	9.1%	8.9%	0.84
1-year mortality	14.5%	13.9%	0.5

ACEIs — angiotensin converting enzyme inhibitors; ASA — acetylsalicylic acid; ARBs — angiotensin II receptor blockers; GP IIb/IIa — glyco-
protein IIb/IIIa; non-IRA — non-infarct related artery; TIMI — Thrombolysis in Myocardial Infarction

prognosis as patients who survive the first month after STEMI treated with primary PCI have only a < 1.5% annual risk of successive cardiac death [18]. There have been major improvements in the delivery of care for STEMI patients including the increased use of PCI and adjunctive therapies, but at the same time some unexpected tendencies in guideline-recommended pharmacotherapy were noted.

Significant reductions were observed of-in hospital D2B delays which is consistent with observations of other authors [19, 20]. Only patients who had PCI performed within 12 h from symptom onset were analyzed, thus the present results have shorter D2B times than most other studies. The medians of D2B times shortened slightly but significantly between 2005 and 2011 — compromising the right direction of changes in management. Women continue to have longer D2B times but the average difference between sexes was only around

1–2 min. It was not statistically significant but remained relatively constant throughout the study period. A study of STEMI patients in Australia analyzing D2B time components have confirmed longer delays in both diagnosis and instituting PCI therapy in women [21]. A potential factor that may contribute to the delay may be related to anatomic factors including smaller diameter of coronary vessels in women [22] and potential technical difficulties in performing the PCI. Possibly for the same reason optimal — TIMI 3 flow after PCI was more often achieved in men during the study period and no significant trend toward reduction of this particular sex discrepancy was noted.

Drug eluting stent compared with BMS are not associated with morality reduction but they improve clinical outcomes by reducing the risk of reintervention [23]. DES is currently preferred over BMS in STEMI patients without contrain-

Table 5. Changes in management and 30-day mortality of percutaneous coronary intervention (PCI)-
-treated men with ST-segment elevation myocardial infarction from 2005 and 2011 matched on
propensity scores.

	2005	2011	P
Door to balloon time [min]	46 (30–72)	41 (30–62)	< 0.001
Additional PCI of non-IRA	8.6%	9.8%	0.032
PCI type:			
Balloon angioplasty	7.8%	5.1%	< 0.001
Bare metal stent	91.6%	75.8%	< 0.001
Drug eluting stent	0.6%	19%	< 0.001
TIMI 3 flow after PCI	91.1%	92.5%	0.013
In-hospital pharmacotherapy:			
ASA	96.3%	90.6%	< 0.001
Clopidogrel	78.3%	98.8%	< 0.001
GP IIb/IIIa inhibitors	28.1%	35.6%	< 0.001
Beta-blockers	81.3%	75.9%	< 0.001
Statins	87.0%	81.3%	< 0.001
ACEIs or ARBs	78.5%	69.8%	< 0.001
In-hospital complications:			
Myocardial reinfarction	2.9%	0.3%	< 0.001
Ischemic stroke	0.2%	0.2%	0.69
Major bleeding	0.6%	0.8%	0.079
Pharmacotherapy at discharge:			
ASA	96.8%	96.5%	0.46
Clopidogrel	43%	93,1%	< 0.001
In-hospital mortality	4.1%	3.3%	0.034
30-day mortality	5.4%	5.5%	0.86
1-year mortality	9.5%	9.7%	0.73

ACEIs — angiotensin converting enzyme inhibitors; ASA — acetylsalicylic acid; ARBs — angiotensin II receptor blockers; GP IIb/IIa — glyco-
protein IIb/IIIa; non-IRA — non-infarct related artery; TIMI — Thrombolysis in Myocardial Infarction

dications to prolonged dual antiplatelet therapy [24]. It was observed that women are less likely to receive DES. The potential gender-related differences in stent type selection might be related to a physician's notion of an increased risk of bleeding in women on prolonged dual antiplatelet therapy, their statistically greater age or other non-specific sex-related disparities. However, the frequency of use of DES has been significantly increasing for both sexes presumably due to better availability and an increasingly established role of DES as a standard mode of treatment in ACS.

In patients with STEMI undergoing infarct-artery PCI benefits of PCI in non-infarct coronary arteries with major stenoses is a subject of debate. European Society of Cardiology (ESC) Guidelines present during the study period did not clearly refer to treating non-infarct related vessels, apart from suggesting treatment of the infarct-related lesion by PCI and perform coronary artery bypass grafting later under more stable conditions. Later, in 2012 ESC Guidelines stated that primary PCI should be limited to the culprit vessel with the exception of cardiogenic shock and persistent ischemia after PCI of the supposed culprit lesion [24]. However, recent CULPRIT-SHOCK trial showed that additional intervention on non infarct-related lesions in cardiogenic shock was associated with higher 30-day risk of unfavorable outcomes [25]. The 2013 American College of Cardiology Foundation/American Heart Association (ACCF/AHA) Guidelines designated a Class III recommendation for multivessel primary PCI in hemodynamically stable patients with STEMI; it has recently been modified to a Class IIb in the 2015 Update [26]. Recently published results of PRAMI [27], CvL-PRIT [28] and DANAMI-3 PRIMULTI [29] trials showed that complete revascularization during the

index admission in patients with STEMI and multivessel disease may be of benefit. Those results have already been incorporated into the current 2017 ESC STEMI Guidelines [30]. An unexpected finding is that the percentage of women undergoing additional PCI of at least one non-IRA almost doubled during the study period whereas a corresponding trend in male patients was over 5 times weaker. Although, in general, women tend to have a more diffused disease, it most probably could not fully explain such a strong trend. Despite a lack of recommendations for routine preventive PCI in STEMI patients, it had been performed in women surprisingly often and only forthcoming trials were to confirm that this is a safe and potentially beneficial approach. It is interesting and warrants further studies of other potential underlying causes.

The frequencies of the use of novel antiplatelet agents (clopidogrel and glycoprotein IIb/IIIa inhibitors) have increased. This is not surprising with regard to the importance of platelet inhibition in PCI-treated patients. In contrast, at the same time the use of ASA and other evidence based medicine-based medications have decreased. Single authors reported similar tendencies in ASA usage in secondary prevention and hypothesized that it may be attributable to a rise in novel antiplatelet agent usage, and physicians being less insistent on dual antiplatelet therapy in patients with a minor intolerance to ASA [11]. However, in the present study the percentages of patients who were recommended ASA at discharge were comparable between 2005 and 2011. On the other hand, some patients who received in-hospital clopidogrel were discharged without this drug, especially in 2005, which might be related to possible economic issues and the use of another thienopyridine (ticlopidine) instead. There may also have been a small number of patients who had STEMI in the mechanism other than atherosclerosis and there was a decision not to prolong aggressive antiplatelet therapy. Significant decreases in the use of beta-adrenolytic agents, ACEI or ARBs and statins was unexpected. Early ACE inhibition was shown to reduce mortality as early as 30 days after STEMI, with most of the benefit observed during the first week [31]. Statins lower both short and long-term mortality in MI patients and is most beneficial when treatment is initiated which was observed early after admission to the hospital [32]. On the other hand, the administration of early beta-blocker therapy in acute MI has failed to prove a net benefit on mortality [33], despite well-established benefits in longer

observations. It has gradually been realized that the greatest benefit of using beta-blockers and ACEI is expected in selected groups of patients (i.e. those with heart failure or left ventricular dysfunction) and our observations may reflect a tendency toward a more discriminating usage of those drugs. Nevertheless, there is no data to confirm this hypothesis as this observation may be related to lower quality of care as well. Especially taking into account that 2012 and 2017 ESC Guidelines presented high IIA Class of recommendation for the routine beta-blocker and ACEI use in all patients without contraindications [24, 30]. A significant decrease in statin use in the present study is alarming and presents an unclear tendency. Optimal medical therapy could be as important as reperfusion therapy in the PCI era [34]. Some pitfalls in this field could explain why no further mortality reduction was observed despite substantial changes in STEMI management. A similar analysis (data not published yet) that included all patients, regardless of treatment strategy, showed better pharmacotherapy standards. This may reflect an improper tendency to pay less attention to concomitant pharmacotherapy in patients who have undergone PCI reperfusion.

In-hospital complication rates considerably declined, which undoubtedly helped to achieve better in-hospital survival rates. Myocardial reinfarctions became less frequent in both sexes. Ischemic strokes were already rare in 2005 in men and their rates significantly decreased in women. The rates of major bleeding during hospitalization showed an insignificant rise in both sexes. This effect was most likely due to an increased use of antiplatelet (and possibly also antithrombotic) agents. However, taking into account significant declines in rates of reinfarctions and ischemic strokes, no significant increase in major bleedings suggest an acceptable safety profile of new management approaches.

There are wide differences in reported mortality rates of STEMI patients and treatment-related statistics across countries [6, 8, 11]. However, in this study mostly data in propensity score matched cohorts allowed for comparing changes between 2005 and 2011 were presented but may not reflect actual frequencies observed in the whole population, so comparisons with other studies are not applicable. The focus herein was mainly on the survival benefit from ongoing changes in treatment among PCI-treated patients and, as mentioned

before, data from other studies which have shown inconsistent results [6–10]. It was believed that each region should be analyzed separately to explore potential factors contributing to variations in outcomes of STEMI patients in the PCI-era.

Limitations of the study

A number of possible limitations of this study should be mentioned. First, it is retrospective in nature using registry data. Participation in PL-ACS Registry is voluntary and participating sites varied during the study period so selection bias cannot be excluded. Some initial patient characteristics were not available (for example data on renal failure or anemia) which might have affected PS model quality. Also some information on treatment strategy (for example data on thrombus aspiration or catheterization access — radial vs. femoral) and data on post-discharge treatment, including pharmacotherapy and the length of dual antiplatelet therapy, were not available. Unavailable records of post-discharge management (compliance to prescribed pharmacotherapy, rehabilitation or the rates of cardioverter-defibrillator implantations) could also be considered important predictors of medium and long-term mortality.

Conclusions

Many changes in PCI techniques and concomitant management in patients with STEMI treated with PCI between 2005 and 2011 in Poland and a significant reduction of their in-hospital mortality rates were noted. However, no significant reduction in 30-day or 1-year mortality was observed. These results have been analogous in male and female populations. The observed trends in treatment strategies have generally presented ongoing improvement which followed current guidelines. There remains room for further improvement in the field of concomitant in-hospital pharmacotherapy among PCI-treated patients. Although randomized control trials have confirmed efficiency of particular interventions, their overall association with medium and long-term mortality reduction at the population level was not confirmed in this study. It could be related to implementation rates of new treatments in a real-life setting and should not be interpreted as calling into question their individual-level of usefulness.

Conflict of interest: None declared

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ORIGINAL ARTICLE

Comparison of short-term clinical outcomes between Resolute Onyx zotarolimus-eluting stents and everolimus-eluting stent in patients with acute myocardial infarction: Results from the Korea Acute Myocardial infarction Registry (KAMIR)

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Abstract

Background: *There are few studies which compare the efficacy and safety of the Resolute Onyx zotarolimus-eluting stent (O-ZES) and everolimus-eluting stent (EES) in patients with acute myocardial infarction (AMI). Therefore, the present study aimed to compare clinical outcomes of O-ZES and EES in patients with AMI undergoing successful percutaneous coronary intervention (PCI).*

Methods: *From January 2016 to December 2016, the Korea Acute Myocardial Infarction Registry (KAMIR) enrolled 3,364 consecutive patients. Among them, O-ZES was used in 402 patients and EES was used in 1,084 patients. The primary endpoint was target lesion failure (TLF), as defined by composite of cardiac death, target vessel myocardial infarction (TV-MI), and ischemic driven-target lesion revascularization (ID-TLR) at 6 month clinical follow-up.*

Results: *At 6 months, the incidence of TLF was not significantly different between O-ZES and EES group (4.0% vs. 3.9%, adjusted hazard ratio [HR] 1.17, 95% confidential interval [CI] 0.58–2.35, p = 0.665). O-ZES also showed similar results of cardiac death (3.7% vs. 3.4%, adjusted HR 1.25, 95% CI 0.59–2.63, p = 0.560), TV-MI (0.2% vs. 0.6%, adjusted HR 0.56, 95% CI 0.07–4.85, p = 0.600), ID-TLR (0.0% vs. 0.3%, p = 0.524), and definite or probable stent thrombosis (0.2% vs. 0.3%, adjusted HR 0.63, 95% CI 0.06–6.41, p = 0.696) when compared with EES.*

Conclusions: *The present study shows that implantation of O-ZES or EES provided similar clinical outcomes with similar risk at 6-month of TLF and definite/probable ST in patients with AMI undergoing successful PCI.* (Cardiol J 2019; 26, 5: 469–476)

Key words: drug-eluting stents, myocardial infarction, percutaneous coronary intervention

Introduction

Compared with bare-metal stents (BMS), drug-eluting stents (DES) have shown better clinical outcomes for patients undergoing percutaneous coronary intervention (PCI) by potent prevention from neointimal hyperplasia [1]. However, early-generation DES produced late thrombotic events, more than 1-year, by delaying arterial healing of stented vessels [2–5]. New-generation DES have developed with thinner stent struts, more biocompatible polymer coatings for drug release, and a variety of antiproliferative agents [6]. This development has led to a significant improvement in the efficacy and safety of early-generation DES. In recent years, new-generation DES replaced early-generation DES because of improved stent design, similar or superior anti-restenotic efficacy, and consistently lower rates of late stent thrombosis (ST) [7, 8]. In fact, currently used DES is a standard treatment in modern clinical procedures and is used by most patients undergoing PCI.

Currently, thin strut and durable polymer-based zotarolimus-eluting stent (ZES) and everolimus-eluting stent (EES) are widely used. Resolute Onyx-ZES (O-ZES), the latest version of ZES, has thinner strut, 81 μm, than Resolute-ZES (R-ZES), prior version of ZES, which had 91 μm of strut thickness. R-ZES and EES have been directly compared in several randomized trials powered for non-inferiority with respect to composite clinical endpoints [9–11]. However, there are few studies to compare the safety and efficacy of O-ZES and EES in patients with acute myocardial infarction (AMI). Therefore, the present study aimed to compare clinical outcomes of O-ZES and EES in patients with AMI undergoing successful PCI.

Methods

Study design and patient population

The Korea Acute Myocardial Infarction Registry (KAMIR) is a prospective multicenter registry providing observational online data collected and designed to examine characteristics, treatment practices, and outcomes in patients presenting AMI with the support of the Korean Circulation Society [12].

Study protocols were approved by the ethics committee at each participating center, and followed principles of the Declaration of Helsinki. Written informed consent was given by each patient. If patients were unable to give consent because of severity, informed consent was obtained from a relative or legal representative.

Among 3,364 patients enrolled in KAMIR between January 2016 and December 2016, a total of 1,486 patients with AMI were selected who had undergone successful PCI with O-ZES (Resolute Onyx™, Medtronic Cardiovascular, Santa Rosa, CA) or EES (XIENCE™, Abbot Vascular Santa Clara, CA / SYNERGY™, Boston Scientific, Natick, MA). 402 patients with AMI were treated with O-ZES and 1,084 patients were treated with EES, 620 of XIENCE™ and 464 of SYNERGY™ (Fig. 1).

Study endpoints, definitions, and interventional procedures

The primary endpoint was target lesion failure (TLF), being defined as a composite of cardiac death, target vessel myocardial infarction (TV-MI), and ischemic driven-target lesion revascularization (ID-TLR) at 6 months clinical follow-up. The secondary endpoints were individual components of the TLF and definite/probable ST as defined by the Academic Research Consortium [13].

Death was regarded as cardiac in origin unless obvious non-cardiac causes could be identified. Myocardial infarction (MI) was defined as either the development of new pathological Q waves ≥ 0.04 s in duration in ≥ 2 contiguous leads or an elevation of creatine phosphokinase levels to > 2 times normal with positive creatine phosphokinase-MB or troponin I or T levels. TV-MI was defined as MI attributable to target vessel. TLR was considered ischemic-driven if associated with a positive functional study, a target lesion stenosis $\geq 50\%$ by core laboratory quantitative analysis with ischemic symptoms or a target lesion stenosis $\geq 70\%$ with or without documented ischemia.

Hypertension was defined as a history of hypertension diagnosed and treated with medication, diet and/or exercise, or blood pressure > 140 mmHg systolic or 90 mmHg diastolic on at least two occasions, or currently on antihypertensive pharmacologic therapy. Diabetes mellitus (DM) was defined as a history of DM, regardless of duration of disease, need for antidiabetic agents, or a fasting blood glucose > 126 mg/dL. Family history of ischemic heart disease was indicated if the patient had any direct blood relatives (parents, siblings, children) who had any of the preceeding, which were diagnosed at age < 55 years.

All patients who underwent PCI received 300 mg acetylsalicylic acid (ASA) and 300 or 600 mg clopidogrel, or prasugrel 60 mg, or ticagrelor 180 mg as a loading dose prior to PCI. After PCI,
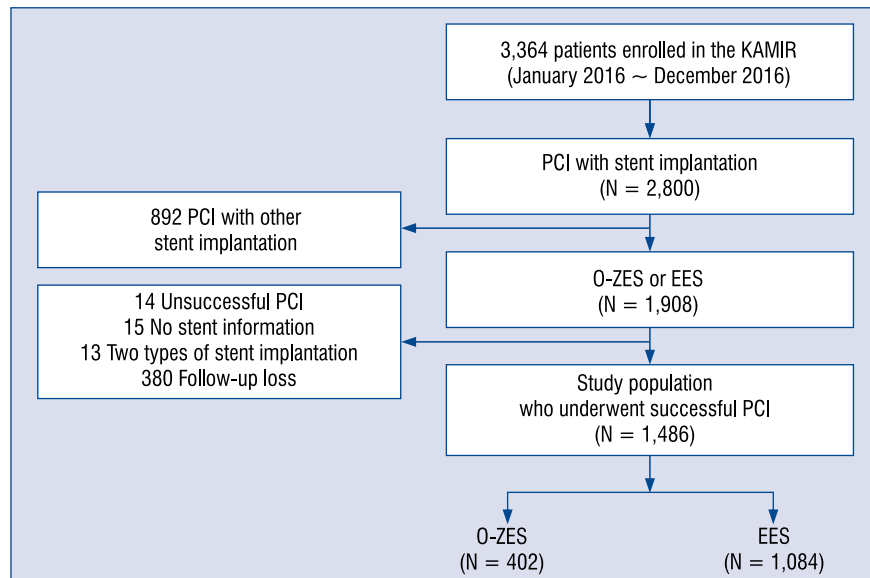
Figure 1. Patients flow chart, EES — everolimus-eluting stent; KAMIR — Korea Acute Myocardial Infarction Registry; O-ZES — Resolute Onyx zotarolimus-eluting stent; PCI — percutaneous coronary intervention.

100–300 mg ASA and 75 mg clopidogrel, or 5 or 10 mg prasugrel one daily or 90 mg ticagrelor twice daily were prescribed for maintenance dose. Medication such as beta-blocker, angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB), and statin were prescribed during hospitalization and after discharge. Coronary artery angiography and stent implantation were performed by standard methods. The selection of PCI timing, medication, vascular access, use of glycoprotein IIb/IIIa inhibitor, use of coronary stents were at physician discretion. Clinical follow-up was done at 6 months after enrollment.

Statistical analysis

All continuous variables were expressed as the mean with standard deviation (SD) or median with interquartile ranges (IQR), when appropriate. All categorical variables were reported as numbers with percentages. The continuous variables were compared using the unpaired t-test or Mann-Whitney U test, as appropriate. The categorical variables were analyzed by using a χ^2 test or Fisher's exact test. The Cox proportional hazard regression modeling (with adjustment for covariates) was used to assess clinical outcomes. A Kaplan-Meier analysis was performed on data from the O-ZES and EES patient groups to compare 6-month TLF and cardiac death, and the difference was determined using a log-rank test. Variables had significance in univariate analysis ($p < 0.100$) for endpoints

which were included in multivariate analysis. The following variables were included in multivariate Cox regression analysis: age ≥ 65, body mass index ≥ 25 kg/m^2, hypertension, DM, dyslipidemia, family history of ischemic heart disease, history of MI, history of angina, history of heart failure, history of cerebrovascular accident, Killip classification III/IV, left ventricular ejection fraction $\leq 50\%$, left main or multivessel disease, image-guided PCI, ACC//AHA B2/C lesion, pre Thrombolysis In Myocardial Infarction (TIMI) flow grade 0/1.

All analyses were two-tailed, and p value < 0.05 was considered to reflect significance. All statistical analyses were performed using SPSS for Windows software (ver. 21.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 1,486 patients with AMI underwent successful PCI were included in the present study. The average age of the total population was 64.1 $\pm$ $\pm$ 12.3 years and 75.4% were men. The mean stent diameter was 3.14 $\pm$ 0.44 mm and the mean stent length was 30.6 $\pm$ 15.1 mm. The average number of stents used per vessel was 1.22 $\pm$ 0.45.

Baseline characteristics and coronary angiographic findings

Mean age was similar for the O-ZES and EES group (64.2 $\pm$ 12.2 vs. 64.0 $\pm$ 12.4, p = 0.802). The O-ZES group had higher prevalence of past his-

Table 1. Baseline clinical and laboratory characteristics of patients in both groups.

Variables	O-ZES (n = 402)	EES (n = 1,084)	P
Demographic:			
Age [years]	64.0 ± 12.4	64.2 ± 12.2	0.802
Male sex	305 (75.9%)	816 (75.3%)	0.813
BMI [kg/m²]	24.2 ± 3.5	24.1 ± 3.4	0.832
Cardiovascular risk factors:			
Hypertension	206 (51.2%)	541 (49.9%)	0.647
Diabetes mellitus	122 (30.3%)	294 (27.1%)	0.218
Dyslipidemia	52 (12.9%)	128 (11.8%)	0.554
Current smoking	158 (39.3%)	446 (41.1%)	0.521
Family history of IHD	35 (8.7%)	106 (9.8%)	0.531
Medical history:			
Angina	36 (9.0%)	70 (6.5%)	0.097
Myocardial infarction	30 (7.5%)	45 (4.2%)	0.010
Heart failure	5 (1.2%)	11 (1.0%)	0.704
Cerebrovascular accident	24 (9.0%)	73 (6.7%)	0.596
Vital sign on admission:			
SBP [mmHg]	131 ± 29	129 ± 29	0.191
DBP [mmHg]	78 ± 18	78 ± 18	0.641
Heart rate [bpm]	79 ±19	78 ± 20	0.474
STEMI	207 (51.5%)	566 (52.2%)	0.805
Killip classification III/IV	58 (14.5%)	151 (14.5%)	0.997
LVEF [%]	52.3 (11.5%)	52.8 (11.2%)	0.388
Laboratory findings:			
Total cholesterol [mg/dL]	174 (146–203)	177 (145–206)	0.965
Triglyceride [mg/dL]	110 (81–151)	110 (78–162)	0.360
HDL-cholesterol [mg/dL]	41 (35–52)	42 (35–49)	0.466
LDL-cholesterol [mg/dL]	109 (86–133)	111 (85–138)	0.461
Creatinine [g/dL]	0.9 (0.8–1.1)	0.9 (0.8–1.1)	0.292
hsCRP [mg/dL]	0.30 (0.14–1.07)	0.30 (0.14–1.20)	0.689
Peak CK-MB [ng/mL]	41 (10–170)	51.3 (10–189)	0.160
Peak troponin-I [ng/mL]	18.9 (4.1–40.0)	22.8 (4.8–40)	0.990

Data are expressed as median (interquartile range), mean ± standard deviation or number (percentage) unless otherwise indicated; O-ZES — Resolute Onyx zotarolimus-eluting stent; EES — everolimus-eluting stent; BMI — body mass index; IHD — ischemic heart disease; SBP — systolic blood pressure; DBP — diastolic blood pressure; STEMI — ST-elevation myocardial infarction; LVEF — left ventricular ejection fraction; HDL — high-density lipoprotein; LDL — low-density lipoprotein; hsCRP — high-sensitivity C-reactive protein; CK — creatine kinase

tory of MI when compared with EES group (7.5% vs. 4.2%, p = 0.010). In laboratory findings, there were no significant differences in either group. In terms of procedural characteristics, pre-PCI TIMI flow grade 0/1 was higher in O-ZES group than in EES group (59.5% vs. 53.2%, p = 0.028). In angiographic findings, O-ZES group more frequently had left main or multivessel disease than in EES group (56.7% vs. 49.0%, p = 0.008). ACEI/ARB was less prescribed in the O-ZES group than in the EES group (73.6% vs. 78.9%, p = 0.032) (Tables 1, 2).

Six-month clinical outcomes

The 6-month clinical outcomes did not differ between the two groups as shown in Table 3. In Cox proportional hazard analysis, O-ZES also showed no statistical differences in the incidence of TLF (4.0% vs. 3.9%, adjusted hazard ratio [HR] 1.17, 95% confidential interval [CI] 0.58–2.35, p = 0.665) (Fig. 2A), cardiac death (3.7% vs. 3.4%, adjusted HR 1.25, 95% CI 0.59–2.63, p = 0.560) (Fig. 2B), TV-MI (0.2% vs. 0.6%, adjusted HR 0.56, 95% CI 0.07–4.85, p = 0.600), ID-TLR (0.0% vs. 0.3%,

Table 2. Characteristics of coronary angiography, procedures and discharge medication between the two groups.

Variables	O-ZES (n = 402)	EES (n = 1,084)	P
Trans-radial access	189 (47.0%)	498 (46.3%)	0.802
Image-guided PCI	123 (30.6%)	359 (33.1%)	0.351
Glycoprotein IIb/IIIa inhibitor	45 (11.5%)	163 (15.2%)	0.070
Pre-PCI TIMI flow grade 0/1	237 (59.5%)	573 (53.2%)	0.028
Infarct-related artery:			0.532
Left anterior descending	186 (46.3%)	528 (48.8%)	
Left circumflex	65 (16.2%)	180 (16.6%)	
Right coronary	140 (34.8%)	337 (31.1%)	
Left main	11 (2.7%)	38 (3.5%)	
Involved vessel type:			0.008
Single vessel	174 (43.3%)	551 (51.0%)	
Left main or multivessel	228 (56.7%)	530 (49.0%)	
ACC/AHA B2/C lesion	354 (90.1%)	952 (89.3%)	0.670
Implanted stent:			
Stent number	1.25 ± 0.46	1.20 ± 0.45	0.077
Stent diameter [mm]	3.12 ± 0.46	3.15 ±0.43	0.222
Stent length [mm]	31.0 ± 14.8	30.4 ± 15.2	0.498
Medical treatment at discharge:			
Acetylsalicylic acid	393 (97.8%)	1,064 (98.2%)	0.626
P2Y12 receptor inhibitor:	393 (97.8%)	1,060 (97.8%)	0.931
Clopidogrel	200 (50.6%)	532 (50.1%)	
Ticagrelor	170 (43.0%)	457 (43.0%)	
Prasugrel	25 (6.3%)	73 (6.9%)	
Statin	371 (92.3%)	1,021 (94.2%)	
ACEI/ARB	296 (73.6%)	855 (78.9%)	0.032
Beta-blocker	314 (78.1%)	863 (79.6%)	0.526
Calcium channel blocker	34 (8.5%)	69 (6.4%)	0.158

Data are expressed as mean ± standard deviation or number (percentage) unless otherwise indicated; O-ZES — Resolute Onyx zotarolimus-eluting stent; EES — everolimus-eluting stent; PCI — percutaneous coronary intervention; TIMI — Thrombolysis In Myocardial Infarction; ACC — American College of Cardiology; AHA — American Heart Association; ACEI — angiotensin-converting enzyme inhibitor; ARB — angiotensin II receptor blocker.

p = 0.524), and definite/probable ST (0.2% vs. 0.3%, adjusted HR 0.63, 95% CI 0.06–6.41, p = 0.696) when compared with EES (Table 3).

Discussion

There have been no studies to directly compare clinical outcomes between O-ZES and EES in specific high-risk groups, such as patients with AMI. This is the first multicenter and currently the largest observational study investigating clinical outcomes of AMI patients undergoing successful PCI with O-ZES or EES. The present study demonstrates that implantation of O-ZES or EES provided similar short-term clinical outcomes in patients with AMI undergoing successful PCI.

There are several studies regarding a comparison of clinical outcomes after PCI with R-ZES. In the RESOLUTE All Comers trial (A Randomized Comparison of a Zotarolimus-Eluting Stent with an Everolimus-Eluting Stent for Percutaneous Coronary Intervention), compared with the EES, the TLF did not significantly differ between R-ZES and EES in complex patients, such as AMI (8.9% in R-ZES group vs. 9.7% in EES group, p = 0.66) at 1-year follow-up [14]. In another randomized TWENTE trial, complex patients treated with R-ZES and EES showed similar TLF during 2-year follow-up (11.7% in R-ZES group vs. 10.9% in EES group, p = 0.68) [15]. In these two randomized trials, however, patients with AMI were only 43.6% and 37.1%, respectively, when compared with the
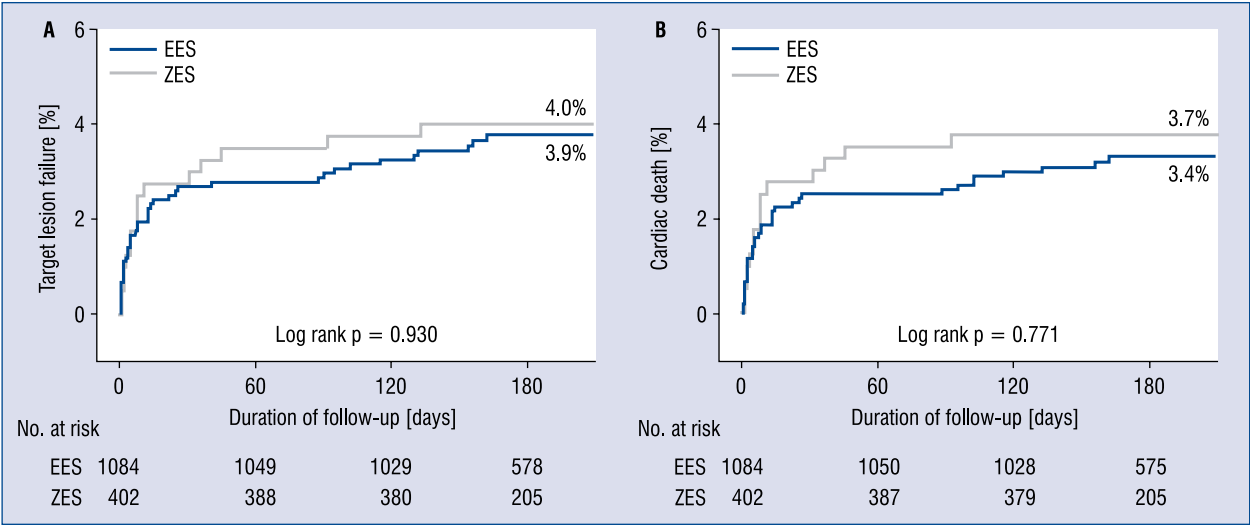
Figure 2. Kaplan-Meier curves for 6-month target lesion failure (**A**) and cardiac death (**B**) in patients with acute myocardial infarction; EES — everolimus-eluting stent; ZES — zotarolimus-eluting stent.

Table 3. Six-month cumulative clinical outcomes in both groups.

	O-ZES (n = 402)	EES (n = 1,084)	Unadjusted			Adjusted		
			HR	95% CI	P	HR	95% CI	P
Target lesion failure	16 (4.0%)	42 (3.9%)	1.03	0.58–1.83	0.930	1.17	0.58–2.35	0.665
Cardiac death	15 (3.7%)	37 (3.4%)	1.09	0.60–1.99	0.771	1.25	0.59–2.63	0.560
TV-MI	1 (0.2%)	6 (0.6%)	0.45	0.05–3.71	0.456	0.56	0.07–4.85	0.600
ID-TLR	0 (0.0%)	3 (0.3%)			0.524			
Definite/probable ST	1 (0.2%)	3 (0.3%)	0.92	0.10–8.79	0.938	0.63	0.06–6.41	0.696

Data are expressed as number (percentage). O-ZES — new developed Resolute Onyx zotarolimus-eluting stent; EES — everolimus-eluting stent; HR — hazard ratio; CI — confidence interval; TV-MI — target vessel myocardial infarction; ischemic driven-target lesion revascularization; ST — stent thrombosis

present study which enrolled all patients with AMI. ST-elevation myocardial infarction (STEMI) patients receiving R-ZES had similar 5-year clinical outcomes as compared with those receiving EES in the RESOLUTE All Comers trial (TLF, 7.6% in R-ZES group vs. 10.4% in EES group, adjusted p = 0.123; definitive/probable ST, 0.8% in R-ZES group vs. 1.3% in EES group, adjusted p = 0.868) [16]. In the RESOLUTE Global Clinical Trial Program comprising 10 prospective trials, R-ZES showed good long-term clinical outcomes. In 7618 patients treated with R-ZES, the 5-year cumulative incidence of TLF was 13.4%, cardiac death 5.0%, TV-MI 4.4%, and ID-TLR 6.3% [17]. In the RESOLUTE Global Clinical Trial Program, STEMI patients treated with R-ZES also had good 3-year clinical outcomes, including TLF 9.8%, cardiac death 2.9%, TV-MI 1.6%, ID-TLR 7.0%, and definite/probable ST 2.8% [16].

Regarding the safety and efficacy of O-ZES, O-ZES showed 1-year TLF rate of 4.4% and similar efficacy and safety compared with most contemporary DES [18]. O-ZES demonstrated superiority for 8-month in-stent late lumen loss compared with the historical control R-ZES in the RESOLUTE ONYX core trial (0.24 ± 0.39 mm with O-ZES vs. 0.36 ± 0.52 mm with R-ZES, p = 0.029) [19]. Moreover, 2.0 mm O-ZES was associated with a low rate of TLF and late lumen loss without definite/probable ST at 12 month follow-up for treatment of coronary lesions with a very small reference vessel diameter, less than 2.25 mm in the recent study [20]. These favorable clinical outcomes including angiographic benefit of O-ZES could be explained by characteristics of O-ZES. O-ZES has a swaged shape and a larger strut width-to-thickness ratio (strut width 91 μm and thickness 81 μm) to maintain radial strength despite thinner strut

when compared with R-ZES. O-ZES also has a dense inner core composed of the platinum-iridium alloy for increased radiopacity. The enhanced radiopacity of O-ZES might have contributed to less geographic miss which was associated with increased target vessel revascularization [21].

Limitations of the study

There were several limitations in the present study. First, it was a retrospective study and there were possibilities for selection bias. Therefore multivariate analysis was undertaken to overcome these limitations. Secondly, the number of patients included in O-ZES group was relatively small, and thus, this study is less robust because of the small sample size. Thirdly, EES group included durable polymer everolimus-eluting stent (XIENCE™) and bioresorbable polymer everolimus-eluting stent (SYNERGY™). Differing stent features can be a possible confounding factor. Finally, interval of follow-up was too short to analyze long-term clinical outcomes. Thus, more extensive and long-term data are needed.

Conclusions

This study shows that implantation of O-ZES or EES provided similar clinical outcomes with similar risk of 6-month TLF and definite/probable ST in patients with AMI undergoing successful PCI.

Acknowledgements

Conflict of interest: None declared

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ORIGINAL ARTICLE

The usefulness of selected biomarkers in aortic regurgitation

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Abstract

Background: *The aim of the study was to investigate the prognostic value of selected biomarkers in patients with aortic regurgitation undergoing valve surgery.*

Methods: *A prospective study was conducted on a group of consecutive patients with hemodynamically significant aortic regurgitation that underwent elective aortic valve surgery. The primary endpoint was 30-day mortality and any major adverse event within 30 days.*

Results: *The study group included 205 consecutive patients who underwent replacement or repair of the aortic valve. The primary endpoint occurred in 72 patients. At multivariate analysis red cell distribution width (RDW; p = 0.03) and high-sensitivity troponin T (hs-TnT; p = 0.02) remained independent predictors of the major complications including death.*

Conclusions: *Elevated preoperative RDW and hs-TnT were associated with a poorer outcome following aortic valve surgery.* (Cardiol J 2019; 26, 5: 477–482)

Key words: aortic regurgitation, biomarkers, EuroSCORE II, risk stratification, high-sensitivity troponin T

Introduction

Troponin T (TnT) is a protein forming part of the contractile apparatus of striated muscle. Moreover TnT is a recognized biomarker of myocardial injury [1]. In the available literature, several studies have reported that elevated high-sensitivity TnT (hs-TnT) is associated with poor outcomes in patients with stable coronary heart disease, acute myocardial infarction (MI), heart failure (HF), dilated cardiomyopathy, atrial fibrillation or significant aortic stenosis [2–9].

Another biomarker, red cell distribution width (RDW), is a measure of the differentiation of the size of red blood cells (anisocytosis). Until now, the RDW parameter has been used mainly as a hematological auxiliary marker indicating an increased destruction of erythrocytes or erythrocytes production dysfunction related to a deficiency of folic acid, vitamin B12, iron or ongoing inflammation.

Recently, numerous publications have demonstrated the usefulness of RDW also as a prognostic factor for various cardiovascular diseases, such as coronary artery disease, chronic HF, perioperative stroke, idiopathic pulmonary hypertension and severe aortic stenosis [10–14]. The usefulness of hs-TnT and RDW in patients with severe symptomatic aortic regurgitation undergoing valve surgery in a 30-day follow-up are not established. As there is a need to complement the tools to determine the risk in patients with aortic regurgitation eligible for valve surgery it was sought to observe the usefulness of these biomarkers in this group of patients.

Methods

This was a prospective study of consecutive patients with hemodynamically significant aortic regurgitation (vena contracta > 6 mm, effective regurgitant orifice area > 30 mm^2 or pressure

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half-time < 200 ms) and without significant atherosclerotic changes in the coronary arteries that were qualified to cardiac surgery and subsequently underwent elective replacement or repair of the aortic valve. The exclusion criteria were: a lack of consent to participate in the study, significant atherosclerotic changes in the coronary arteries identified by angiography, patients under 18 years of age, autoimmune diseases, chronic inflammatory bowel, active neoplastic diseases and active infective endocarditis. Patients who were eligible for surgery but subsequently refused the procedure, or did not undergo surgery for logistic or other reasons were not included in the study. The following data were collected: age, gender, body mass index (BMI), comorbidities (coronary artery disease, MI, hypertension, chronic obstructive pulmonary disease, stroke, diabetes), the results of echocardiography findings and the assessment of the coronary arteries. The risk of surgery using EuroSCORE II was calculated for each patient. The day before surgery a blood sample for biomarkers was collected from each patient. Complete blood count was performed with K2-EDTA samples, using a Cobas 6000 electronic counter (Roche, Mannheim, Germany). The plasma levels of cardiac TnT (cTnT) concentrations were measured with the Troponin T hs-STAT (Roche). All procedures were performed through a midline sternotomy incision under general anaesthesia in a normothermia. All patients were given cold blood cardioplegia at the initial dose of 15–20 mL/kg followed by booster doses of 5–10 mL/kg every 20 min. The primary end-point was death from all causes as well as: hemodynamic instability (defined as the need for a supply of catecholamines more than 48 h after completing the cardiopulmonary bypass surgery or the need to resupply), perioperative MI (defined as the development of new Q waves in two or more leads on an electrocardiogram, or alterations of myocardial contractility that did not previously exist in echocardiography), stroke (evidence of a new neurological deficit or a transient ischemic attack, confirmed by an imaging test), perioperative renal failure (requiring renal replacement therapy), prolonged mechanical ventilation (either mechanical ventilatory support lasting longer than 24 h, or the need for reintubation) and the occurrence of multiple-organ failure (the dysfunction of two or more organs — based on laboratory parameters and/or the need to use organ replacement therapy). In all patients the follow up was conducted through direct observation during hospitalization, telephone interviews, or clinic visits for 30 days after surgery. The study was conducted at the Institute of Cardiology, Warsaw, Poland. The protocol was approved by The Institutional Ethics Committee.

Statistical analysis

A statistical analysis was performed using SAS version 9.2. Data are presented as the mean ± standard deviation and the frequency (%). Intergroup comparisons were made using the Mann-Whitney U test, the Pearson's χ^2 test or Student t-test. Logistic regression was used to assess relationships between variables. The following preoperative covariates: age, atrial fibrillation, BMI, chronic kidney disease, chronic obstructive airway disease, coronary artery disease, creatinine, high-sensitivity C-reactive protein (hs-CRP), hs-TnT, hematocrit, hemoglobin, hypertension, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular ejection fraction (LVEF), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, New York Heart Association (NYHA) classes, N-terminal pro-hormone of B-type natriuretic peptide (NT-proBNP), peripheral atherosclerosis, platelets, pulmonary blood pressure, red blood cell count, RDW, stroke history, tricuspid annulus plane systolic excursion and white blood cell count were investigated for association with the endpoints in univariate analysis. Significant determinants (p < 0.05) identified from univariate analysis were subsequently entered into multivariate models. Receiver operating characteristic (ROC) curves were plotted for the EuroSCORE II alone and for the model combined of EuroSCORE II, hs-TnT and RDW for 30-day survival following aortic regurgitation surgery. The additional predictive value of RDW and hs-TnT was assessed by a comparison of the areas under the ROC of the respective curves.

Results

The study included 205 patients who underwent aortic valve surgery with or without concomitant procedures. In 97 patients a mechanical aortic valve prosthesis was implanted, and in 77 a biological valve. The mean age in the study group was 58.7 ± 7.7. Seven (3.4%) of the patients in the study had a previous MI, but currently none of the patients had significant atherosclerotic changes in the coronary arteries. Sixteen (8%) patients had significantly impaired left ventricular systolic function (LVEF ≤ 35%). The mean plasma preoperative hs-TnT level was

20.7 ± 13.9 ng/L. Patients in NYHA class II, III and IV had a significantly higher levels of hs-TnT compared to patients in NYHA class I. Baseline characteristics of the patients are presented in Table 1.

Six patients died during the follow-up period as a result of gradually increasing multi-organ failure (2 patients with LVEF ≤ 35%, 3 patients with LVEF 36–50%, 1 patient with LVEF > 50%). The actual mortality was 2.9% vs. the mortality 3.5% predicted by the EuroSCORE II model. The primary end-point occurred in 72 patients: perioperative renal failure in 11 patients, prolonged mechanical ventilation in 21 patients, stroke in 7 patients and catecholamine infusion for over 48 h in 57 patients. Multi-organ failure was observed in 17 patients. Myocardial infarction occurred in 6 patients. In the postoperative period, 4 patients developed pneumonia. In addition, 6 patients experienced sternal wound infections. Statistically significant predictors of major complications including death at univariate analysis are presented in Table 2. At multivariate analysis RDW (odds ratio [OR] 1.526; 95% confidence interval [CI] 1.011–2.331; p = 0.03) and hs-TnT (OR 1.384; 95% CI 1.082–1.686; p = 0.02) remained independent predictors of the primary endpoint. A positive correlation was found between the level of hs-TnT and LVEF (r = –0.37; p = 0.001), moreover between RDW and CRP (r = 0.42; p = 0.0007). Patients with concomitant chronic kidney disease hadn't significantly higher preoperative hs-TnT levels (p = 0.36) compared with patients without chronic kidney disease. The optimal cut-of points for primary end-point were calculated at the hs-TnT level of 27.5 ng/L and the RDW 14.2%. RDW, hs-TnT and combined with EuroSCORE II was a better predictor of 30-day major complications including death in patients with aortic regurgitation undergoing valve surgery (area under receiver operator characteristic curve [AUROC] = 0.802; 95% CI 0.716–0.886) compared with EuroSCORE II alone (AUROC = 0.699; 95% CI 0.601–0.797).

Figure 1 shows the areas under receiver operator characteristic curves of EuroSCORE II and the combined model RDW + hs-TnT + EuroSCORE II for 30-day major complications.

Discussion

The present paper demonstrated the prognostic significance of hs-TnT and RDW in predicting major complications including death in patients with severe symptomatic aortic regurgitation

Table 1. Baseline characteristics of the study population (n = 205).

Parameters	Values
Preoperative characteristics of patients	
Age [years]	58.7 ± 7.7
Male: men	154 (75%)
Previous myocardial infarction	7 (3.4%)
Stroke in history	5 (2.4%)
Atrial fibrillation	62 (30%)
Peripheral atherosclerosis	4 (1.9%)
Diabetes mellitus	22 (11%)
Hypertension	115 (56%)
Current smoker	37 (18%)
Hyperlipidemia	56 (27%)
Body mass index [kg/m^2]	26.8 ± 3.9
Chronic obstructive airways disease	4 (1.9%)
Chronic kidney disease (GFR < 60 mL/min/1.73 m^2)	48 (23.4%)
LVEF > 50%	149 (73%)
LVEF 50–36%	40 (19%)
LVEF ≤ 35%	16 (8%)
LVEDD [mm]	65 ± 13
LVESD [mm]	46 ± 12
Pulmonary BP [mmHg]	39.1 ± 15.6
NYHA I class	6 (3%)
NYHA II class	112 (55%)
NYHA III class	82 (40%)
NYHA IV class	5 (2%)
EuroSCORE II [%]	3.5 ± 3.1
Hemoglobin [g/dL]	14.1 ± 1.6
RDW [%]	13.8 ± 0.4
Plateletes [1000/μL]	193 ± 51
Hs-TnT [ng/L]	20.7 ± 13.9
Hs-TnT I [ng/L]	7.3 ± 3
Hs-TnT II [ng/L]	18.4 ± 9
Hs-TnT III [ng/L]	22.2 ± 11
Hs-TnT IV [ng/L]	26.1 ± 12
NT-proBNP [pg/mL]	1922 ± 1017
GFR [mL/min/1.73 m^2]	69.7 ± 17.7
Aortic regurgitation severe	205 (100%)
Aortic stenosis mild	26 (13%)
Aortic stenosis moderate	20 (10%)
Aortic stenosis severe	0 (0%)
Postoperative characteristics of patients	
Aortic cross-clamp time [min]	93 ± 36
Cardiopulmonary bypass time [min]	122 ± 53
Time at the ICU [days]	6.4 ± 4.5
Time in the hospital [days]	14.7 ± 5.7

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Table 1 (cont.). Baseline characteristics of the study population.

Parameters	Values
Drainage after 12 h [mL]	552 ± 240
Drainage after 24 h [mL]	780 ± 365
Number of RBC units transfused	4.5 ± 3
RDW I	13.8 ± 0.4
RDW II	14.0 ± 0.5
Main procedures	
AVR	153 (74.6%)
AVP	28 (13.6%)
AVR + supracoronary ascending aortic replacement	11 (5.4%)
Bentall procedure	10 (4.9%)
David procedure	3 (1.5%)

Values are represented by number (percentage) or mean ± and a measure of the variation of the internal standard deviation. AVR — aortic valve replacement; AVP — aortic valve plasty; BP — blood pressure; GFR — glomerular filtration rate; Hs-TnT — high-sensitivity troponin T; Hs-TnT I, II, III, IV — high-sensitivity troponin T in NYHA class I, II, III, IV, respectively; ICU — intensive care unit; LVEF — left ventricular ejection fraction; LVEDD — left ventricular end-diastolic diameter; LVESD — left ventricular end-systolic diameter; NT-proBNP — N-terminal pro-hormone of B-type natriuretic peptide, NYHA — New York Heart Association; RDW — red cell distribution width (preoperative); RDW I — red cell distribution width measured immediately after surgery; RDW II — red cell distribution width measured one day after surgery

Table 2. Univariate analysis of predictive factors for the occurrence of the primary endpoint.

Variable	Odds ratio	95% CI	P
Age [years]	1.028	1.006–1.051	0.01
NT-proBNP [pg/mL]	1.023	1.011–1.034	0.006
Hs-TnT [ng/L]	1.095	1.033–1.134	0.004
LVEF [%]	0.959	0.935–0.983	0.001
LVEDD [mm]	1.264	1.124–1.421	0.008
Hemoglobin [g/dL]	0.663	0.543–0.811	0.006
RDW [%]	1.699	1.281–2.253	0.002

CI — confidence interval; Hs-TnT — high sensitivity troponin T; LVEDD — left ventricular end-diastolic diameter; LVEF — left ventricular ejection fraction; NT-proBNP — N-terminal pro-hormone of B-type natriuretic peptide; RDW — red cell distribution width

undergoing aortic valve surgery in postoperative 30-day follow-up.

Troponin T is a polypeptide that is part of the striated contractile muscle apparatus. The TnT function in all types of striated muscles is the same. As one of the three proteins included in the troponin complex, it performs key functions in the process of muscle contraction. A very important aspect, from a diagnostic point of view, is the fact that the sequence of troponins of cardiac origin differs from the sequence of skeletal muscle troponins.

Thanks to this, after obtaining specific monoclonal antibodies, it became possible to use them in the diagnosis of ischemic heart disease. Currently, it is believed that cTnT is the best laboratory parameter in the early diagnosis of acute MI. Considering the fact that cytoplasm of cardiomyocytes contains a small amount of free troponins TnT and TnI, even small damage to the cell membrane (e.g. in the initial phase of myocardial ischemia) causes their release and the ability to be detected in the blood sample under study [15, 16]. High sensitivity tests, which have been available for several years, are able to detect very low levels of troponin T present in the blood with high reliability [17]. Significant heart valve defects are often the result of pressure or volume overload of the heart cavities. Hypertrophy and remodeling of the myocardium is a response to increasing overload. This mechanism initially restores and maintains the tension of the left ventricular wall. However, long-lasting additional burden on the myocardium causes progressive degenerative changes of the myocardium, which are accompanied by slow processes of necrosis and fibrosis, which may be a reason for the presence of TnT in the blood [18–20].

The predictive power of the preoperative TnT for death in 30-days follow-up in a group of 224 patients with surgically treated severe aortic stenosis was previously demonstrated [9]. Moreover, in a small group of 60 patients with severe aortic valve stenosis undergoing aortic surgery, preoperative TnT was an important predictor of serious post-operative complications in long-term follow-up [21]. Also, in a study of a group of 24 patients undergoing mitral valve replacement surgery, elevated values of postoperative TnT significantly correlated with the length of postoperative hospital stay [22]. On the other hand, Piekarska et al. [23] didn't show a significant correlation between postoperative TnT level and the results of surgical treatment of patients with severe aortic stenosis. It has also not been shown that the preoperative troponin improved the result of the Society of Thoracic Surgeons score in patients undergoing aortic valve replacement [24].

Red cell distribution width is a simple, cheap and widely available parameter, determined in each patient during the standard blood test. So far, the predictive capacity of the RDW in the area of patients with valvular heart disease has been described in a few publications [10–14]. The predictive value of the RDW for serious complications and death in patients with severe aortic stenosis has previously been described, and the occurrence
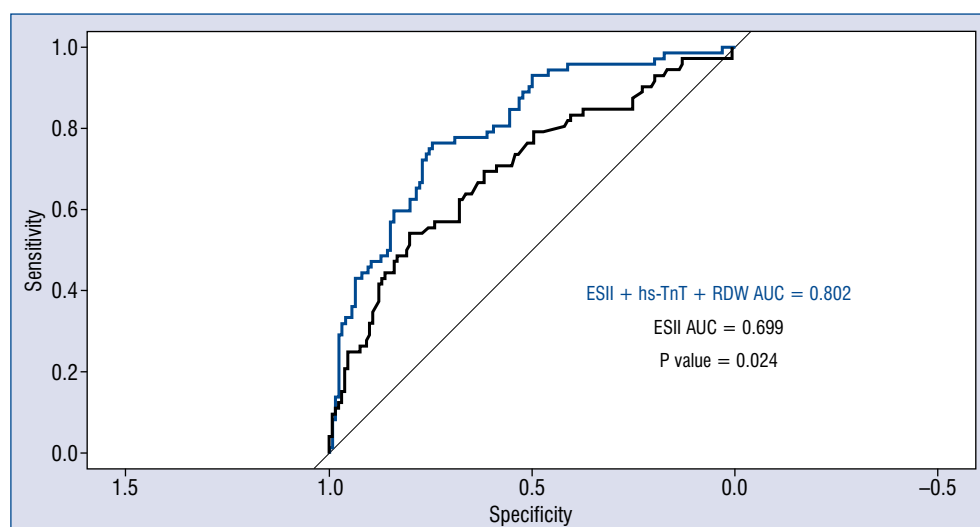
Figure 1. Areas under receiver operator characteristic curves of EuroSCORE II (ESII) and combined model Euro-SCORE II + high sensitivity troponin T (hs-TnT) + red cell distribution width (RDW) for 30-day major complications including death following aortic valve surgery; AUC — area under curve.

of perioperative stroke of the central nervous system in the group of patients undergoing heart valve surgery [12, 14]. In turn, two studies on groups of 250 and 175 patients with severe aortic valve stenosis who underwent transcatheter aortic valve implantation showed a significant correlation between elevated RDW values and the occurrence of an increased risk of death and serious complications in long-term observation [25, 26]. So far, the relationship between value of RDW and worse results in numerous clinical observations hasn't been fully understood and explained. In the literature the hypotheses available combine higher RDW values with the occurrence of inflammation and treat them as a marker of oxidative stress. This hypothesis can be confirmed by a significant correlation between the CRP value, determined by the high sensitivity method, with the RDW parameter (r = 0.42; p = 0.0007) as demonstrated in the presented study.

The results of the presented study indicate the usefulness of two laboratory parameters commonly used in clinical practice in a group of patients with significant aortic valve regurgitation undergoing valve surgery. Hs-TnT, which indicates damage to the myocardium and RDW, which according to some authors indicate the physiological reserve of humans, may be important in making therapeutic decisions in patients with aortic regurgitation. It is worth noting that multi-organ failure which occurred in 17 patients in the early post-operative period was the cause of all 6 deaths.

Conclusions

It was a single-center study with a limited number of patients. Enlargement of the group of participants may allow confirmation of the results obtained and to develop a risk calculator using selected biomarkers. Further studies are needed regarding the suitability of other biomarkers as predictors of complications in patients with aortic regurgitation undergoing cardiac surgery. The results of the presented studies may be helpful in the perioperative risk stratification in patients with aortic regurgitation. It is worth noting that hs-TnT levels in combination with RDW and EuroSCORE II were better predictors of postoperative major complications including death compared to Euro-SCORE II alone.

Conflict of interest: None declared

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ORIGINAL ARTICLE

Severe degenerative aortic stenosis with preserved ejection fraction does not change adipokines serum levels

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Abstract

Background: *The role of the adipokines in the pathogenesis of aortic stenosis (AS) is not well established. The aim was to evaluate the relationship between adipokines and clinical characteristics as well as echocardiographic indices and noninvasive markers of vascular remodeling in patients with severe AS with preserved ejection fraction (EF).*

Methods: *Sixty-five patients (F/M: 38/27; age: 68.3 ± 9.0 years; body mass index [BMI]: 29.6 ± 4.3 kg/m², with severe AS with preserved EF: 33 patients with paradoxical low-flow low-gradient AS (PLFLG AS) and 32 patients with normal flow high-gradient AS (NFHG AS) were prospectively enrolled into the study. Twenty-four subjects (F/M: 14/10; age: 65.4 ± 8.7 years; BMI: 29.6 ± 4.3 kg/m²) who matched as to age, sex, BMI and coronary artery disease (CAD) constituted the control group (CG). Clinical data and markers of vascular remodeling were related to the serum adipokines.*

Results: *There were no differences in the adipokines concentrations in the AS/CG. Patients with AS and coexisting CAD were characterized by decreased serum adiponectin (9.9 ± 5.5 vs. 12.7 ± 5.8 µg/mL, p = 0.040) and leptin (8.3 ± 7.8 vs. 21.6 ± 17.1 ng/mL, p < 0.001) levels compared to subjects without CAD. There were no differences in the serum adipokines concentrations between patients with PLFLG AS and NFHG AS. Systemic hypertension, diabetes, hyperlipidemia or markers of vascular remodeling did not discriminate adipokines concentrations. Multivariate regression analysis indicated that age (F = 3.02; p = 0.015) and E/E' index (F = 0.87, p = 0.032) were independent predictors of the adiponectin level in the AS group.*

Conclusions: *The presence of AS with preserved EF did not change the adipokine serum profile. Adipokines levels were modified by coexisting atherosclerosis but not the typical cardiovascular risk factors or the hemodynamic type of AS.* (Cardiol J 2019; 26, 5: 483–492)

Key words: echocardiography, aortic stenosis, atherosclerosis, adipokines, vascular remodeling

Introduction

Adipokines are involved in various inflammatory and metabolic processes, including atherosclerosis, hypertension and dyslipidemia. Their standard panel includes adiponectin, leptin resistin and visfatin.

Adiponectin has antiatherogenic and anti-inflammatory effects on endothelial cells and macrophages [1, 2], which are caused by an increase in

nitrix oxide (NO) production, a reduction of adhesion molecules in the endothelial cells, inhibition of macrophages cytokine production and foam cell formation supression [3–5]. Clinical studies implicate hypoadiponectinemia in the pathogenesis of diabetes mellitus (DM) type 2, arterial hypertension (HA), coronary artery disease (CAD) and left ventricular (LV) hypertrophy [6–9].

Leptin, in turn, may have an impact on cardiac hypertrophy and may be associated with an increased cardiovascular risk as plasma leptin concentrations were shown to be elevated in patients with LV dysfunction and heart failure [10–12].

Resistin, which has primarily been suggested as a hormone that potentially links obesity to DM, was later proved to regulate the processes of the atherosclerosis and cardiovascular disease as well [13, 14].

Visfatin, which was originally described as a protein secreted by visceral fat that mimics insulin effects, turned out to have a relationship with cardiovascular diseases. It can promote foam cell formation and angiogenesis [15–17]. Elevated visfatin serum levels were found in patients with acute myocardial infarction [18].

Adipokines may thus be considered to be a network that influences metabolic states and has the potential to impact directly upon the metabolic homeostasis of a system. Their role in other pathological cardiovascular conditions is not yet well known. Assuming that there are some similarities between the pathophysiology of calcific aortic valve (AV) disease (AVD) and atherosclerosis, those proteins may likewise play a potential role in its development. Moreover, in aortic stenosis (AS) with a low-gradient and preserved ejection fraction (EF), a clinical profile of patients with a higher incidence of obesity, HA and increased peripheral vascular resistance may especially be associated with adipokines abnormalities [19, 20].

Thus, the aim of this study was to evaluate the relationship between adipokines and the clinical characteristics as well as echocardiographic indices and noninvasive markers of vascular remodeling in patients with severe AS with preserved EF.

Methods

Population

Sixty-five patients (F/M: 38/27; age: 68.3 ± ± 9.0 years; body mass index [BMI]: 29.6 ± 4.3 kg/m^2) with severe degenerative AS (New York Heart Association [NYHA] I/II/III: 20/37/8) with preserved LVEF (> 50%) (Table 1) — 33 patients with paradoxical low-flow/low-gradient AS (PLFLG AS)

and 32 patients with normal flow/high-gradient AS (NFHG AS) were prospectively enrolled into the study. Twenty-four healthy subjects (F/M: 14/10; age: 65.4 ± 8.7 years; BMI: 29.6 ± 4.3 kg/m^2) who matched as to age, sex and BMI constituted the control group (CG). Clinical data, body composition, echocardiography, coronary angiography and non-invasive markers of vascular remodeling were examined. The above data were related to the serum adiponectin, leptin, resistin and visfatin levels. The trial was conducted between 2011 and 2013 in the Departments of Cardiology and Cardiac Surgery at the Silesian Medical Center in Katowice and included patients with severe AS and any symptoms that are related to AS. The exclusion criteria included actual indications for coronary revascularization, segmental wall motion abnormalities, a bad acoustic window (5 or more segments that could not be analyzed), non-sinus rhythm, a bicuspid AV, moderate/severe mitral regurgitation, acute and chronic inflammatory diseases including myocarditis and endocarditis (in the 3 preceding months), NYHA IV, dilatation of the aortic root, Marfan syndrome, acute coronary syndromes, recurrent supraventricular and ventricular arrhythmias, acute and chronic kidney disease (glomerular filtration rate < 60 mL/min/1.73 m^2), malignancies, autoimmune diseases, immunosuppressive therapy, coexisting psychiatric or neurological disorders and alcohol or drug abuse. The study protocol was approved by the local Bioethics Committee. Each patient gave written consent to participate in the study.

Clinical data

The clinical characteristics of the patients in the study included their clinical status (NYHA/CCS class), anthropometric data (height, BMI, waist to hip ratio [WHR]), a physical examination and medical history (concomitant diseases, pharmacotherapy and smoking status). Body composition was determined by an impedance analysis using Bodystat.

Laboratory tests

Blood samples (10 mL) were drawn from the peripheral vein from patients in the supine decubitus position in the morning after an overnight fast. Total cholesterol, high- and low-density lipoprotein cholesterol fractions, triglycerides and creatinine serum concentrations were measured using routine methods.

Serum levels of adipokines (intra-assay variability ranged: 6–9%) were measured using an enzyme-linked immunosorbent assay. A single-use kit was used in order to avoid inter-assay variability.

Coronary angiography

Coronary angiography was performed in all patients. A diagnosis of CAD was established in cases of a ≥ 70% diameter narrowing in at least one of the three major epicardial coronary arteries.

Ultrasound assessment

Two-dimensional transthoracic echocardiography was performed in all of the patients by an experienced sonographer according to guidelines of the European Society of Echocardiography [21]. An ultrasound system (GE Vivid 9) equipped with a 3.5–1.75 MHz transthoracic transducer was used for all of the patients.

Left ventricular geometry and function

Two-dimensional M-mode echocardiography was used to measure LV dimensions in the left parasternal long-axis view. The LV end-diastolic diameter (LVEDD), posterior wall (PW) and septal wall thickness (IVS) were measured at the end-diastole (d). The LV end-systolic diameter (LVESD) was also obtained. The LV mass (LVM) was calculated according to the following formula: $LVM = 1.04 \times \times [(LVEDD + IVS + PW)^3 - LVEDD^3] - 13.6$ and indexed for body surface area (BSA) to obtain the LVM index (LVMI). The values of LV end-diastolic (LVEDV) and end-systolic volume (LVESV) were measured using the Simpson method; LVEF, stroke volume (SV) and LV cardiac output (CO) were also obtained. The values of the LVM, SV and CO were indexed for body surface area (BSA).

Severity of aortic valve stenosis/effective orifice area of the implanted prosthesis

Maximal (Pmax) and mean (Pmean) transvalvular pressure gradients were obtained using a modified Bernoulli equation and the effective orifice area (EOA) was measured using the continuity equation. The LV outflow tract (LVOT) dimension was measured three to four times and finally the mean LVOT value was used in the automatically calculated EOA. In order to avoid any mistakes, we compared EOA calculated by VTI and the Vmax and SV measured by Simpson's formula and this was obtained using the Bernoulli equation. The values of the EOA were indexed for BSA. The valvuloarterial impedance (Zva) was calculated according to the recommended equation [21].

Noninvasive markers of vascular remodeling
Flow-mediated dilatation (FMD)

The examinations were performed in the morning in a room at 22°C after a 15-min rest and an overnight fast. Smoking cigarettes was not allowed for the previous 24 h. The subjects lay supine with their heads slightly extended. All of the measurements of brachial arteries (BA) were performed above the antecubital fossa in the longitudinal plane using a sphygmomanometric cuff on the proximal portion of the arm. The right BA was scanned at rest, during reactive hyperemia and following the administration of sublingual nitroglycerin (0.5 mg). Reactive hyperemia was induced with the inflation of a sphygmomanometer cuff to 200 mm Hg in order to occlude arterial inflow for 3 min. BA diameter (BAd) and blood flow were obtained during the 50–60 s after cuff deflation. Blood flow was measured from the pulsed Doppler signal and arterial diameters were taken from the anterior to the posterior "M" line at the end diastole. Images were acquired with electrocardiogram gating with the end of diastole corresponding to the onset of the R wave. The baseline and after-cuff deflation measurements were used for the FMD calculation (percent increase of the artery diameter compared to the baseline results) estimation. Endogenous vasodilatory capability, independent of BAd, was defined as the FMD × BAd index and calculated for allsubjects.

Pulse wave velocity (PWV) assessment

Two pulse waves were obtained transcutaneously at the base of the neck for the right common carotid artery and over the right femoral artery. Transit time was measured as the time between the foot of the pulse wave and the foot of the R wave. Pulse transit time was determined as the average of 10 consecutive beats. Time delay (t) was calculated as the difference between these two transit times. The distance (d) traveled by the pulse wave was measured over the body surface as the distance between two recording sites. Pulse wave velocity was calculated as PWV = d (meters)/t (seconds).

Augmentation index (AIx)

The examination was performed using the applanation tonometry — Applanation (SphygmoCor system created by the Australian company AtCorMedical).

The test was performed in a lying position after a 5-min adaptation with the inner side of the hand facing upward. The tonometer head was placed on the wrist at the most palpable pulse. The system processed a registered pulse wave of the radial artery and marked the pulse curve in the ascending aorta.

Table 1. Characteristics of the study group and controls.

Variable	AS patients (n = 65)	Controls (n = 24)	P
Women/men	38 (58%) / 27 (42%)	14 (58%) / 10 (42%)	0.7
Age [years]	68.3 ± 9	69.1 ± 8.1	0.9
NYHA class I/II/III	20 (31%) / 37 (57%) / 8 (12%)	6 (25%) / 17 (71%) / 1 (4%)	0.9
CCS class 0/1/2/3	15 (23%) / 20 (31%) / 28 (43%) / 2 (3%)	4 (17%) / 8 (33%) / 12 (50%) / 0	0.9
Height [cm]	164 ± 10	165 ± 11	0.9
Weight [kg]	78.0 ± 13.7	77.1 ± 14.1	0.9
BMI [kg/m^2]	29.6 ± 4.3	28.3 ± 4.3	0.5
BSA [m^2]	1.87 ± 0.21	1.86 ± 0.22	0.9
WHR [cm]	0.93 ± 0.1	0.92 ± 0.06	0.9
Body fat (%)	33.1 ± 2.8	32.8 ± 3.1	0.8
Heart rate [bpm]	68.0 ± 10.4	67.3 ± 8.1	0.9
Systolic BP [mmHg]	127.1 ± 21.7	124.2 ± 17.8	0.9
Diastolic BP [mmHg]	82.6 ± 12.1	74.5 ± 7.4	0.7
Coronary artery disease	26 (40%)	12 (50%)	0.7
Hypertension	53 (82%)	17 (71%)	0.8
Diabetes	16 (25%)	8 (33%)	0.7
Current smoking	6 (9%)	4 (17%)	0.7
Smoking history	12 (18%)	7 (29%)	0.7
Triglycerides [mg/dL]	138 ± 48	142 ± 55	0.7
Total cholesterol [mg/dL]	188 ± 51	184 ± 47	0.8
HDL cholesterol [mg/dL]	45 ± 12	48 ± 14	0.8
LDL cholesterol [mg/dL]	112 ± 38	117 ± 37	0.9
Glomerular filtration rate [mL/min]	87.8 ± 12.3	89.6 ± 8.3	0.9

Data are presented as the mean ± standard deviation or number (percentage); AS — aortic stenosis; BMI — body mass index; BSA — body surface area; BP — blood pressure; CCS — Canadian Cardiovascular Society grading of angina pectoris; HDL — high-density lipoprotein cholesterol; LDL — low-density lipoprotein cholesterol; NYHA — New York Heart Association functional classification; WHR — waist-to-hip ratio

The analyzed susceptibility of the vessels was defined by the following parameters: central systolic and diastolic blood pressure levels (aortic systolic and diastolic pressure), central pulse pressure (aortic pulse pressure) and the value of the index gain (AIx).

Statistical analysis

Statistical analysis was performed using Med-Calc for Windows, version 10.0. All of the text and table results are expressed as means ± standard deviation (SD) or as a number (percentage). The normal distribution result was analyzed using the Kolmogorov-Smirnov test. In the case of abnormal distribution, a logarithmic transformation was used.

The baseline clinical parameters and the results of the ancillary investigations were compared using the two-sample t-tests for normally distributed continuous variables (Student's t-test); in the case of an abnormal distribution, the Mann-Whitney U test was used. Categorical variables were compared using the χ^2 test. The Spearman rank-order test or Pearson correlations were used to determine the relationship between the variables. Multivariable linear regression was used to assess the independent predictors of the adipokine levels; parameters that were associated with plasma adipokines at the level of p < 0.1 on the univariate analysis were analyzed. A value of p < 0.05 was considered statistically significant.

Results

Clinical data

The clinical characteristics of AS patients and controls did not reveal any significant differences (Table 1).

There were no significant differences in LVESV, LVEDV, LVEF as well as in CO and the cardiac index (CI) between the patients with AS and the CG. LVMI was obviously significantly higher in the AS group (150.4 ± 32.6 vs. 115.2 ± 21.7 g/m^2).

Table 2. Results of transthoracic echocardiography in the study group and controls.

Variable	AS patients (n = 65)	Controls (n = 24)	P
LVESV [mL]	47.0 ± 19.1	47.8 ± 17.1	0.92
LVEDV [mL]	115.9 ± 32.6	118.4 ± 39.1	0.91
LVEF [%]	60.5 ± 6.5	60.4 ± 6.4	0.95
CO [L/min]	4.4 ± 1.2	4.8 ± 1.7	0.87
CI [L/min/m^2]	2.7 ± 0.7	2.8 ± 0.8	0.91
LVMI [g/m^2]	150.4 ± 32.6	115.2 ± 21.7	0.009
AV anulus [mm]	21.5 ± 1.9	22.9 ± 2.1	0.013
Pmax [mmHg]	95.8 ± 24.7	–	–
Pmean [mmHg]	58.6 ± 16.6	–	–
iEOA [cm^2/m^2]	0.37 ± 0.08	–	–
Zva [mmHg/mL/m^2]	5.6 ± 1.1	–	–

Data are presented as the mean ± standard deviation; AS — aortic stenosis; AV — aortic valve; CO — cardiac output; iEOA — indexed effective orifice area; LVEF — left ventricular ejection fraction; LVEDV — left ventricular end-diastolic volume; LVESV — left ventricular end-systolic volume; LVMI — left ventricular mass index; Pmax — maximum gradient across aortic valve; Pmean — mean gradient across aortic valve; Zva — valvuloarterial impedance

Table 3. Baseline vascular parameters of the study groups.

	AS patients (n = 65)	Controls (n = 24)	P
FMD [%]	11.57 ± 5.8	13.22 ± 5.2	0.205
BAd [mm]	3.46 ± 0.5	3.58 ± 0.6	0.799
FMD × BAd	36.28 ± 8.5	43.51 ± 9.8	0.043
PWV [m/s]	9.9 ± 2.7	7.8 ± 3.2	0.089
Aortic SP [mmHg]	123.2 ± 10.5	131.2 ± 11.2	0.744
Aortic DP [mmHg]	69.3 ± 15.2	72.5 ± 11.6	0.804
AP [mmHg]	49 ± 14.1	56.4 ± 12.3	0.462
AIx [%]	33.0 ± 12.8	33.0 ± 12.8	0.281

Data are presented as the mean ± standard deviation; AS — aortic stenosis; AIx — augmentation index; AP — arterial pressure; BAd — brachial artery diameter; DP — diastolic pressure; FMD — flow-mediated dilatation; PWV — pulse wave velocity; SP — systolic pressure

The AV annulus was significantly smaller in the AS group (21.5 ± 1.9 vs. 22.9 ± 2.1 mm, p = 0.013). The mean maximum AV gradient (Pmax) in the AS group was 95.8 ± 24.7 mmHg while the mean gradient (Pmean) was 58.6 ± 16.6 mmHg. The indexed EOA was calculated for 0.37 ± 0.08 cm^2/m^2 in the AS group (Table 2).

There were no significant differences observed in the FMD value between the AS and the CG. There were also no differences in the BAd. However, the FMD × BAd index remained significantly higher in the CG.

There were also no significant differences in the PWV between the two examined groups. Aortic systolic, diastolic pressure and pulse were insignificantly higher in the CG. Value of the index and the gain remained same in both groups (Table 3).

Clinical data — PLFLG AS vs. NFHG AS

The mean age of both groups remained similar (68.5 ± 7.9 for PLFLG AS vs. 68.1 ± 10.2 for NFHG AS) as well as BMI (29.48 ± 4.2 vs. 29.8 ± ± 4.6 kg/m^2), WHR (0.9 ± 0.1 vs. 0.9 ± 0.1), systolic (133.9 ± 22.6 vs. 140.3 ± 20.5 mmHg) and diastolic (82.9 ± 12.3 vs. 82.3 ± 12.1 mmHg) blood pressure.

There were significant differences between the PLFLG AS and NFHG AS groups in CO (3.7 ± ± 0.8 vs. 5.6 ± 1.6 L/min, p < 0.001) and CI (2.1 ± ± 0.4 vs. 2.9 ± 0.6 L/min/m^2, p < 0.001), LVMI (153.2 ± 26.7 vs. 136.1 ± 31.2 g/m^2, p = 0.032) and Zva (6.2 ± 1.2 vs. 4.8 ± 0.9 mmHg/mL/m^2, p < 0.001). Differences were observed in Pmean values, which were significantly lower in the PLFLG AS group (51.1 ± 16.9 vs. 60.4 ± 16.3 mmHg,

Table 4. Serum adipokines levels in the study group and controls.

	AS patients (n = 65)	Controls (n = 24)	P
Adiponectin [µg/mL]	11.57 ± 5.8	11.59 ± 4.2	0.674
Leptin [ng/mL]	16.28 ± 13.5	21.51 ± 19.8	0.44
Visfatin [ng/mL]	2.93 ± 2.2	2.80 ± 2.2	0.734
Resistin [ng/mL]	6.37 ± 5.5	7.13 ± 4.6	0.281

Data are presented as the mean ± standard deviation; AS — aortic stenosis

p = 0.02). The mean values of AV annulus, Pmax and iEOA were similar.

There were no noticeable differences in PWV (7.5 ± 3.3 vs. 6.5 ± 2 m/s) and in FMD (15.3 ± 11 vs. 18.6 ± 10%).

No significant differences were observed between all adipokine levels in patients with PLFLG AS vs. NFHG AS.

Serum adipokines levels in the study group and controls

There were no significant differences in the serum adiponectin, leptin, visfatin and resistin concentrations in the AS and CG (Table 4).

Serum adipokines levels in the study group regarding clinical characteristics

Patients with AS and co-existing CAD were characterized by decreased serum adiponectin (9.9 ± ± 5.5 vs. 12.7 ± 5.8 µg/mL, p = 0.040) and leptin (8.3 ± 7.8 vs. 21.6 ± 17.1 ng/mL, p < 0.001) levels compared to subjects without CAD. There were no significant differences in visfatin (2.4 ± 2.0 vs. 3.3 ± 2.4 ng/mL, p = 0.074) and resistin levels (5.00 ± 4.2 vs. 7.28 ± 6.1 ng/mL, p = 0.102) (Table 5).

There were no significant differences in serum adipokines concentrations between patients with concomitant HA. Patients with AS and DM had an insignificantly lower adiponectin level (10 ± 5.9 vs. 12.26 ± 5.6, p = 0.052 µg/mL) (Table 5).

There were also no significant differences in the serum adipokine concentrations between patients with PLFLG AS and NFHG AS (Table 5).

Regression analysis

The adiponectin levels correlated with age (r = 0.464, p < 0.001), BMI (r = –0.334, p = = 0.006), body fat percentage (r = –0.315, p = 0.011), LVMI (r = –0.256, p = 0.039), LV SVi (r = –0.327, p = 0.008) and the E/E' index (r = –0.268, p = 0.022) (Table 6).

Leptin levels correlated with BMI (r = 0.456, p < 0.001), body fat percentage (r = 0.522, p < 0.001) and iEOA (r = –0.290, p = 0.019) (Table 6).

Multivariate regression analysis indicated age (F = 3.02; p = 0.015) and the E/E' index (F = 0.87, p = 0.032) as independent predictors of the adiponectin level in the AS group.

Discussion

In the population with AS with preserved EF, some clinical and metabolic indices may play a pivotal role for the progression of the disease and its symptomatology. This is why the serum adipokines concentrations were compared between AS patients and the controls — there were no differences observed. Moreover, the hypothesis that the CAD, HA, DM, vascular parameters influenced the adipokine serum levels was verified. This verification was a negative value in regard to the HA, DM and vascular indices. Patients with AS and co-existing CAD were characterized by decreased adiponectin and leptin levels compared to the subjects without CAD. This is an important finding of the study, especially that low serum levels of adiponectin have been recognized as a risk factor for atherosclerotic disease [22]. It should be noted that the protective mechanism of adiponectin, which has an influence on the progression of atherosclerosis and is associated with increasing apolipoprotein A1 and high-density lipoprotein cholesterol, which have beneficial effects on lipid metabolism [23].

When discussing the role of adiponectin in AS patients, we may take into account the data that describes the adiponectin levels in non-AS patients. In the literature, there are mainly data on adiponectin levels in patients with CAD [24, 25] or metabolic disorders [26] but limited data on the role of adiponectin in AS pathogenesis. Low adiponectin concentrations are well documented in patients with CAD [24]. Low adiponectin contributes to coronary plaque vulnerability [25]. In this study, patients with AS and co-existing CAD were characterized by decreased serum adiponectin and leptin levels compared to subjects without CAD. It was suspected that the same CAD-related mechanism of hypoadiponectinemia was present in the presented group.

Table 5. Serum adipokines levels in the study group regarding clinical characteristic.

Coronary artery disease (CAD)	CAD (+)	CAD (–)	P
Adiponectin [µg/mL]	9.87 ± 5.5	12.7 ± 5.8	0.040
Leptin [ng/mL]	8.34 ± 7.8	21.56 ± 17.1	0.001
Visfatin [ng/mL]	2.38 ± 2.0	3.31 ± 2.4	0.074
Resistin [ng/mL]	5.00 ± 4.2	7.28 ± 6.1	0.102
Arterial hypertension (AH)	**HA (+)**	**HA (–)**	**P**
Adiponectin [µg/mL]	11.64 ± 5.8	11.26 ± 5.9	0.754
Leptin [ng/mL]	16.35 ± 15.3	15.95 ± 13.9	0.741
Visfatin [ng/mL]	2.99 ± 2.1	2.70 ± 2.1	0.227
Resistin [ng/mL]	6.40 ± 5.4	6.23 ± 6.1	0.750
Diabetes mellitus (DM)	**DM (+)**	**DM (–)**	**P**
Adiponectin [µg/mL]	9.95 ± 5.9	12.26 ± 5.6	0.052
Leptin [ng/mL]	10.62 ± 8.9	18.13 ± 16.9	0.183
Visfatin [ng/mL]	2.87 ± 2.4	2.96 ± 2.2	0.957
Resistin [ng/mL]	7.53 ± 6.7	5.99 ± 4.6	0.654
Stroke volume index (SVi)	**SVi < 35 mL/m² (PLFLG AS)**	**SVi ≥ 35 mL/m² (NFHG AS)**	**P**
Adiponectin [µg/mL]	12.19 ± 5.4	10.92 ± 6.2	0.259
Leptin [ng/mL]	17.50 ± 14.1	15.02 ± 6.9	0.311
Visfatin [ng/mL]	3.09 ± 2.4	1.78 ± 2.1	0.507
Resistin [ng/mL]	6.76 ± 4.6	5.96 ± 3.9	0.718

Data are presented as the mean ± standard deviation; PLFLG AS — paradoxical low-flow low-gradient aortic stenosis; NFHG AS — normal flow high gradient aortic stenosis

Table 6. Regression analysis in the study group with adipokines as dependent variables.

	Correlation coefficient	P
Adiponectin		
Age	0.464	< 0.001
Body mass index	–0.334	0.006
Body fat%	–0.315	0.011
Left ventricular mass index	–0.256	0.039
Stroke volume index	–0.327	0.008
E/E′	–0.268	0.022
Leptin		
Bod mass index	0.456	< 0.001
Body fat%	0.522	< 0.001
Indexed effective orifice area	–0.290	0.019
Resistin		
Pulse wave velocity	0.269	0.033

The available data on the role of adipokines in AS are limited to some problems. Recently, Gucuk Ipek et al. [27] showed, similar to the present findings, that there is no relationship between the adiponectin levels and calcific AVD. In contrast, Kolasa-Trela et al. [28] published an article suggesting that adipokines may be involved in the progression of AS. In 2010, Mohty et al. [29] observed that older patients with AS had a higher plasma level of resistin, which was associated with the degree of valvular calcification and inflammation. In their next paper, they showed that adiponectin may play a protective role against the inflammatory process and progression of calcific AVD [30]. They hypothesized that adiponectin might be partly responsible for the association between metabolic syndrome and calcific AVD. Concentrations of adiponectin were persistently reduced in patients with metabolic syndrome and this was the first demonstration of its negative association with the progression of AS. This observation is in accordance with the present findings and from a practical point of view — the reduced circulating level of adiponectin may be associated with a more rapid progression of AS stenosis and enhanced valvular inflammation.

The next step of analysis involved the hemodynamic profiles of patients with AS and preserved EF.

Well-documented literature data as well as our previous findings indicated differences in demographics, the frequency of concomitant disease and systemic vascular resistance between populations with NFHG and PLFLG AS. Patients with PLFLG AS are characterized by a higher frequency of females, HA, LV hyperthrophy, increased systemic vascular resistance and finally lower SVi [19, 20]. Although our subpopulation with PLFLG AS had a limited number of patients, it was representative of the above-mentioned characteristics. Regardless of different clinical characteristics of NFHG and PLFLG patients, there were no differences in adipokines concentrations in these groups.

Adiponectin, and also leptin concentrations, correlated with standard obesity indices (BMI, body fat%). This observation is in concordance with previous data. It was demonstrated that low-level adiponectin subjects were characterized by a significantly higher prevalence of some cardiometabolic comorbidities (obesity, visceral obesity, DM, insulin resistance, LV hypertrophy, metabolic syndrome, CAD) [26, 31, 32].

Interesting relationships were observed with regard to the adiponectin levels. Adiponectin levels correlated positively with age and negatively with BMI, body fat, LVMI, SVi and the E/E' index. Multivariate regression analysis confirmed that age and the E/E' index were independent predictors of the adiponectin level in the AS group. Thus, the diastolic dysfunction that is typical for AS influenced adiponectin concentration.

Stojanovic et al. [31], similar to the present findings, found that adiponectin levels correlated with age, BMI and the E/E' index in patients with metabolic syndrome and/or CAD. On the other hand, in patients with hypertrophic cardiomyopathy, plasma adiponectin levels were associated with an impaired LV systolic function but only slightly with E/E' index [33].

Positive correlations were found in leptin levels with BMI and body fat%. Generally, leptin, which is a mediator of the long-term regulation of energy balance, suppresses food intake [34]. Subjects with high plasma leptin levels have a better prognosis, thus suggesting a protective role of leptin in overweight/mild obesity [35]. On the other hand, overweight and obesity are associated with hyperleptinemia secondary to an impaired sensitivity of leptin receptors [36]. Recently, Karmazyn et al. [37] concluded that leptin is a cardiac hypertrophic factor and hyperleptinemia is associated with cardiovascular risk, especially as it pertains to heart failure. This can be of importance in regards to AS-mediated hypertrophy and/or CAD. As presented above, the presented subjects with AS and CAD were characterized by increased serum leptin levels compared to AS patients without CAD. Further studies are needed to evaluate these interesting concepts.

Regarding adipokines levels, it should also be taken into account the specificity of the population herein — relatively older subjects with AS. Schautz et al. [38] concluded that age-related changes in leptin and adiponectin levels are opposed to each other and are partly independent of adiposity and body fat distribution.

Limitations of the study

A limitation of the current study could be the relatively small number of patients. It should be stressed, however, that the study was conducted in a prospective fashion, which undoubtedly represents its methodological strength. It is also agreed that the division of the AS cohort into the subgroups PLFLG AS and NFHG AS had limitations related to the number of subjects. However, a well-known PLFLG AS clinical characteristic suggested an abnormal adipokine profile compared to NFHG AS.

An echocardiographic evaluation, especially in older, obese patients, has limitations regarding the LVOT measurements, and finally the AS severity calculation. These problems were apparent, this is why all echocardiographic measurements were done very precisely by an experienced sonographer. Vascular response to sublingual nitroglycerin was not assessed because of the severity of AS. Moreover, adipokines levels were measured at one point only.

Conclusions

Severe degenerative AS with preserved EF is not associated with any change in the adipokine serum profile. Adipokines serum levels are modified by co-existing atherosclerosis but not the typical cardiovascular risk factors or the hemodynamic type of AS. The adiponectin serum level in patients with severe AS with preserved EF is related to age and LV diastolic dysfunction.

Conflict of interest: None declared

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ORIGINAL ARTICLE

How does the risk of cardiovascular death and cardiovascular risk factor profiles differ between socioeconomic classes in Poland: A country in transition

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Abstract

Background: *Socioeconomic status (SES) is an important factor for cardiovascular diseases (CVD) development. A decline in death rate from CVD among subjects with high SES is observed in developed countries. The aim of this study was to assess differences in cardiovascular risk (CV) between socioeconomic classes in Poland, a country currently in transition.*

Methods: *A sample of 15,200 people was drawn. A three stage selection was performed. Eventually, 6170 patients were examined (2013/2014). Data was collected using a questionnaire in face-to-face interviews, anthropometric data and blood tests were also obtained. Education was categorized as incomplete secondary, secondary and higher than secondary school. Monthly income per person was categorized as low (≤ 1000 PLN), medium (1001–2000 PLN) and high (≥ 2001 PLN). Education and income groups were analyzed by prevalence of CVD risk factors and high CVD risk (SCORE ≥ 5%).*

Results: *Higher education was associated with lower prevalence of all analyzed CVD risk factors (p < 0.001), having the highest income with lower prevalence of hypertension, currently smoking, obesity and lower high density lipoprotein cholesterol. Multivariable analysis showed that frequency of high CVD risk decreased with increasing education level (OR 0.61; 95% CI 0.49–0.76; p < 0.01), a similar favorable impact of higher income on high CVD risk was demonstrated in the whole group (OR 0.81; 95% CI 0.67–0.99; p = 0.04).*

Conclusions: *Socioeconomic status is an independent predictor of high CV risk of death. A favorable impact on the prevalence of high CV risk was demonstrated for education and partly for income in the whole group. It may reflect a transition being undergone in Poland, moreover, it predicts how socioeconomic factors may generate health inequalities in other transitioning countries.* (Cardiol J 2019; 26, 5: 493–502)

Key words: socioeconomic, education, income, cardiovascular risk factors, cardiovascular disease

Introduction

Cardiovascular diseases (CVD) remain a leading cause of morbidity and mortality, despite improvements in treatment outcomes. Age-adjusted coronary artery disease (CAD) mortality has declined since the 1980s, particularly in high-income regions in Europe. However, inequalities between countries persist and prevalence of many risk factors, particularly obesity and diabetes mellitus (DM), have been increasing substantially [1]. It is estimated that ≥ 80% of all CVD mortality now occurs in developing countries [2].

Cardiovascular diseases morbidity and mortality are affected by social, environmental and economic factors. Socioeconomic status (SES) focused attention as an important factor related to CVD. During the past decades a widening of the relative gap in death rates between upper and lower socioeconomic groups has been reported for several European countries [3]. In Poland, the social gradient of CVD mortality has increased since the onset of economic transition. At the beginning of this period (1991–1993), the mortality of men with primary education was 2.2 times higher than for men with higher education, but in the (years) 2010–2012 it became 4.2 times higher. Additionally, the death rate of men with higher education decreased by 62% while for men with primary education by only 28% [4]. In epidemiological studies, education and income are determinants commonly used in SES evaluation. Education is the most widely used SES indicator as it is constant throughout life and its measurement is relatively easy, moreover, objective. Income is also an important indicator as it determines access to material goods and services, including medical care.

Recently published data indicate that low SES is related to increased morbidity and CVD mortality [5, 6]. An unhealthy lifestyle and prevalence of premature CVD are more common in lower socioeconomic groups [7].

To estimate the risk of CVD development, various models of multifactorial risk assessment have been proposed, Systematic Coronary Risk Evaluation (SCORE) algorithm is the most widely used [8]. The high or very high risk group included subjects with a likelihood of CVD death within 10 years ≥ 5%, and with significantly increased values of single risk factors, DM and moderate to severe chronic kidney disease (CKD) (glomerular filtration rate < 60 mL/min/1.73 m^2) and those with already diagnosed CVD [1]. Poland, which is considered a high risk country, using current CVD mortality rates and data on major CVD risk factors, prevalence in the Polish population, has recently updated SCORE tables (Pol-Score 2015) [9].

In this paper the aim was to examine the relationship between SES and cardiovascular risk assessed by SCORE algorithm and assess differences in CVD risk between socioeconomic classes in Poland, the first Eastern Bloc country which adopted political and economic changes and was still in transition.

Methods

Study population and design

This Multi-center National Population Health Examination Survey (WOBASZ II study) was carried out in Poland in 2013 and 2014. The WOBASZ II study is a cross-sectional study consisting of a random sample of 15,200 Polish residents above the age of 19. A sample of both genders was drawn from the national, electronic population register (PESEL) at the Department of State Registers of the Ministry of the Interior. The selection was performed as a three stage sampling, stratified according to administrative units (voivodships), type of urbanization and gender. For each voivodeship: 2 small communities (below 8000 citizens), 2 medium communities (8000–40, 000 citizens) and 2 large communities (over 40, 000 citizens) were selected. In each community persons above the age of 19, 70 women and 70 men were drawn. The total drawn sample size was 15,120 men and women. Finally, 6170 participants (2752 men and 3418 women) were examined, response rate exceeded 45% [10]. Additionally, a subpopulation was distinguished as free of CVD (CAD, ischemic stroke and/or transient ischemic attack, peripheral artery disease), diabetes and CKD. In this group, consisting of 2482 subjects (1078 men and 1404 women), cardiovascular risk was evaluated based on the SCORE algorithm for high risk countries. The selection process is shown in Figure 1. All participants provided written consent and the study was approved by the Bioethical Committee.

The project consisted of a survey questionnaire, physical examination (blood pressure and heart rate measurements, anthropometric measurements: height, weight, waist circumference and hip circumference) and biochemical tests. Body mass index (BMI) [kg/m^2] and waist-to-hip ratio (WHR) were calculated. Subsequent to fasting, blood was collected from a vein to a disposable, vacuum tube, then centrifuged and frozen. Serum samples were transported on dry ice to a Central
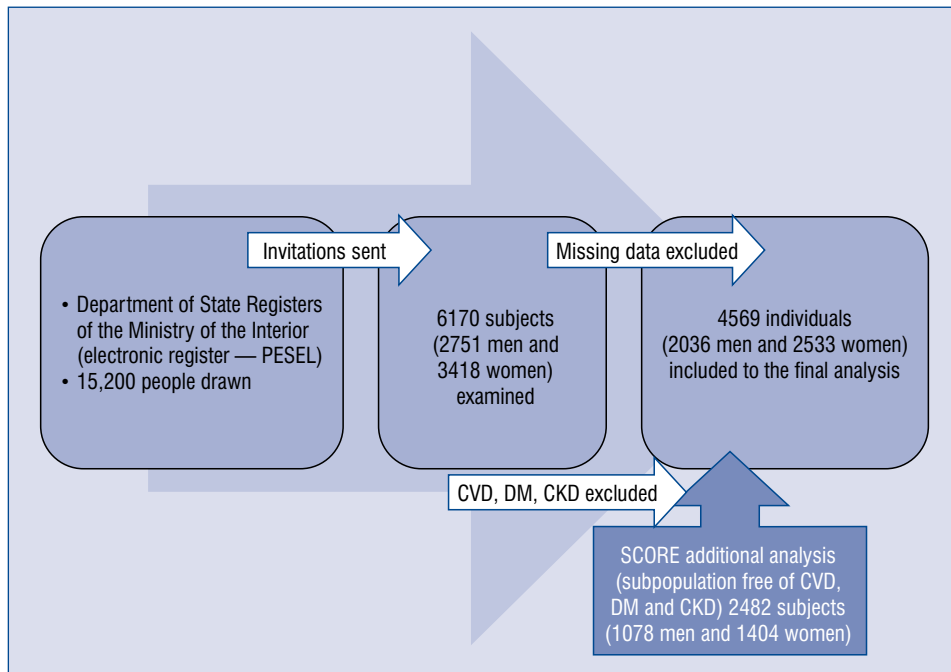
Figure 1. Patient flow chart; CKD — chronic kidney disease, CVD — cardiovascular diseases, DM — diabetes mellitus.

Laboratory, where all biochemical tests (glucose during fasting, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were conducted. All biochemical analyses were performed using analyzer Cobas 6000, by Roche. The project has been described in detail before [10].

Data analysis

One of the basic research tools used in the project was a comprehensive questionnaire, which included data on demographics, health knowledge, working status, education, income, physical activity, cigarette smoking, and a detailed medical history.

In the present study, data on education and income was used. The questionnaire included 9 categories of education (lack of education, primary, middle school, vocational after primary school, vocational after high school, high school/technical, secondary, bachelor degree and higher and 7 income categories determined by monthly net income per person in the household: less than 500 PLN, 501–1000 PLN, 1001–1500 PLN, 1501–2000 PLN, 2001–2500 PLN, 2501–3000 PLN, above 3000 PLN. Three groups of education were distinguished for analysis: incomplete secondary, secondary and higher than secondary. Regarding monthly net income per person in the household,

3 income groups were distinguished: low (below 1000 PLN), medium (1001–2000 PLN) and high (above 2001 PLN). The lower limit of income was determined by the value of social minimum, which, according to the Central Statistical Office, in 2013 amounted to 1061 PLN. In the defined education and income groups, the prevalence of classic CVD risk factors were analyzed.

Smoking status was defined as follows: current smokers included individuals who smoked at least 1 cigarette a day, ex-smokers were considered as subjects who smoked cigarettes regularly for at least 1 year in the past, but currently do not smoke, non-smokers included participants who have never smoked or smoked cigarettes for less than 1 year in the past. Hypertension diagnosis was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and//or diastolic blood pressure (DBP) $\geq$ 90 mmHg, previously diagnosed hypertension and currently under antihypertensive treatment. Obesity and overweight were defined by BMI (overweight: BMI 25.0–29.9 kg/m^2; obesity: BMI $\geq$ 30.0 kg/m^2). Abdominal obesity was diagnosed according to the WHR ($\geq$ 0.90 men, $\geq$ 0.85 women).

The lipid disorder diagnosis was based on the European Guidelines on cardiovascular disease prevention in clinical practice [1] and included hypercholesterolemia (total cholesterol $\geq$ 5 mmol/L), high LDL-C (LDL levels $\geq$ 3 mmol/L), low HDL-C

(HDL levels < 1 mmol/L in men and < 1.2 mmol/L in women), hypertriglyceridemia (triglyceride ≥ 1.7 mmol/L) and actual lipid-lowering treatment. Diagnosis of diabetes was based on medical history, medication use, and fasting serum glucose level above 126 mg/dL.

Additionally, in a subpopulation free of CVD consisting of 2482 subjects, evaluated cardiovascular risk was based on the SCORE algorithm. Then, in defined education and income groups, the prevalence of high and very high CVD risk was analyzed (SCORE ≥ 5%). The high risk group included both "high" and "very high" risk according to SCORE.

Statistical analysis

Categorical variables were summarized by counts and percentages. Statistical significance of between-group differences was calculated by χ^2 test. The Cochran-Armitage test for trends was used to test the trend in contingency tables. To examine significance of CVD risk factor interactions and the independent influence of education and income on high CVD risk (SCORE ≥ 5%) in age and gender categories, multivariable analysis for gender, education and income status was performed, using multiple logistic regression. Logistic odds ratio (OR) and their 95% confidence intervals (95% CI) adjusted for gender, education and income status were calculated. All statistical analyses were computed using SAS, version 9.4 (SAS Institute Inc., Gary, NC), with the statistical significance level at $\alpha = 0.05$.

Results

Study sample characteristics

After excluding subjects with missing data, 4569 individuals (2036 men and 2533 women) were included in the final analysis. High prevalence of classic CVD risk factors was observed in the whole sample. It is a remarkable that hypercholesterolemia, including increased LDL-C (OR = 58.1%; 95% CI 56.6–59.5% and OR = = 50.5%; 95% CI 49.1–52.0%) and abdominal obesity (OR = 56.3%; 95% CI 54.9–57.8% in the whole sample and OR = 71.6%; 95% CI 69.6– –73.5% in men) were found in more than a half of participants (Table 1). Higher education was substantially more frequent in women in comparison with men (OR = 24.5%; 95% CI 22.8– –26.2% vs. OR = 18.8%; 95% CI 17.1–20.5%; p < 0.01). Men more frequently than women declared the highest income OR = 17.8%; 95% CI

16.1–19.4% vs. OR = 11.3%; 95% CI 10.1–12.5%; p < 0.01) (Table 1).

CVD risk factors and education

Prevalence of CVD risk factors by education level is presented in Table 2. There was a strong relationship between CVD risk factors and education. Higher education was associated with a lower prevalence of all CVD risk factors taken into account (p < 0.001). CVD risk factors were the most common in the incomplete secondary education group. The prevalence of hypertension and obesity (by BMI) in persons with incomplete secondary education was twice that compared to the higher education group (OR = 51.6%; 95% CI 49.4–53.9% vs. OR = 24%; 95% CI 21.4–26.7% and OR = 32%; 95% CI 30–34.2% vs. OR = 16%; 95% CI 13.7–18.2%, respectively, p < 0.0001), and almost five times higher for those with DM (OR = = 16.7%; 95% CI 15–18.3% vs. OR = 3.5%; 95% CI 2.4–4.6%; p < 0.0001) (Table 2).

CVD risk factors and income

Distribution of CVD risk factors in the income groups was ambiguous, compared to education-related ones. The highest income was associated with a lower prevalence of hypertension, current smoking, obesity and lower HDL-C but with a higher prevalence in former smokers, overweight and non-HDL lipid disorders, however results for dyslipidemias were not statistically significant (border significant for increased LDL-C, p = 0.06) (Table 3).

High CVD risk (SCORE) in categories of education and income

In a subgroup of participants free of CVD, DM and CKD, SCORE risk was evaluated. Higher education was associated with a lower frequency of high CVD risk of death (Fig. 2), results for income were not statistically significant (p = 0.14).

Multivariable logistic regression model. Odds ratio for CVD risk factors prevalence according to age, gender, education and income

Almost all of analyzed CVD risk factors (except hypercholesterolemia) were more common in men, especially smoking (OR = 3.25; 95% CI 2.79–3.78 for former smoker and OR = 2.29; 95% CI 1.97–2.66 for current smoker; p < 0.001) and abdominal obesity (OR = 3.57; 95% CI 3.12–4.09; p < 0.001). Similarly, almost all of them (except current smoker) were more common in older

Table 1. Study group characteristics.

Variable	Whole group	Men	Women	P
Gender	4569 (100%)	2036 (44.5%)	2533 (55.5%)	
Age [years]:				
19–29	568 (12.4%)	283 (13.9%)	285 (11.2%)	0.06
30–39	809 (17.7%)	357 (17.5%)	452 (17.8%)	
40–49	778 (17.0%)	353 (17.3%)	425 (16.7%)	
50–59	979 (21.4%)	419 (20.5%)	560 (22.1%)	
60–69	878 (19.2%)	395 (19.4%)	483 (19.0%)	
≥ 70	557 (12.1%)	229 (11.2%)	328 (12.9%)	
CVD risk factors				
Former smoker	1198 (26.2%)	645 (31.7%)	553 (21.8%)	< 0.01
Current smoker	1143 (25.0%)	685 (33.7%)	458 (18.0%)	
Hypertension	1877 (41.1%)	866 (42.5%)	1011 (39.9%)	0.07
Overweight (BMI ≥ 25 kg/m^2)	1700 (37.2%)	888 (42.5%)	812 (32.0%)	< 0.01
Obese (BMI ≥ 30 kg/m^2)	1233 (26.8%)	533 (26.2%)	690 (27.2%)	
Abdominally obese (WHR)	2574 (56.3%)	1457 (71.6%)	1117 (44.1%)	< 0.01
Hipercholesterolemia	2653 (58.1%)	1164 (57.2%)	1489 (58.8%)	0.27
Increased LDL-C	2310 (50.5%)	1076 (52.8%)	1234 (48.7%)	< 0.01
Decreased HDL-C	939 (20.5%)	450 (22.1%)	489 (19.3%)	0.02
Hypertriglyceridemia	1259 (27.5%)	712 (35.0%)	547 (21.6%)	< 0.01
Diabetes	507 (11.1%)	250 (12.3%)	257 (10.1%)	0.02
SCORE ≥ 5% (n = 2482)	644 (25.9%)	464 (43.0%)	180 (12.8%)	< 0.01
Education groups				
Incomplete secondary	1902 (41.6%)	942 (46.2%)	960 (37.9%)	< 0.01
Secondary	1665 (36.4%)	712 (35.0%)	953 (37.6%)	
Higher than secondary	1002 (21.9%)	382 (18.8%)	620 (24.5%)	
Income groups				
Low (< 1000 PLN)	2074 (45.4%)	862 (42.3%)	1212 (47.8%)	< 0.01
Medium (1001–2000 PLN)	1848 (40.4%)	813 (39.9%)	1035 (40.9%)	
High (≥ 2001 PLN)	647 (14.2%)	361 (17.8%)	286 (11.3%)	

BMI — body mass index; CVD — cardiovascular diseases; HDL-C — high density lipoprotein cholesterol; LDL-C — low density lipoprotein cholesterol; SCORE — systematic coronary risk evaluation; WHR — waist-to-hip ratio

Table 2. Prevalence of cardiovascular diseases risk factors according to education level.

Risk factor	Education			P (a) vs. (b) vs. (c)
	Incomplete secondary (a)	Secondary (b)	Higher (c)	
Hypertension	982 (51.6%)	654 (39.3%)	241 (24.0%)	< 0.0001
Former smoker	519 (27.3%)	415 (24.9%)	209 (20.8%)	< 0.0001
Current smoker	564 (29.6%)	457 (27.4%)	177 (17.7%)	< 0.0001
Overweight (BMI ≥ 25 kg/m^2)	723 (38.0%)	626 (37.6%)	351 (35.0%)	< 0.0001
Obese (BMI ≥ 30 kg/m^2)	610 (32.0%)	453 (27.2%)	160 (16.0%)	< 0.0001
Abdominally obese (WHR)	1292 (67.9%)	886 (53.2%)	396 (39.5%)	< 0.0001
Diabetes	317 (16.7%)	155 (9.3%)	35 (3.5%)	< 0.0001
Hipercholesterolemia	1153 (60.6%)	978 (58.7%)	522 (52.1%)	< 0.0001
Hypertriglyceridemia	613 (32.2%)	447 (26.8%)	199 (19.9%)	< 0.0001
Increased LDL-C	1015 (53.3%)	849 (51.0%)	446 (44.5%)	< 0.0001
Decreased HDL-C	439 (23.0%)	322 (19.3%)	178 (17.7%)	0.001

BMI — body mass index; HDL-C — high density lipoprotein cholesterol; LDL-C — low density lipoprotein cholesterol; WHR — waist-to-hip ratio

Table 3. Cardiovascular disease risk factors according to income groups.

Risk factor	Income			P (a) vs. (b) vs. (c)
	Low (a)	Medium (b)	High (c)	
Hypertension	819 (39.5%)	816 (44.1%)	242 (37.4%)	0.001
Former smoker	471 (22.7%)	492 (26.6%)	180 (27.8%)	< 0.0001
Current smoker	609 (29.3%)	439 (23.8%)	150 (23.2%)	0.0001
Overweight (BMI ≥ 25 kg/m²)	735 (35.4%)	692 (37.4%)	273 (42.2%)	0.014
Obese (BMI ≥ 30 kg/m²)	555 (26.8%)	514 (27.8%)	154 (23.8%)	0.014
Abdominally obese (WHR)	1187 (57.2%)	1032 (55.8%)	355 (54.9%)	0.49
Diabetes	238 (11.5%)	209 (11.3%)	60 (9.3%)	0.27
Hipercholesterolemia	1204 (58.0%)	1053 (57.0%)	396 (61.2%)	0.17
Hypertriglyceridemia	564 (27.2%)	500 (27.0%)	195 (30.1%)	0.28
Increased LDL-C	1047 (50.5%)	910 (49.2%)	353 (54.5%)	0.06
Decreased HDL-C	469 (22.6%)	357 (19.3%)	113 (17.5%)	0.04

BMI — body mass index; HDL-C — high density lipoprotein cholesterol; LDL-C — low density lipoprotein cholesterol; WHR — waist-to-hip ratio
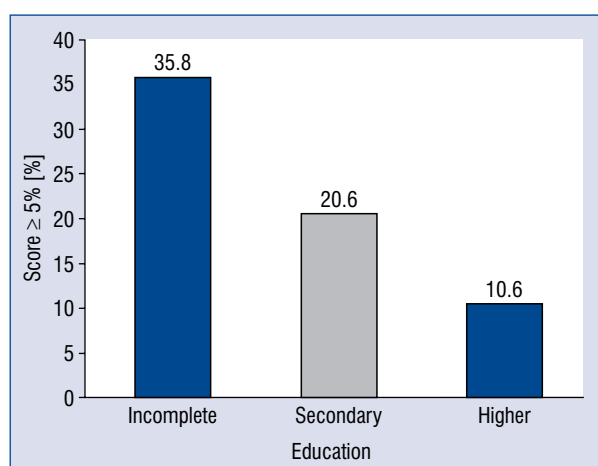
Figure 2. Prevalence of high cardiovascular disease risk (SCORE ≥ 5%) according to education level.

subjects. Higher education was independently associated with a lower prevalence of almost all investigated CVD risk factors, results for increased LDL-C were border significant (OR = 0.84; 95% CI 0.70–1.01; p = 0.06) (Table 4). Participants with higher income, showed an increased prevalence of overweight, obese (BMI ≥ 30), hypercholesterolemia, hypertriglyceridemia and increased LDL-C (p < 0.05) (Table 4), however this association was inverse for decreased HDL-C (OR = 0.74; 95% CI 0.57–0.95; p = 0.009) (Table 4).

High SCORE risk according to education and income

Higher education was independently associated with a lower prevalence of high CVD risk ac-

cording to SCORE (OR = 0.46; 95% CI 0.29–0.74; p = 0.01) (Table 5). Results for high SCORE risk according to income were not statistically significant (p = 0.5) (Table 5). High CV risk was also more common in men and in older subjects (Table 5).

SCORE high risk, education and income in different age and gender groups

We found important correlation between education and high CVD risk in the whole group and in the subgroups of men and women. The prevalence of high CVD risk decreased with increasing education level in the whole group (OR = 0.61; 95% CI 0.49–0.76; p < 0.01) and in subgroups of men (OR = 0.52; 95% CI 0.39–0.70, p < 0.01) and women aged 50–59 years (OR = 0.37; 95% CI 0.14–0.99, p = 0.05) (Fig. 3). Results for other subgroups were not statistically significant.

A significant, favourable impact of income on prevalence of high CVD risk was also demonstrated in the whole group (OR = 0.81; 95% CI 0.67–0.99; p = 0.04) and in the subgroup of men (OR = 0.69; 95% CI 0.53–0.90, p < 0.01), which means that subjects declaring a higher income have decreased prevalence of high CVD risk (Fig. 4). The analysis of the women subgroup and other age subgroups showed no significance (p ≥ 0.05).

Discussion

Socioeconomic status is an important factor related to CVD. However, the fact that it may be assessed in many different ways caused uncertainty as to which SES indicators would be the

Table 4. Odds ratio for cardiovascular disease risk factors prevalence according to age, gender, education and income.

Variable	Age			Gender (male vs. female)			Education (higher vs. incomplete)			Income (high vs. low)		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Hypertension	1.06	1.06–1.07	< 0.0001	1.20	1.05–1.37	0.007	0.68	0.55–0.85	0.005	1.32	1.06–1.66	0.05
Former smoker	1.01	1.01–1.02	< 0.0001	3.25	2.79–3.78	< 0.001	0.73	0.57–0.93	0.01	1.26	0.99–1.62	0.06
Current smoker	0.98	0.97–0.98	< 0.0001	2.29	1.97–2.66	< 0.001	0.32	0.25–0.41	< 0.001	1.04	0.81–1.33	0.74
Overweight (BMI ≥ 25)	1.03	1.03–1.04	< 0.0001	1.91	1.65–2.21	< 0.001	0.89	0.71–1.11	0.30	1.45	1.15–1.84	0.001
Obese (BMI ≥ 30)	1.04	1.04–1.05	< 0.0001	1.36	1.16–1.64	0.001	0.56	0.43–0.73	< 0.001	1.45	1.11–1.89	0.006
Abdominally obese (WHR)	1.03	1.03–1.04	< 0.0001	3.57	3.12–4.09	< 0.001	0.53	0.44–0.65	< 0.001	1.10	0.89–1.37	0.22
Diabetes	1.06	1.05–1.07	< 0.0001	1.30	1.07–1.59	0.007	0.41	0.27–0.62	0.001	1.35	0.96–1.91	0.10
Hypercholestero-lemia	1.02	1.01–1.02	< 0.0001	0.92	0.81–1.05	0.21	0.82	0.68–0.99	0.01	1.33	1.08–1.63	0.001
Hypertrigly-ceridemia	1.02	1.01–1.02	< 0.0001	1.92	1.69–2.22	< 0.001	0.60	0.48–0.75	< 0.001	1.38	1.11–1.72	0.002
Increased LDL-C	1.02	1.01–1.02	< 0.0001	1.17	1.04–1.33	0.008	0.84	0.70–1.01	0.06	1.31	1.07–1.61	0.001
Decreased HDL-C	1.01	1.01–1.02	0.0006	1.22	1.05–1.40	0.009	0.95	0.75–1.21	0.99	0.74	0.57–0.95	0.009

BMI — body mass index; CI — confidence interval; HDL-C — high density lipoprotein cholesterol; LDL-C — low density lipoprotein cholesterol; OR — odds ratio; WHR — waist-to-hip ratio

most objective. Most epidemiological studies use a single SES indicator such as education, income, wealth or professional status. In the current paper the aim was to investigate CVD risk factors profile and CVD risk in a large population of Polish citizens using both education and income. This study revealed a significant and clear relationship of higher education being associated with lower prevalence of all analyzed CVD risk factors. This results directly in the subsequent level of overall CVD risk. The negative relationship between education and CVD risk remained significant after multivariable adjustment. It was found that higher education was independently associated with lower prevalence of high CVD risk of death. These findings are consistent with previous data [11–19]. The biggest differences in CVD risk factor frequency in education categories were found for actual smokers and obese, similarly as in the Tromso study [13].

The CVD risk gradient is distinctly affected by factors related to lifestyle. There is substantial evidence in the literature confirming that negative health-related behaviors are more frequent in lower SES groups. Mejean et al. [14] demonstrated that diet and lifestyle factors explained more than 70% of educational differences in CAD. Healthy lifestyle among subjects with higher social status may also be partially explained by higher health awareness in this group [11]. Evidence in the literature revealed that CVD risk might also be affected by psychosocial risk factors, like depression, marital status, lack of social support or chronic work stress [11].

In western countries higher education usually involves professional and financial benefits. In former communist countries of Central and Eastern Europe, including Poland, there was an observed weak association between income and education, suggesting that education attainment effects are less likely to be mediated by underlying differences in financial resources [20]. Kozakiewicz et al. [11] demonstrated in the WOBASZ I study, performed 10 (years) prior to the present study, that SES was defined as a combination of education and income categories and was an independent predictor of high CVD risk, but only in young men and women aged 30–39 years. Herein demonstrated a significant, favorable impact of both education and income on the prevalence of high CVD risk in the entire investigated group, which may reflect the undergoing transition in Poland.

The association between income and CVD is not as clear as with education. It was demonstrated that participants with higher income, reported higher prevalence of overweight, obesity (BMI ≥ 30),

Table 5. Odds ratio for high cardiovascular (CV) risk according to education and income adjusted for age and gender.

	High CV risk related to education					High CV risk related to income			
	OR	95%	CI	P		OR	95%	CI	P
Higher vs. incomplete	0.46	0.29	0.74	0.01	High vs. low	0.77	0.52	1.15	0.5
Age	1.36	1.33	1.40	< 0.0001	Age	1.37	1.34	1.41	< 0.0001
M vs. F	27.58	19.97	38.08	< 0.0001	M vs. F	28.20	20.43	38.91	< 0.0001

CI — confidence interval; F — female; M — male; OR — odds ratio
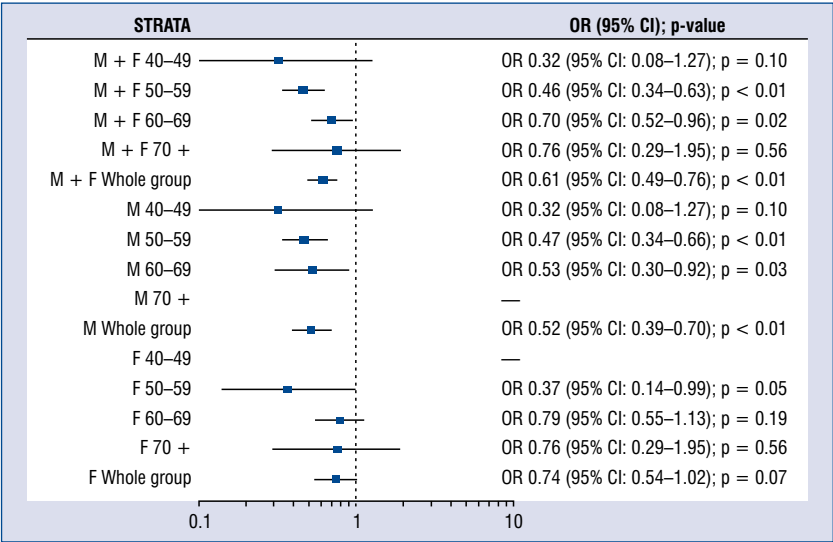
Figure 3. High cardiovascular disease risk according to higher education presented in age and gender subgroups. Odds ratio (OR) with confidence intervals (95% CI); F — female; M — male.
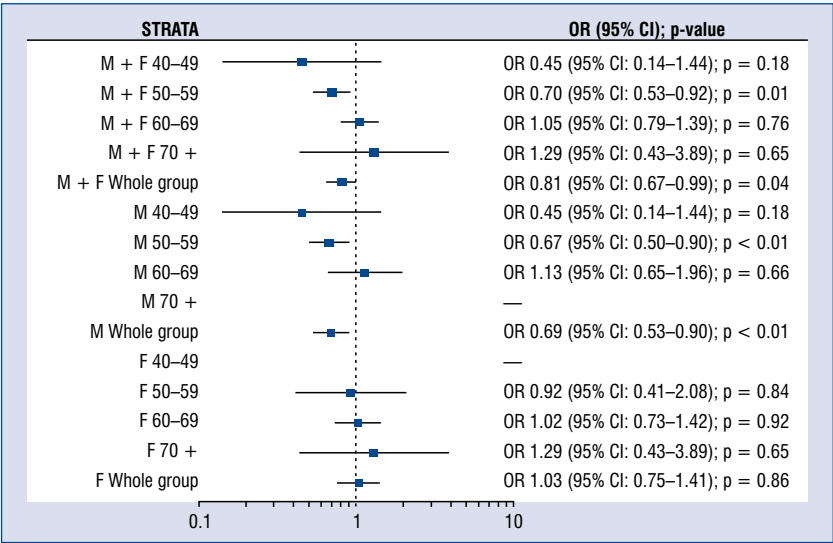
Figure 4. High cardiovascular disease risk according to high income presented in age and gender subgroups. Odds ratio (OR) with confidence intervals (95% CI); F — female; M — male.

hypercholesterolemia, hypertriglyceridemia, increased LDL-C, hypertension and former smoking but the inverse association for decreased HDL-C. However, when the subsample of participants free of CVD, DM and CKD diagnosed was analyzed, it was found that subjects declaring higher income have a lower prevalence of high CVD risk.

Results of other studies on the relation between SES and income and CVD risk factors are ambiguous. Stelmach et al. [20] showed that lower economic status did not affect CVD risk factors, similarly as in the Bobak and Marmot study [21]. Results of the Moli-sani study presented that healthy behaviors are strongly linked to material resources, even in a high-income country. Even small income differences produce gradient in modifiable risk factors, with more disadvantaged persons having not only more risk factors but also fewer protective factors [22]. According to Robert and House [23], financial assets remain associated with health until late in life and become more important relative to education.

Limitations of the study

The examined sample may not be representative of the whole population of Poland due to low response rate. Low response rates are a problem in many epidemiological studies, results of analysis regarding participation rates from the 1970s demonstrated that response rates decrease gradually [24]. It was found that study participants have better health than non-respondents [25]. Another limitation is the cross-sectional character of presented data, and in a consequence a problem of causality cannot be addressed.

Some estimates were based on interviews, and the answers may be inaccurate. This refers to the assessment of income, where people might have rated their income higher or lower. In this study, psychosocial factors were not taken into consideration, which could have affected cardiovascular risk.

Conclusions

This study, based on a large population of Polish citizens, showed that SES assessed by education and income is a significant and independent predictor of high cardiovascular risk of death as estimated by SCORE. Moreover, a favorable impact of education and income (in subgroup analysis) on the prevalence of high cardiovascular risk was demonstrated not only in younger subjects, as had been shown in previous Polish studies, but in the whole group investigated, which may reflect the fact that Poland

was undergoing a socioeconomic transition. Data on SES and CVD interactions from Poland, the first Eastern Bloc country which had to adopt political and economic changes and was still in transition, may clarify and predict how socioeconomic factors generate inequalities in health in other transitioning countries. Considering the strong association between education and CVD, it would be beneficial to include it into cardiovascular risk estimations and screening tools along with reducing socioeconomic inequalities and developing effective prevention strategies focused on lower socioeconomic groups.

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ORIGINAL ARTICLE

Percutaneous renal artery denervation in patients with chronic systolic heart failure: A randomized controlled trial

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Abstract

Background: *Renal denervation (RDN) is as an effective treatment for heart failure (HF), but its effects on cardiac function of patients with HF are not well documented. Here, the aim was to investigate RDN's effect on patients with chronic systolic HF, by conducting a single-center, prospective, randomized, and controlled study.*

Methods: *Sixty patients with chronic systolic HF were randomly assigned to the RDN or control groups, receiving percutaneous catheter-based RDN with radiofrequency ablation and drug treatment, respectively. All patients performed a 6-minute walk test, echocardiography, blood pressure measurement, and biochemical test, at both baseline and in a 6-month follow up.*

Results: *Over 6-month follow up, patients in RDN group showed a decrease in N-terminal pro-B-type natriuretic peptide (440.1 ± 226.5 pg/mL vs. 790.8 ± 287.0 pg/mL, p < 0.001, Cohen's d = 1.14), an increase in left ventricular ejection fraction (39.1 ± 7.3% vs. 35.6 ± 3.3%, p = 0.017, Cohen's d = 0.61), improved New York Heart Association class assessment (p = 0.01, Cohen's d = 0.66), and decreased blood pressures (p < 0.001, Cohen's d = 0.91), without reporting hypotension and syncope amaurosis. No significant between-group difference was observed for glomerular filtration rate and heart rate.*

Conclusions: *Renal denervation which effectively and safely improves patient's cardiac function as well as exercise tolerance, could be considered as an effective treatment for chronic systolic HF.* (Cardiol J 2019; 26, 5: 503–510)

Key words: renal denervation, sympathetic nervous system, heart failure, blood pressure, cardiology

Introduction

Chronic heart failure (HF) is a common disease suffered by around 100 million people all over the world. There were 5.1 million people in United States at present a number which increases at a rate of 800,000 cases per year whosuffer from this disease. HF is believed to be a major cause of sudden cardiac death, and previous studies have shown that the death rate for patients with chronic HF is 6–9 times higher than those in the normal population. Around 300,000 deaths are caused by HF every year in United States [1]. The medical expense on HF reachedas much as $30 billion in 2012,and has kept growing in recent years [2].

Excessive activity of the sympatheic nerve system is believed as one of the major causes of the HF progression. Sympathetic activation involves efferent and afferent pathways to regulate cardiovascular functions such as blood pressure (BP) and heart rate, in response to acute stress like volume depletion or excessive vasodilatation [3]. Exces-

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sive activity of the sympathetic nervous system has a direct adverse consequence for cardiovascular diseases, particularly for HF and hypertension [4]. A proper suppression of the sympathetic nervous system activity may be able to improve the conditions of patients with HF. Particularly, catheter-based renal sympathetic denervation (RDN), which can specificly and effectively reduce the activity of sympatheic nervous system [3, 5], which is considered a proper treatment for HF.

Renal denervation was implemented by selective sympathetic denervation of the human kidney with radiofrequency energy ablation. It was firstly proposed to treat resistant hypertension [6, 7], but its treatment effect for this specific disease was not as good as had been imagined. HTN-3 study found that there was no significant difference in BP between the RDN and sham groups [8]. Even though, its ability in suppressing the activity of the sympathetic nervous system suggests it is a possible application in the treatment of HF.

To explore the treatment effect of RDN on HF, several animal models, e.g.pig and rat models, were established [9–11] and the performance of RDN on these animal models were quite promissing. However, these animal based results were insufficient to be considered as direct evidence for the application of RDN in clinical practice; more experiments on humans should be conducted to eliminate the concerns regarding its effectiveness and safety to patients with HF. A first-in-man study of chronic systolic HF has already been conducted [12], and suggests that patient exercise tolerances were improved after the intervention of RDN. Nevertheless, small sample size and its single-group non-blinded and non-randomized nature limited the validity of these research findings. Thus, the aim presently was to comprehensively investigate the effectiveness and safety of catheter-based RDN in the treatment of chronic systolic HF, by conducting a randomized controlled trial with a larger sample size.

Methods

Patients

Sixty patients with chronic systolic HF (New York Heart Association [NYHA] class II or III) were recruited in this study. All patients were not less than 18 years old. The inclusion criteria were: (1) left ventricular ejection fraction (LVEF) should be smaller than 40% at echocardiography or N-terminal pro-B-type natriuretic peptide (NT-proBNP) should be larger than 125 pg/mL; (2) glomerular

filtration rate (GFR) should not be smaller than 45 mL/min/1.73 m^2 and systolic BP should not be smaller than 100 mmHg. The exclusion criteria were: (1) patients with renal artery stenosis (in history or revealed by imaging), type I diabetes, severe heart valvar disease, and myocardial infarction or cerebrovascular accident 6 months prior were excluded; (2) patients who were or would have been in pregnancy during the study.

The study was approved by the ethics committee of Putuo Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. All patients had been informed in advance and signed a consent form.

Experimental design

This was a single-centre, prospective, randomized and controlled study. The 60 recruited patients were equally and randomly assigned to the intervention group undergoing catheter-based RDN and the control group receiving drug treatment with the random envelope method, there were 30 patients in each group. All involved patients were followed up for 6 months.

RDN intervention

Before the RDN intervention, patients were given enteric-coated acetylsalicylic acid (300 mg) or clopidogrel (300 mg) by chewing. During the operation, patients were given heparin (6000–8000 U) by intravenous injection. Femoral artery puncture were conducted after skin preparation in right fold inguen and disinfection; then 7 F vascular sheath was imbedded. Renal arteriography was conducted in both left and right with a JR4 catheter. Spiral ablation was performed with an imbedded 6 F radiofrequency ablation catheter (Ablation instrument [39D-72X]: Johnson Medical Instrument Co. Ltd.) in temperature control mode (8–10 W, 50°C) to both left and right renal arteries. There were 4–6 ablation points in the left and right renal arteries respectively. The effective ablation time for each point was 60 s and the interval between two neighbouring points was 0.5 cm. Renal arteriography was conducted after operation.

Study assessment

Efficacy endpoint. All patients accepted NT-proBNP test, echocardiographic LVEF assessment and NYHA class assessment both before the operation and after 6-month follow up. Six-minute walk test was also conducted both preoperatively and monthly during the following period. Changes in these tests were treated as the efficacy endpoint in this study.

Safety endpoint. All patients accepted the office BP measurement both preoperatively and monthly during the follow up. Heart rate and GFR were also measured both before the operation and after 6-month follow up. Changes in these measurements were treated as safety points in this study.

Pharmacological therapies

All patients underwent pharmacotherapy for HF with a maximal tolerated dose prior to denervation, including beta-blockers, angiotensin converting enzyme (ACE) inhibitors or endothelin receptor antagonist, and spironolactone. No change of medications was permitted prior to denervation. During the following, patients were given standard HF care and the physicians could freely adjust the dosage of pharmacotherapy according to patient condition, e.g., changes in BP and heart rate.

Statistical analysis

Statistical analyses were carried out with the SPSS 21.0 statistical analysis package (SPSS Inc., New York, USA). All measurement data were presented as mean ± standard deviation (SD); while, the enumeration data were presented as a percentage number. The comparison of measurement data was achieved by the student independent t-test with a prior checking of dataset normality by Shapiro-Wilk test; and for enumeration data, they were compared by χ^2 test or the Fisher exact test. Statistical difference was defined as the situation where p-value was smaller than 0.05.

Results

Baseline clinical characteristics

Sixty patients (13 females and 47 males) with a mean age of 60.2 ± 11.6 years were enrolled in this randomized controlled study between January 2014 and July 2015. Their mean body mass index was 26.7 ± 2.7 kg/m². Among these patients, 65.0% were with hypertension, 58.3% were with coronal heart disease, 11.7% were with atrial fibrillation, and 25% were with type 2 diabetes. All patients were diagnosed as chronic HF (NYHA class II or III, NT-proBNP level is 791.2 ± 363.7 pg/mL, 6-minute walk distance is 213.8 ± 65.9 m, and the LVEF is 34.9 ± 3.2% during the echocardiogram). There was a mean GFR of 100.6 ± 33.9 mL/ /min/1.73 m², mean office systolic BP was 142.6 ± ± 22.6 mmHg, mean diastolic BP was 80.8 ± 12.6 mmHg, and mean heart rate was 69.1 ± 7.3 bpm. In addition, 95% of patients were treated with ACE inhibitors or angiotensin II receptor blockers, 78.3% with beta-blockers, 16.7 % with aldosterone antagonists, 43.3% with calcium-channel blockers, 6.7% with digoxin, and 50% with loop diuretics.

Bilateral renal denervation was successfully performed in all 30 patients in the RDN group. No statistically significant difference was found between the two groups in age, sex, most comorbidities, reported duration spent on pharmacotherapy for HF, and assessments of effectiveness and safety endpoints (Table 1).

Efficacy endpoint

All patients in both groups were followed up for 6 months. Results of the Student independent t-test on NT-proBNP indicates that the NT-proBNP levels in RDN group (440.1 ± 226.5 pg/mL) were significantly lower (p < 0.001, Cohen's d = 1.14) than those in the control group (790.8 ± 287.0 pg/ /mL) in the 6th month follow up (Fig. 1).

The effects of RDN on cardiac functions of patients with chronic HF can be indicated by the results of independent t-tests on echocardiographic parameters between the two groups (Table 2). It is seen that LVEF significantly increased (p = 0.017, Cohen's d = 0.61) in the RDN group (39.1 ± 7.3%) when compared with the control group (35.6 ± 3.3%) at the end of the 6-month follow up. Further, left ventricular end systolic diameters in the RDN group (46.4 ± 4.7 mm) were significantly smaller (p < 0.001, Cohen's d = 0.87) than those in the control group (50.2 ± 3.1 mm); nevertheless, no statistically significant difference was found in left ventricular end diastolic diameter and interventricular septal thickness between the two groups (p > 0.05).

After 6-month follow up, patients in the RDN group showed an improvement of cardiac function, with 7 patients showing better results of NYHA class assessment (Fig. 2). The 6-minute walk distance in the RDN group (301.2 ± 139.5 m) was significantly increased (p = 0.01, Cohen's d = 0.66) when compared with the control group (227.2 ± ± 65.0 m) in the 6th month (Fig. 3).

Safety endpoint

In the 6th month, the office BP of patients in the RDN group (123.3 ± 0.9 / 68.5 ± 7.0 mmHg) significantly decreased (p < 0.001, Cohen's d = 0.91) when compared with those in the control group (139.8 ± 20.7 / 80.2 ± 11.8 mmHg), as shown in Figure 4; nevertheless, no patient reported symptoms of hypotension syncope or amaurosis during the follow up period. No significant differ-

Table 1. Baseline patient characteristics, demographics, background medications, efficacy and safety endpoints parameters in renal denervation and control groups.

	Renal denervation group (n = 30)	Control group (n = 30)	P
Age [years]	59.0 (12.1)	61.3 (11.1)	0.446
Sex [male]	25 (83.3%)	22 (73%)	0.347
Body mass index [kg/m²]	26.8 (2.6)	26.7 (2.8)	0.939
Medical history:			
Hypertension	21 (70.0%)	18 (60.0%)	0.417
Coronary heart disease	17 (56.7%)	18 (60.0%)	0.793
Atrial fibrillation	4 (13.3%)	3 (10.0%)	0.688
Type 2 diabetes	8 (26.7%)	7 (23.3%)	0.766
Patients receiving drug class:			
ACE or ARB	29 (96.7%)	28 (93.3%)	1.000
Beta-blockers	23 (76.7%)	24 (80.0%)	0.754
Aldosterone antagonists	6 (20.0%)	4 (13.3%)	0.488
Calcium-channel blockers	14 (46.7%)	12 (40.0%)	0.602
Digoxin	3 (10%)	1 (3.3%)	0.612
Loop diuretics	14 (46.7%)	16 (53.3%)	0.606
Hemoglobin	119.1 (25.9)	112.3 (24.5)	0.299
Efficacy endpoint:			
NT-proBNP [pg/mL]	797.7 (356.1)	784.7 (377.1)	0.892
Six minute walk test [m]	217.5 (69.5)	210.0 (63.0)	0.666
NYHA class:			
Class II	17 (56.7%)	16 (53.3%)	0.795
Class III	13 (43.3%)	14 (46.7%)	0.795
Echocardiographic LVEF [%]	35.0 (3.2)	34.8 (3.2)	0.872
Safety endpoint:			
GFR [mL/min/1.73 m²]	104.8 (35.9)	96.4 (31.8)	0.337
Symbolic BP [mmHg]	142.0 (24.8)	143.2 (20.7)	0.844
Diastolic BP [mmHg]	79.8 (12.6)	81.7 (12.9)	0.579
Heart rate [bpm]	68.7 (7.9)	69.4 (6.9)	0.728

Data are mean (standard deviation) or number (%). ACE — angiotensin-converting enzyme inhibitor; ARB — angiotensin II receptor blocker; BP — blood pressure; GFR — glomerular filtration rate; LVEF — left ventricular ejection fraction; NT-proBNP — N-terminal pro-B-type natriuretic peptide; NYHA — New York Heart Association

ence was found for heart rates (p = 0.062, Cohen's $d = 0.49$) and GFR (p = 0.209, Cohen's $d = 0.33$) between the two groups after 6-month follow up, even though there was a slight decrease in heart rate and an increase in GFR were observed (Figs. 5, 6).

Discussion

This was a prospective randomized and controlled study to explore the effectiveness and safety of cathedral-based RDN on patients with chronic systolic HF. The improvement of exercise tolerance, decrease of NT-proBNP level, and increase of LVEF at echocardiography, suggest the feasibility and effectiveness of RDN in treatment of chronic systolic HF. In addition, no amaurosis and syncope in the RDN group, as well as no significant difference between-group of GFR further indicate the safety of RDN treatment.

Renal denervation has never been proposed as an effective way to treat resistant hypertension [6, 7], but its effect on this specific disease was inconclusive due to disappointing results acquired in the SIMPLICITY HTN-3 study, no significant difference was found in reduced BP between the RDN and sham groups [8]. Even though, recent studies proposed that RDN might be an effective way for the treatment of HF [12], due to its abilities in
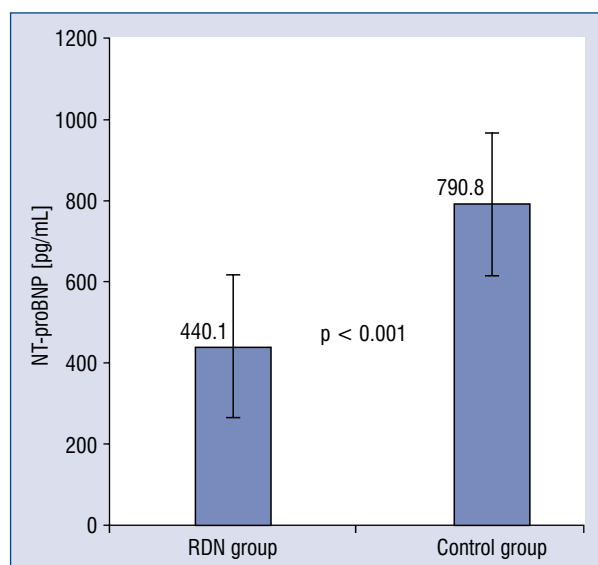
Figure 1. Mean N-terminal pro B-type natriuretic peptide (NT-proBNP) level at 6 months after renal denervation (RDN) in the RDN and control groups. A significant decrease in the RDN group was observed. Error bars indicate standard errors.
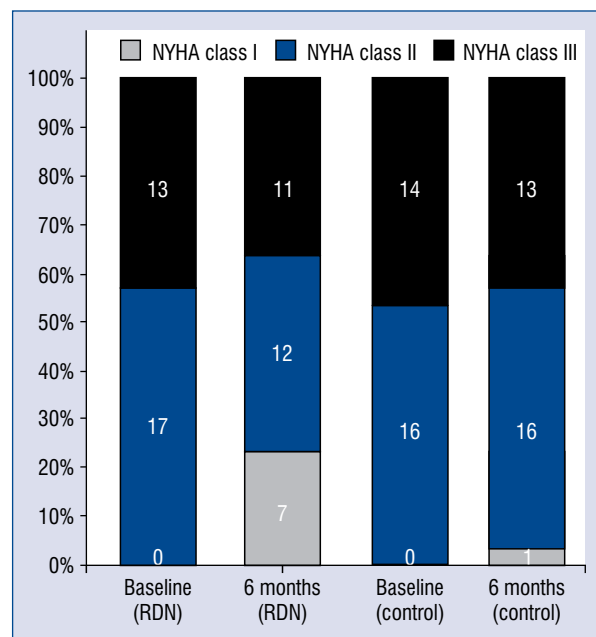
Figure 2. New York Heart Association (NYHA) class assessment in the renal denervation (RDN) and control groups at baseline and at 6 months after RDN. Black bars indicate NYHA class III. Blue bars indicate NYHA class II. Gray bars indicate NYHA class I.

suppressing activities of the sympathetic nervous system and renin–angiotensin–aldosterone system (RASS) by blocking the renal sympathetic nerve. It should be noted that excessive activation of the sympathetic nerves and the over expression of RASS are believed to be major contributors to the cause and development of HF [13]. Actually, excessive activation of the renal sympathetic nerves can lead to renal vasoconstriction and increase renin secretion as well as proximal tubular sodium re-absorption [14]. This kind of chronic stimulation caused by excessive activation of the sympathetic nerves can lead to volume overloading, myocardial remodeling and cardiovascular function deterioration [15].

Accumulating animal evidence suggests that RDN is able to bring hemodynamic changes and improve cardiac functions. For example, RDN showed an effective inhibition on RASS in a porcine model of pacing-induced HF [11]. After pacing, the RDN group has shown a significantly higher LVEF at echocardiography and a significantly lower plasma concentration of renin, relative to the control group. In contrast, although a significant elevated plasma concentration of aldosterone was reported in the control group, no significant change in the aldosterone level was observed in the RDN group. Similar results have been found in a rat model of

Table 2. Changes in echocardiographic parameters before and 6 months after randomization in the renal denervation (RDN) and control groups.

Parameter	Baseline			6 months		
	RDN	Control	P	RDN	Control	P
LVEF [%]	35.0 ± 3.2	34.8 ± 3.2	0.872	39.1 ± 7.3	35.6 ± 3.3	0.017
LVESD [mm]	50.3 ± 3.3	51.4 ± 2.4	0.156	46.4 ± 4.7	50.2 ± 3.1	< 0.001
LVEDD [mm]	63.3 ± 4.5	63.6 ± 4.7	0.082	60.0 ± 6.7	62.5 ± 4.5	0.106
IVST [mm]	10.3 ± 1.3	10.2 ± 1.3	0.848	9.5 ± 0.9	9.6 ± 0.9	0.575

Data are mean ± standard deviation. LVEF — left ventricular ejection fraction; LVESD — left ventricular end systolic diameter; LVEDD — left ventricular end diastolic diameter; IVST — interventricular septal thickness
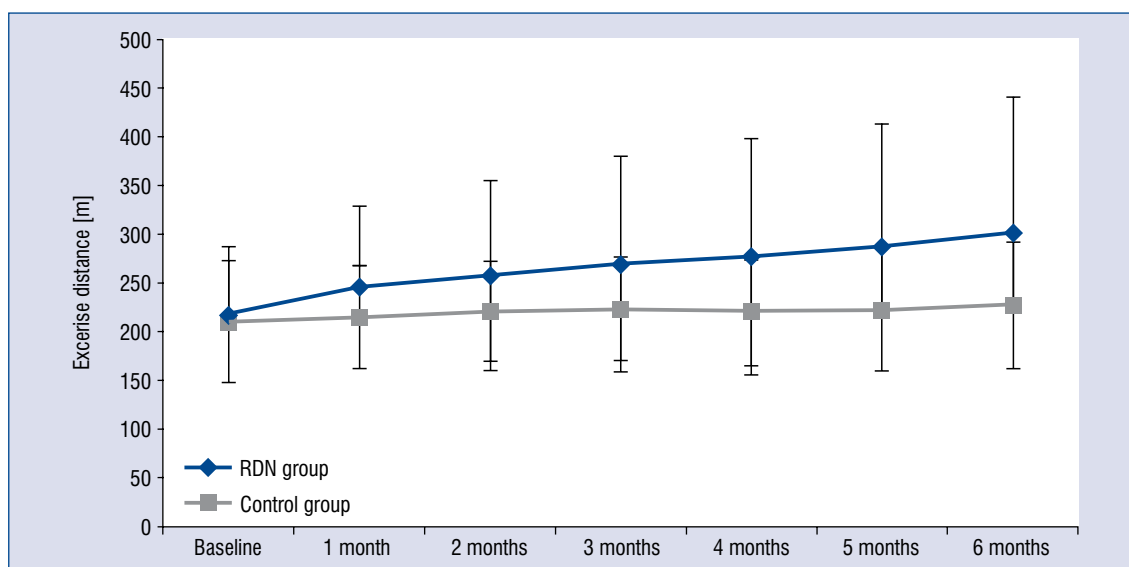
Figure 3. Change in 6 minute walk test before and after renal denervation (RDN) for RDN and control groups. A 6 minute walk test was measured at baseline and over a 6 month follow-up. A significant improvement in 6 minute walking distance was observed in the RDN group over 6 months. Error bars indicate standard errors.
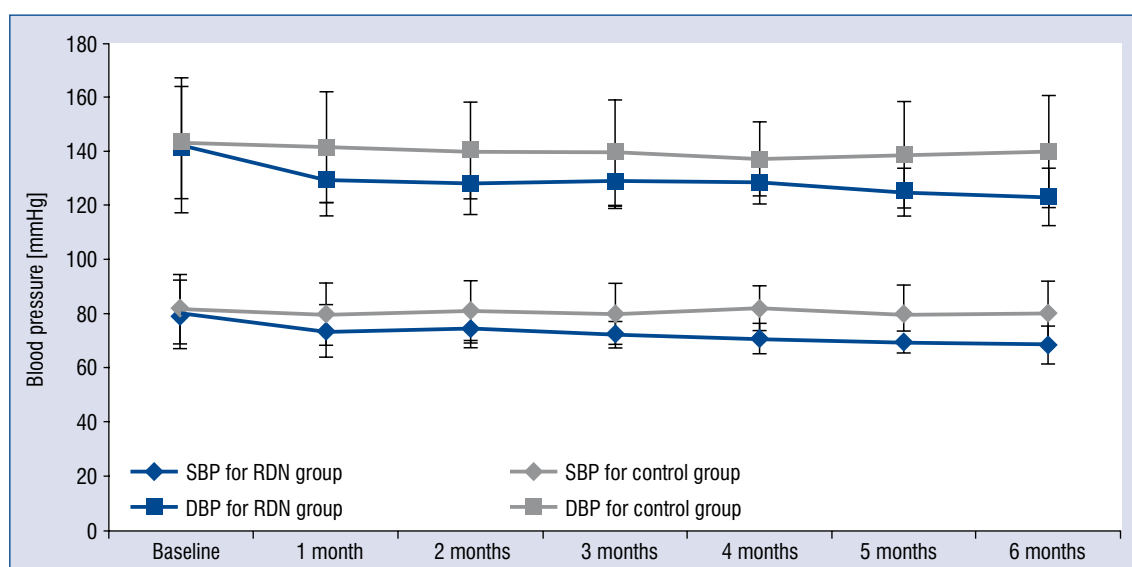
Figure 4. Paired changes of systolic blood pressure (SBP) and diastolic blood pressure (DBP) before and after renal denervation (RDN) for RDN and control groups. Blood pressure was measured at baseline and over a 6 month follow-up. Significant reductions in blood pressure were observed in the RDN group over 6 months. Error bars indicate standard errors.

isoproterenol induced chronic HF. It was reported that RDN down-regulated the protein expression of angiotensin II in myocardial tissue of rats left atrial, and thereby improved the cardiac function, shown by an increased LVEF [10]. Moreover, RDN was reported to be able to improve the cardiac function and inhibit myocardial remodeling in rats with post-myocardial infarction [9]. Further animal study has suggested that denervation intervention can ameliorate progression of left ventricular hypertrophy in spontaneously hypertensive rats, probably due to the reduction of BP and lower expression of inflammatory factors (e.g., TLR4, NF-κB, TNF-α, and IL-6) in myocardial tissue [16].
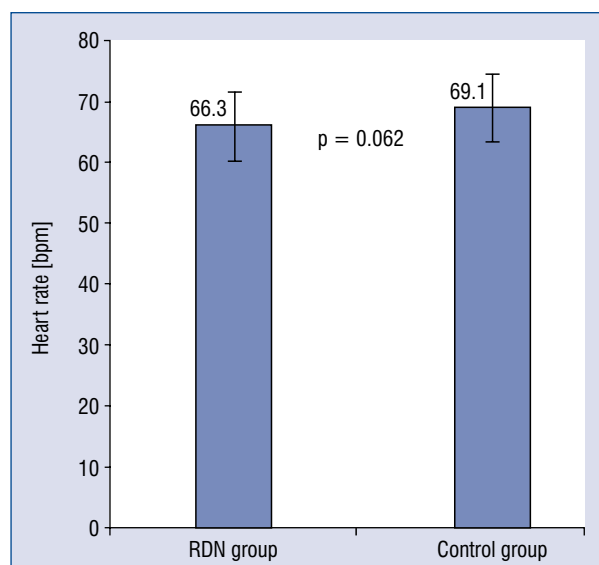
Figure 5. Mean heart rate at 6 months after renal denervation (RDN) in the RDN and control groups. No significant between-group differences were observed. Error bars indicate standard errors.
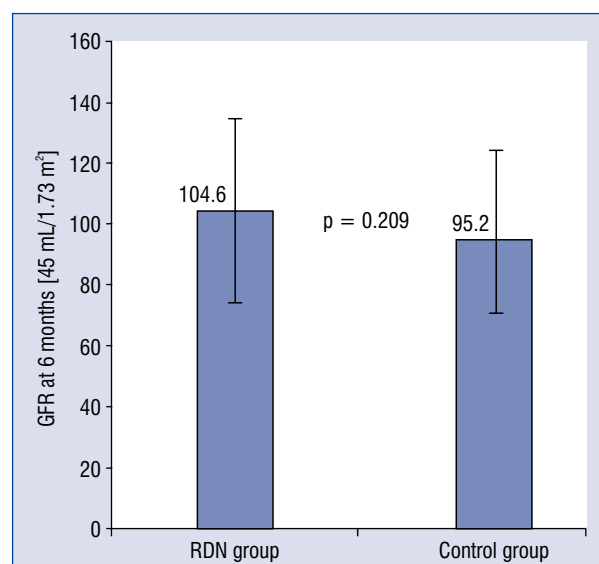
Figure 6. Mean glomerular filtration rate (GFR) at 6 months after renal denervation (RDN) in the RDN and control groups. No significant between-group differences were observed. Error bars indicate standard errors.

Though the positive results have been well confirmed in animal studies, the effects of RDN on patients with HF are still not well documented, and some indirect evidence were reported in studies on treatment of resistant hypertension. For example, a study including 72 patients reported

that RDN could ameliorate the cardiac function, indicated by an improvement in left ventricular mass index, left ventricular mass/body surface area ratio, left ventricular wall stress, as well as LVEF values [17]. Another study also reported that the resistant hypertension patients were also accompanied with left ventricular hypertrophy and diastolic dysfunction [18]. These results together suggest that RDN would be promising treatment for HF, especially for that accompanied with hypertension. Actually, the REACH-Pilot study has once investigated the effects of RDN on patients with HF and reported that RDN could improve patient exercise tolerances [12]. Nevertheless, this study was limited by its small sample size and its single-group non-blinded and non-randomized nature [19]. Here, more patients were recruited and a randomized controlled trial was performed for more convincing results. Obtained results verfied the values of RDN in improving the cardiac function and treating patients with HF.

The RDN group in this study had shown a reduction in BP of approximately 20 mmHg over 6-month follow up. However, such changes in BP did not accompany any symptoms of hypotension, indicating that this intervention did not lead to symphonic problems in patients with chronic systolic HF. Furthermore, patients receiving RDN showed no changes in GFR and heart rate before and after the procedures. The reduction of BP has been suggested to potentially dampen the impairment of renal function, as a decreased BP may help reduce the sympathetic outflow to the kidney [6]. In this study, the hemodynamic changes and normal renal function show no adverse effects on the kidneys of patients with chronic systolic BP, suggesting the safety of RDN.

Limitations of the study

Several limitations need to be noted in this study. First, the number of patients with systolic HF for RDN procedure was not large, thereby limiting statistical power. However, the improvement of cardiac function in the present population is in line with that of previous studies in hypertension with a large sample size. Second, non-blinded or sham-procedure group was not provided. The results, therefore, may contain bias. A randomized controlled trial with a sham group should be done to further investigate the effectiveness of RDN in HF. Third, whether RDN treatment in chronic systolic HF could improve a patients' diuretic need or resistance warrants further investigation.

Conclusions

Catheter-based RDN with radiofrequency ablation has shown to be very promising in improving cardiac function and exercise tolerance for patients with chronic systolic HF, in the present single-center, randomized, controlled trial. No adverse effects on the kidneys of patients should also be noted. Thus, this innovative technique can be an effective and safe new therapy for chronic systolic HF, even though more long-term experimental and clinical evidence should be provided in the future studies.

Acknowledgements

Conflict of interest: None declared

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ORIGINAL ARTICLE

VIA MEDICA

Lipoprotein(a) screening in young and middle-aged patients presenting with acute coronary syndrome

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Abstract

Background: *Elevated lipoprotein(a) [Lp(a)] is an independent risk factor for coronary artery disease (CAD). However, its role in real-world practice and implications for clinical care remains limited. Under investigation herein, are the clinical characteristics associated with increased Lp(a) levels in patients presenting with acute coronary syndrome (ACS).*

Methods: *Lp(a) was measured at admission in patients ≤ 65 years of age presenting with ACS in a single center. Logistic regression model was used to determine the independent association of clinical characteristics with elevated Lp(a).*

Results: *A total of 134 patients were screened for Lp(a); 83% males, mean age 52 ± 8 years. Median Lp(a) level was 46 nmol/L (interquartile range [IQR] 13–91). Elevated Lp(a) > 72 nmol/L (30 mg/dL) was documented in 32% and associated with younger age at CAD diagnosis. In a multiple logistic regression model, premature CAD (odds ratio [OR] 3.85, 95% confidence interval [CI] 1.48–10.07, p = 0.06), previous revascularization (OR 2.56, 95% CI 1.17–5.59, p = 0.019) and probable/definite familial hypercholesterolemia (FH) (OR 3.18, 95% CI 1.10–9.21, p = 0.033), were independently associated with elevated Lp(a). In contrast, Lp(a) levels were not associated with other traditional cardiovascular risk factors, previous statin treatment, C-reactive protein level or ACS type.*

Conclusions: *In young and middle-aged patients presenting with ACS, premature CAD, previous revascularization and FH were independently associated with elevated Lp(a), indicating progressive CAD and higher cardiovascular risk. These results, are in accordance with guideline based recommendations for Lp(a) screening, and may be of importance in addressing residual cardiovascular risk in young ACS patients, in light of the novel emerging therapies targeting Lp(a).* (Cardiol J 2019; 26, 5: 511–518)

Key words: lipoprotein(a), acute coronary syndrome, coronary artery disease, familial hypercholesterolemia

Introduction

Lipoprotein(a) [Lp(a)] consists of an apolipoprotein B containing low-density lipoprotein (LDL) like particle, covalently linked to plasminogen-like glycoprotein apo(a) [1]. Lp(a) is mainly determined genetically by the LPA gene, and is considered proatherogenic, proinflammatory and potentially antifibrinolytic [2]. Evidence from epidemiological and clinical analyses in both primary and secondary prevention populations show an independent asso-ciation between Lp(a) and risk for cardiovascular disease (CVD) and death [3–8], results that are further supported by genetic studies indicating that Lp(a) has a causal role in the development of coronary artery disease (CAD) [9–11]. Nevertheless, despite these associations, the value of Lp(a) as a prognostic biomarker remains controversial and is incompletely defined due to lack of standardized assays [12], the limited therapeutic options for significantly lowering Lp(a) and the need of outcome data showing the benefit of lowering Lp(a) levels [13].

Although screening for Lp(a) is recommended by professional societies in selected patients [14], there is wide variation in the clinical utility of Lp(a) measurement among health care providers, and real-life data regarding the screening for Lp(a) levels in patients with established CAD is limited. It is therefore important to identify clinical characteristics and risk factors associated with elevated Lp(a), as well as high-risk populations in whom future preventive strategies and emerging therapies will be applied [15]. In addition, screening for Lp(a) in the younger population presenting with acute coronary syndrome (ACS) may serve as an opportunity to identify residual cardiovascular risk, with long-term implications.

In light of these considerations, the aim of the current study was to investigate the clinical features associated with elevated Lp(a) in young and middle-aged patients ≤ 65 years presenting with ACS. Moreover, as Lp(a) levels were suggested to be related to pro-inflammatory conditions [16, 17], their association with C-reactive protein (CRP) levels at presentation with ACS will be analyzed.

Methods

Study design

This study is a retrospective observational cohort analysis performed in a single center at Lady Davis Carmel Medical Center, Haifa, Israel. 134 patients were included, aged 65 years and under who presented to the Cardiology Department with ACS between June 2016 to November 2017 and were tested for Lp(a) levels. Blood analysis was performed at a single laboratory with samples collected within 24 h of hospital admission. Laboratory blood tests included Lp(a) levels, routine lipid panel, kidney function tests and CRP levels. LDL cholesterol was calculated by the Friedwald formula. Lp(a) was measured using a particle-enhanced quantitative turbidimetric immunoassay (PETIA) (Tina-quant® Lipoprotein (a) Gen.2, Roche Diagnostics International Ltd.), on a COBAS automated chemistry analyzer. Lp(a) levels were reported in nmol/L units, according to recent recommendations [13]. Levels above 72 nmol/L were considered elevated (estimated conversion factor from molar to mass based concentration: 1 nmol/L × 0.4167 = mg/dL), consistent with traditional thresholds for elevated Lp(a) above 30 mg/dL which approximate the 75th percentile in white populations, and also reflect epidemiological data of CVD risk thresholds [4, 18].

Additional demographic and clinical characteristics as well as traditional cardiovascular risk factors were recorded from computerized data of patient files. Patients were assessed for clinical indications to Lp(a) measurement, as recommended by customary guidelines [14], including (1) premature CAD (male age < 55 years and female age < 60 years), (2) family history of premature CAD, (3) familial hypercholesterolemia (FH) and (4) markers of progressive CAD including previous revascularization, presence of multi-vessel CAD and need for cardiac surgery. The clinical diagnosis of FH was established using the Dutch Lipid Clinic Network (DLCN) algorithm [19]. Peak LDL cholesterol level documented in each patient's history was used to calculate the DLCN score. FH was considered probable or definite if the total score was ≥ 6 points. The study was approved by the Lady Davis Carmel Medical Center Institutional Ethics Committee in Haifa, Israel, with a waiving of the need for individual patient consent.

Data analysis

Continuous data are presented as means ± ± standard deviation or median and interquartile range (IQR), and categorical variables as numbers and percentages. The independent-samples T-test or Mann-Whitney test was used to compare continuous variables and the χ^2 test to compare categorical variables. The Fisher exact test was used in cases of small sample size. Information on covariates was complete except for CRP levels, missing in 3 patients. Spearman's correlation coefficient was used to investigate the relationship between Lp(a) and CRP levels at admission.

Multivariate logistic regression model was used to determine the independent association between clinical characteristics and elevated Lp(a), defined as > 72 nmol/L. Included in the multivariable model were variables with a significance level < 0.20 in the univariate analysis. Odds ratio were further adjusted for age, gender and statin treatment prior to hospitalization. Lp(a) levels were additionally analyzed according to distribution into tertiles. The results were considered statistically significant when the 2-sided p-value was < 0.05. SPSS statistical software version 20.0 was used to perform all statistical analyses.

Results

Lipoprotein(a) was measured in 134 patients aged 65 years and under presenting with ACS. Un-
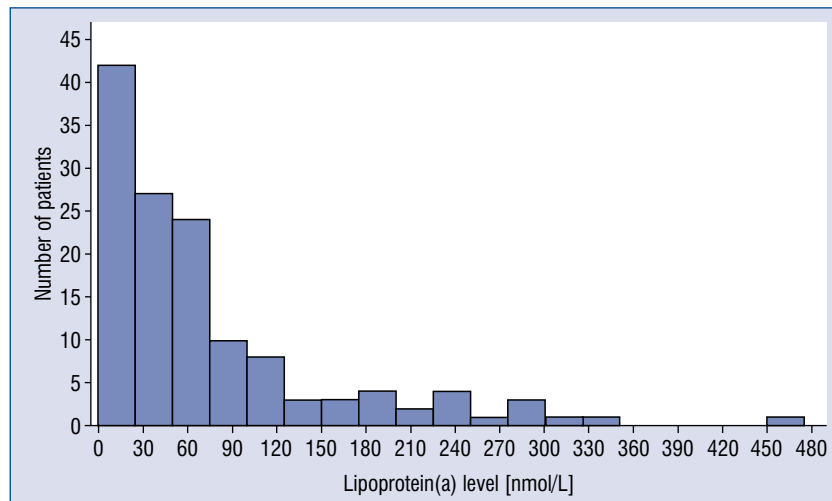
Figure 1. Lipoprotein(a) level distribution in study population.

stable angina was diagnosed in 11% of the patients, non ST-segment elevation myocardial infarction (NSTEMI) in 58%, and ST-segment elevation myocardial infarction (STEMI) in 31%. Mean age was 52 ± 8 years and 83% were males. Mean LDL cholesterol level at admission with ACS was 123 ± 52 mg//dL, and high-density lipoprotein (HDL) cholesterol 35 ± 9 mg/dL. Median Lp(a) level was 46 (IQR 13–91) nmol/L. Lp(a) level distribution in the study population is presented in Figure 1, showing a skewed distribution with a tail towards the highest levels. Younger patients under 45 years of age (n = 24) had significantly higher Lp(a) levels than middle-aged patients between 45 and 65 years (n = 110): mean 105 ± 119 nmol/L, median (IQR) 61 (24–120) nmol/L vs. mean 65 ± 70 nmol/L, median (IQR) 40 (11–83) nmol/L, p = 0.027, respectively. Similarly, their mean LDL-cholesterol levels were higher: 143 ± 66 mg/dL vs. 119 ± 48 mg/dL, p = 0.037, respectively.

Elevated Lp(a) > 72 nmol/L was documented in 43 patients with ACS (32%) and associated with younger age and premature CAD (men < 55 years and women < 60 years) (Table 1). In addition, elevated Lp(a) was associated with previous revascularization (42% vs. 22%, p = 0.017) and more prevalent clinical diagnosis of probable/definite FH (21% vs. 8%, p = 0.027). In contrast, elevated Lp(a) was not related to other traditional risk factors such as hypertension, diabetes, smoking, chronic kidney disease, as well as family history of premature CAD; nor was it associated with previous statin treatment or ACS type (Table 1). Triglyceride and cholesterol levels at admission were comparable in both Lp(a) groups. In addition, performance rates of cardiac surgery and angiographic evidence of 3 ves-

sel CAD were also similar between patients with and without elevated Lp(a) levels. In a multiple logistic regression model, previous revascularization, premature CAD and probable/definite FH remained independently and significantly associated with high Lp(a) levels, after additional adjustment to age, gender and previous statin therapy (Table 2). These independent risk markers of progressive CAD were also associated with Lp(a) levels stratified by tertiles (Fig. 2). Moreover, an increase in 20 nmol/L in Lp(a) was associated with significant increase in the adjusted odds ratio (OR) for premature CAD (OR 1.237, 95% confidence interval [CI] 1.014–1.509, p = 0.036), but not the other two risk predictors.

C-reactive protein levels measured at admission of patients with ACS were not correlated with elevated Lp(a), both when analyzed as a continuous variable (Spearman's correlation coefficient 0.136, p = 0.120) or as a categorical variable (high CRP levels observed in 27% of those with elevated Lp(a) compared to 23% with normal Lp(a), p = 0.630). Repeat Lp(a) was measured ≥ 2 months after discharge in 5 patients with significantly high admission Lp(a) levels, and remained elevated in all subjects (Fig. 3).

Discussion

In the present study of patients presenting with ACS, elevated Lp(a) was evident in a third of the population and was associated with younger age, premature CAD and previous revascularization indicating progressive CAD. Furthermore, high Lp(a) was related to clinical diagnosis of probable/

Table 1. Patient characteristics according to lipoprotein(a) [Lp(a)] level.

Variable	Total (n = 134)	Lp(a) < 72 nmol/L (n = 91)	Lp(a) > 72 nmol/L (n = 43)	P
Age [years] (range 29–65)	52.2 ± 8.0	53.1 ± 7.8	50.3 ± 8.3	0.056
Age at CAD diagnosis	49.9 ± 9.0	51.3 ± 8.9	47.1 ± 8.6	0.010
Gender (male)	111 (83%)	75 (82%)	36 (84%)	0.852
Family history of premature CAD	75 (56%)	51 (56%)	24 (54%)	0.980
Dyslipidemia	100 (75%)	69 (76%)	31 (72%)	0.643
Hypertension	71 (53%)	46 (51%)	25 (58%)	0.411
Diabetes mellitus	42 (31%)	27 (30%)	15 (35%)	0.544
Obesity (BMI > 30 kg/m^2)	51 (39%)	397 (43%)	12 (29%)	0.105
Current smoking	69 (52%)	51 (56%)	18 (42%)	0.125
Chronic kidney disease	13 (10%)	7 (8%)	6 (14%)	0.348
Stroke	5 (4%)	4 (4%)	1 (2%)	0.484
Previous revascularization	38 (28%)	20 (22%)	18 (42%)	0.017
LDL cholesterol [mg/dL]	123 ± 52	123 ± 52	124 ± 52	0.909
Triglycerides [mg/dL]	225 ± 218	223 ± 201	228 ± 251	0.902
HDL cholesterol [mg/dL]	35 ± 9	34 ± 9	37 ± 10	0.116
Peak LDL cholesterol [mg/dL]	165 ± 46	163 ± 47	171 ± 44	0.364
Probable/definite FH	16 (12%)	7 (8%)	9 (21%)	0.027
Previous statin therapy	65 (49%)	41 (45%)	24 (56%)	0.245
Cardiac surgery	23 (17%)	16 (17%)	7 (16%)	0.341
Unstable angina	15 (11%)	8 (9%)	7 (16%)	
NSTEMI	77 (58%)	55 (60%)	22 (51%)	0.381
STEMI	42 (31%)	28 (31%)	14 (33%)	
Three-vessel CAD	53 (40%)	35 (39%)	18 (42%)	0.707
Premature CAD	93 (69%)	56 (61%)	37 (86%)	0.004

BMI — body mass index; CAD — coronary artery disease; FH — familial hypercholesterolemia; HDL — high-density lipoprotein; LDL — low--density lipoprotein; NSTEMI — non-ST-segment elevation myocardial infarction; STEMI — ST-segment elevation myocardial infarction

Table 2. Multivariate regression analysis showing independent associations between clinical characteristics and elevated lipoprotein(a)*.

Variable	Odds ratio	95% CI	P
Previous revascularization	2.56	1.17–5.59	0.019
Premature CAD	3.85	1.48–10.07	0.06
Probable/definite FH	3.18	1.10–9.21	0.033

*Multivariable logistic regression model was adjusted to age, gender and previous statin therapy. CAD — coronary artery disease; CI — confidence interval; FH — familial hypercholesterolemia

definite FH. In contrast, traditional cardiovascular risk factors were not associated with elevated Lp(a), and no correlation was observed between admission CRP levels during ACS and Lp(a).

Plasma levels of Lp(a) are similar in men and women and show a skewed distribution in the population with a tail towards the highest levels. Lp(a) concentration is lower in non-Hispanic Caucasians and Asian populations, and higher in Hispanic and Black ethnic populations [18]. Individual studies used different thresholds to define elevated Lp(a), with common thresholds of 30 mg/dL and 50 mg/dL, corresponding to the 75th and 80th percentiles in the general population. In the setting of a large referral center, Lp(a) levels > 30 mg/dL and > 50 mg/dL were shown to be fairly common, present in 35% and 24% of the subjects, respectively [20]. This is in line with the current study, in which 32% of the patients presenting with ACS at a relatively young age had Lp(a) levels above a cutoff equivalent to 30 mg/dL.

Although there is an exponential relationship between Lp(a) levels and cardiovascular risk, in epidemiological and Mendelian randomization studies increased cardiovascular risk starts at a level as low as 20 mg/dL or 50 nmol/L, especially when
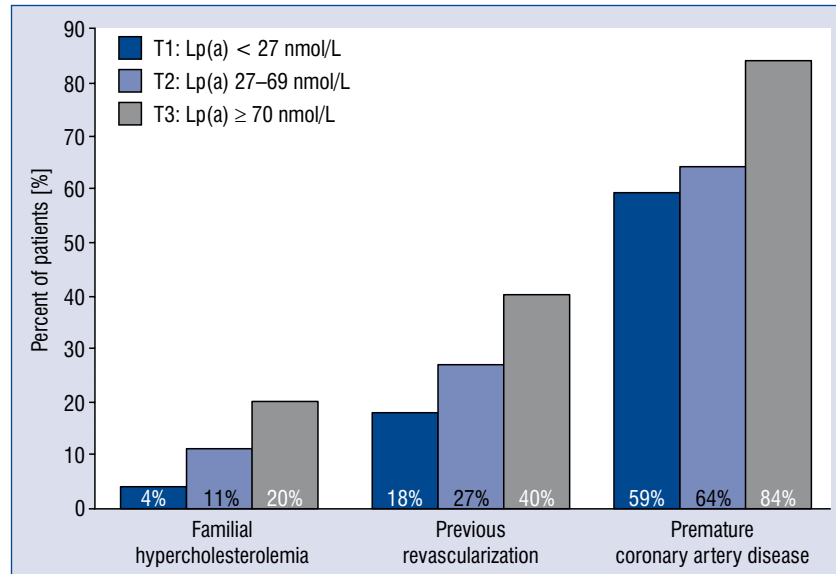
Figure 2. Prevalence of independent clinical risk markers according to lipoprotein(a) [Lp(a)] tertiles.
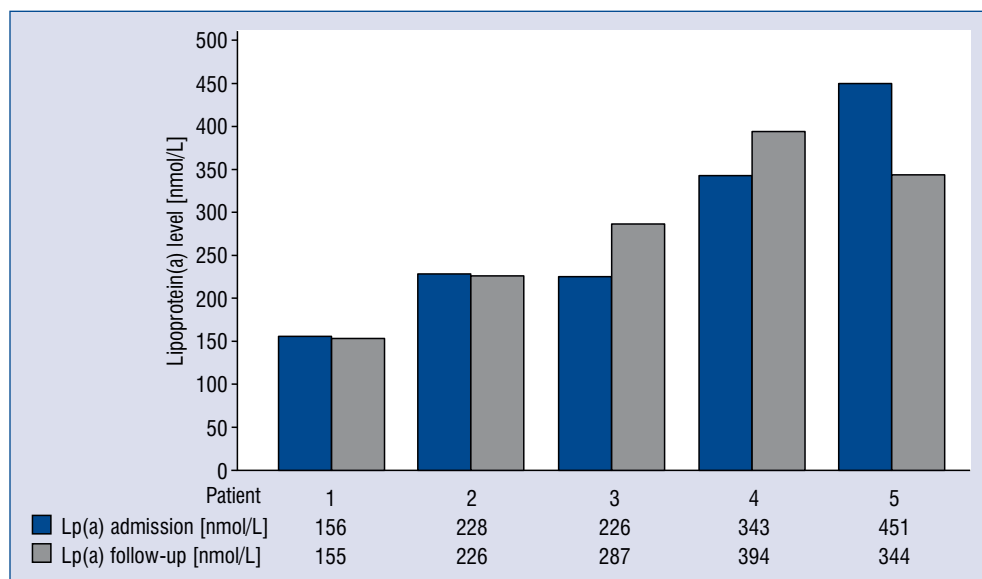
Figure 3. Admission versus post-hospitalization repeat lipoprotein(a) [Lp(a)] levels in 5 patients with acute coronary syndrome and elevated lipoprotein(a).

evaluated in primary care populations [10, 13, 18, 21]. In the setting of ACS, there are non-conclusive findings. Past studies have demonstrated an association between baseline Lp(a) concentrations and increased risk of cardiac death in patients admitted with ACS [22]. Lp(a) was also shown to be independently associated with ACS and subsequent cardiovascular events in younger and middle aged individuals below 60 years old [23–25]. However, data from sub-analyses of large prospec-

tive randomized trials of lipid-modifying therapies in patients with ACS or established CAD, showed conflicting results with some reporting no association between Lp(a) concentration and adverse cardiovascular outcomes [26–28], while others have demonstrated that Lp(a) was associated with increased cardiovascular risk [8, 29, 30]. Future studies with antisense oligonucleotides targeting apo(a), recently shown to reduce Lp(a) levels by 80% in phase 2 trials, may further shed light on

the impact of Lp(a) reduction on cardiovascular outcomes in patients with CAD [15]. The present findings of a stepwise association between tertiles of Lp(a) with premature and progressive CAD support the role of Lp(a) as a risk marker also in patients with ACS.

The European Society of Cardiology/European Atherosclerosis Society has given a Class IIa recommendation for measuring Lp(a) in patients with premature CVD, FH, family history of premature CVD or elevated Lp(a), as well as in those with recurrent CVD despite optimal lipid-lowering therapy, and also for risk reclassification in subjects with borderline risk [14]. Nevertheless, in many countries assays for Lp(a) measurement are not routinely available in clinical practice, often performed only at dedicated lipid clinics, and there is low awareness for the risk associated with high Lp(a). Current results are consistent with the above recommendations, demonstrating an independent association between the majority of these risk groups and high Lp(a) also in the setting of ACS. However, although the plasma level of Lp(a) is, to a major extent, genetically determined, no similar association was observed between Lp(a) and family history of premature CAD. This may have been affected by the use of an electronic chart diagnosis for defining positive family history and not by directly questioning the patients and preparing a family tree when appropriate.

Familial hypercholesterolemia is an autosomal co-dominant genetic disorder associated with raised concentrations of LDL cholesterol from birth and an elevated risk of premature CVD [31]. Concentrations of Lp(a) are raised in patients with FH compared with individuals with normal lipid levels, and data in patients with FH shows that high Lp(a) levels further increase cardiovascular risk [32, 33]. Prospective data from 46,200 individuals from the Copenhagen General Population Study showed that the risk of myocardial infarction (MI) was highest in patients classified as having both FH and high Lp(a) values, concluding that high Lp(a) concentrations represent a novel risk factor for clinical FH, and suggesting that all individuals with FH should have their Lp(a) measured in order to identify those with the highest concentrations, and as a result, the highest risk for MI [34]. The present results, demonstrate that a clinical diagnosis of probable/definite FH is independently associated with elevated Lp(a) and premature CAD in patients presenting with ACS, are compatible with a recent investigation concluding that the combination of elevated Lp(a) and phenotypic FH is commonly encountered in patients with premature CAD admitted to the coronary care unit [35]. Overall, this data supports the routine screening for both FH and elevated Lp(a) in young patients hospitalized in cardiac units for evaluation or treatment of CAD. This will also serve as an opportunity to perform cascade-screening of relatives of identified index-cases, due to the genetic nature of both disorders [36].

Past small-scale studies have reported conflicting findings regarding the associations between Lp(a) levels and inflammatory markers following MI, with both increases or no change in Lp(a) levels as a function of increasing CRP levels [16, 17, 37]. In patients with rheumatoid arthritis, high Lp(a) levels were related to an active inflammatory disease [38, 39]; while following an acute ischemic stroke, Lp(a) levels were shown to remain stable [40]. A more recent, far larger analysis, in the setting of the general population, reported only minimal increases in Lp(a) with increasing CRP levels [41]. Furthermore, the ability of elevated Lp(a) to predict ischemic heart disease and MI was not affected by markers of inflammation. In the current study, no correlation between CRP and Lp(a) concentration was found at admission of patients with ACS. In addition, in a small number of patients with significantly high Lp(a), repeat levels measured more than 2 months from the acute event remained high. These findings should be confirmed in larger studies, and could be clinically relevant, as Lp(a) measurement performed during the acute phase may lead to intensification of treatment such as with proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies and possibly future apo(a) antisense therapy, in addition to a more aggressive management of modifiable cardiovascular risk factors [15].

Limitations of the study

Several limitations of the current study should be acknowledged. This is a retrospective analysis of a single center, with a relatively small sample size. Nevertheless, Lp(a) levels are not routinely measured in the study region, and there is low awareness of the health care providers to Lp(a) and its associated risk. In addition, as Lp(a) level varies among different races, and results may not be generalizable to other races or geographical areas. No genetic testing was performed for diagnosing FH, although a customary algorithm for phenotypically diagnosing probable and definite FH was used. Finally, it should be noted that theindependent associations between clinical variables

and Lp(a) levels described in this analysis do not prove causation.

Conclusions

In young and middle-aged patients ≤ 65 years of age presenting with ACS, previous revascularization, premature CAD and FH were independently associated with elevated Lp(a). These findings, limited by a small sample size, are in accordance with guideline based recommendations for Lp(a) screening, and suggest that testing for Lp(a) in young patients in the setting of ACS may address residual cardiovascular risk, with potential clinical benefit in light of the novel emerging therapies targeting Lp(a).

Conflict of interest: None declared

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VIA MEDICA

ORIGINAL ARTICLE

Impact of left atrial appendage closure on cardiac functional and structural remodeling: A difference-in-difference analysis of propensity score matched samples

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Abstract

Background: *Although the safety and efficacy of left atrial (LA) appendage (LAA) closure (LAAC) in nonvalvular atrial fibrillation (NVAF) patients have been well documented in randomized controlled trials and real-world experience, there are limited data in the literature about the impact of LAAC on cardiac remodeling. The aim of the study was to examine the impact of LAAC on cardiac functional and structural remodeling in NVAF patients.*

Methods: *Between March 2014 and November 2016, 47 NVAF patients who underwent LAAC were included in this study (LAAC group). A control group (non-LAAC group) was formed from 141 NVAF patients without LAAC using propensity score matching. The difference-in-difference analysis was used to evaluate the difference in cardiac remodeling between the two groups at baseline and follow-up evaluations.*

Results: *The LAAC group had a larger increase in LA dimension, volume and volume index than the non-LAAC group (+3.9 mm, p = 0.001; +9.7 mL, p = 0.006 and +5.9 mL/m², p = 0.011, respectively). Besides, a significant increase in E and E/e' ratio was also observed in the LAAC group (+14.6 cm/s, p = 0.002 and +2.3, p = 0.028, respectively). Compared with the non-LAAC group, left ventricular (LV) ejection fraction and fractional shortening decreased in LAAC patients, but were statistically insignificant (–3.5%, p = 0.109 and –2.0%, p = 0.167, respectively).*

Conclusions: *There were significant increases in LA size and LV filling pressure among NVAF patients after LAAC. These impacts of LAAC on cardiac functional and structural remodeling may have some clinical implications that need to be addressed in future studies.* (Cardiol J 2019; 26, 5: 519–528)

Key words: left atrial appendage closure, atrial fibrillation, stroke prevention, diastolic function, systolic function, cardiac remodeling, difference-in-difference analysis

Introduction

Thrombus formed inside the left atrial (LA) appendage (LAA) is the most common cause of ischemic stroke in nonvalvular atrial fibrillation (NVAF) patients [1–3]. Although oral anticoagulant (OAC) has been shown to be effective in reducing the incidence of ischemic stroke, it also increases the risk of hemorrhage complications in these patients [4–6]. Percutaneous LAA closure (LAAC) is considered to be effective both in decreasing the risk of stroke and lowering the bleeding complica-

tion of OAC in NVAF patients with concomitant high bleeding risk [7–9].

Left atrial appendage has several important mechanical and endocrine functions regarding unique anatomical and physiological properties [10, 11]. When LA volume or pressure overload happens, LAA becomes a significant reservoir chamber due to its distensible ability [12]. LAA removal resulted in increased LA size, LA pressure and decreased cardiac output in animals [13]. The removal may be particularly harmful with existing heart failure because it would further reduce the cardiac output and promote heart failure. Besides, LAA is also known as the source of atrial natriuretic peptide (ANP) in the human heart. The concentration of ANP is much higher in LAA walls than in the rest of the atrial free wall and in the ventricles [11]. Patients with LAA removal were found to have a significantly lower ANP secretion and concomitant increase in salt and water retention [14, 15]. Interestingly, percutaneous LAAC with devices for ischemic stroke prevention has been shown to be associated with a significant reduction in plasma ANP [16, 17]. Therefore, both LAA mechanical and endocrine functions will be altered after LAAC. These changes can further affect the cardiac function and structure.

Although the safety and efficacy of LAAC in NVAF patients are well documented, there are limited data in the literature about the impact of percutaneous LAAC on cardiac functional and structural remodeling.

Methods

Patients

The data of NVAF patients, who underwent LAAC between March 2014 and November 2016 (LAAC group), were retrospectively collected before LAAC (baseline) and 12 months after the procedure (follow-up). All of these patients had permanent atrial fibrillation (AF,) high risk of ischemic stroke (CHA$_2$DS$_2$-VASc score $\geq$ 2), and contraindication to OAC or high risk of bleeding (HAS-BLED score $\geq$ 3). A control group (non-LAAC group) was formed by including NVAF patients without LAAC that were being followed-up at the documented institute. For each case in the LAAC group, three control subjects were matched (1:3 style) based on sex, age and ejection fraction (EF) using propensity score matching. Patients with incomplete data or with the following conditions were excluded from the study: device embolization, significant residual peri-device leak ($\geq$ 5 mm) detected on follow-up

transesophageal echocardiography (TEE), mitral valve stenosis, proximal AF or AF that was converted to sinus rhythm during follow-up, prosthetic valve, atrial septal defect, dilated cardiomyopathy, and moderate to severe mitral valve regurgitation.

Devices

The devices used for LAAC were either Cardiac Plug or Amulet (St. Jude Medical, St. Paul, Minnesota, USA). Cardiac Plug is a self-expanding device that is made from nitinol wire mesh with a lobe and a disc connected via a waist. The design is aimed at sealing the body and ostium of the LAA. The lobe is usually implanted 10 mm inside the LAA body, and the anchoring mechanism is aided by stabilizing wires. The Amulet (or Cardiac Plug 2) is the second generation of the Cardiac Plug, which retains the basic structure of Cardiac Plug with some modifications for better performance and sealing (Fig. 1).

Procedure

Before the procedure, TEE was performed to assess LAA size, morphology and to confirm the absence of thrombus in LA and LAA. After transseptal puncture, heparin was administered to achieve an active clotting time of 250–350 s [18]. At least 2 standard projections (RAO cranial and RAO caudal) were performed to obtain good visualization of the LAA. Fluoroscopy and TEE (or intra-cardiac echocardiography) imaging were used to re-evaluate the LAA and to select an appropriate device for each patient. The device size was chosen to be at least 20% larger than the measured diameter at the landing zone [18]. TEE (or intra-cardiac echocardiography) was used to check for the compression, positioning, stability of the device, peri-device leak and the relationship between device and adjacent structures. Transthoracic echocardiography, electrocardigraphy and chest X-ray were performed within 24 h after the procedure to rule out complications. All patients were discharged with dual antiplatelet therapy. The follow-up echocardiography was performed at 1 and 6 months, and then every 12 months after the procedure.

Echocardiography

Echocardiography was performed using the Philips EPIQ 7 ultrasound system for cardiology (Philips Corporation, MA, USA) with an S8-3 sector array transducer. All acquired data were stored in digital files for offline interpretation and were then evaluated by a single experienced expert in cardiovascular imaging. The measurement and quantification followed the recommendations from

Feature	Ampalzer™ Cardiac Plug								Amplazer™ Amulet							
Size	16	18	20	22	24	26	28	30	16	18	20	22	25	28	31	34
Disc diameter	Lobe + 4 mm				Lobe + 6 mm				Lobe + 6 mm				Lobe + 7 mm			
Lobe length	6.5 mm								7.5 mm				10 mm			
Waist length	4 mm								5.5 mm				8 mm			
SW diameter	0.006 inch								0.0065 inch							
SW pairs	6								6				8		10	
Sheath (Fr)	9				10		13		12				14			
Proximal disc end-screw	Protruding								Recessed							
Preparation	Partly pre-load								Fully pre-load							
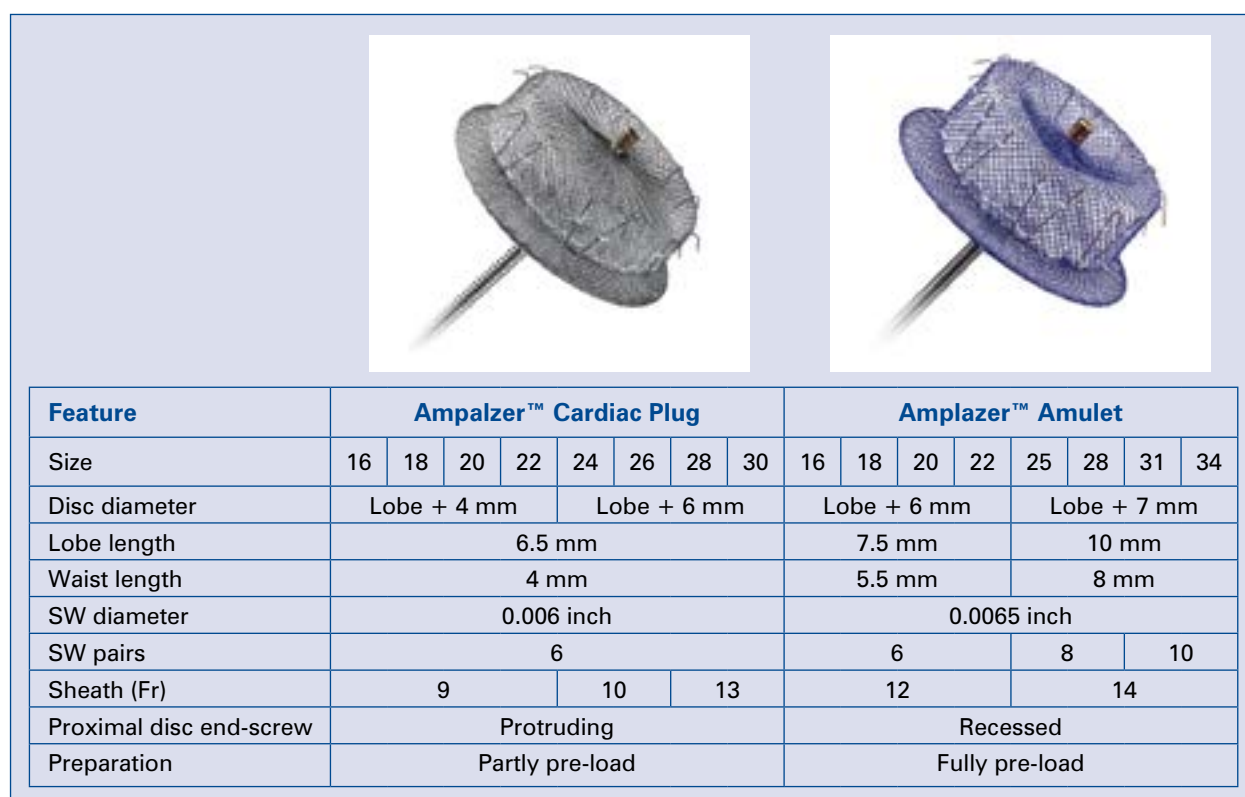
Figure 1. Ampalzer™ Cardiac plug and Amplazer™ Amulet; SW — stabilising wire.

the American Society of Echocardiography [19, 20]. The parameters were obtained by averaging five consecutive cardiac cycles. In this study, baseline and follow-up echocardiography data were collected in both LAAC and non-LAAC groups.

In the M-mode, the following parameters were measured at parasternal long axis view: left ventricular internal dimension at end diastole (LVDd), left ventricular internal dimension at end systole (LVDs), interventricular septum thickness at end diastole (IVSd), interventricular septal thickness at end systole (IVSs), left ventricular posterior wall thickness at end diastole (LVPWd), left ventricular posterior wall thickness at end systole (LVPWs), left atrial dimension (LAD). The Teichholz method was used to calculate EF (the M-mode left ventricular ejection fraction). Cube formula was used to estimate left ventricular mass (LVM) and left ventricular mass indexed to body surface area (LVMI). Tricuspid annular plane systolic excursion (TAPSE) was measured on apical four-chamber views.

Pulse-wave Doppler was used to measure E (peak velocity of early diastolic trans-mitral flow) and deceleration time of early diastolic trans-mitral flow (DT). Tissue Doppler was used to measure e'

(peak velocity of early diastolic mitral annular motion). The E/e' ratio was then calculated.

In two-dimensional (2D) mode, biplane left ventricular long-axis length at end diastole (LVLd) and biplane left ventricular long-axis length at end systole (LVLs) were calculated by averaging measurements from apical two-chamber and apical four-chamber views. The biplane technique of disk summation (modified Simpson's rule) was used to calculate left ventricular end diastolic volume (EDV), left ventricular end systolic volume (ESV), left ventricular stroke volume (SV) and left ventricular ejection fraction (EF) in the 2D mode [20]. Right atrial dimension (RA), right ventricular basal and mid cavity dimensions (RVb and RVm, respectively) were measured on apical four-chamber view that was slightly focused on the right heart chambers. left atrial volume (LAV) and right atrial volume (RAV) were calculated using the area length method [20, 21]. The change of LAV index (LAVI) was defined as the difference between follow-up and baseline LAVI (ΔLAVI = follow-up LAVI – baseline LAVI). Similarly, the change of the E/e' ratio was the difference between follow-up and baseline E/e' ratio (ΔE/e' = follow-up E/e' – baseline E/e').

Table 1. The baseline characteristics of participants.

Variable	LAAC group (n = 47)	Non-LAAC group (n = 141)	P
Age [year]	75.1 ± 9.9	74.7 ± 9.7	0.799
Male gender	20 (42.6%)	60 (42.6%)	1.000
BMI [kg/m^2]	24.4 ± 3.8	24.3 ± 3.7	0.953
HAS-BLED score	2.55 ± 1.41	2.23 ± 1.34	0.156
CHA$_2$DS$_2$-VASc score	3.83 ± 1.98	3.32 ± 1.65	0.082
Labile INR	6 (12.8%)	20 (14.2%)	0.807
Bleeding	8 (17.0%)	23 (16.3%)	0.910
Stroke or TIA	21 (44.7%)	49 (34.8%)	0.223
Hypertension	32 (68.1%)	92 (65.2%)	0.722
Diabetes mellitus	6 (12.8%)	21 (14.9%)	0.719
Congestive heart failure	14 (29.8%)	35 (24.8%)	0.502
Chronic kidney disease	5 (10.6%)	13 (9.2%)	0.775
Vascular diseases	7 (14.9%)	14 (9.9%)	0.349
Heart rate [bpm]	78.0 ± 16.9	80.4 ± 18.1	0.429
Cardiothoracic ratio	0.58 ± 0.06	0.59 ± 0.07	0.538
Left ventricular ejection fraction	56.7 ± 10.8	55.4 ± 11.7	0.425

LAAC — left atrial appendage closure; BMI — body mass index; INR — international normalized ratio; TIA — transient ischemic attack

Statistical analysis

To reduce the time-modified confounding effect, the difference-in-difference (DID) estimator was adopted to evaluate the impact of percutaneous LAAC on cardiac remodeling among NVAF patients [22, 23]. The analysis focused on comparing cardiac functional and structural changes that occurred between baseline and follow-up in both intervention (LAAC group) and control (non-LAAC group) groups. The model that was used for this DID regression is written as: $Y_{it} = \beta_0 + \beta_1 G_i + \beta_2 T_t + \beta_3 G_i T_t + \varepsilon_{it}$, where, Y_{it} is the parameter Y in participant i measured at time t (baseline or follow-up); β_0 is a constant; G_i is a dummy that indicates whether this participant was in the LAAC group ($G_i = 1$) or in the non-LAAC group ($G_i = 0$); T_t is a dummy that indicates whether the parameter was measured at the follow-up ($T_t = 1$) or at the baseline ($T_t = 0$); ε_{it} represents the controlled variables. The main parameter of interest was β_3 (the DID estimator), which indicated whether LAAC patients had more cardiac functional and structural changes over time than the NVAF patients without LAAC. The DID estimator revealed the real effect of LAAC on cardiac functional and structural remodeling after removing the effect of factors related to the time trend such as aging, disease progression, social life change, new medication, and other factors.

Statistical analyses were performed using SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as the mean ± standard deviation. Categorical variables were presented as frequencies and percentages. The χ^2 test was used to compare categorical variables, and for independent samples T-test was used to compare continuous variables. Multiple regression with the stepwise method was used to identify independent predictors of cardiac remodeling in the LAAC group, which had a significant association recognized using univariate analysis. A two-sided p value < 0.05 was considered statistically significant.

Results

Out of 188 NVAF patients who were included in this study, 47 were in the LAAC group, and 141 were in the non-LAAC group. The baseline characteristics of the participants are summarized in Table 1. There were no statistically significant differences in age, sex, body mass index, EF and cardiothoracic ratio between the two groups. The HAS-BLED score, the CHA$_2$DS$_2$-VASc score and the rate of stroke history were higher in the LAAC group than that in the non-LAAC group. However, these differences were also statistically insignificant. The mean procedure time was 94.2 ± 42.9 min,

Table 2. Changes on cardiac configuration evaluated by difference-in-difference (DID) analysis.

Parameters	LAAC group (n = 47)		Non-LAAC group (n = 141)		DID estimator	95% CI	P
	Baseline	Follow-up	Baseline	Follow-up			
M-mode							
LAD [mm]	48.2 ± 8.7	53.6 ± 9.0	49.9 ± 8.5	51.3 ± 8.6	+3.9	1.9; 5.8	**0.001**
LVDd [mm]	51.1 ± 5.5	50.8 ± 5.7	51.1 ± 6.3	50.7 ± 6.5	+0.7	−1.1; 1.7	0.685
LVDs [mm]	34.6 ± 6.2	35.7 ± 6.7	34.1 ± 7.0	33.5 ± 7.5	+1.7	−0.1; 3.5	0.064
IVSd [mm]	9.5 ± 1.3	9.2 ± 1.0	10.0 ± 1.6	9.7 ± 1.6	-0.2	−0.6; 0.3	0.447
IVSs [mm]	13.5 ± 2.1	13.0 ± 1.5	14.2 ± 2.5	14.1 ± 2.6	−0.4	−1.2; 0.4	0.298
LVPWd [mm]	9.3 ± 1.2	8.9 ± 0.9	9.3 ± 1.2	9.5 ± 3.2	−0.4	−1.4; 0.6	0.398
LVPWs [mm]	14.1 ± 2.5	13.4 ± 2.1	14.5 ± 2.4	14.4 ± 2.4	−0.6	−1.5; 0.3	0.209
2D-mode							
LVLd [mm]	73.9 ± 8.1	72.4 ± 6.2	74.5 ± 9.2	74.2 ± 9.1	−0.9	−2.9; 1.1	0.383
LVLs [mm]	64.6 ± 7.8	62.2 ± 6.2	65.1 ± 8.3	64.3 ± 8.2	−1.6	−3.9; 0.6	0.154
RVb [mm]	33.3 ± 8.3	34.5 ± 4.6	34.0 ± 5.0	35.0 ± 6.9	+0.2	−2.4; 2.8	0.887
RVm [mm]	22.3 ± 3.4	22.7 ± 3.0	22.2 ± 2.7	23.5 ± 4.9	−0.9	−2.6; 0.7	0.262
RAD [mm]	40.8 ± 8.8	40.7 ± 5.7	43.3 ± 8.4	44.0 ± 9.1	−0.9	−3.9; 2.4	0.589
Volume and mass							
EDV [mL]	99.4 ± 29.4	101.3 ± 29.2	104.6 ± 37.3	107.6 ± 46.4	−1.6	−10.5; 7.3	0.722
ESV [mL]	39.1 ± 19.9	41.0 ± 29.1	42.3 ± 23.0	43.5 ± 29.5	+0.3	−5.8; 6.4	0.926
SV [mL]	47.0 ± 16.0	46.8 ± 12.2	48.4 ± 18.3	49.8 ± 18.5	−1.5	−6.5; 3.4	0.540
LAV [mL]	90.3 ± 36.7	108.4 ± 37.7	95.9 ± 35.9	104.2 ± 44.9	+9.7	3.6; 15.9	**0.006**
LAVI [mL/m²]	54.7 ± 23.0	68.1 ± 23.5	60.0 ± 24.4	67.5 ± 31.4	+5.9	1.7; 9.8	**0.011**
RAV [mL]	67.1 ± 39.1	62.3 ± 17.8	78.4 ± 40.6	76.1 ± 50.8	−1.8	−13.8; 10.2	0.764
LVM [g]	173.6 ± 46.3	162.1 ± 33.8	181.5 ± 50.1	176.4 ± 49.5	−6.4	−17.3; 4.5	0.246
LVMI [g/m²]	107.2 ± 26.3	100.2 ± 21.6	110.4 ± 31.0	107.9 ± 29.2	−4.6	−10.8; 3.1	0.280

LAAC — left atrial appendage closure; CI — confidence interval; LAD — left atrial dimension; LVDd/LVDs — left ventricular internal dimension at end diastole/systole; IVSd/IVSs — interventricular septum thickness at end diastole/systole; LVPWd/LVPWs — left ventricular posterior wall thickness at end diastole/systole; LVLd/LVLs — biplane left ventricular diastolic/systolic length; RVb/RBm — right ventricular base/middle dimension; RAD — right atrial dimension; EDV/ESV — biplane left ventricular volume at end diastole/systole; SV — biplane left ventricular stroke volume; LAV — left atrial volume; LAVI — left atrial volume indexed to body surface area; RAV — right atrial volume; LVM — left ventricular mass; LVMI — left ventricular mass index

and Cardiac Plug was used in 30 (66.0%) patients.

The impact of LAAC on cardiac structural remodeling is demonstrated in Table 2. There was no significant difference in structural change of left ventricle, right ventricle and right atrium between the two groups. However, the LAAC group had a higher relative increase in LAD, LAV and LAVI than the non-LAAC group with positive DID estimators (+3.9 mm, p = 0.001; +9.7 mL, p = 0.006 and +5.9 mL/m², p = 0.011, respectively).

The impact of LAAC on cardiac functional remodeling is presented in Table 3. In comparison with the non-LAAC group, EF and fractional shortening had more relative decrease among LAAC patients with a negative DID estimator, but these changes were statistically insignificant (−3.5%,

p = 0.109 and −2.0%, p = 0.167, respectively). Additionally, there was a significant relative increase in E and E/e' ratio observed in patients with LAAC (+14.6 cm/s, p = 0.002 and +2.3, p = 0.028, respectively).

Univariate analysis showed that the relative LAVI change in the LAAC group was associated with baseline LAD ($\beta = -0.56$, p < < 0.001), IVSd ($\beta = -0.33$, p < 0.05); RVm ($\beta = -0.35$, p < 0.05), E ($\beta = -0.38$, p < 0.05) and pulmonary arterial systolic pressure ($\beta = -0.29$, p < 0.05). Besides, the relative change of the E/e' ratio was correlated with IVSd ($\beta = -0.34$, p < 0.05), LVPWd ($\beta = -0.49$, p < 0.001), LVM ($\beta = -0.29$, p < 0.05) and RVm ($\beta = -0.30$, p < 0.05). In multiple linear regression with the stepwise method, the predictor of the relative LAVI change was baseline LAD ($\beta = -0.94$, p < 0.001,

Table 3. Changes on cardiac function and pulmonary arterial pressure evaluated by difference-in-difference (DID) analysis.

Parameters	LAAC group (n = 47)		Non-LAAC group (n = 141)		DID estimator	95% CI	P
	Baseline	End-line	Baseline	End-line			
Systolic function							
FS [%]	32.3 ± 7.7	31.2 ± 7.1	33.4 ± 8.2	34.3 ± 8.3	–2.0	–4.9; 0.9	0.167
EF [%]	63.1 ± 12.1	60.1 ± 11.2	64.3 ± 12.3	64.8 ± 12.2	–3.5	–7.7; 0.8	0.109
EF$_{2C}$ [%]	58.9 ± 10.9	56.6 ± 9.5	55.3 ± 12.4	55.8 ± 11.1	–2.9	–6.6; 0.7	0.111
EF$_{4C}$ [%]	56.5 ± 12.4	55.2 ± 9.9	55.2 ± 11.0	55.6 ± 10.2	–1.4	–4.8; 1.9	0.404
EF$_{BP}$ [%]	57.7 ± 10.8	55.8 ± 9.2	55.4 ± 11.7	55.7 ± 10.1	–2.2	–5.3; 0.9	0.164
TAPSE [mm]	11.7 ± 2.2	12.0 ± 1.7	11.9 ± 2.3	11.9 ± 2.4	+0.4	–0.7; 1.4	0.476
Diastolic function							
DT [ms]	183.5 ± 73.1	181.0 ± 73.1	182.8 ± 75.3	182.7 ± 71.8	–3.79	–36.8; 29.3	0.819
E [cm/s]	85.6 ± 28.4	106.1 ± 27.3	89.5 ± 26.9	95.3 ± 28.8	+14.6	6.18; 22.9	**0.002**
E/e' ratio	14.3 ± 6.9	18.4 ± 7.8	14.4 ± 7.3	16.2 ± 7.3	+2.3	0.3; 4.3	**0.028**
Pulmonary arterial pressure							
PAPs	39.1 ± 11.0	40.1 ± 9.5	39.2 ± 10.9	40.0 ± 12.8	+0.24	–3.7; 4.2	0.903

LAAC — left atrial appendage closure; CI — confidence interval; FS — left ventricular fractional shortening; EF — left ventricular ejection fraction on M-mode; EF$_{2C}$ — left ventricular ejection fraction on two chamber view; EF$_{4C}$ — left ventricular ejection fraction on four chamber view; EF$_{BP}$ — biplane left ventricular ejection fraction measured; TAPSE — tricuspid annular plane systolic excursion; DT — deceleration time of early diastolic trans-mitral flow; E — peak velocity of early diastolic trans-mitral flow; e' — peak velocity of early diastolic mitral annular motion; PAPs — pulmonary arterial systolic pressure

R = 0.55), and the predictor of the relative E/e' ratio change was baseline LVPWd (β = –2.23, p < 0.01, R = 0.477).

Discussion

The present study showed that there were several impacts of LAAC on cardiac functional and structural remodeling in NVAF patients. Significant increases were identified in LA size and LV filling pressure in this group of patients 12 months after the procedure.

Percutaneous LAAC is sometimes the only option for ischemic stroke prevention, especially in NVAF patients with contraindication for long-term OAC or high risk of bleeding. Although the safety and efficacy of this treatment have been demonstrated in randomized controlled trials and real-world experience [5–7, 24, 25], the data about the impacts of LAAC on cardiac remodeling are not well established. To evaluate the impact of this therapy on cardiac functional and structural remodeling, changes were measured and compared of echocardiographic parameters between LAAC and non-LAAC groups before and 12 months subsequent to the procedure.

A remarkable finding in this study was noted, a significantly higher increase in LAD (+3.9 mm),

which may predictably lead to a higher increase in LAV (+9.7 mL) and the LAVI (+5.9 mL/m^2) among LAAC patients after the procedure in comparison with non-LAAC patients (Fig. 2A, B). This LA remodeling may be explained by the alteration of LAA function after LAAC. Previous studies have shown that there was a decrease in ANP concentration after percutaneous LAA device closure for ischemic stroke prevention [15, 17]. Even though the mechanism of this phenomenon has not been fully understood, the insufficiency of this endocrine hormone may let the LA suffer more from pressure and volume overload. Besides, LAA is more compliant than the LA main chamber and plays an important role in the presence of LA pressure and/or volume overload [26, 27]. The separation between LAA and LA after complete endothelialization of the device results in the disappearance of this LAA reservoir function [14]. An animal study has shown that LA compliance decreased after removal of the LAA, and the change in compliance was associated with decreased atrial reservoir function, which was manifested by smaller reservoir volume alterations [13]. Furthermore, LAA clamping during coronary artery bypass grafting or mitral valve surgery indicated a significant increase in LA maximal dimension [14]. A recent study that evaluated the impact of LAAC on LA mechanical
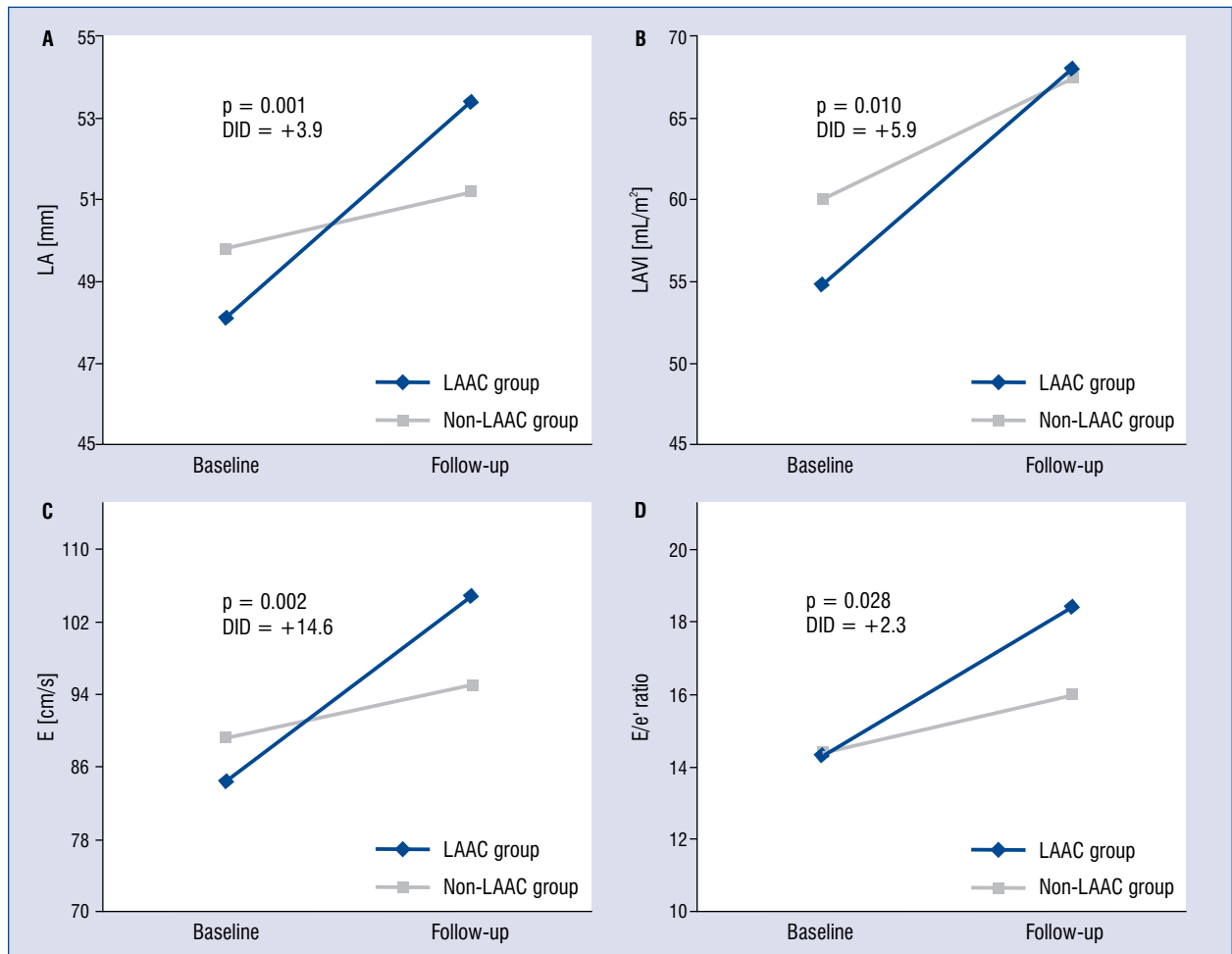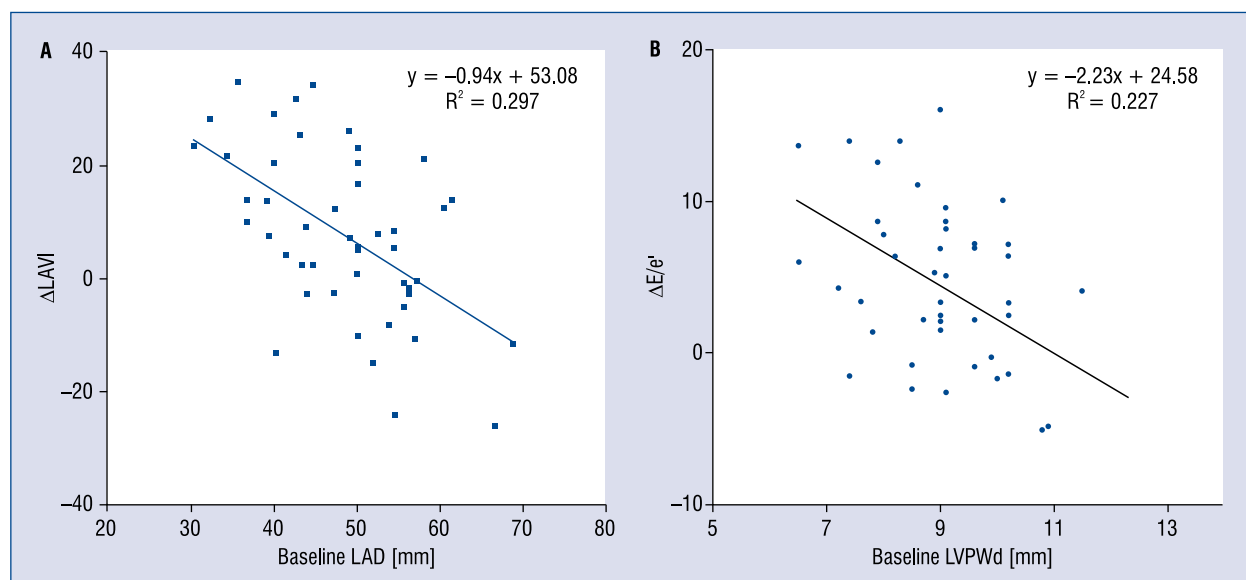
Figure 2. A–C. Changes in cardiac function and structure between baseline and follow-up; LA — left atrial; LAVI — left atrial volume indexed to body surface area; E — peak velocity of early diastolic trans-mitral flow; e' — peak velocity of early diastolic mitral annular motion; DID — difference-in-difference analysis; LAAC — left atrial appendage closure.

function also found that there was an increase in maximum LAV, LAVI and expansion index among NVAF patients who received this treatment [28]. However, these studies presented the alteration of LAD and LAV in a short time period, ranging from hours to 60 days. Another study used 3D-echocardiographic measurements in patients 6 months after LAAC with Watchman device and showed that LAV increased significantly after interventional LAAC. However, the LA enlargement did not correlate with clinical progression of heart failure [29]. In the present study, a similar consequence of LAA isolation, related to the increase in LA size, could be observed 12 months after LAAC procedure. Furthermore, multiple linear regression with the stepwise method showed that the predictor of LAVI change was baseline LAD ($\beta = -0.94$, p < 0.001, R = 0.545). Interestingly, the negative correlation suggested that if LA had had

a more normal appearance at the baseline (smaller LA size), then it would be more impacted after the LAAC (Fig. 3A). Thus, clinicians should be more careful in selecting patients with a less remodeled LA for LAAC. In addition, it may be necessary to pay more attention to medical therapy and follow-up for this subgroup of patients after the procedure to reduce LA adverse remodeling.

Another important finding in the present study was a significant increase in the E/e' ratio, which indicated an increase in LV filling pressure among patients with LAAC (Fig. 2D). In AF, atrial contraction is lost, Doppler assessment of LV filling pressure is limited by the variability in cycle length and the absence of organized atrial activity [19, 30]. However, echocardiographic parameters that are independent of atrial influence can be utilized to assess LV filling pressure in AF patients. Among these parameters, the E/e' ratio was documented

Figure 3. A, B. Correlation between cardiac function and configuration changes with corresponding independent predictors; LAVI — left atrial volume indexed body surface area; e — peak velocity of early diastolic transmitral flow; e' — peak velocity of early diastolic mitral annular motion; LAD — left atrial dimension; LVPWd — left ventricular posterior wall thickness at end diastole; ΔLAVI = follow-up LAVI – baseline LAVI; E/e' = follow-up E/e' – baseline E/e'.

to have a most reliable correlation with invasive assessment of LV filling pressure [19, 31, 32]. Thus, the increase of the E/e' ratio in the present study was a reliable manifestation of LV filling pressures elevation among AF patients after LAAC. This may be a consequence of inadequate volume and pressure regulation in LA because of LAA function loss following LAAC procedure. Previous studies confirmed that clamping or surgical excision of the appendage resulted in an immediate increase in mean atrial pressure [13, 27]. Besides, the elevated LV filling pressure in AF patients, which was estimated by E/e', was also found to be independently associated with atrial remodeling [33]. Therefore, the elevation of LV filling pressure was a good explanation for the increase in LAD and LAV after LAAC in this study [34–36]. Further analysis with multivariable regression showed that the change of LV filling pressure in LAAC group, represented by the E/e' ratio, was independently associated with LVPWd ($\beta = -2.23$, p < 0.01, R = 0.477) (Fig. 3B). Besides, the correlation of the E/e' ratio with IVSd, LVPWd, LVM in univariate analysis suggested the important role of baseline LV wall thickness in the alteration of LV filling pressure after LAAC.

The result of this study also showed no significant change in LV and RV systolic function. However, all of the negative DID estimators of EF and fractional shortening change may elicit the decrease in systolic function among NVAF patients after LAAC. Along with increase in LA size and LV filling pressure, these changes may have some long-term physiological and clinical implications that need to be addressed.

Limitations of the study

This was a retrospective study with a limited number of patients in the LAAC group. Observed changes may be caused by chance in this small study cohort. Furthermore, because only some relevant indicators related to cardiac remodeling were collected, the alteration of other parameters as well as clinical presentations may have happened and were not observed in this study. Similarly, changes in medication that may affect cardiac remodeling was not evaluated. Besides, patients were not matched for LA size at baseline, which may differently affect cardiac remodeling. Because the study population had a normal EF and elevated LV filling pressure at baseline, the results may only be specific to this population.

Conclusions

The present study showed that there was a significant increase in LA size and LV filling pressure among NVAF patients after LAAC. These impacts of LAAC on cardiac functional and structural re-

modeling observed on echocardiography may have clinical implications that need to be addressed in future studies.

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It is acknowledged that all authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest: None declared

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VIA MEDICA

ORIGINAL ARTICLE

Does the use of cardiopulmonary resuscitation feedback devices improve the quality of chest compressions performed by doctors? A prospective, randomized, cross-over simulation study

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Abstract

Background: *The aim of the study was to compare the quality of chest compressions (CCs) carried out with and without the use of the TrueCPR device during simulated cardiopulmonary resuscitations conducted by trainee doctors.*

Methods: *The study was a prospective, randomized, cross-over simulation study. The study involved 65 trainee doctors who were tasked with performing a 2-min cycle of uninterrupted CCs under conditions of a simulated cardiopulmonary resuscitation of adults. CC were carried out in two scenarios: with and without TrueCPR chest compression support. Participants did not have experience in the use of CCs prior to this study.*

Results: *The depth of compressions in regard to CC techniques were varied by 45 mm (IQR 43–48) for manual CC and 53 mm (IQR 51–55) for the TrueCPR device (p < 0.001). The incidence of CCs with and without TrueCPR was: 112 (IQR 103–113) vs. 129 (IQR 122–135) compressions (p = 0.002). The degree of complete chest relaxation with the TrueCPR device was 95% (IQR 76–99) and without the device, 33% (IQR 29–38) (p < 0.001).*

Conclusions: *In the simulation study performed, the use of the TrueCPR device resulted in a significant improvement in the quality of CCs in relation to frequency and depth of CCs and correctness of chest relaxation.* (Cardiol J 2019; 26, 5: 529–535)

Key words: cardiopulmonary resuscitation, chest compressions, quality, medical simulation, doctor

Introduction

Sudden cardiac arrest is a challenge for modern medicine, resulting not only from the scale of the phenomenon, but also the social and economic burden of the health care system [1, 2]. In Europe, it is indicated that the annual incidence of EMS--treated out-of-hospital cardiopulmonary arrests for all rhythms is 38 per 100,000 population [3].

Current guidelines for cardiopulmonary resuscitation indicate high quality chest compressions (CCs) as an element affecting the effectiveness of

cardiopulmonary resuscitation. The quality of CCs is made up of factors such as minimization of CCs, CCs to the appropriate depth and with appropriate frequency, full chest relaxation and correct positioning of the hands on the chest [4–6]. Minimizing breaks in CCs has a direct impact on increasing the perfusion pressure during resuscitation procedures, both in adults and pediatric patients [7]. As Ewy et al. [8] research indicates, the first CCs are ineffective in the context of the perfusion pressure, only compression 7–8 is effective. This situation is repeated each time there is a break in the CCs, which includes performing rescue breaths in the case of resuscitation in a cycle of 30 compressions to 2 rescue breaths. Minimizing breaks in CCs can be achieved by instrumental protection of the airways by means of an endotracheal tube or supraglottic ventilation device and asynchronous resuscitation, during which there is no need to take breaks for rescue breaths [6, 7]. For such a solution, Ewy et al. [8] and many other authors [9, 10] point to an optimal method of resuscitation. In the case of adult CCs, the guidelines of the European Resuscitation Council (ERC) [11], as well as the American Heart Association (AHA) [4], indicate that it should be between 50 and 60 mm. In addition, CCs should be carried out with a frequency of 100–120 compressions per minute (cpm). Faster CCs may result in a higher perfusion pressure; however, a faster time causes greater fatigue to the chest compressor, which in the case of prolonged resuscitation can lead to a significant deterioration of the quality of CCs. Another important component responsible for the quality of CCs is the correctness of chest relaxation. Performing CCs at an appropriate depth and then leading to full chest relaxation results in the creation of an appropriate pressure difference in the chest, which determines the perfusion pressure. Incomplete chest relaxation will result in a reduction of perfusion pressure, thereby reducing chances of spontaneous circulation returning [12–14].

The correctness of hand position on the chest also plays an important role in the context of quality of CCs. The current guidelines [4, 5] recommend that the hands be placed in the middle of the chest on the sternum, in addition, the person pressing on the chest should take such a position relative to the patient that his upper limbs are at right angles to the patient's chest. Bad hand placement on the chest may cause damage to the patient's chest [15]. Performing CCs based on the above recommendations determines the highest quality of CCs,
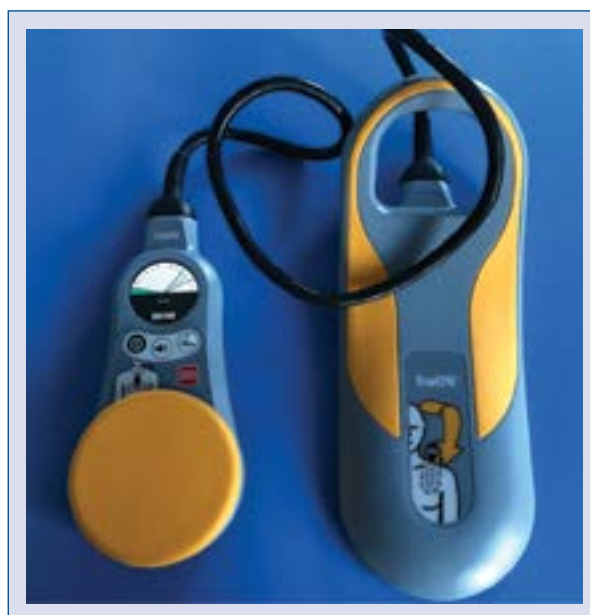
Figure 1. TrueCPR cardiopulmonary resuscitation feedback device.

translating directly to the resuscitation outcome in the form of a return of spontaneous circulation [16].

The aim of the study was to compare the quality of CCs performed unmanageaged and using the TrueCPR device (Fig. 1) during simulated cardiopulmonary resuscitation conducted by trainee doctors.

Methods

The study was a randomized, cross-over study and was conducted under conditions of medical simulation. The study protocol was accepted by the Institutional Review Board of the Polish Society of Disaster Medicine (approval no. 17/05/2017). The study was conducted from June to July 2017. The doctors qualified the trainees participating in emergency medicine courses organized by the Department of Emergency Medicine at the Medical University of Warsaw. The inclusion criteria included: completing medical studies and having the status of trainee, lack of previous experience in the use of cardiopulmonary resuscitation feedback devices, and voluntary consent to join the study.

Among the exclusion criteria were: spine injury or wrist injury during the month preceding the examination preventing CCs, pregnancy, or non-fulfillment of inclusion criteria. Ultimately, 68 physicians were enrolled in the study, but only
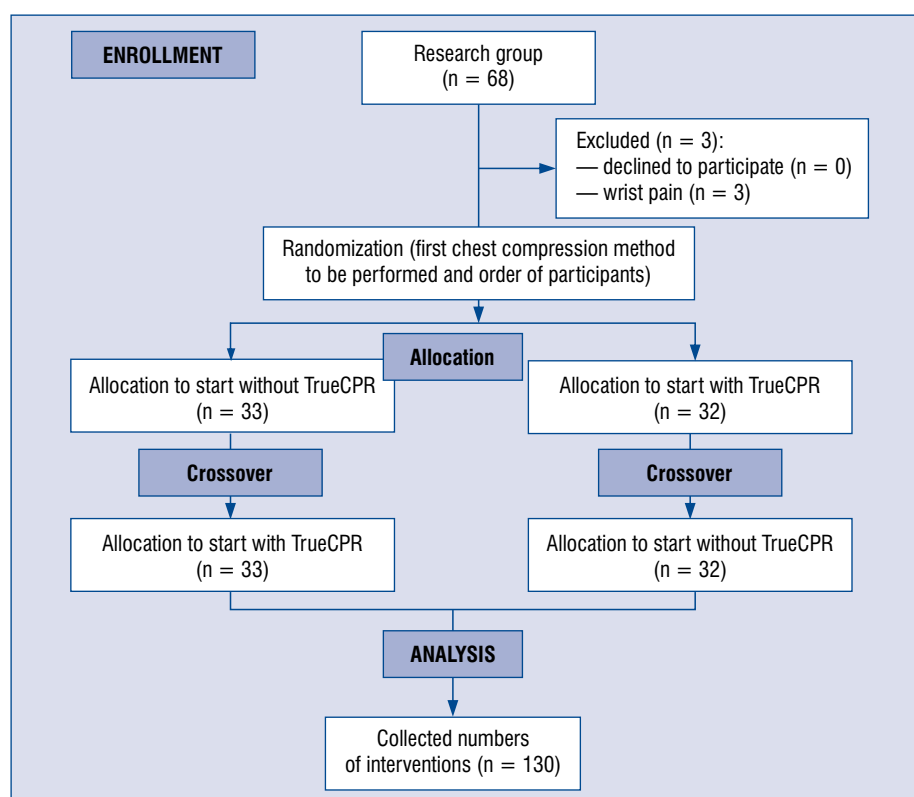
Figure 2. Randomization flow chart.

65 physicians completed the study. Three people withdrew from the study due to wrist pain (Fig. 2).

Prior to the study, all persons participated in the training module in the field of basic resuscitation procedures based on the guidelines of the American Resuscitation Council [2, 3]. The correct CCs were shown once again in a non-instrumental manner, as well as performing cardiopulmonary resuscitation based on the TrueCPR device, which, owing to information displayed on the depth of compressions, the frequency of compressions, as well as the degree of chest relaxation, allowing for correction of quality of CCs in real time. After instruction, participants of the study had 10 min to read the TrueCPR device, however, practical exercises with this device were not done in advance. On the following day, participants during the target study were asked to perform CCs continuously for 2 min with and without the TrueCPR device. Cardiopulmonary resuscitation was carried out based on one rescuer. Both the order of the participants and the methods of CCs was random. For this purpose, the coin-toss technique was used. A detailed randomization procedure is presented on Figure 2. To simulate a patient in need of CCs, an

adult simulator, Resusci Anne Simulator (Laerdal, Stavanger, Norway) was used.

During the study, only the parameters concerning the quality of CCs such as the depth of compressions, the frequency of compressions, the degree of chest relaxation and the correctness of hands on the chest during compressions were evaluated. Parameters were measured using SimPad®PLUS (Laerdal, Stavanger, Norway), which is an operating device used to control Laerdal simulators. Additionally, after completing the scenarios, the participants assessed the 100-degree scale on the level of self-confidence in the correctness of chest CCs (1 — uncertain; 100 — confident), as well as indicating which of the CC techniques they would prefer to use under real resuscitation conditions.

Sample size calculation was performed using G*Power 3.1 with a two-tailed t-test (Cohen's d: 0.8, alpha error: 0.05, power: 0.95). According to the calculation, a minimum of 51 participants were necessary.
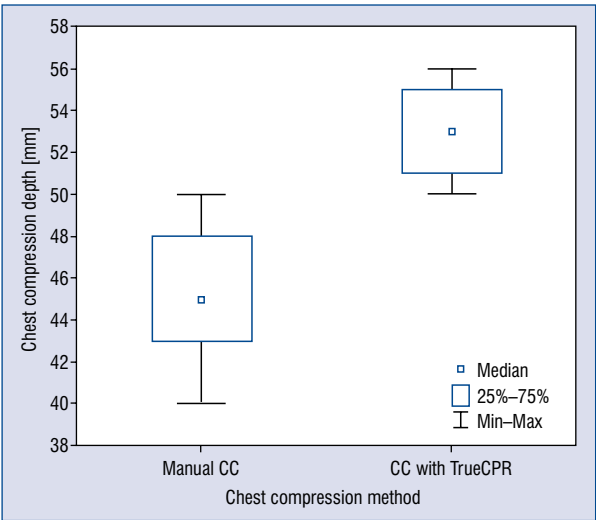
Statistical analysis

The statistical package Statistica 13.0 EN (StatSoft, Tulusa, OK, USA) was used for all sta-
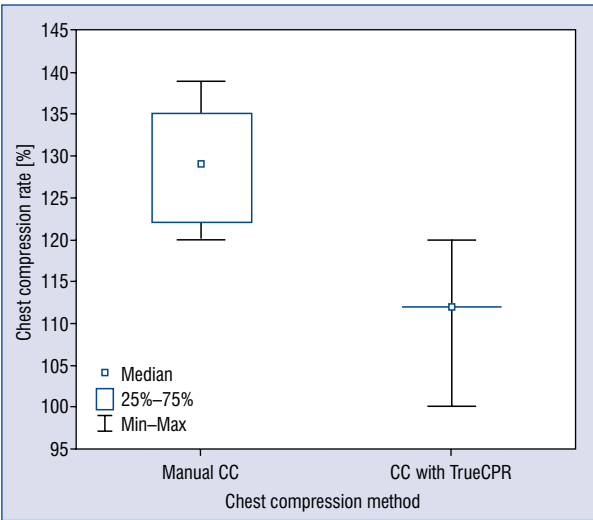
Table 1. Chest compression (CC) parameters with and without TrueCPR device.

Parameter	Manual CC	CCs with TrueCPR	P
CC rate [n × min⁻¹]	129 [IQR 122–135]	112 [IQR 103–113]	**0.002**
CC depth [mm]	45 [IQR 43–48]	53 [IQR 51–55]	**< 0.001**
Full release [%]	33 [IQR 29–38]	95 [IQR 76–99]	**< 0.001**
Correct hand position [%]	94 [IQR 82–96]	95 [IQR 89–98]	0.185

IQR — interquartile range

Figure 3. Median chest compression (CC) depth.

Figure 4. Median chest compression (CC) rate.

tistical analysis. Normal distribution was confirmed by the Kolmogorov-Smirnov test. When the data did not follow normal distribution, non-parametric tests were used. The participants' subjective opinions were compared with the use of the Stuart--Maxwell test. Data were presented as medians and interquartile range (IQR) or percentages (%). The results were considered significant at p < 0.05.

Results

The study involved 65 trainee doctors (27 female, 41.5%), whose median age was 25 (IQR 24.5–26) years. None of the participants had previous clinical or experimental experience in cardiopulmonary resuscitation feedback devices.

Detailed data on the parameters of CCs are presented in Table 1. The depth of compressions varied in relation to the CC technique tested, with 45 mm (IQR 43–48) for manual CCs and 53 mm (IQR 51–55) for the TrueCPR device. This difference was statistically significant (Fig. 3, p < 0.001).

The frequency of CCs when the TrueCPR device was used resulted in 112 cpm (IQR 103–113)

and achieved statistical significance which was lower than when not using the device during CCs, resulting in 129 cpm (IQR 122–135) (Fig. 4, p = 0.002).

The degree of complete chest relaxation with the TrueCPR device was 95% (IQR 76–99) and without a device it was 33% (IQR 29–38). The difference in the degree of correctly performed chest relaxation between the tested devices was statistically significant (Fig. 5, p < 0.001).

In the case of proper hand positioning on the chest, no statistically significant differences were observed in the examined devices (Table 1).

The degree of self-confidence of correct CCs in the absence of a device was 77 (IQR 56–87) and when using the TrueCPR device, the degree of self-confidence was higher, at 87 (IQR 73–99) (p = 0.024).

Fifty-seven people, which accounted for 87.7% of the whole research group, declared that they would choose the TrueCPR device during routine cardiopulmonary resuscitation in their professional practice. The remaining 12.3% of people opted for the device-less CCs as their preferred method of cardiopulmonary resuscitation.
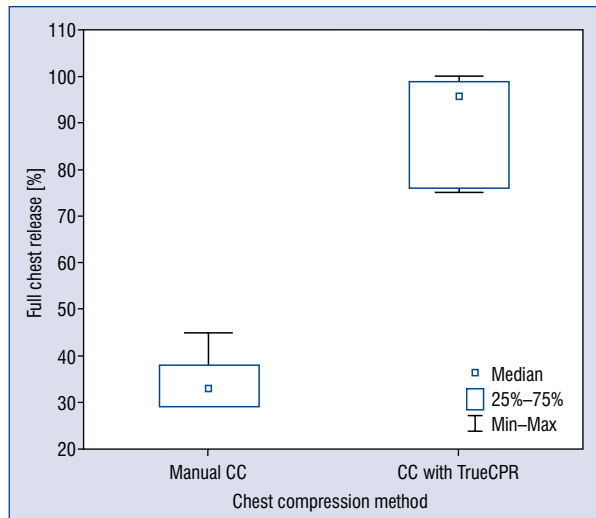
Figure 5. Degree of chest compression (CC) relaxation.

Discussion

In this simulation study, the impact of using the cardiopulmonary resuscitation feedback device versus TrueCPR on the quality of CCs performed by trainee doctors was assessed. The results obtained indicate an advantage of the method of using the device over not using the device in CCs.

Cardiopulmonary resuscitation is one of the most stressful situations that a physician can encounter during their daily work experience [17]. It is dictated by the necessity of implementing advanced resuscitation activities and to conduct them until the arrival of an emergency medical team or the arrival of a resuscitation team [18].

In the present study, a statistically significant difference was found between the depth of CCs with and the depth without the TrueCPR device (53 vs. 45 mm, respectively). Current guidelines for cardiopulmonary resuscitation recommends that the depth of CCs in adults is between 50 and 60 mm [4]. As shown by studies conducted by Lampe et al. [19], deeper CCs redirect several hemodynamic parameters. Iskrzycki et al. [20] conducting studies on the effectiveness of cardiopulmonary resuscitation carried out by water rescuers, demonstrated that the use of visual real-time feedback devices has improved the quality of cardiopulmonary resuscitation in relatively unexperienced cardiopulmonary resuscitation providers. In the case of prolonged resuscitation, when the person performing CCs becomes fatigued, the use of feedback devices that are able to correct the quality of CCs is all the more justified. Buléon et al. [21] indicated that the

real-time feedback device delivers longer, effective, and steadier CCs over time. Other authors have also come to similar conclusions [4, 6, 22–24].

Another important factor influencing the return of spontaneous circulation is the frequency of CCs [16]. Field et al. [25] showed that a CC rate of $100–120/\text{min}^{-1}$ for 2 min is feasible whilst maintaining adequate CC quality in terms of depth, duty-cycle, leaning, and decay in compression performance. These are the parameters currently recommended by guidelines for cardiopulmonary resuscitation [4]. In turn, studies by Lee et al. [26] showed that the frequency of compressions over 120/min was associated with a higher depth of compressions than the frequency of compressions indicated in the guidelines for resuscitation [4]. Zou et al. [27] indicated the most optimal frequency of CCs is a frequency of 120/min [26, 27]. In the present study, the compression rate performed without the device by trainee doctors was 129 cpm, while in the case of using the TrueCPR device it was 112 cpm. The reduction in frequency using cardiopulmonary resuscitation feedback devices may be dictated by the fact that these devices (including TrueCPR) display the current pressure of the compressions performed by the rescuer, thanks to which they are able to adapt to the frequency recommended by the resuscitation guidelines. Wee et al. [28] indicate that the use of feedback devices helps improve the quality of cardiopulmonary resuscitation during training. This opinion is also shared by other authors dealing with the issue of improving the effectiveness of resuscitation [20, 29–32].

Full chest relaxation after each compression also plays a significant role in the quality of cardiopulmonary resuscitation and the frequency of return of spontaneous circulation and is one of the recommendations of the AHA as well as the ERC [4, 11]. Luire points out that complete chest wall recoil improves hemodynamics during cardiopulmonary resuscitation by generating relatively negative intrathoracic pressure, which draws venous blood back to the heart, and provides cardiac preload prior to the next CC [33]. In the study, the intern doctors tended to perform full chest relaxation just to the recommended level. Thanks to the use of the feedback device, this percentage increased to 95%. The TrueCPR device, due to the scales on the display, indicates whether the person performing CCs fully relaxes it, in regards to the frequency and depth of compressions, making it possible to correct these parameters if needed. Numerous studies comparing CCs with compressions

using feedback devices or mechanical CC devices indicate that a tendency towards incomplete chest relaxation during resuscitation concerns not only physicians but other professional groups as well [12, 28, 34, 35].

In the study conducted, the participants indicated that their confidence in the correctness of CCs increased in the case of using a cardiopulmonary resuscitation feedback device. This may be related to the fact that the device, so to say, tells people performing CCs what parameters to pay attention to and how to improve them to achieve optimal quality of performed procedures.

Limitations of the study

The study has specific limitations resulting from the methodology. Firstly, the study was conducted under simulated conditions, not real cardiopulmonary resuscitation, however, this choice was intentional, because the medical simulation allows for full standardization of conditions for procedures performed, moreover, it was the only one that allowed cross-randomized trials without potential damage to a patient's health [36–39]. Additionally, thanks to the use of advanced simulators, it was possible to obtain the data regarding quality of CCs performed without an accompanying feedback device. The second limitation was to perform CCs in a 2-min cycle, however, such a period of CCs is recommended by the guidelines for resuscitation [13, 39, 40] and after this time there should be a change in the person performing CCs. The strength of the study is its randomized, cross-over nature, and the use of one of the most advanced feedback devices available

Conclusions

In the simulation test conducted, the use of the TrueCPR device resulted in a significant improvement in the quality of CCs in relation to the frequency and depth of CCs and the correctness of chest relaxation performed by trainee doctors.

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ORIGINAL ARTICLE

VIA MEDICA

Schoolteachers as candidates to be basic life support trainers: A simulation trial

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Abstract

Background: *The aim was to assess future schoolteachers' basic life support (BLS) knowledge and willingness to include this content in school lessons. The aim was also to determine the learning effect of a brief BLS hands-on training session, supported by real-time feedback.*

Methods: *A convenience sample of 98 University students of Educational Sciences and Sports were recruited. The training program consisted of brief theoretical and hands-on interactive sessions with a 2/10 instructor/participants ratio. Knowledge and willingness was assessed by means of a survey. Chest compressions (CC) and ventilation quality were registered in 47 cases during 1 min cardiopulmonary resuscitation (CPR) tests.*

Results: *Fifty-eight percent of subjects declared to know how to perform CPR, 62% knew the correct chest compression/ventilation ratio but only one in four knew the CC quality standards. Eighty-eight percent knew what an automated external defibrillator (AED) was; willingness to use the device improved from 70% to 98% after training. Almost half of CCs were performed at an adequate rate. Men performed deeper compressions than women (56.1 ± 4.03 mm vs. 52.17 ± 5.51 mm, p = 0.007), but in both cases the mean value was within recommendations. Full chest recoil was better in women (72.2 ± 32.8% vs. 45.4 ± 32.9%, p = 0.009). All CCs were delivered with correct hand positions.*

Conclusions: *Brief hands-on training supported by real-time feedback of CPR quality helps future schoolteachers improve their knowledge, self-confidence and CPR skills. BLS training should be implemented in University curricula for schoolteachers in order to promote their engagement in effective BLS training of schoolchildren.* (Cardiol J 2019; 26, 5: 536–542)

Key words: teachers, basic life support, cardiopulmonary resuscitation, automated external defibrillation, training

Introduction

The European Resuscitation Council (ERC) guidelines endorsed the recommendation that all citizens should be taught cardiopulmonary resuscitation (CPR) [1–3]. At least, half of out-of-hospital cardiac arrests (OHCA) are witnessed [4, 5], but although bystander resuscitation could improve survival and outcomes [6], the actual rate of CPR initiated by bystanders remains very low in most countries [7].

Schools had been pointed out as a perfect environment to start CPR training [8]; in fact, children are considered to be an ideal target group to train in basic life support (BLS) because they are also situated in a vital stage of easy learning [9, 10].

The inclusion of schoolteachers as a key element of schoolchildren BLS training has been endorsed by international initiatives like Kids Save Lives [11–13], which emphasise the teacher role as facilitator and/or trainer due to their pedagogic abilities.

Previous studies have reported that teachers have willingness to provide this instruction and it seems that even a very brief BLS training program might be enough to improve their knowledge, skills and self-confidence [14, 15]. This kind of training could ease formation access as well as regular retraining [2, 16, 17] without significant interference or changes to the regular scholar curriculum. In addition, feedback and self-instructed learning seem to be useful tools to strengthen CPR learning [18, 19].

Thus, the aim of the present study was to assess future schoolteachers' BLS knowledge and willingness to include this content in school lessons, as well as to determine the learning effect of a brief BLS training session, supported by real-time feedback by means of quantitative measurement of CPR performance.

Methods

Participants

A convenience sample of 98 University students (62 men and 36 women) of Physical Activity and Sport Sciences at the University of Vigo (Spain) were recruited for this study.

Study design

The training program consisted of theoretical and hands-on sessions and was conducted by instructors certified in basic and advanced life support. The theory session lasted 30 min and content included the chain of survival (how to recognise a cardiac arrest, call of medical emergency service, how to perform BLS, call for an automated external defibrillator [AED] and the importance of public access of defibrillation by means of AED). For practice, the sample was distributed into groups with a 10/2 participants/instructors ratio. The hands-on session lasted 1 h, and participants trained 15 min "chest compressions (CC) only" CPR, 15 min pediatric CPR, 15 min real time quality feedback CPR (compressions and ventilations) and 15 min

for AED use. After practice, CPR skills of 47 participants were assessed by means of a practical test consisting of 1-min CPR (CC and ventilations). Participants used a Laerdal Mini Anne manikin and Laerdal Resusci Anne manikin (Laerdal, Stavanger, Norway) for CPR practice with a Laerdal AED trainer (Fig. 1).

Participation was voluntary and no personal incentive for participation was given. The study respected the Helsinki Declaration and was approved by the local institutional review board (Research Ethics Committee of the University School of Education and Sports Sciences, University of Vigo, Spain).

Measurement tools

First of all, a questionnaire was given to all participants. The survey included questions about personal prior training or experiences and basic knowledge in CPR and AED management. The same questionnaire was completed after hands on training.

Twenty-three questions formed the survey and the main topics were: Prior training in BLS (questions 1–4); CPR knowledge and prior experience as a bystander (questions 5–13); CPR quality standards and willingness to teach BLS (questions 14–17); AED knowledge and willingness to use the device (questions 18–21); and a personal opinion about the importance of BLS training programs (questions 22–23). Test included dichotomous, multiple choice answer and subjective opinion by means of a Likert scale. Questionnaires were encoded to preserve the anonymity of participants.

Cardiopulmonary resuscitation quality was assessed through the Laerdal Resusci Anne manikin and Laerdal PC Skill Reporting Software, version 2.4, which measures chest compression and ventilation quality. Goals were set according to the 2015 quality standard established by the ERC [1].

Cardiopulmonary resuscitation quality metrics included mean compression rate (in CC per minute), percentage of CC at adequate rate, mean compression depth (in millimetres), percentage of compressions at adequate depth, percentage of CC with full-chest recoil, percentage of CC with adequate hands position, and mean estimated tidal volume (in millilitres).

Statistical analysis

Categorical data were described as absolute numbers and percentages. Continuous data were described by mean and standard deviation (SD). The Kolgomorov-Smirnov test was used to study
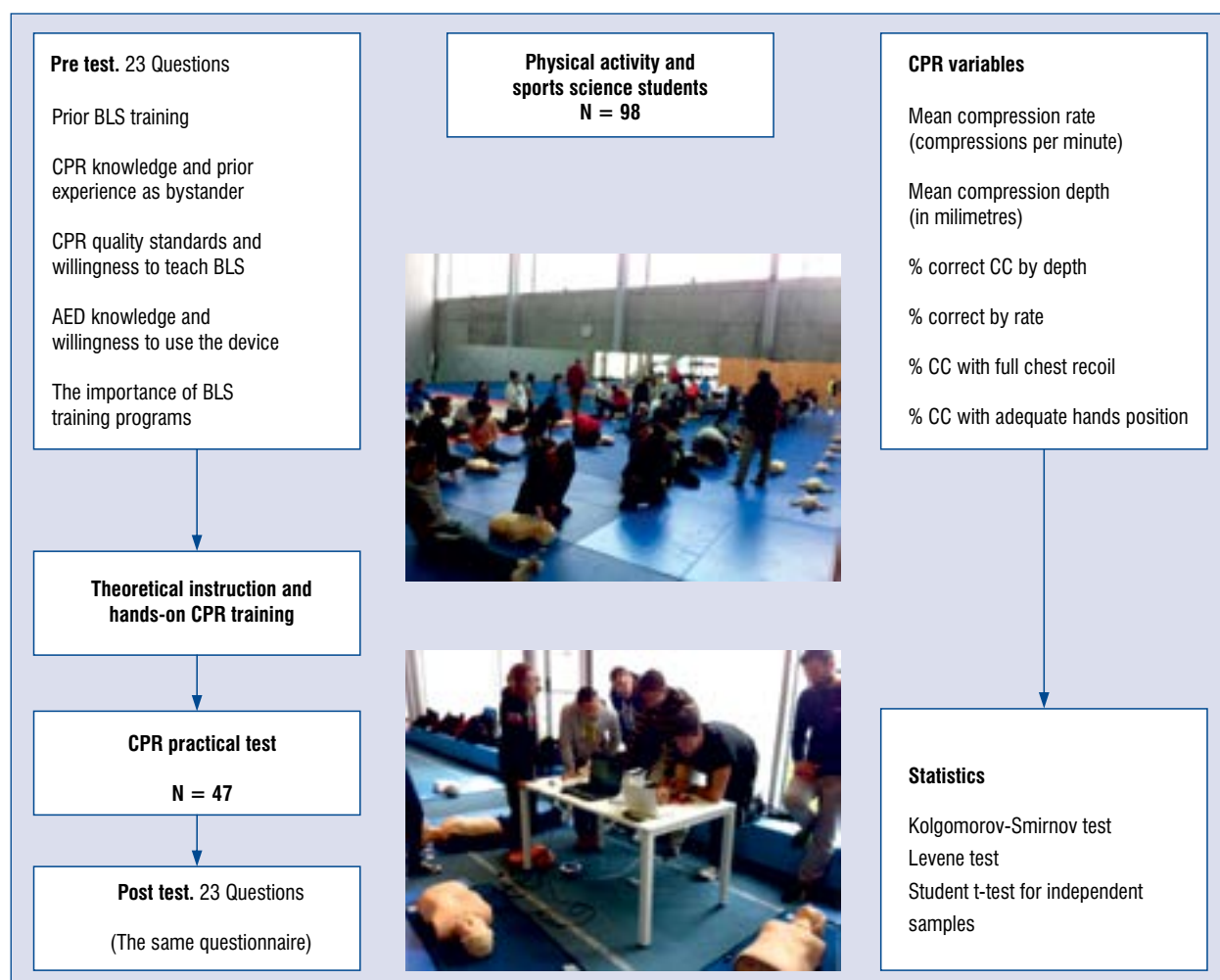
Figure 1. Study design. Stages of sample knowledge evaluation and practical test. Cardiopulmonary resuscitation (CPR) variables and statistics; AED — automated external defibrillator; BLS — basic life support; CC — chest compressions.

normal distribution of continuous variables. The equality of variances was determined using the Levene test. Correlations between continuous data were assessed using the Student t-test for independent samples to assess quality CPR differences between the Men Group and Women Group as well as between two response groups of question 6. SPSS Statistics 20.0 Software was used for statistical analyses. In all analyses, a significance level of $p < 0.05$ was considered.

Results

The study included 98 participants, 62 (63.3%) men and 36 (36.7%) women. Mean age was 23.66 ± 5.79 years.

Prior training data of the sample are shown in Table 1. All participants affirmed to know what CPR is. Fifty-eight percent declared to know how to per-

form CPR on an adult victim, a figure that increased to 100% after training. More than half of participants (62.2%) knew the correct chest compressions/ventilations ratio in an adult but only 22.4% answered correctly to the question about the target compression rate and 23.5% of the recommended compression depth. After taking part in the training program, 99% participants knew the correct chest compressions/ventilations ratio; 92.9% knew the correct CC rate and 85.7% the adequate CC depth.

Fifty-nine (60.2%) participants declared to have been trained in CPR before the study, 50% of them knew the correct chest compressions/ventilations ratio, 35% knew CC recommended rate and 33% the correct CC depth goal (Table 2).

With regard to their prior response as bystanders, only 8/98 (8.2%) had witnessed an emergency situation and 4.1% had participated in actual resuscitation manoeuvres.

Table 1. Prior cardiopulmonary resuscitation (CPR) training characteristics and CPR quality questions answered by participant who had received prior CPR training.

Parameters		Frequency (%)
Prior CPR training characteristics	Participants with prior CPR training	59 (60.2%)
	CPR training as part of a specific subject	30 (30.6%)
	Training in the last 3 months	50 (51%)
	Participants who performed real time feedback CPR	51 (52%)
Participants with prior training (n = 59)	Chest compressions/ventilations rate (30:2)	50 (87.7%)
	Chest compressions per minute (100–120 cpm)	20 (35.1%)
	Chest compressions depth (50–60 mm)	19 (33.3%)

cpm — compressions per minute

Table 2. Comparison of cardiopulmonary resuscitation quality variables by sex of the participant.

	Men (n = 29)	Women (n = 18)	P*
Mean compression rate [cpm]	112.8 ± 9.16	111 ± 7.42	0.488
Mean compression depth [mm]	56 ± 4.03	52 ± 5.51	0.007
Correct chest compressions by depth [%]	49.01 ± 36.31	54.4 ± 30.80	0.603
Correct chest compressions by rate [%]	83.6 ± 11.26	87.9 (9.41	0.599
Chest compressions with full chest recoil [%]	45.4 ± 32.9	72.2 ± 32.85	0.009
Chest compressions with adequate hand positions [%]	100 ± 0.00	100 ± 0.00	

*Student T test for independent samples; cpm — compressions per minute

The percentage of participants who knew what an AED increased from 87.8% before to 100% after training, and the number who declared to know how to use an AED (pre-test 38.8% vs. post-test 100%). Most subjects declared the willingness to use an AED in an eventual emergency situation both pre-test (70.4%) and post-test (98%).

Eighty-five percent of participants considered that a specific First Aid subject is important for their academic training and 78.6% stated that this subject should be mandatory for all Physical Activity and Sports Science students, most of them would be willing to include this content in projects or didactic units with their students (pre-test 71.4% and post-test 76.5%). The number of participants who had considered their previous training as very efficient decreased from 48% to 34.7% after the current training. On the other hand, 34.7% participants considered their CPR skills as very insufficient initially, but after training 43.9% students described their CPR skills as "enough" and 29.6% as "effective".

Results of CPR quality metrics by sex are shown in Table 2 and Figure 2. During the 1-minute test, more than 80% of CCs were performed at an adequate rate. Mean compression depth goal (50–60 mm) was achieved by both groups with
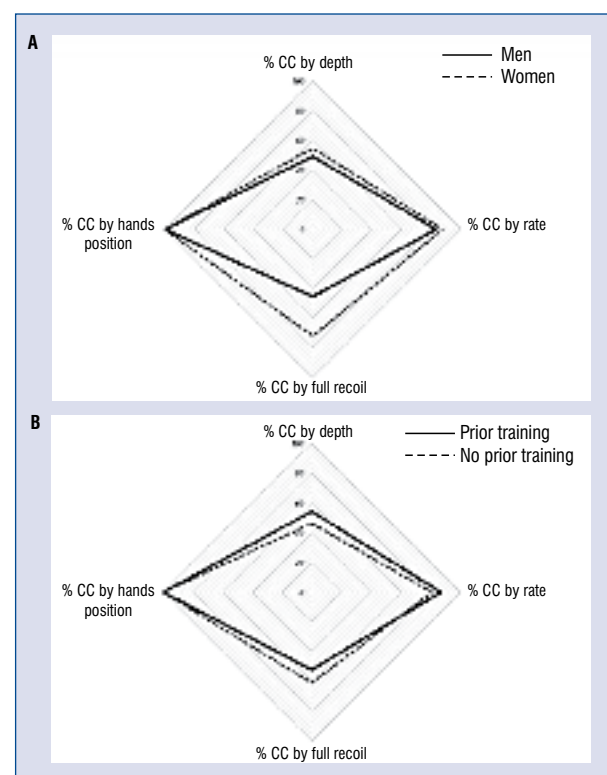
Figure 2. Cardiopulmonary resuscitation (CPR) quality standards. Comparisons by sex (**A**) and prior training (**B**); CC — chest compresions.

deeper CC performed by men (56.1 ± 4.03 mm vs. 52.17 ± 5.51 mm, p = 0.007). Full chest recoil was better in women (72.2 ± 32.8%) than in men (45.4 ± 32.9%, p = 0.009). All CCs were delivered with correct hand positions.

No significant differences were observed regarding prior CPR training of participants. Data are shown in Figure 2.

Discussion

Layperson CPR training is essential to increase bystander CPR rates and OHCA outcome [20]. Moreover, it is worth remembering that teaching how to act in life-threatening situations, including cardiac arrest resulting from myocardial dysfunction or arrhythmias, is a key element of public safety [21]. Although schools are seen as an ideal environment to involve citizens in BLS training, it is not clear however, which professionals are more suitable for teaching schoolchildren [12, 14, 22]. In the present study, it was found that a very brief BLS training program had a positive effect on Physical Activity and Sports Science student knowledge and willingness to include this topic in school lessons. Also, after a brief hands-on practice with quality feedback, most of them were able to perform CPR that fulfils quality standard goals.

In spite of more than half of the present sample (60.2%) having declared to have received prior CPR training, less than 25% knew the correct CPR quality standards (CC per minute and CC depth). Results are comparable with those obtained by Bogle et al. [23] in a survey responded to by 267 University students (only 46.1% met CPR quality standards). Observations revealed that a training session lasting less than 2 h (30 min theory and 1-h hands-on practice) was enough to improve CPR performance of 85% of subjects involved.

Very brief training programs could be an effective formative strategy for both adults and schoolchildren. Thus, 45 min training appears to be enough for 8th grade students to improve their CPR and AED knowledge and skills [24]. This knowledge and skill retention was maintained for 2 months, getting worse at 4 months for participants who had not re-trained previously; this fact endorses the importance of periodic re-training [25, 26]. A contemporary study has shown how opportunistic 5 min CC feedback training was, and whether it was enough for laypeople to be able to surpass a 70% goal for most of the technical parameters in 2-min CC test [27]. In the present sample, brief training with quality feedback was effective in accomplishing a mean compression rate and depth quality standard as well as correct hand positions.

In countries where CPR is a mandatory part of school curriculum, bystander CPR is performed in more than 40% of OHCA and has been associated with double to triple survival rates [22]. The need of certified instructors could mean a practical and financial barrier for BLS training implementation. Thus, BLS training conducted by teachers could help to overcome this barrier. Prior studies have shown that primary school teachers, previously trained by medical staff, can teach BLS effectively [13, 22]. Most of the present participants (93%) considered that First Aid training is important for their education, more than 90% thought their academic curriculum should include this specific subject and for 78% it should be mandatory. Thus, for future teachers, a good BSL training during their academic education seems to be relevant and could improve their self-confidence and willingness [28]. To include these contents in University student curricula could ease implementation of strategies that endorse the role of teachers in school BLS training as with the Kids Saves Lives initiative [9, 11, 12].

Lukas et al. [12] have shown that BLS training provided by trained teachers is as effective as the training provided by emergency physicians, additionally, schoolchildren trained by teachers accomplished better knowledge marks. It can be assumed that teachers have practical expertise in youngster education and can obtain better results than instructors with a non-educational background. The use of school teacher staff for BLS training has many advantages, such as the ease of implementation for this instruction at school centres, to act as role models and to act as facilitator of instruction [11, 12]. In the last several years self-instruction models like the Relieve Game proposed by Semeraro et al. [11] have been seen as relevant for schoolchildren training; in this kind of training, teachers act as facilitators or guides for instruction but they do not play the trainer role.

Although it is not clear at what age schoolchildren are capable of effectively learning different aspects of First Aid, previous studies have pointed out that the age of 13 is the minimum age to be able to perform CPR with a similar quality to an adult [29]. Whereas around 9 years old, children can start to be trained in the knowledge and use of AED [30, 31]. Regardless, it seems to be positive to familiarize children with BLS from an early age [9]. Child's retention is good 1 year after a 1-h BLS course and this retention is better than in adults regarding CPR

compressions/ventilation ratio knowledge, this endorsed the idea that training schoolchildren is a good investment for the future [32].

Conclusions

Brief hands-on training helps to improve knowledge and self-confidence in BLS and CPR skills of future schoolteachers. BLS training should be implemented in the University curricula for schoolteachers to promote the engagement of these professionals in effective BLS training of schoolchildren as supported by initiatives such as Kids Save Lives.

Conflict of interest: None declare

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ORIGINAL ARTICLE

The subcutaneous implantable cardioverter--defibrillator: A tertiary center experience

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Abstract

Background: *The aim of the study was to evaluate subcutaneous implantable cardioverter-defibrillator (S-ICD) patients with regard to underlying etiology, peri-procedural outcome, appropriate/inappropriate shocks, and complications during follow-up.*

Methods: *All patients who underwent S-ICD implantation from February 2013 to March 2017 at an academic hospital in Vienna were included. Medical records were examined and follow-up interrogations of devices were conducted.*

Results: *A total of 79 S-ICD patients (58.2% males) with a mean age of 44.5 ± 17.2 years were followed for a mean duration of 12.8 ± 13.7 months. A majority of patients (58.2%) had S-ICD for primary prevention of sudden cardiac death. The most common of the 16 underlying etiologies were ischemic cardiomyopathy, non-ischemic cardiomyopathy, and idiopathic ventricular fibrillation. The lead was implanted to the left sternal border in 96.2% of cases, between muscular layers in 72.2%. Mean implant time was 45 min, 3 patients were induced, and all patients except one were programmed to two zones. Six (7.6%) patients experienced at least one appropriate therapy for ventricular arrhythmias and the time to first event ranged from 1 to 52 months. Seven patients experienced inappropriate shocks due to T-wave oversensing, atrial tachycardia with rapid atrioventricular conduction, external electromagnetic interference, and/or baseline oversensing due to lead movement. Four patients underwent revision for lead repositioning (n = 1), loose device suture (n = 1), and infection (n = 2).*

Conclusions: *While S-ICDs are a feasible and effective treatment, issues remain with inappropriate shock and infection.* (Cardiol J 2019; 26, 5: 543–549)

Key words: arrhythmia, complication, subcutaneous implantable cardioverter--defibrillator, sudden cardiac death

Introduction

The entirely subcutaneous implantable cardioverter-defibrillator (S-ICD) offers an alternative to the transvenous/epicardial system in an effort to prevent sudden cardiac death. The advantages of implantation outside the thoracic cavity (Fig. 1) include: avoidance of cardiac complications (arrhythmias, perforation, tricuspid valve damage),

vessel-related problems (arterial puncture causing hematoma, venous thrombosis/obstruction), and tissue damage (pneumothorax, nerve palsus, shoulder dysfunction) [1]. Only brief fluoroscopy is needed to verify proper lead placement. The defibrillator lead is more robust and is expected to provide better long-term outcomes than transvenous leads, which manifest a 20% failure rate over 10 years and which extraction (if necessary) can bring serious complica-
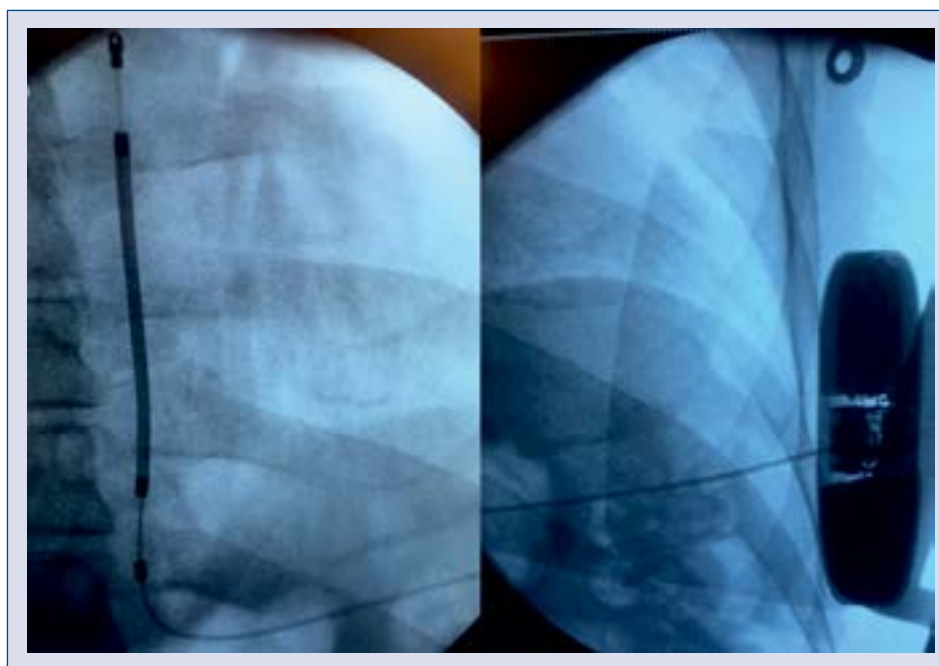
Figure 1. X-ray of the entirely subcutaneous implantable cardioverter-defibrillator system. Lead in the left sternal position and device in the left mid-axillary line.

tions, including death [2]. The use of an S-ICD is limited by its inability to provide antitachycardia pacing, cardiac resynchronization therapy, and bradycardia pacing, except for an immediate post-shock period; S-ICD systems are also comparatively expensive [1]. The pooled data from the landmark IDE and EFFORTLESS trials have proven the overall effectiveness of S-ICDs [3]. These promising results have been verified in external cohorts but further study in different settings is needed to justify more widespread use of S-ICD therapy [4–14].

The aim of this study was to evaluate patients who were implanted with an S-ICD at a tertiary center with regard to underlying etiology, periprocedural outcome, appropriate/inappropriate shocks, and complications during follow-up.

Methods

Setting

The complete records of all S-ICD implants at Allgemeine Krankenhaus Wien, a University Hospital in Vienna, Austria were extracted from the database of the Medical University of Vienna, Department of Surgery, Division of Cardiac Surgery, Vienna, Austria. The first implant was performed in February, 2013 and the last in March, 2017.

Data collection

Medical records were used to validate patient characteristics including the underlying etiology and follow-up. All device interrogations were stored in the database provided by Boston Scientific and evaluated for appropriate and inappropriate shocks.

Ethics

The study complies with the Declaration of Helsinki and the local ethical committee approved the study.

Variables

An appropriate therapy was defined as detection of ventricular tachycardia (VT) or ventricular fibrillation (VF) and subsequent shock. Inappropriate shocks were due to false classification of the arrhythmia (i.e. supraventricular tachycardias, oversensing of external signals, T-wave oversensing, or baseline drift due to movement of the lead tip). Prophylaxis after surviving cardiac arrest/ /VF or VT with hemodynamic compromise was a secondary prevention. Patients with a primary prevention indication were judged to be at an increased risk for life-threatening ventricular arrhythmias but without having had one.

Statistical analysis

Numeric data were expressed as frequencies, percentages, means, and percentiles. Continuous variables were summarized as means, standard deviations (SDs), percentiles, and compared using t-tests. Fisher's test was used for categorical variables. A two-sided p-value of < 0.05 was considered statistically significant. The database in Excel 2010 (Microsoft Corporation, Redmond, WA) was imported into SPSS version 22 (IBM, Armonk, NY).

Results

Patient characteristics

A total of 79 patients had an S-ICD implanted and were followed for a combined total of 1015 months (84.6 years). The follow-up time ranged from 3 days (3 patients were lost to follow-up) to 4.4 years, with a median of 7.0 months (mean 12.8 ± ± 13.7 months). A majority were males (n = 46; 58.2%). The median age at implant was 45 years (25th percentile 30 years and 75th percentile 57 years); mean age was 44.5 ± 17.2 years with no significant sex difference (males 46.5 years and females 41.8 years; p = 0.217).

Coronary artery disease was diagnosed in 21 (26.6%) patients and 11 (13.9%) patients had a history of atrial fibrillation at baseline or during follow-up. The underlying cardiac etiologies were cardiomyopathy in 45 patients (ischemic, non-ischemic dilated, peripartal, arrhythmogenic right ventricular, hypertrophic, and Takotsubo cardiomyopathy), amyloidosis in 1 patient, ion-channelopathies in 10 patients (Brugada syndrome and long QT syndrome), congenital disease in 5 patients (Ebstein's anomaly, Duchenne muscular dystrophy, Carnitine transporter deficiency), acquired structural heart disease in 2 patients (sarcoidosis, myocarditis), and idiopathic VF or nonsustained VT with syncope in 16 patients. Each etiology category with regard to primary (n = 46; 58.2%) and secondary (n = 33; 41.8%) indication prevention is reported in Table 1. The most common cause of implant for men was ischemic cardiomyopathy followed by dilated cardiomyopathy and for women idiopathic VT/ /VF and ischemic cardiomyopathy, respectively. Sex distribution with regard to primary (males 65.2%; n = 30/46 and females 34.8%; n = 16/46) and secondary indication — 48.5% (n = 16/33) males and 51.5% (n = 17/33) females which was not statistically different (p = 0.168).

Implant procedure

The subcutaneous lead was tunneled parallel to the left sternal border in 76 (96.2%) patients and the remaining 3 cases were tunneled to the right based on preimplant screening. The S-ICD device was implanted between the muscular layers in 57 (72.2%) patients versus above the pectoral fascia in 22 (27.8%) patients. Early in the study, the 3-incision technique was sometimes used, but the 2-incision technique was used more frequently as the study progressed (n = 55, 69.6%).

Although the manufacturer recommends defibrillation threshold testing, it was performed in 1 of 3 patients. In all 3 cases, VF could be successfully induced (idiopathic VF, long QT syndrome, and ischemic cardiomyopathy).

Both the mean and median procedure times (skin-to-skin) were 45 min when performed without additional interventions such as bradycardia pacemaker implant, device extraction, epicardial leads, or concomitant tricuspid valve surgery.

Programming

With one exception, all patients had two-zone programming. The lower zone was typically 200 bpm but was set to 190 bpm in 1 patient and 210 to 230 bpm in 14 patients, while the higher zone ranged 220–250 bpm. The primary vector was used in 57% (n = 45), second vector in 32.9% (n = 26), and alternative vector in 10.1% (n = 8) upon hospital discharge. Notably, 10 patients switched from the primary to secondary vector after discharge, 2 patients from second to first vector, and 1 patient from alternate to primary vector. In 3 patients, the zones were changed during follow-up.

Appropriate shock

Six (7.6%) patients experienced at least one appropriate therapy of VT/VF. In one of these patients there was an additional appropriate therapy. The annual incidence was 8.3% (7 episodes during 84.6 years, 83 events per 1000 years). In 6 out of 7 episodes the VT/VF converted at first attempt but in one episode a second shock (with reversed polarity) was needed.

The underlying etiologies of the 6 patients were ischemic cardiomyopathy (n = 3), idiopathic VF (n = 2), and arrhythmogenic right ventricular cardiomyopathy (n = 1). In 5 of these patients the indication for the S-ICD was secondary prevention and in one, primary prevention. The time to first event ranged from 1 to 52 months.

Inappropriate shock and complications

Seven patients experienced inappropriate shocks due to T-wave oversensing, atrial tachycardia with rapid atrioventricular conduction,

Table 1. Underlying etiology and primary versus secondary indication of 79 patients with an subcutaneous implantable cardioverter-defibrillator (S-ICD).

Etiology	Primary (n = 46)	Secondary (n = 33)	Total (n = 79)
Ischemic cardiomyopathy	16	6	22 (27.8%)
Non-ischemic dilated cardiomyopathy	7	4	11 (13.9%)
Peripartum cardiomyopathy	1	0	1 (1.3%)
Arrhythmogenic right ventricular cardiomyopathy	4	1	5 (6.3%)
Hypertrophic cardiomyopathy	4	1	5 (6.3%)
Takotsubo cardiomyopathy	0	1	1 (1.3%)
Amyloidosis	1	0	1 (1.3%)
Duchenne muscular dystrophy	1	0	1 (1.3%)
Carnitine transporter deficiency	0	1	1 (1.3%)
Ebstein's anomaly	2	1	3 (3.8%)
Sarkoidosis	1	0	1 (1.3%)
Myocarditis	1	0	1 (1.3%)
Idiopathic ventricular fibrillation	0	15	15 (19.0%)
Idiopathic non-sustained ventricular tachycardia	1	0	1 (1.3%)
Long QT syndrome	3	3	6 (7.6%)
Brugada syndrome	4	0	4 (5.1%)

Table 2. Inappropriate subcutaneous implantable cardioverter-defibrillator (S-ICD) shocks: patient characteristics and causes.

Sex, age at shock	Etiology	Prevention	Reason
Female, 51 years	Idiopathic ventricular fibrillation	Secondary	T-wave oversening due to decreased R-wave and change in morphology
Male, 69 years	Ischemic cardiomyopathy	Primary	Atrial tachycardia with rapid atrioventricular conduction
Female, 13 years	Hypertrophic cardiomyopathy	Secondary	T-wave oversense due to decreased R-wave and change in morphology
Female, 31 years	Idiopathic ventricular fibrillation	Secondary	Atrial tachycardia with rapid atrioventricular conduction after appropriate shock of ventricular tachycardia
Male, 41 years	Cardiac sarcoidosis	Primary	External electromagnetic inference in the bathroom
Male, 18 years	Long QT syndrome	Secondary	Oversensing of myopotentials due to lead movement (two incision technique)
Male, 54 years	Ischemic cardiomyopathy	Primary	Baseline oversensing of myopotentials due to lead movement (two incision technique)

external electromagnetic interference, and baseline oversensing of myopotentials due to lead movement (Table 2). Notably, in the 2 cases of baseline oversensing, the lead tip moved and they were both implanted using the two-incision technique (Table 1).

Four patients underwent revision of the S-ICD system. In 1 case lead position was checked on X-ray and deemed unacceptable. The incisions were closed so it had to be re-opened in order to reposition the lead. In another case, the device suture in the pocket ripped off and had to be re-sewn. Furthermore, there were 2 cases of infection requiring explantation; 1 patient had no known risk factors for infection and the other was a diabetic on dialysis. One patient with severe amyloidosis died 2 months after implant but no post-mortem interrogation was performed.

Discussion

This large sample confirms the age- and sex distribution of S-ICD reported in trials and other cohorts [3–14]. This is expected in a tertiary center sample where the underlying etiologies can vary and include rare diagnoses.

The relatively young age of S-ICD cohorts may be explained by the fact that physicians may be more likely to recommend these devices to patients with long life expectancies, who are able to pay higher device costs, and who may want to avoid adding hardware to the vasculature.

The lower percentage of females has been noted in several S-ICD studies and this cohort shares this finding [3–14].

S-ICD therapy is effective

The 6 patients who experienced therapy were all converted in 7 episodes of VT/VF, which emphasizes the efficacy demonstrated by all major S-ICD cohorts [3–14]. This 7.6% proportion of patients during a mean follow-up of 12.8 months is in line with previous findings from diverse cohorts and the EFFORTLESS pooled data reported 5.3%, 7.9%, and 11.8% at 1, 2, and 3 years cumulative incidence of first appropriate therapy, respectively [3]. However, it should be remembered that not every appropriate therapy is indeed lifesaving, as ventricular arrhythmias may be self-terminating. Based on the MADIT-RIT study of transvenous ICDs, antitachycardia pacing was delivered to 22% of patients with conventional programming, 8% with high-rate programming, and 4% with delayed programming. However there was no difference among these three groups over 1.4 years with respect to the rate of shock therapy [15].

In the S-ICD, the 18/24 interval is fixed and the time to therapy is longer than in transvenous-ICDs, with overall time to shock therapy being about the same as modern transvenous-ICD programming.

Inappropriate shocks do occur but may be avoided

Seven (8.9%) patients were affected by inappropriate shocks. This is in the lower range of other reported S-ICD cohorts [3–14]. The pooled analysis of IDE and EFFORTLESS registries reported 13.1% inappropriate shocks at 3 years; notably, 11.7% inappropriate shocks in dual-zone programming and 20.5% in single-zone programming [3]. The dual-zone allows discrimination based on QRS morphology in order to prevent shock due to supraventricular tachycardias [16]. The lower incidence of inappropriate shocks in our cohort may be the result of adherence to manufacturer recommendations of prescreening and use of high-specificity sensing algorithms for supraventricular tachycardias [16].

Hypertrophic cardiomyopathy patients may fail the prerequisite of providing a QRS and T-wave morphology template and, in fact, 15% of patients in another study were ruled ineligible for this reason [16, 17]. However, T-wave oversensing remains a problem (2 patients in the present study) and was the most frequently encountered reason (39%) for inappropriate shock in the EFFORTLESS registry [3] and its risk is increased with hypertrophic cardiomyopathy [10]. There are ways to reduce the likelihood of T-wave oversensing, such as using exercise test settings and monitoring the reprogramming from secondary to primary (or alternative vector) configurations. T-wave oversensing is more likely with a low R/T ratio, bundle branch block, and repolarization abnormalities, which are most likely to occur during exercise [16]. A thorough preoperative screening is warranted and further improvement in the sensing algorithm would possibly decrease T-wave oversensing [18].

Different spectrum of complications

The EFFORTLESS registry reports a 6.4% (1.7% infections) implant-related complications requiring surgical interventions during a mean follow-up of 558 days. Interestingly, there seems to be a learning curve in S-ICD implantation technique suggested by a decrease in complications over time [3].

Renal failure is prevalent in this population and is a known risk factor for infection in transvenous ICD recipients. In some S-ICD cohorts, 20% or more patients are on dialysis, a finding supported by a United States registry [19]. Note that compared to transvenous leads, S-ICD leads may be extracted with less risk to the patient.

Even though vascular access is not needed, surgical skills are important for proper lead placement and pocket formation. Intramuscular implant using blunt dissection in order to avoid skin erosion and discomfort [1, 20] is advocated herein. In the present cohort, mean implant time of 45 min was due to the fact that 96.2% of patients did not undergo defibrillation testing. In a study by Winter et al. [20], the average implant time was 65 minutes.

The two-incision technique was performed in 69.6% and has previously been described [1]. Nevertheless, the 2 cases of inappropriate shocks due to baseline oversensing called for careful attention in order to secure optimal long-term lead
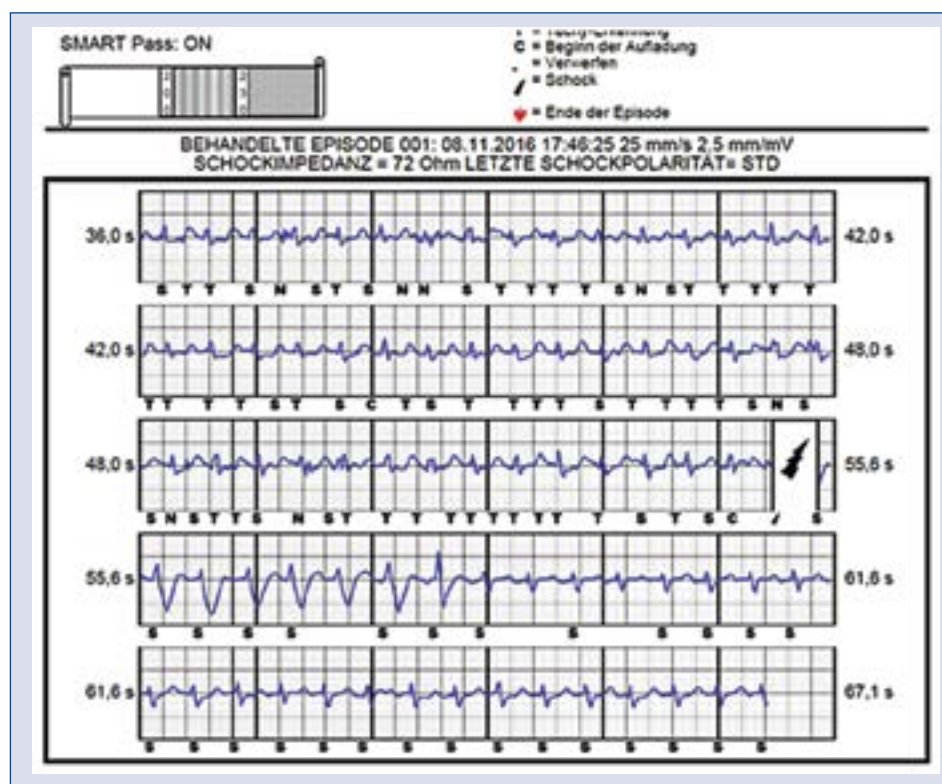
Figure 2. Inappropriate subcutaneous implantable cardioverter-defibrillator (S-ICD) shock due to baseline oversensing caused by lead movement (two-incision technique).

placement (Fig. 2). This is similar to transvenous lead failure, which may impede appropriate shock but may also give rise to inappropriate shocks. Transvenous leads are susceptible to complications not only during implant but, unfortunately, increasingly so over time [2]. While there is still a risk of infection with an S-ICD system, endocarditis and myocardial/vessel damage may be ruled out. For this reason, an S-ICD may be a good choice for patients who had to have a transvenous ICD extracted due to infection.

Strengths and weaknesses

This study supports the use of S-ICD in selected patients who are at risk for sudden cardiac death, but short follow-up time remains a major limitation. To further compare S-ICD systems with transvenous ICDs, randomized controlled trials are needed to overcome the limitations of comparisons using historical cohorts or matched-controlled groups with heterogeneity in etiology, age, and comorbidities.

Conclusions

This patient cohort from a single tertiary center demonstrates the implant feasibility and therapeutic efficacy of the S-ICD, but inappropriate shocks and infection remain problems.

Acknowledgements

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ORIGINAL ARTICLE

Morning and afternoon serum cortisol level in patients with post-myocardial infarction depression

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Abstract

Background: *Post-myocardial depression is a highly prevalent condition which worsens the course and prognosis of coronary artery disease. One possible pathogenetic factor is dysregulation of the hypothalamic-pituitary-adrenal axis, resulting in cortisol profile disturbances.*

Methods: *Thirty seven patients hospitalized due to a first myocardial infarction (MI) were enrolled in this study. The Beck Depression Inventory (BDI) was used to rate the severity of their depressive symptoms. Morning and afternoon serum cortisol samples were taken on the fifth day of the MI.*

Results: *Depression, defined as BDI ≥ 10, was present in 34.4% of the patients. A statistically significant difference was observed between the mean morning and the evening plasma concentrations in patients with depression compared to the no-depression group: $F_{(1.29)} = 5.0405$, $p = 0.0328$.*

Conclusions: *Patients with depressive symptoms directly after MI have a flattened diurnal serum cortisol profile. This is particularly expressed in patients with longer lasting symptoms.* (Cardiol J 2019; 26, 5: 550–554)

Key words: depression, myocardial infarction, cortisol, hypothalamic pituitary adrenal axis

Introduction

Approximately 1 in 5 patients after myocardial infarction (MI) develop depression during their initial hospitalization [1]. Depending on the diagnostic method and criteria used, the prevalence of post-MI depression varies from 7.2% to 47%.

The pathogenesis of post-MI depression is complex. Apart from psychological and psychosocial factors, biological elements play a significant role. They include immune, endocrine, autonomous and nutritional aspects [2–5]. Post MI depression can be defined as an "acute sickness response" triggered by MI [6]. This response includes the activation of proinflammatory cytokines, the autonomous nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which in some cases may prolong dysregulation in these systems. One of the effects of this dysregulation is probably the selective dysfunction of the prefrontal cortex and anterior cingulate gyrus, precipitating depressive symptoms [7]. Studies involving the immune and endocrine (mainly HPA axis) profiles in patients with post-MI depression may elucidate the pathophysiology of post-MI depression and its effect on the course and prognosis of the condition in patients after MI [7]. Unfortunately there remain very few studies in this area.

The aim of this study was to evaluate morning and afternoon serum cortisol concentrations as parameters of the HPA axis function in patients with depression after MI. The comparison group was composed of patients after MI without depression.

Methods

Thirty-seven patients (8 women; 22%) admitted to the First Cardiology Department at the Medical University of Gdansk were enrolled, 32 patients completed the study. They were hospitalized due to a first myocardial infarction with ST elevation (STEMI). The left ventricular ejection fraction (LVEF) was ≥ 40%, and mean body mass index 26.9 kg/m^2. All patients had a cardiovascular intervention and received standard pharmacological treatment. Exclusion criteria were endocrine diseases such as diabetes, hypo- or hyperthyroidism, severe renal or hepatic failure, hormone therapy, active addiction to psychoactive substances and the presence of psychiatric disorders other than depression.

The study was approved by the Independent Ethics Committee of the Medical University of Gdansk (approval number NKEBN/205/2006). For each participant written consent was obtained.

All patients were diagnosed with the Structured Clinical Interview for DSM-IV Axis I Disorders [8] and rated with the Beck Depression Inventory (BDI) [9]. The evaluation took place 3 times over a 6 month period: on the fifth day (during the first 5 days) and on the third and sixth month after MI. Medical history of 9 patients revealed depression in the past — none of them suffered from the disorder directly before MI. All patients included in the depression group met the criteria of post-MI depression.

Blood samples for cortisol analysis were collected on the fifth day of the MI twice. First between 8 and 10 am and later between 3 and 5 pm. Both times, two samples were taken at least 20 min apart: two samples in the morning and two samples in the afternoon. The blood was centrifuged immediately and the serum was frozen at –80°C for batch analysis.

The cortisol concentration was measured with a chemiluminescent microparticle immunoassay (CIMA) for the quantitative determination of cortisol in the human serum, on the Architect system (Architect®, Abbott, DE) kit. The intra-assay coefficient of variation (CV) ranged from 2.1% to 5.5% and total CV values ranged from 2.5% to 7.7%. The sensitivity was 22.07 nmol/l (0.8 μg/dL). The cortisol concentration units were nmol/L and the result was an average of two measurements. All protocol requirements (6-month observation, complete blood sampling) being fulfilled, 32 patients (including 7 women — 22%) qualified for further analysis. These patients were divided into two groups depending on their BDI score on the

fifth day after MI: < 10 (non-depressed) and ≥ 10 (depressed).

Statistical analysis

Statistical analysis was performed with the use of Statistica v.12.5.1920 and StatsDirect v.3.0.183. Depending on the distribution (Wilk-Shapiro test), comparison was done with the help of the Student t-test or Mann-Whitey U test. ANOVA with repeated measures was used when the changes between the morning and afternoon cortisol levels were compared between the two groups. All tests were two-tailed with an alpha = 0.05.

Results

Demographic, clinical and hormonal characteristics of the two groups are presented in Table 1.

There were no differences in the morning and afternoon cortisol concentrations between depressed and non-depressed groups (Table 1).

The changes of cortisol levels between the morning and afternoon in the two groups are shown in Figure 1. A statistically significant difference was found between the groups (ANOVA with repeated measurement).

Post-hoc analysis (Bonferroni test) showed a significant difference between the morning and afternoon cortisol levels only in the non-depressed group (p = 0.0004). The presence of depressive episodes in the past did not change these results (F = 5.04, p = 0.0328 ANOVA with repeated measures).

No differences in cortisol concentrations were observed between women (n = 6) and men (n = 7) in the depressed group (F(1.9) = 0.067, p = 0.8019).

No significant differences between morning and afternoon levels of cortisol were found in both subgroups based on the length of depression. The longer lasting depression group (≥ 3 months) differed significantly from the no depression group in the morning and afternoon ratio (Table 2)

In the depression group (BDI ≥ 10), patients with the diagnosis of major depression (n = 4) did not differ in their cortisol concentrations from the rest of depression group patients (n = 7) (unpaired t-test).

Discussion

In this study the BDI was used to measure depression. The cut-off score of 10 was used to differentiate between depressed and non-depressed

Table 1. Demographic and clinical variables.

	All patients	Beck Depression Inventory (BDI)	
		≥ 10	< 10
N	32	11 (34.4%)	21 (65.6%)
Women	7 (22%)	6 (55%)	1 (5%)
Age [years]#	54.7 (51.9, 57.5)	53.4 (47.0, 59.7)	55.4 (52.3, 58.5)
Weight [kg]#	81.2 (75.4, 87.0)	80.0 (68.2, 91.8)	81.8 (74.7, 88.9)
Body mass index [kg/m²]#	26.9 (25.5, 28.3)	25.5 (23.1, 27.9)	27.6 (25.8, 29.4)
Tail circumference [cm]#	96.2 (92.1, 100.2)	94.2 (85.7, 102.7)	97.3 (92.5, 102.1)
Waist to hip ratio#	0.96 (0.93, 0.98)	0.95 (0.89, 1.00)	0.96 (0.93, 0.99)
Depression in the past	9	5	4
BDI score (3rd day)##	5 (1, 12)	14* (12, 21)	2 (1, 5)
BDI (3rd month)## Incidence	6	6	0
BDI (6th day)## Incidence	3	2	1
Major depressive disorder (DSM IV-TR)+	4	4	0
Morning plasma cortisol level [nmol/L]#	366.3** (327.7, 404.8)	368.8 (299.1, 438.4)	364.9*** (314.4, 415.5)
Afternoon plasma cortisol level [nmol/L]#	292.8 (245.3, 340.4)	351.6 (261.5, 441.6)	262.0 (206.1, 317.9)
Morning/afternoon cortisol ratio#	1.55 (1.32, 1.78)	1.21 (0.94, 1.49)	1.73**** (1.42, 2.03)

+DSM IV-TR — Diagnostic and Statistica Manual of Mental Disorders Fourth Edition Text Revision
#mean (95% confidence interval [CI])
##median (interquartile range)
*vs. group (< 10): p < 0.0001; Mann-Whitney U test, difference between medians = 12 (9, 17)
**vs. afternoon cortisol: p = 0.0006; paired t-test, difference between means (95% CI) = 73.4 (34.5, 112.3)
***vs. afternoon cortisol: p = 0.0001 paired t-test, difference between means (95% CI) = 102.9 (57.9, 148.0)
****vs. group (≥ 10): p = 0.0228; Student's t-test, mean of differences (95% CI) = 0.34 (0.06, 0.97)
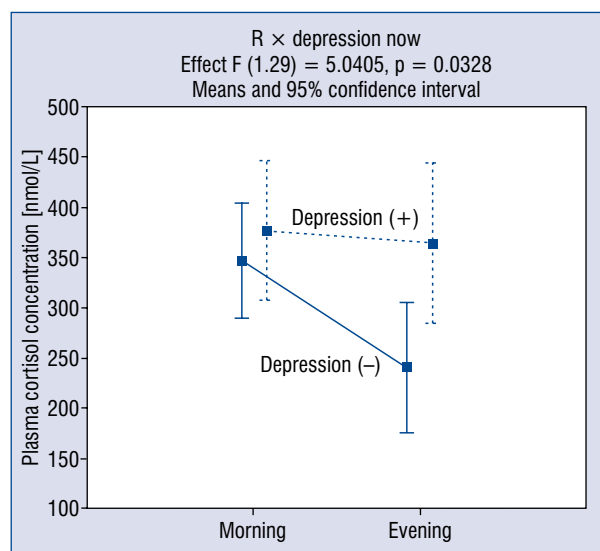
Figure 1. Diurnal change of plasma cortisol level and the presence of depression (Beck Depression Inventory ≥ 10): ANOVA with repeated measures.

patients. It is this criterion that is most commonly used in post-myocardial depression studies. Many studies have shown that depression as defined by a BDI ≥ 10 is related to increased cardiovascular risk, including death [10–14].

A BDI score ≥ 10 was observed in 11 (34.4%) patients. Four (12.5%) patients met criteria of having suffered a major depressive episode according to DSM-IV-TR. These data correspond with other studies [1, 10, 11].

An immediate increase in cortisol after MI has been previously reported [15, 16]. In the (aforementioned) studies cortisol concentration normalised during the first 72 h after MI. In the present study, serum cortisol was measured on the fifth day of MI. Morning as well as afternoon concentrations did not exceed physiological values. The depressed and non-depressed groups did not differ from each other in either morning or afternoon cortisol concentration, which is in line with the results published by Whitehead et al. [17]. However, a significant difference was found in the diurnal profile (morning–afternoon) of the cortisol level between these two groups. The non-depressed group showed a normal cortisol secretion rhythm; the morning cortisol level was significantly higher than the afternoon level. Such a significant difference was not recorded in the de-

Table 2. Clinical variables and length of depression (BDI ≥ 10)

	Presence of depression (BDI ≥ 10) after myocardial infarction		
	NO {1}	≥ 3 months {2}	< 3 months {3}
N	21 (65.6%)	6 (18.8%)	5 (15.6%)
BDI score (3rd day)##	2 (1, 5)	14* (12, 18)	15** (11, 21)
BDI score (3rd month)##	3 (1, 5)	12*** (12, 14)	4 (4, 6)
BDI score (6th day)##	2.5 (1, 5)	10§ (8, 12)	3.0 (3, 5)
Major depressive episode (DSM IV-TR)+	0	3	1
Morning plasma cortisol level [nmol/L]#	364.9§§ (314.4, 415.5)	353.0 (348.3, 457.7)	387.7 (242.9, 532.5)
Afternoon plasma cortisol level [nmol/L]#	262.0 (206.1, 317.9)	344.8 (239.2, 450.5)	359.7 (137.3, 582.2)
Morning/afternoon cortisol ratio#	1.73 (1.42, 2.03)	1.07§§§ (0.66, 1.48)	1.38 (0.89, 1.88)

+DSM IV-TR — Diagnostic and Statistica Manual of Mental Disorders Fourth Edition Text Revision
#mean (95% confidence interval [CI])
##median (interquartile range)
*vs. group {1}: p = 0.00001; Mann-Whitney U test 12 (9, 17)
**vs. group {1}: p = 0.00003; Mann-Whitney U test 12 (8, 20)
***vs. group {1}: p = 0.00004; Mann-Whitney U test (95% CI) = 10 (7, 14); {3}: p = 0.008; Mann-Whitney U test (95% CI) = 8 (4, 17)
§vs. group {1}: p = 0.002; Mann-Whitney U test (95% CI) = 7 (3, 10); {3}: p = 0.016; Mann-Whitney U test (95% CI) = 6 (2, 10)
§§vs. afternoon cortisol: p = 0.0001; paired t-test (95% CI) = 102.9 (57.9, 148.0)
§§§vs. group {1}: p = 0.0331; Student's t-test (95% CI) = –0.31 (–1.25, –0.06)

pressed group, which suggests that flattened daily rhythm of cortisol is more expressed in patients with a longer (≥ 3 months) duration of depression.

According to available research there have been no previous studies on diurnal cortisol profile in patients with depression shortly after MI.

These observations may have important clinical implications. A flattening of the diurnal rhythm of cortisol has been observed in young patients with major depressive disorder [18] and in adolescence patients after major depression [19]. This had a negative impact on health [20]. In a healthy population it is connected with a decline in cognitive functions [21] which could lead to a lowered ability in dealing with stress.

The absence of normal cortisol diurnal rhythm in the present post-infarct depression group could influence abilities in dealing with stress and, as a result, maintain depressive symptoms and increase the risk of somatic complications. In the presented study patients with longer depression (lasting at least 3 months) had a particularly low morning to afternoon cortisol concentration ratio. A flatter diurnal rhythm of cortisol secretion has been observed before in patients with coronary artery disease (CAD) who scored ≥ 10 points in BDI [22].

A flatter diurnal cortisol slope is related to a worse prognosis in patients after coronary artery bypass graft surgery [23], and it can be also responsible for the progression of atherosclerosis in patients with depression and CAD [24]. No

somatic complications were observed in either the depressed or non-depressed group during the 6-month study. The interrelations however, between post-MI depression, the course of CAD and diurnal cortisol rhythm disturbance needs further study.

Limitations of the study

This study has a number of limitations. Firstly, the study group is quite small. Secondly, cortisol concentration was only tested on 1 day. Additional samples on subsequent visits after 3 and 6 months, could broaden interpretation of the results. Thirdly, to some extent, the lack of a control group is also a limitation, although the aim of this study was to determine the cortisol profile in patients with depression compared to patients without depression on the fifth day after MI. Another shortcoming is the small number of women in the study group (22%) and the imbalance between the number of women in the depressed (6) and the non-depressed group (1). Preliminary findings of this study need further confirmation in larger groups with comparisons to healthy controls.

Conclusions

The presence of depressive symptoms (BDI ≥ 10) directly after MI is related to a flattening of the diurnal serum cortisol profile. This seems to be expressed particularly in patients with longer lasting (≥ 3 months) depression.

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VIA MEDICA

ORIGINAL ARTICLE

High mobility group box-1 in hypothalamic paraventricular nuclei attenuates sympathetic tone in rats at post-myocardial infarction

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Abstract

Background: *Inflammation is associated with increased sympathetic drive in cardiovascular diseases. The paraventricular nucleus (PVN) of the hypothalamus is a key regulator of sympathetic nerve activity at post-myocardial infarction (MI). High mobility group box-1 (HMGB1) exhibits inflammatory cytokine-like activity in the extracellular space. Inflammation is associated with increased sympathetic drive in cardiovascular diseases. However, the role of HMGB1 in sympathetic nerve activity at post-MI remains unknown. The aim of the present study is to determine the role and mechanism of HMGB1 in the PVN, in terms of sympathetic activity and arrhythmia after MI.*

Methods: *Sprague-Dawley rats underwent left anterior descending coronary artery ligation to induce MI. Anti-HMGB1 polyclonal antibody or control IgG was bilaterally microinjected into the PVN (5 μL every second day for seven consecutive days). Then, renal sympathetic nerve activity (RSNA) was recorded. The association between ventricular arrhythmias (VAs) and MI was evaluated using programmed electrophysiological stimulation. After performing electrophysiological experiments in vivo, immunohistochemistry was used to detect the distribution of HMGB1, while Western blot was used to detect the expression of HMGB1 and p-ERK in the PVN of MI rats.*

Results: *HMGB1 and p-ERK were upregulated in the PVN in rats at post-MI. Moreover, bilateral PVN microinjection of anti-HMGB1 polyclonal antibody reversed the expression of HMGB1 and p-ERK, and consequently decreased the baseline RSNA and inducible VAs, when compared to those in sham rats.*

Conclusions: *These results suggest that MI causes the translocation of HMGB1 in the PVN, which leads to sympathetic overactivation through the ERK1/2 signaling pathway. The bilateral PVN micro-injection of anti-HMGB1 antibody can be an effective therapy for MI-induced arrhythmia.* (Cardiol J 2019; 26, 5: 555–563)

Key words: HMGB1, ERK, hypothalamic paraventricular nucleus, sympathetic nerve, myocardial infarction

Introduction

Increasingly more attention has been given to the role of proinflammatory cytokines in cardiovascular and cardiovascular-related injuries.

High-mobility group box 1 (HMGB1) is a member of the HMGB protein family, which is a highly conserved non-histone nuclear protein in-volved in damage response. HMGB1 was a critical co-factor of somatic cell transcriptional regulation [1]. It plays roles in a wide variety of processes, including inflammation, immune responses, apoptosis and responses to injury [2, 3]. Furthermore, HMGB1 can be passively released from damaged cells to exacerbate inflammation through receptors, including the receptor for advanced glyca-

tion end products (RAGE) and toll-like receptors (TLR2 and TLR4) [4–6]. These receptors activate multiple intracellular signaling pathways, including mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK)1/2, and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (AKT). Phosphorylated (p-) ERK1/2 has numerous cytoplasmic and nuclear effects [7, 8]. Among its nuclear effects, p-ERK1/2 can activate multiple nuclear transcription factors [7–10], and its products may include RAS components and inflammatory mediators [9, 11, 12].

The paraventricular nucleus (PVN) is an integrative site in regulating sympathetic outflow and cardiovascular activity [13, 14]. Previous studies have shown that PVN is involved in excessive sympathetic activation and enhanced cardiac sympathetic afferent reflex in chronic heart failure [15–19]. However, it remains unknown how inflammatory mediators form in the PVN and enhance sympathetic nervous system activity in acute myocardial infarction (AMI) rats. In the present study, a putative mechanism was examined. It was determined whether myocardial infarction (MI) could lead to HMGB1 release and ERK1/2 MAPK signaling activation in the PVN, thereby contributing to sympathetic activation. The present results indicate that HMGB1 and ERK1/2 MAPK signaling contribute to its generation and sympathetic activation, which therefore can be reduced by HMGB1 antagonists. These findings show that this can be a potential target for AMI therapy.

Methods

Animals

All experimental procedures were conducted in accordance with the Shandong University Institutional Animal Care and Use Committee guidelines for animal experiments, and were approved by the University's Committee. Adult male Sprague–Dawley rats (Charles River, Beijing, China), weighing 250–280 g, were used for all experiments. These rats were housed in a temperature-controlled room (23 ± 2°C) with light-controlled animal quarters, and were provided free access to laboratory chow and water.

Location and catheterization of the PVN

Sixty rats were anesthetized *via* intraperitoneal injection of chloral hydrate (40 mg/kg). Then, the rats were fixed on the brain stereotactic locator (RWD Life Science Co., Shenzhen, China), a skin incision was performed along the sagittal suture to expose the skull, and the anterior and posterior fontanelles were adjusted to the same level. The stereotaxic coordinates for the PVN were 1.8 mm caudal from the bregma, 0.4 mm lateral to the midline and 7.9 mm ventral to the dorsal surface. A stainless-steel casing with a core outer diameter of 0.6 mm and an inner diameter of 0.4 mm (RWD Life Science Co., Shenzhen, China) was implanted into the skull using a gripper instrument (RWD Life Science Co., Shenzhen, China). The cannula was fixed with dental cement, and penicillin powder was sprayed on the top of the skull. Then, the skin incision was sutured. Rats were fed in a single cage. After 1 week of recovery, 49 rats implanted with a cannula survived for further testing.

The MI model

The 49 rats that were successfully implanted with a cannula were anesthetized *via* intraperitoneal injection of chloral hydrate (40 mg/kg). The animals underwent a thoracotomy and pericardiotomy, and the left anterior descending coronary artery was ligated to establish the MI, as previously reported [20]. Sham rats underwent a thoracotomy and pericardiotomy without coronary artery ligation. Rats underwent electrocardiography (ECG) monitoring using an animal biological function experiment system (BL-420S, TaiMeng, China) during the MI surgery. The infarction was confirmed by ST segment elevation, regional cyanosis and wall motion abnormalities. The ST segment (from the end of the QRS wave to the beginning of the T wave) elevation after ligation of coronary artery is one of the evaluation of the MI model (Fig. 1A). With respect to clinical importance, only rats with moderate infarct size (30–50%) were enrolled.

PVN microinjection

Rats received bilateral PVN microinjections of chicken anti-HMGB1 polyclonal antibody (Shino-Test Corporation, Tokyo, Japan; 10 μg in 10 μL of 10 mM of Tris-buffered saline, pH 7.4, 5 μL every second day for seven consecutive days), a dose of anti-HMGB1 polyclonal Ab similar to that used in previous studies, or an equivalent volume of control IgG. The PE pipe with a length of 15–20 cm and the casing pipe with a diameter of 0.4 mm were connected, and these were subsequently connected to a microsyringe. The bilateral PVN microinjections were carried out using a micropump injection device (RWD Life Science Co., Shenzhen, China). The bilateral PVN microinjection volume was 5 μL for each site. The injection rate was 0.5 μL/ /min. After the injection, the syringe was left for
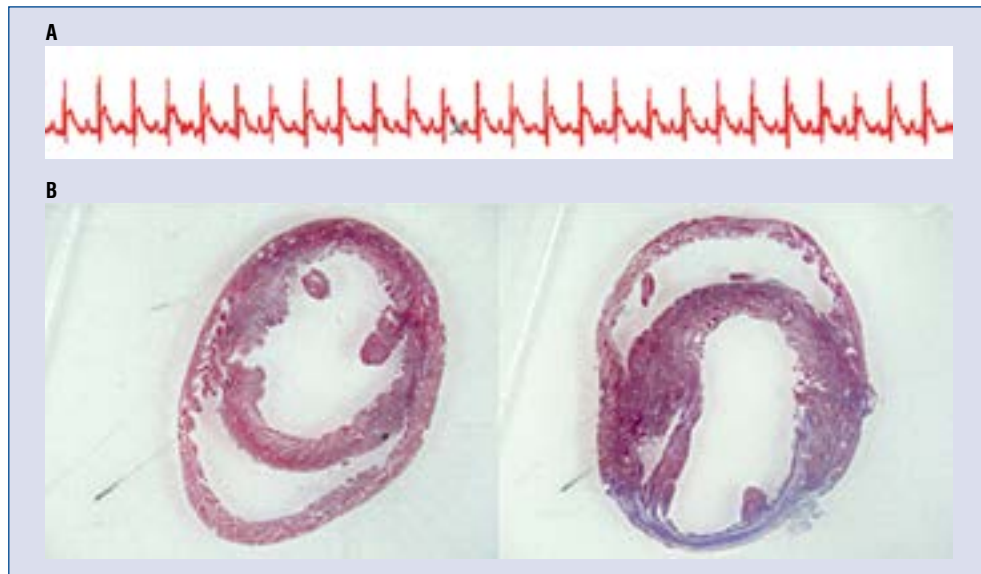
Figure 1. Evaluation of the myocardial infarction (MI) model; **A**. Electrocardiogram before (up) and after (down) ligation of coronary artery. The ST segment elevated after ligation of coronary artery; **B**. Representative histologic image of the heart stained with Massion's trichrome. Sections from the sham operation (left) and MI (right) rat hearts, respectively. Myocytes are red and fibrotic tissues are blue.

an additional 5 min before it was slowly retracted. At the end of the experiment, 50 nL of Evans Blue (2%) was injected into the microinjection site for histological identification. Only the data from rats, in which the microinjection sites were within the boundaries of the PVN were used for analysis. Rats with microinjection sites outside the PVN or at the margin of the PVN were excluded from the data analysis.

Electrophysiological experiments

After 7 days, the rats were anesthetized, as previously described. Then, a second thoracotomy was performed. The protocol for programmed electrophysiological stimulation was similar to that described in a previous study of the investigators [21]. Programmed electrical stimulation was performed to measure the ventricular effective refractory period (VERP), while inducing ventricular arrhythmias (VAs). Briefly, the stimulation intensity was twice the threshold, and stimulus length was 5 ms. In order to induce VAs, the pacing involved a cycle length of 180 ms (S_0), followed by 1–3 extrastimuli (S_1, S_2 and S_3) at shorter coupling intervals. In order to determine the VERP, a single extrastimulus was introduced at progressively shorter intervals. The VERP was longest at the S_1–S_2 interval, which did not evoke a premature ventricular depolarization. The experiment was typically completed within 10 min. The results were classified as follows:

(1) inducible sustained monomorphic ventricular tachycardia (MVT); (2) inducible polymorphic ventricular tachycardia (PVT), and ventricular fibrillation (VF); (3) no inducible ventricular tachycardia VT/VF. All rats underwent 6-lead ECG.

RSNA recording and measurement

A retroperitoneal incision was made for the isolation of the left renal sympathetic nerve. The nerve was cut distally to eliminate its afferent activity, and placed on a pair of silver electrodes, which were immersed in warm mineral oil. The renal sympathetic nerve activity (RSNA) was amplified with a four channel AC/DC differential amplifier (DP-304; Warner Instruments, Hamden, CT, USA) with a high pass filter at 100 Hz and a low pass filter at 3000 Hz. The RSNA was integrated at a time constant of 100 ms. Background noise was determined, as previously reported [22]. Basal nerve activity (baseline) was determined by efferent RSNA at the beginning of the experiment. The RSNA activity during the experiment was calculated by subtracting the background noise from the recorded value. The RSNA responses were expressed as a percentage change from the basal value.

Heart tissue preparation

After the electrophysiological study, rats were sacrificed, and the hearts were rapidly removed.

A sample of fresh cardiac tissue was immersed in 10% formalin for 24 h, embedded in paraffin, cut into 10-μm sections, and stained with Masson's trichrome (Fig. 1B). Then, corresponding heart positions were sampled in sham rats. Sections from sham group (Fig. 1A), MI group (Fig. 1B). Myocytes are red, and fibrotic tissues are blue.

ELISA

A rat noradrenaline (NA) ELISA Kit (Catalog Number CSB-E07022r, CUSABIO) was used to detect serum NE concentrations, according to manufacturer instructions. The intra- and inter-sample variability for each kit was < 8%.

Western blot analysis

The expression of HMGB1 and p-ERK in the PVN were detected by Western blot analysis. For immunoblot analyses, proteins were isolated using a protein extraction kit (Beyotime Institute of Biotechnology, Jiangsu, China). The extracted protein was measured using a BCA protein assay reagent kit (Pierce, Madison, WI, USA). Equal amounts of total protein (80 μg of protein/lane) were resolved on 5–10% SDS-PAGE gels, and transferred onto polyvinylidene difluoride membranes. Then, the membranes were blocked with 5% non-fat dry milk in phosphate buffered saline (PBS) containing 0.05% Tween 20, and incubated overnight at 4°C with the respective primary antibodies against HMGB1 (ab172730-Rabbit monoclonal IgG; 1:20,000; Abcam) and primary antibodies against p-ERK (1:2,000; 9101; Cell Signaling Technology). Next, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibodies (1:10,000; Zhongshan Golden Bridge Biotechnology). The blots were developed using an enhanced chemiluminescence detection kit (Millipore, Billerica, MA), and visualized using a FluorChem E Imager (Protein-Simple, Santa Clara, CA). The densities relative to β-actin were analyzed using NIH ImageJ software.

Immunohistochemistry

After the electrophysiology studies, the brain was rapidly removed, and samples were immersed in 10% formalin for 24 h, embedded in paraffin and cut into 5-μm thick slices. The paraffin sections were deparaffinized, rehydrated and soaked in 0.1 M of citric acid buffer for 15 min at 92–98°C in a microwave oven, and washed with PBS. Then, the sections were incubated with the primary antibodies of anti-HMGB1 (ab172730-Rabbit monoclonal IgG,

1:5,000; Abcam) overnight at 4°C. Subsequently, the samples were incubated with horseradish peroxidase-conjugated secondary antibodies of rabbit anti-sheep IgG (KPL, Gaithersburg, MD, USA) and goat anti-mouse IgG (Zhongshan Golden Bridge Biotechnology) for 1 h at 37°C. Immunore-activity was developed with 3,3'-diaminobenzidine tetrahydrochloride (Zhongshan Golden Bridge Biotechnology). Finally, the sections were counterstained with hematoxylin, mounted and examined by microscopy.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The significance of differences in mean values was analyzed by the unpaired t-test. Analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The differences were considered significant at $p < 0.001$.

Results

The expression of HMGB1 in the PVN

Using immunohistochemistry, the expression of HMGB1 in the PVN of the MI group and MI + Anti-HMGB1 polyclonal antibody group were measured. Few HMGB1 appeared in control and sham-operated PVN (Figs. 2A, B). Compared with sham-operated PVN, MI increased the density of HMGB1-positive nucleus in the PVN (Fig. 2C). In addition, density of HMGB1-positive nucleus was lower in MI PVN with anti-HMGB1 polyclonal antibody treatment, than in MI alone (Fig. 2D).

Effects of anti-HMGB1 polyclonal antibody on the expression of HMGB1 and p-ERK in the paraventricular nucleus

HMGB1 levels in the PVN were upregulated in MI rats, when compared with sham-operated rats ($p < 0.001$, Fig. 1). Moreover, HMGB1 levels were lower in MI with MI + Anti-HMGB1 polyclonal antibody, than in MI alone ($p < 0.001$, Fig. 1).

The nuclear protein levels of p-ERK in the PVN were higher in rats with MI, than in sham-operated rats ($p < 0.001$, Fig. 1). Moreover, anti-HMGB1 polyclonal antibody treatment significantly reduced the MI-induced nuclear expression of p-ERK ($p < 0.001$, Fig. 3).

Effects of anti-HMGB1 polyclonal antibody on baseline RSNA

Anti-HMGB1 polyclonal antibody (5 μL, *qod*, 7 consecutive days) was microinjected into the PVN after MI, and RSNA was expressed as a percentage
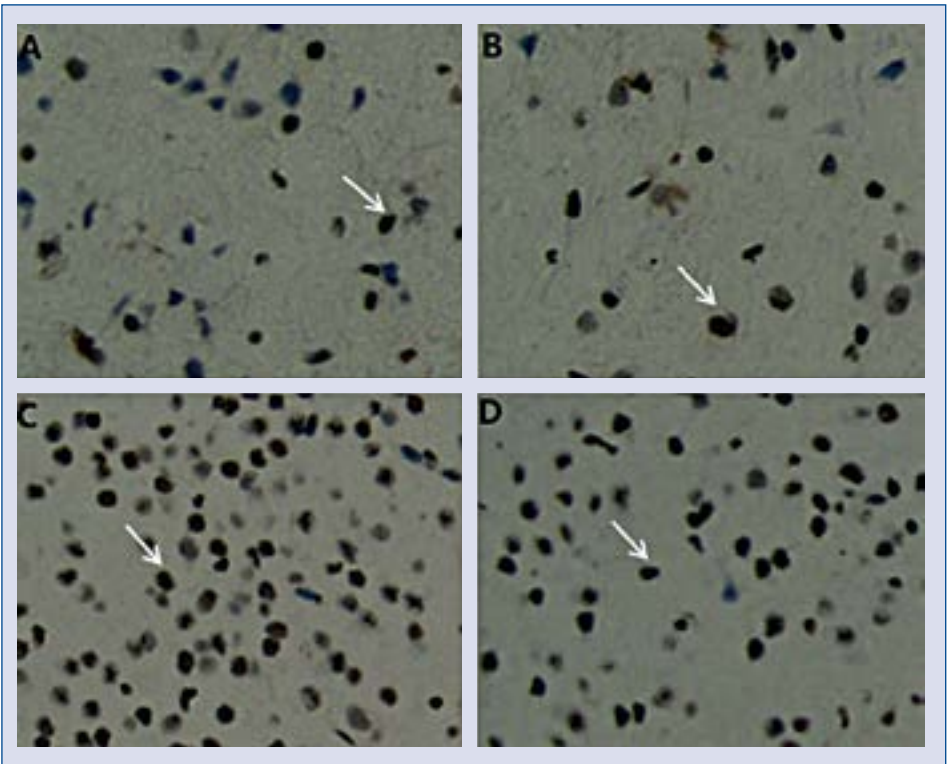
Figure 2. A–D. Representative immunostaining of high mobility group box-1 (HMGB1) in the paraventricular nucleus from sham-operated, myocardial infarction (MI), and MI+Anti-HMGB1 polyclonal antibody treated rats (magnification, ×400). Brown represents the positive staining (arrows). Bar = 100 μm.
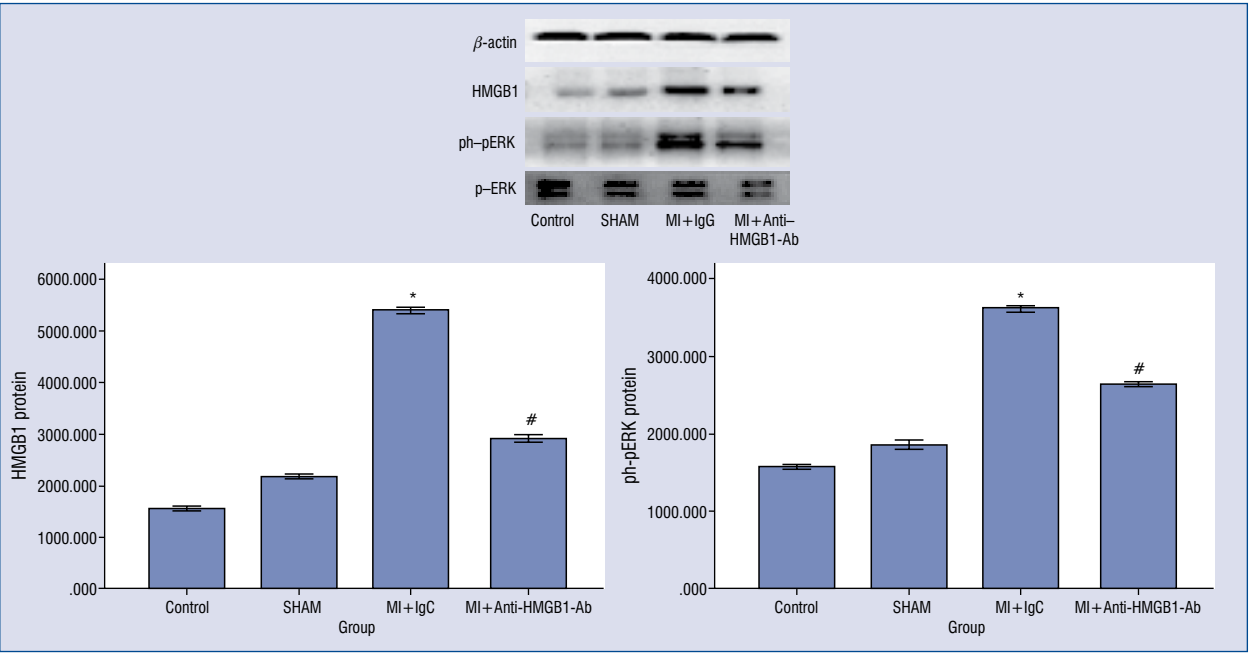
Figure 3. Western blot analysis of the protein levels of high mobility group box-1 (HMGB1) (25 kDa) and ph-pERK (42 kDa) in cytoplasmic protein homogenates of the paraventricular nucleus. The quantification is relative to β-actin levels. Data are presented as mean ± standard deviation; *p < 0.001 vs. sham; #p < 0.001 vs. MI+Anti-HMGB1 polyclonal antibody.
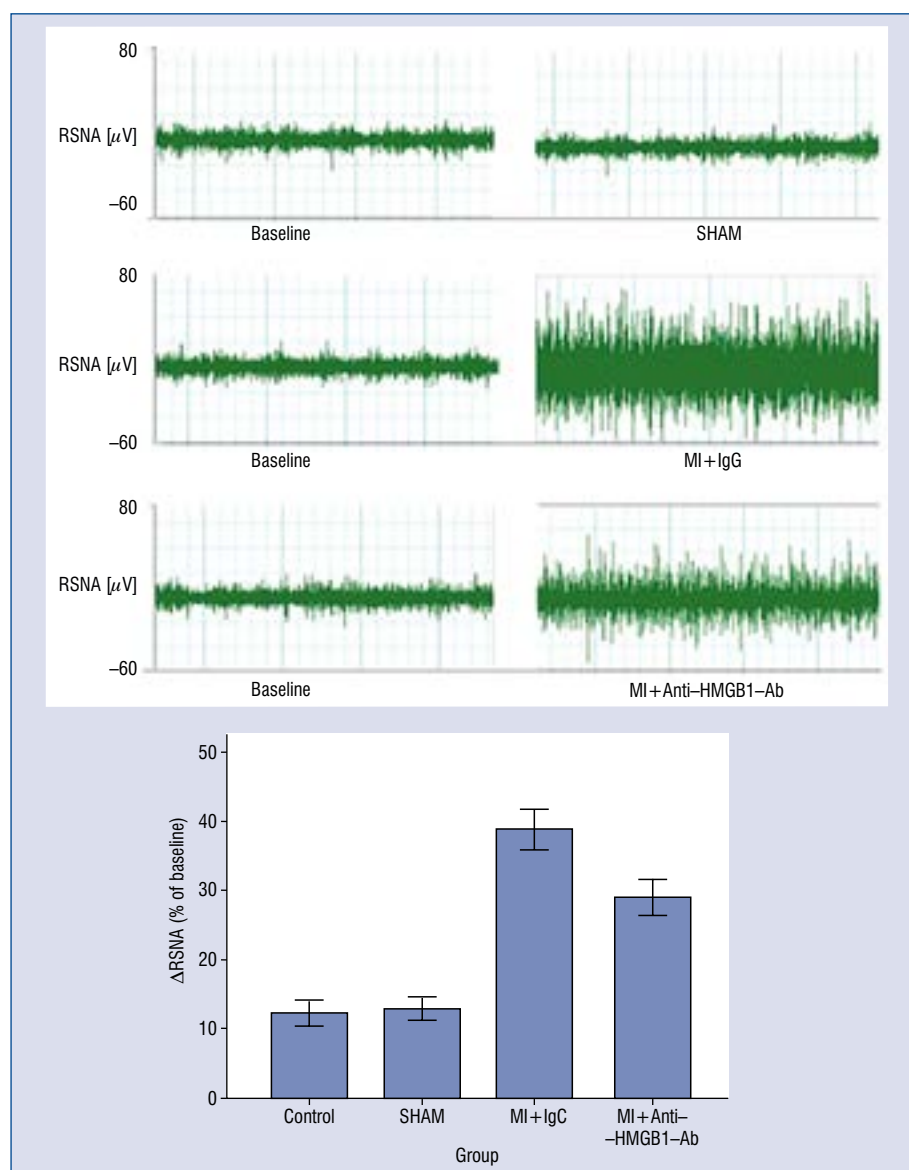
Figure 4. The segments of the original recordings obtained from individual rats in each experimental group show the responses of the renal sympathetic nerve activity (RSNA). Mean changes in RSNA after the microinjection of anti-HMGB1 polyclonal antibody into the paraventricular nucleus vs. the sham group; p < 0.001; MI+Anti-HMGB1 polyclonal antibody treatment group vs. the MI group; p < 0.001.

change from the basal value. As shown in Figure 4, compared with the AMI group, RSNA responses decreased in the MI+Anti-HMGB1 polyclonal antibody groups.
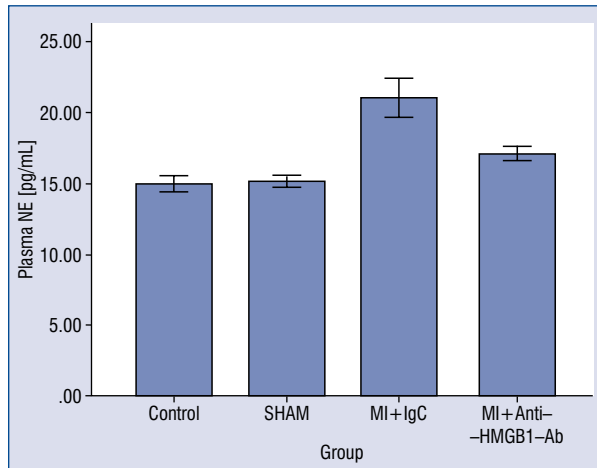
Serum content of norepinephrine

In order to further examine the action of sympathetic activity, norepinephrine (NE) levels in serum were measured by ELISA. As shown in Figure 5, the NE level in MI+Anti-HMGB1 polyclonal antibody rats was higher than in rats in the control group (17.101 ± 0.490 vs. 14.949 ± 0.562;

p < 0.001), and was lower in MI rats (21.047 ± ± 1.358 pg/mL; p < 0.001).

Electrophysiological characterization

In order to further elucidate an association of VAs and MI in rats, programmed electrophysiological stimulation was performed. No rat experienced spontaneous VAs during the placement of the electrodes, and none of the rats died during the electrophysiological study. VAs occurred in 1/10 (10.0%) rats in the control group, which was significantly fewer than those in the MI+Anti-

Figure 5. Norepinephrine content in serum of rats. ELISA results of the NE content in serum obtained from rats in the control group, myocardial infarction (MI) group and MI+Anti-HMGB1 polyclonal antibody group. Data are expressed as mean ± standard deviation.
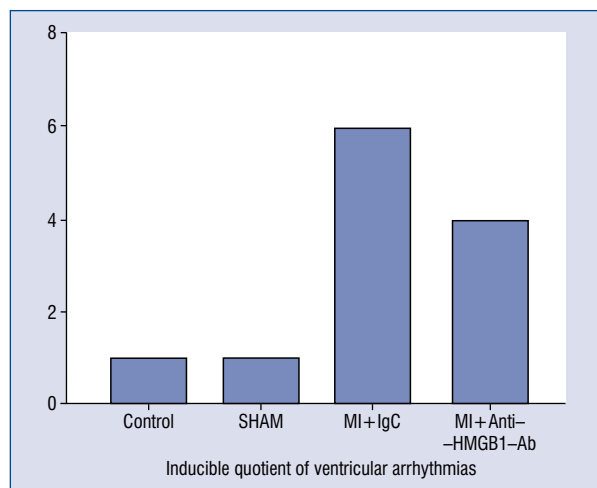
Figure 6. Comparisons of the arrhythmia score between the four groups at 7 days post-myocardial infarction (MI).

-HMGB1 polyclonal antibody (4/11, 36.4%) and MI (6/12, 50%) groups. The recordings of inducible VAs are presented in Figure 6.

Discussion

According to available research, the present study was the first to explore the role and mechanism of HMGB1 in the PVN in the development of sympathetic overaction and ventricular arrhythmia in MI rats.

There are several mechanisms between peripheral sympathetic and ventricular arrhythmo-

genesis. The PVN of the hypothalamus is a critical site of autonomic and neuroendocrine regulation [23]. Inflammation is associated with increased sympathetic drive in cardiovascular diseases. In particular, recent studies have established a causal relationship between inflammation and the activation of the sympathetic nervous system [24, 25]. However, the mechanism of inflammatory mediators in sympathetic overactivity remains unknown. HMGB1 is normally found in the nucleus. When cell damage occurs, HMGB1 is translocated to the cytoplasm and released by the cell to act as a multifunctional cytokine with roles in infection, organ dysfunction, inflammation, and immune responses [26, 27]. Consistently, it was found that the expression of HMGB1 significantly increased in the PVN after MI in rats. The significant increase in HMGB1 levels in the PVN after MI and the HMGB1 expression demonstrated by immunohistochemistry support the finding that a considerable amount of HMGB1 was released into the extracellular space.

Recent studies have revealed that the pharmacological manipulation of neuronal activity within the PVN can markedly alter sympathetic nerve activity [28]. Therefore, the investigators determined the effect of HMGB1 in the PVN on sympathetic activity and arrhythmia after MI, as well as its possible mechanism through the bilateral PVN microinjection of anti-HMGB1 polyclonal antibody, which is an HMGB1 antagonist.

One novel finding in the present study was that levels of HMGB1 and p-ERK were significantly elevated in the PVN, which was accompanied by high RSNA and high-risk VAs, in rats after MI.

In addition, another new finding in the present study was that the administration of anti-HMGB1 polyclonal antibody in the PVN effectively inhibited the expression of HMGB1 and p-ERK in MI rats. In addition, it was found that the administration of anti-HMGB1 polyclonal antibody in the PVN prevented an increase in RSNA, and reduced a high risk of VAs in MI rats. Therefore, it was speculated that HMGB1 in the PVN after MI in rats may result in sympathetic overaction through the ERK1/2 signaling pathway.

There are at least two mechanisms by which ERK1/2 signaling might contribute to sympathetic excitation. Once activated, p-ERK1/2 can stimulate several transcription factors, such as activator-protein 1, Elk-1, nuclear factor kappa B and cyclic AMP response element-binding protein [7–10], in which the downstream products may include key excitatory elements, such as angiotensinogen [29], the precursor of ANG II, AT_1R [11, 12], tumor necrosis factor α and interleukin-1β [9, 30, 31], and COX-2 [32],

the inducible enzyme that produces PGE$_2$. A second mechanism by which ERK1/2 signaling may contribute to increased sympathetic activation is by disinhibiting presympathetic neurons [33].

In summary, HMGB1 in the PVN in rats after MI regulate RSNA through ERK1/2 signaling, which may well-contribute to generation of excitatory and inflammatory mediators in the PVN in MI rats. This is an important mechanism in sympathetic activation and VAs in rats after MI.

Conclusions

Overall, these results indicate that the expression of HMGB1 in the PVN in MI rats and ERK1/2 signaling may contribute to the generation of excitatory and inflammatory mediators, which may participate in regulating the RSNA and increase the risk of VAs in MI rats. Manipulations designed to inhibit HMGB1 activation in the PVN may be an effective method for the present treatment of VAs after MI. These results may provide a fundamental mechanism and therapeutic method for the high incidence of VAs in patients after MI in the future.

Conflict of interest: None declared

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VIA MEDICA

ORIGINAL ARTICLE

The long noncoding RNA THRIL knockdown protects hypoxia-induced injuries of H9C2 cells through regulating miR-99a

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Abstract

Background: *Myocardial infarction (MI) is a leading cause of disease with high morbidity and mortality worldwide. Recent studies have revealed that long non-coding RNAs (lncRNAs) are involved in heart disease pathogenesis. This study aimed to investigate the effect and the molecular basis of THRIL on hypoxia-injured H9C2 cells.*

Methods: *THRIL, miR-99a and Brahma-related gene 1 (Brg1) expressions in H9C2 cells were altered by transient transfections. The cells were subjected to hypoxia for 4 h, and then the levels of THRIL, miR-99a and Brg1 were investigated. Cell viability, migration and invasion, and apoptotic cells were respectively measured by trypan blue exclusion assay, transwell migration assay and flow cytometry assay. Dual luciferase reporter assay was conducted to verify the interaction between miR-99a and THRIL. Furthermore, levels of apoptosis-, PI3K/AKT and mTOR pathways-related factors were measured by western blotting.*

Results: *Hypoxia induced an increase of THRIL but a reduction of miR-99a and Brg1. THRIL inhibition significantly attenuated hypoxia-induced cell injuries, as increased cell viability, migration and invasion, and decreased cell apoptosis. THRIL negatively regulated miR-99a expression through sponging with miR-99a binding site, and miR-99a inhibition abolished the protective effects of THRIL knockdown against hypoxia-induced injury in H9C2 cells. Furthermore, miR-99a positively regulated the expression of Brg1. Brg1 inhibition promoted hypoxia-induced cell injuries, while Brg1 overexpression alleviated hypoxia-induced cell injuries. Moreover, Brg1 overexpression activated PI3K/AKT and mTOR pathways.*

Conclusions: *This study demonstrated that THRIL inhibition represented a protective effect against hypoxia-induced injuries in H9C2 cells by up-regulating miR-99a expression. (Cardiol J 2019; 26, 5: 564–574)*

Key words: THRIL, miR-99a, Brg1, myocardial infarction, hypoxia

Introduction

Ischemic heart disease, such as myocardial infarction (MI), poses a major threat to human health and is one of the leading causes of diseases with high morbidity and mortality worldwide [1]. MI is mainly caused by a life-threatening interruption of blood supply to part of the heart [2]. Despite rapid progress obtained in targeted therapy strategies

and improvement of life quality of patients, there still remain limitations in the clinical treatment and prevention of MI. Thus, it is of great significance to understand further mechanisms of physiological and pathological processes in MI.

Non-coding RNAs (ncRNAs) are classified into small ncRNAs and long ncRNAs (lncRNAs) according to their size. Small ncRNAs, such as short interfering RNAs (siRNAs), piwi-interactingRNA

(piRNAs), and microRNAs (miRNAs) that have a length of less than 200 nucleotides (nt) [3]. LncRNAs are nonprotein coding transcripts with length longer than 200 nucleotides, and many of which are emerged as an important class of regulatory molecules in governing fundamental biological processes [4]. The mechanism of gene regulation by lncRNAs are involved in activation or inhibition of gene expression and modulation of chromatin architecture [5]. Recent studies have revealed that a number of lncRNAs have significant roles in a diverse range of cellular functions including development, differentiation, and cell fate as well as disease pathogenesis [3, 6, 7]. In the cardiovascular system, it has been reported that several lncRNAs, such as Novlnc6 and Mhrt, are involved in acute MI and heart failure; whereas others (such as CARL, CHRF, Novlnc6 and et al.) control hypertrophy, mitochondrial function and apoptosis of cardiomyocytes [8]. THRIL (TNFα and hnRNPL related immunoregulatory lincRNA) is first reported to regulate lipopolysaccharide-induced tumor necrosis factor alpha (TNFα) by interacting with heterogenous nuclear ribonucleoprotein L (hnRNPL), and plays a crucial role in the regulation of physiological and pathological inflammatory immune responses [9]. However, its biological role and regulatory mechanism in hypoxia-induced injury of H9C2 cells are poorly defined.

In the present study, the role of THRIL in the survival and metastasis of H9C2 cells against hypoxia stimulus as well as the underlying mechanism is investigated. It was found that THRIL knockdown attenuated hypoxia-induced cell injuries in H9C2 cells. THRIL negatively regulated miR-99a expression through sponging with its banding site. In addition, we also proved that Brahma-related gene 1 (Brg1) expression was positively regulated by miR-99a and Brg1 was involved in the hypoxia-induced injuries in H9C2 cells. This study will provide new insight into the fundamental information and novel pharmacological target for therapeutic approaches of cardiovascular disease.

Methods

Cell culture and hypoxia treatment

H9C2 cells, obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA), were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Life Technologies Corpo-

ration, Carlsbad, CA, USA) at 37°C in an atmosphere of 95% air and 5% CO_2. When cells reached about 80% of confluence in appropriate culture dishes, cells were pre-starved using DMEM supplemented with 0.5% FBS for 1 h and then were incubated in hypoxic incubator containing 94% N_2, 5% CO_2, and 1% O_2 for 4 h to simulate hypoxia injury.

Transfection and generation of stably transfected cell lines

Short-hairpin RNA (shRNA) directed against human lncRNA THRIL was ligated into the U6/GFP/Neo plasmid (GenePharma, Shanghai, China) and was referred as to sh-THRIL. The plasmid PGPU6/GFP/neo-shControl (GenePharma) was used as the negative control (NC) and encoded with a nonsense sequence, and was referred as to sh-NC. For the analysis of the Brg1 functions, the full-length Brg1 sequences and shRNA directed against Brg1 were constructed in pEX-2 and U6/GFP/Neo plasmids (GenePharma), respectively. And they were referred as to pEX-Brg1 and sh-Brg1. The plasmid carrying a non-targeting sequence was used as a NC of pEX-Brg1 and sh-Brg1, which were referred as to pEX and sh-NC. The lipofectamine 3000 reagent (Life Technologies Corporation) was used for cell transfection according to the manufacturer instructions. The stably transfected cells were selected by the culture medium containing 0.5 mg/mL G418 (Sigma-Aldrich, St. Louis, MO, USA). After approximately 4 weeks, G418-resistant cell clones were established. miR-99a mimic, inhibitor and their respective NC were synthesized (Life Technologies Corporation) and transfected into cells in the study. Since the highest transfection efficiency occurred at 48 h, thus 72 h post-transfection was considered as the harvest time in the subsequent experiments.

Quantitative real-time PCR

Total RNA was extracted from cells using TRIzol reagent (Life Technologies Corporation) according to the manufacturer instructions. RNA was reverse transcribed to cDNA using a Reverse Transcription Kit (Takara, Dalian, China). THRIL and Brg1 expression levels were determined by quantitative real time polymerase chain reaction (qRT-PCR) using the SYBR Green Master Mix (Takara). The expression of miR-99a was measured using Taqman MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) according to manufacturer instructions. GAPDH and U6 were used for the normalization of mRNA, lncRNA and miRNA. The results were presented as fold changes rela-

tive to U6 or GAPDH and were calculated using the $2^{-\Delta\Delta CT}$ method. The primer sequences used in qRT-PCR analysis were shown as follows: lncRNA THRIL (Forward: 5'-GAG TGC AGT GGC GTG ATC TC-3', Reverse: 5'-AAA ATT AGT CAG GCA TGG TGG TG-3'); miR-99a (Forward: 5'-CGG AAC CCG TAG ATC CGA T-3', Reverse: 5'-CAG TGC AGG GTC CGA GGT-3'); Brg1 (Forward: 5'-CCT CTC TCA ACG CTG TCC AAC TG-3', Reverse: 5'-ATC TTG GCG AGG ATG TGC TTG TCT T-3').

Cell viability assay

For cell viability assay, 1×10^5 cells were seeded in duplicate in 60-mm dishes. After 4 h for hypoxia followed by 72 h transfection, cells were washed with phosphate buffered saline (PBS) and living cell numbers were determined by trypan blue exclusion (Beyotime Biotechnology, Shanghai, China) as previously described [10].

Apoptosis assay

For apoptosis assay, cells were stained with propidium iodide (PI) and fluorescein-isothio-cyanate (FITC) — conjugated Annexin V using an Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime Biotechnology). Briefly, the cells were harvested after transfection in 72 h and 4 h for hypoxia incubation and then centrifuged at 800 g for 5 min at 4°C. Cells were washed with PBS 3 times and resuspended with 500 μL 1 × binding buffer. Then cells were mixed with 5 μL Annexin V-FITC and 5 μL PI for 15 min in the dark at 37°C. Flow cytometry analysis was done using a FACS can (Beckman Coulter, Fullerton, CA, USA). The data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Migration and invasion assay

Cell migration and invasion was measured using a transwell chamber (8 μm, 24-well format; Corning, Lowell, MA, USA). For migration assay, 1×10^5 cells in 0.2 mL of serum-free medium were plated on the upper compartment of 24-well transwell culture chamber, and 0.6 mL of medium containing 10% FBS was added to the lower chamber. For invasion assay, 1.5×10^5 cells were planted on the upper chamber which is pre-coated with 20 μg of Matrigel (BD Biosciences, Bedford, MA, USA). After 48 h of incubation, the migrated and invaded cells in the lower chamber were fixed in 100% methanol and stained with 0.1% crystal violet (Sigma-Aldrich) and cells that did not migrate or invade through the pores were removed by cotton swabs. The migrated and invaded cells were counted in 5 random fields and expressed as the average number of cells per field. These experiments were done in triplicate and performed a minimum of three times.

Reporter vectors constructs and luciferase reporter assay

The segment from the 3'-UTR of the THRIL containing the predicted miR-99a binding site was amplified by PCR and then cloned into a pmirGlO Dual-luciferase miRNA Target Expression Vector (Promega, Madison, WI, USA) to form the reporter vector THRIL-wild-type (THRIL-wt). To mutate the putative binding site of miR-99a in the THRIL, the sequence of putative binding site was replaced and was named as THRIL-mutated-type (THRIL--mt). Then the vector and miR-99a mimic were co-transfected into H9C2 cells with lipofectamine 3000 (Life Technologies Corporation), and the Dual-Luciferase Reporter Assay System (Promega) were used for detecting the luciferase activity.

Western blot

Cells were homogenized in RIPA protein lysis buffer (Beyotime Biotechnology) supplemented with protease inhibitors (Roche, Basel, Switzerland) at 4°C for 30 min before centrifugation at 12,000 g for 15 min at 4°C. The protein concentration was quantified using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Denatured samples (20–40 μg of protein) were loaded into each well, separated by 10–12% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE), and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). Subsequently, the membrane was blocked in 5% BSA (Roche) in TBST at room temperature for 1 h. Primary antibodies: anti-Bcl-2 (#4223), anti-Bax (#5023), anti-caspase-3 (#9662), anti-caspase-9 (#9502), anti-GAPDH (#2118), anti-Brg1 (#49360), anti-PI3K (#4249), anti-p-PI3K(#4228), anti-AKT (#4685), anti-p-AKT (#4060), anti-mTOR (#2983), anti-p-mTOR (#5536), anti-p70S6K (#2708), and anti-p-p70S6K (#9204, Cell Signaling Technology, Beverly, MA, USA) were prepared in 5% BSA at a dilution of 1:1000. The blot was incubated respectively with primary antibodies overnight at 4°C. After washing with TBST for 3 times, the membranes were further incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG or anti-mouse IgG (Sigma-Aldrich) at a 1:5000 dilution for 2 h at room temperature. The blots were developed with ECL solution (Pierce) and visualized by using Image Lab™ Software (Bio-Rad, Hercules, CA, USA).
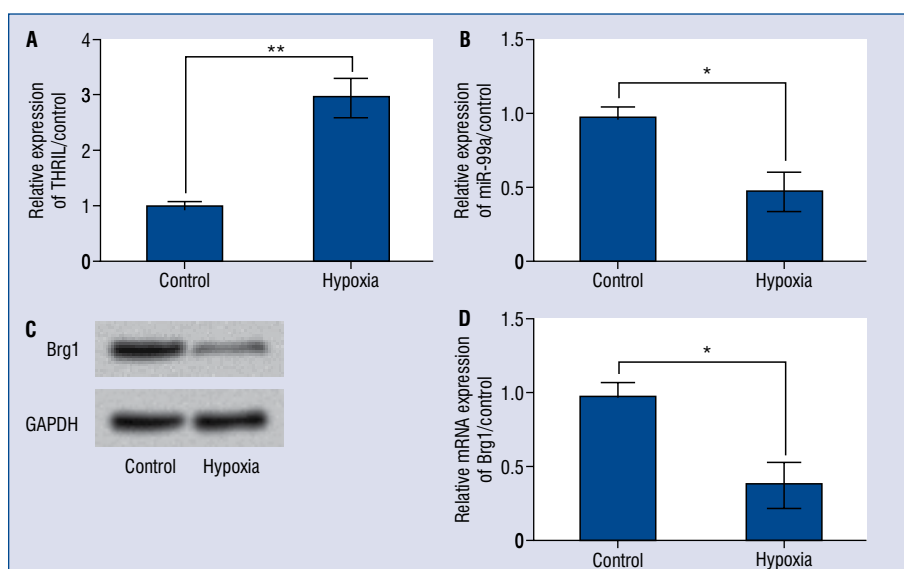
Figure 1. Hypoxia induced an increase of THRIL but the reduction of miR-99a and Brahma-related gene 1 (Brg1). H9C2 cells were incubated in hypoxia incubator for 4 h to induced hypoxia injury. Then, the expression levels of (**A**) THRIL and (**B**) miR-99a were measured by quantitative real time polymerase chain reaction (qRT-PCR). The protein (**C**) and mRNA expression of Brg1 (**D**) was respectively determined by Western blotting and qRT-PCR; *p < 0.05; **p < 0.01.

Statistical analysis

All experiments were repeated 3 times. The results of multiple experiments are presented as the mean ± standard deviation (SD). Statistical analyses were performed using Graphpad 6.0 statistical software (GraphPad Software, San Diego, CA, USA). The p-values were calculated using a one-way analysis of variance (ANOVA). A p-value of < 0.05 was considered to indicate a statistically significant result.

Results

Hypoxia induced the increase of THRIL but the reduction of miR-99a and Brg1

H9C2 cells were exposed to hypoxia incubator for 4 h to stimulate hypoxia injury. As shown in Figure 1A, the expression of THRIL was significantly up-regulated by hypoxia treatment in H9C2 cells (p < 0.01). However, hypoxia treatment induced a significant reduction of miR-99a expression (p < < 0.05; Fig. 1B). In addition, the protein and mRNA expression of Brg1 was also down-regulated by hypoxia injury in H9C2 cells (p < 0.05; Fig. 1C, D).

THRIL knockdown attenuated hypoxia--induced cell injuries in H9C2 cells

To analyze the potential role of THRIL knockdown in hypoxia-induced injuries in H9C2 cells, we transfected THRIL targeted shRNA (sh-THRIL)

into H9C2 cells to induce silence of THRIL and the efficiency of transfection was performed by qRT-PCR analysis (Fig. 2A). As expected, the expression level of THRIL was significantly reduced after transfection with sh-THRIL in H9C2 cells (p < 0.01). As the migration of cardiomyocytes into the injury site has been reported to be regulated independently of proliferation, and that coordination of both processes is necessary for heart regeneration [11], thus not only the effect of THRIL knockdown on cell viability and apoptosis was investigated, but also the migration and invasion of H9C2 cells were examined. As shown in Figure 2B–E, there was a significant reduction of cell viability, relative migration and invasion (all p < 0.01), while an increase of cell apoptotic rate in H9C2 cells after hypoxia treatment (p < 0.001). Consistently, hypoxia treatment induced the up-regulation of pro-apoptotic proteins including Bax, Cleaved capase-3 and Cleaved caspase-9, and the down-regulation of Bcl-2 (Fig. 2F). Interestingly, THRIL knockdown significantly increased the cell viability, migration and invasion, and declined apoptotic cell rate after hypoxia treatment (all p < 0.05) (Fig. 2B–E). Besides, THRIL inhibition decreased pro-apoptotic proteins levels and increased Bcl-2 levels in hypoxia-exposed H9C2 cells (Fig. 2F). Taken together, these results indicated that THRIL inhibition exerted a protective effect on hypoxia-induced cell injuries in H9C2 cells.
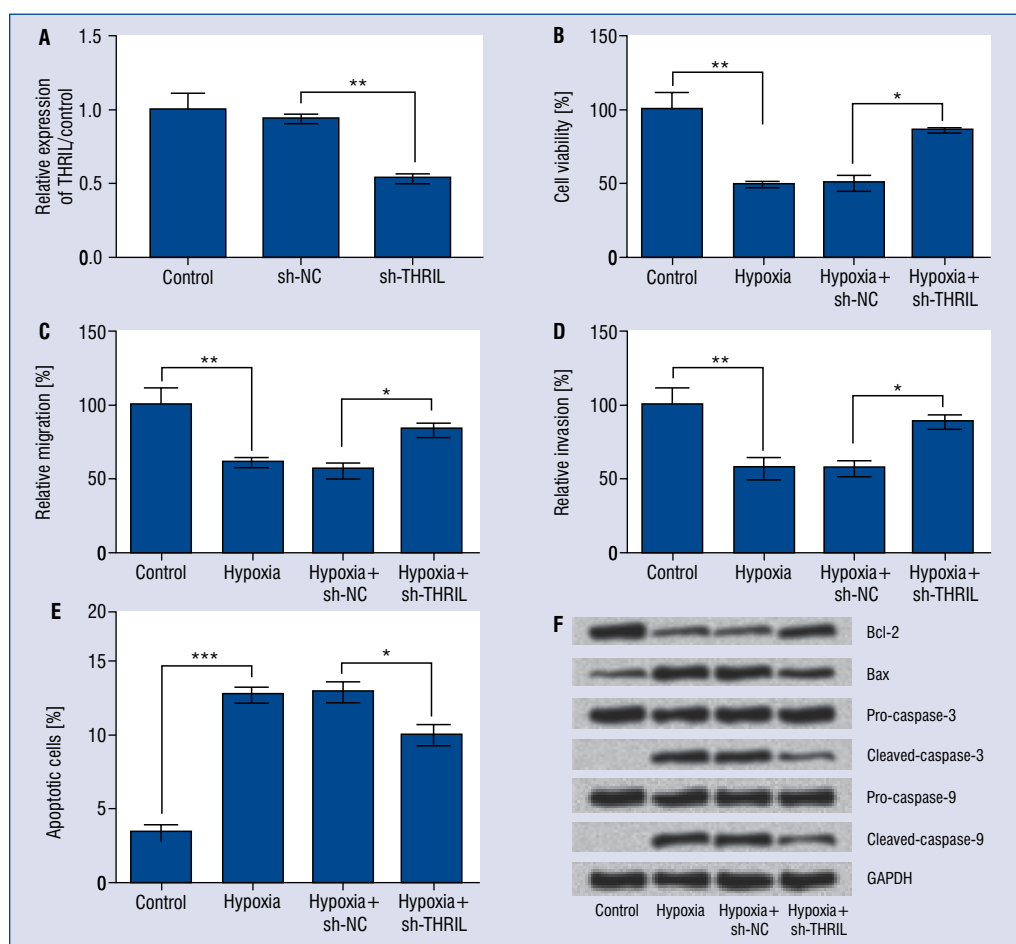
Figure 2. THRIL knockdown attenuated hypoxia-induced cell injuries in H9C2 cells. H9C2 cells were transfected with sh-THRIL or sh-NC and then were incubated in hypoxic incubator for 4 h to simulate hypoxia. **A**. The transfection efficiency was verified by quantitative real time polymerase chain reaction (qRT-PCR). Cell viability (**B**), relative migration (**C**) and invasion (**D**), apoptotic cells rate (**E**), and the expression of apoptosis-related factors (**F**) were respectively assessed by trypan blue exclusion assay, transwell analysis, flow cytometry, and Western blotting; *p < 0.05; **p < 0.01; ***p < 0.001.

miR-99a was negatively regulated by THRIL

As it was found that hypoxia treatment up-regulated expression of THRIL but down-regulated the expression of miR-99a, it was hypothesized as to whether there was a relationship between those two genes. By seeking bioinformatics online databases including TargetScan (http://www.targetscan.org/vert_71/) and NCBI (https://www.ncbi.nlm.nih.gov/), and using the bioinformatics method, lncRNA THRIL was predicted to bind to miR-99a. The predicted banding sequences were shown in Figure 3A. Thus, it was speculated that there might be an interaction between THRIL and miR-99a in H9C2 cells. To verify whether THRIL has a direct interaction with miR-99a, a dual-luciferase reporter system was employed by co-transfection of miR-99a and luciferase reporter plasmids containing 3'-UTR of THRIL, or mutated THRIL (bearing deletions of the putative miR-99a target sites). The dual-luciferase reporter assay revealed that co-transfection of miR-99a mimic and THRIL-wt specifically decreased the luciferase activity (p < 0.05), and there was no significant difference between co-transfection with miR-99a mimic and THRIL-mt in H9C2 cells (p > 0.05) (Fig. 3B), which suggested that THRIL might function as endogenous sponge RNA to interact with miR-99a. As shown in Figure 3C, THRIL inhibition up-regulated the expression of miR-99a in H9C2 cells (p < 0.001). These findings indicated that THRIL negatively regulated the expression of miR-99a through sponging with miR-99a in H9C2 cells.
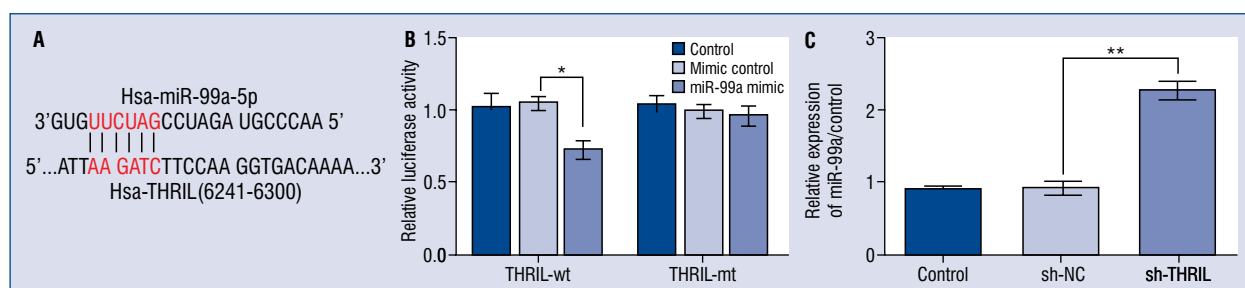
Figure 3. miR-99a was negatively regulated by THRIL. **A.** The predicted banding sequences of THRIL and miR-99a; **B.** H9C2 cells were co-transfected with the luciferase reporter plasmid containing wild-type or mutant THRIL and miR-99a mimic or mimic control. Dual luciferase activity assay was performed to detect the interaction between miR-99a and THRIL; **C.** The expressions of miR-99a in H9C2 cells were assessed by quantitative real time polymerase chain reaction after transfection with sh-THRIL or sh-NC; *p < 0.05; **p < 0.01.

THRIL knockdown alleviated hypoxia--induced cell injuries through up-regulation of miR-99a in H9C2 cells

Further, exploring whether THRIL regulated hypoxia-induced cell injuries through targeting miR-99a. miR-99a expression in H9C2 cells were altered by transfection with miR-99a mimic or miR-99a inhibitor. As shown in Figure 4A, B, the expression miR-99a was dramatically up-regulated after transfection with miR-99a mimic, but was down-regulated with miR-99a inhibitor (p < 0.01, or p < 0.001). miR-99a inhibitor blocked the protective effects of THRIL on hypoxia-induced cell injuries, as miR-99a inhibition significantly decreased cell viability (p < 0.01; Fig. 4C), suppressed cell migration and invasion (p < 0.01; Fig. 4D, E), and promoted apoptotic cell rates (p < 0.05; Fig. 4F). Furthermore, the effect of THRIL suppression on apoptosis related proteins was reversed by miR-99a inhibition in H9C2 cells (Fig. 4G). Overall, these results indicate that THRIL knockdown alleviated hypoxia-induced cell injuries through up-regulation of miR-99a in H9C2 cells.

Brg1 was positively regulated by miR-99a

Given that, Brg1 is required for cell proliferation to form the compact and septal myocardium [12]. Cross-regulation was detected between miR-99 and Brg1, to further reveal the mechanism(s) via which miR-99a modulated H9C2 cells. It was found that miR-99a inhibition declined both the protein and mRNA levels of Brg1 (p < 0.05; Fig. 5A, C). Of contrast, miR-99a mimic elevated Brg1 levels in H9C2 cells (p < 0.01; Fig. 5B, D). Taken together, these results indicated that Brg1 was positively regulated by miR-99a in H9C2 cells.

Brg1 was involved in hypoxia-induced cell injuries in H9C2 cells

To investigate whether Brg1 was required for hypoxia-induced cell injuries in H9C2 cells, H9C2 cells were transfected with overexpressing-vector and shRNA specific targeted Brg1. As shown in Figure 6A, B, the protein and mRNA levels of Brg1 were significantly up-regulated in H9C2 cells after transfection with pEX-Brg1 (p < 0.001), while were down-regulated in cells which were transfected with sh-Brg1 (p < 0.001). Results revealed that Brg1 inhibition promoted hypoxia-induced cell injuries, as decreased cell viability (p < 0.05; Fig. 6C), cell migration and invasion (p < 0.05, or p < 0.01; Fig. 6D, E), and induced apoptosis (p < 0.01; Fig. 6F, G). Of contrast, inverse regulations were found in cells which were transfected with pEX-Brg1 (all p < 0.05; Fig. 6C, G). These results suggested that Brg1 was involved in hypoxia-induced cell injuries in H9C2 cells.

Brg1 regulated PI3K/AKT and mTOR signaling pathways

miR-99a has been identified to regulate PI3K//AKT and mTOR signaling pathways [13, 14]. Here, the effect of Brg1 overexpression on PI3K//AKT and mTOR signaling pathways in H9C2 cells was explored. As shown in Figure 7A, hypoxia suppressed the activation of PI3K and AKT. However, Brg1 overexpression increased the levels of p-PI3K and p-AKT, indicating the activated effect of PI3K//AKT signaling pathway. Furthermore, it was found that Brg1 also activated mTOR signaling pathway, as increased expression of p-mTOR and p-p70S6K (Fig. 7B).
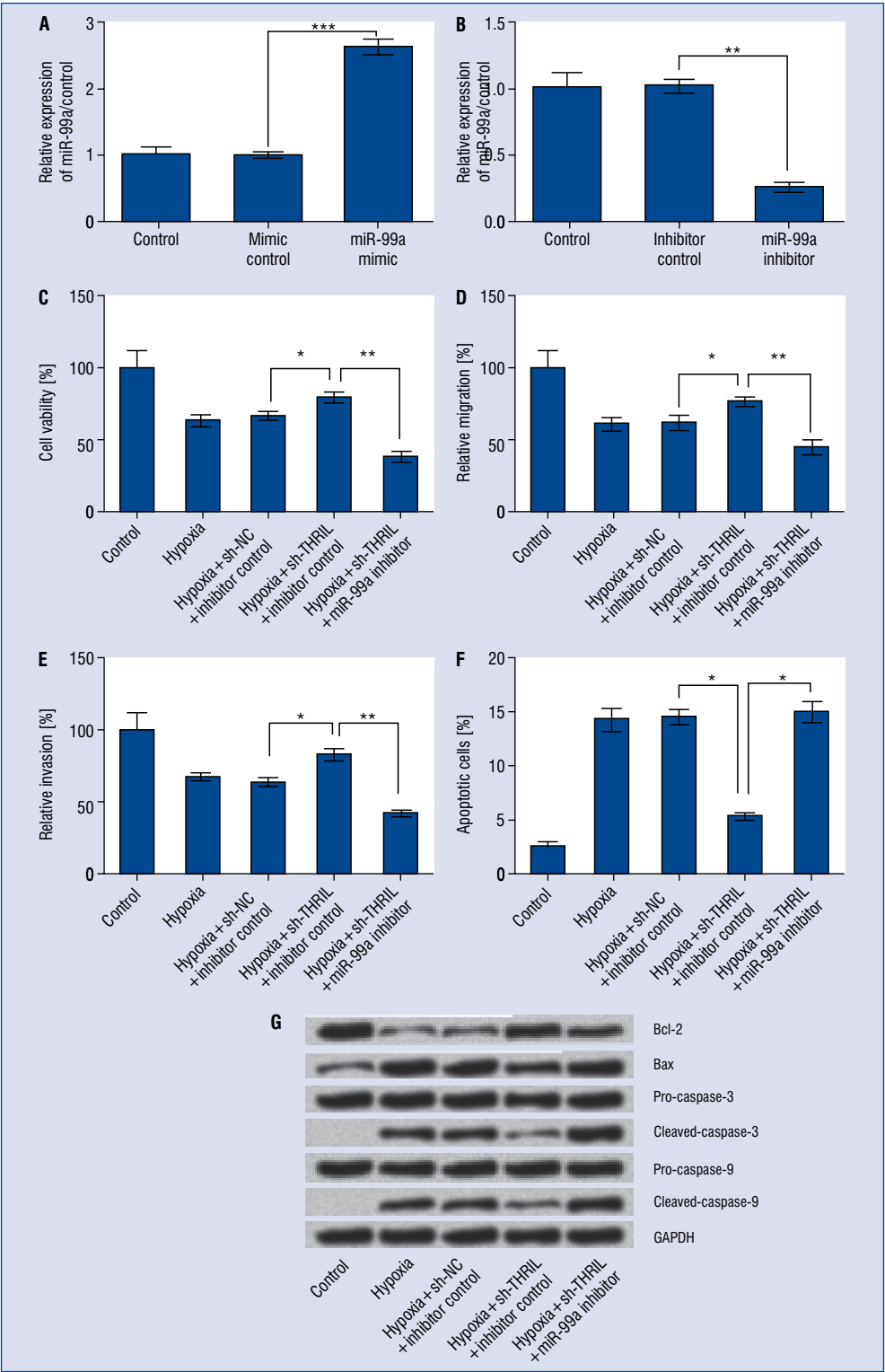
Figure 4. THRIL knockdown alleviated hypoxia-induced cell injuries through up-regulation of miR-99a in H9C2 cells. H9C2 cells were transfected with miR-99a mimic, miR-99a inhibitor or their corresponding controls, i.e., mimic control and inhibitor control, or co-transfected with 99a inhibitor and sh-THRIL. Cells were incubated in hypoxic incubator for 4 h to simulate hypoxia. **A, B.** The expression of miR-99a was assessed by quantitative real time polymerase chain reaction. Cell viability (**C**), relative migration (**D**) and invasion (**E**), apoptotic cells rate (**F**), and the expression of apoptosis-related factors (**G**) were respectively assessed by trypan blue exclusion assay, transwell analysis, flow cytometry, and Western blotting; *p < 0.05; **p < 0.01, ***p < 0.001.
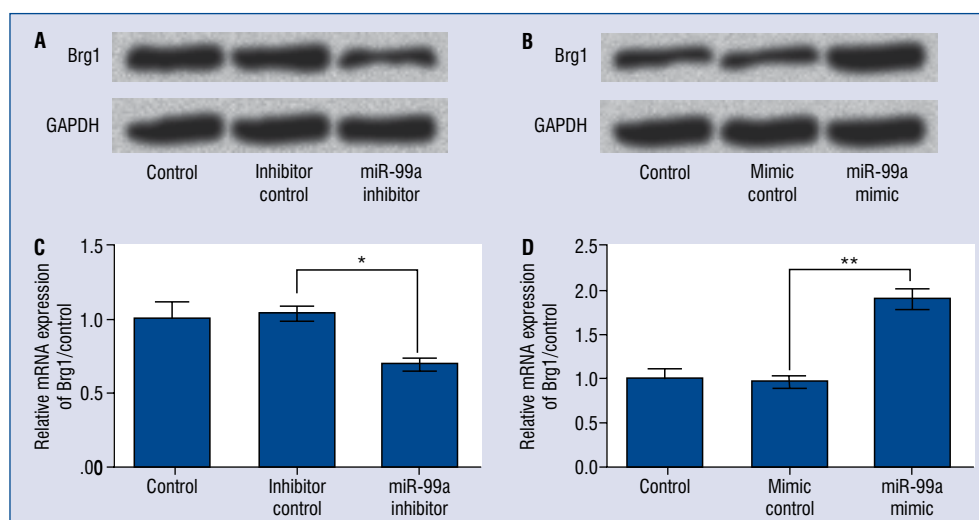
Figure 5. Brahma-related gene 1 (Brg1) was positively regulated by miR-99a. H9C2 cells were transfected with miR-99a mimic, miR-99a inhibitor or their corresponding controls, i.e., mimic control and inhibitor control; then the (**A, B**) mRNA and (**C, D**) protein expressions of Brg1 were assessed by quantitative real time polymerase chain reaction and Western blotting; *p < 0.05, **p < 0.01.

Discussion

Myocardial hypoxia triggers cell injuries, which is associated with the pathogenesis of many cardiovascular diseases including heart failure, MI, myocardial ischemia as well as reperfusion injury [15]. The present data indicated that miR-99a was negatively regulated by THRIL, and THRIL knockdown alleviated hypoxia-induced cell injuries through up-regulation of miR-99a in H9C2 cells. Moreover, it was shown that Brg1 was positively regulated by miR-99a, and was involved in hypoxia-induced cell injuries in H9C2 cells. These data suggested that THRIL may play a crucial role in hypoxia-induced cell injuries via regulating miR-99a in H9C2 cells.

LncRNAs have been defined to have important functions in various cellular processes such as genomic imprinting, cell fate determination, RNA processing, chromatin modification, modulation of apoptosis and invasion, and is important for development, differentiation and metabolism [16–19]. Emerging evidences have reported that lncRNAs are essential for the development of cardiomyocytes [20–22]. For example, the lateral mesoderm-specific lncRNA Fendrr is an essential regulator of the fate of lateral mesoderm derivatives, specifically the heart and the body wall in mice [23]. APF regulates autophagy and MI by targeting miR-188-3p [18]. Recent evidence suggests that THRIL is associated with TNFα regulation

and may contribute to other common inflammatory diseases [9]. Another report showed that THRIL regulates helicobacter pylori cagA induced-inflammation in gastric cancer cells via inhibition of NF-κB translocation [24]. However, the potential role of THRIL in regulating hypoxia-induced injuries in H9C2 cells have not been elucidated. The present results revealed that THRIL knockdown functioned as a protector against hypoxia-induced injuries in H9C2 cells, as increased cell viability, cell migration and invasion, and reduced cell apoptosis.

Recently, lncRNAs have been identified as competing endogenous RNAs to sponge miRNAs thus modulating the depression of miRNA targets and imposing an additional level of post-transcriptional regulation [25, 26]. miR-99a has been demonstrated to play an important role in cardiomyogenesis. Recent evidence showed that miR-99a expression significantly declined in patients with MI, and overexpression of miR-99a attenuated ventricular remodeling, cardiac hypertrophy and improved cardiac performance after MI [27–29]. In the present study, it was found that THRIL negatively regulated the expression of miR-99a, and THRIL acted as a sponge of miR-99a in H9C2 cells. Furthermore, we also found that THRIL inhibition alleviated hypoxia-induced cell injuries through up-regulation of miR-99a in H9C2 cells.

Brg1 is one of the central ATPase catalytic subunits, which is essential for zygote genome activation, erythropoiesis, cardiac development
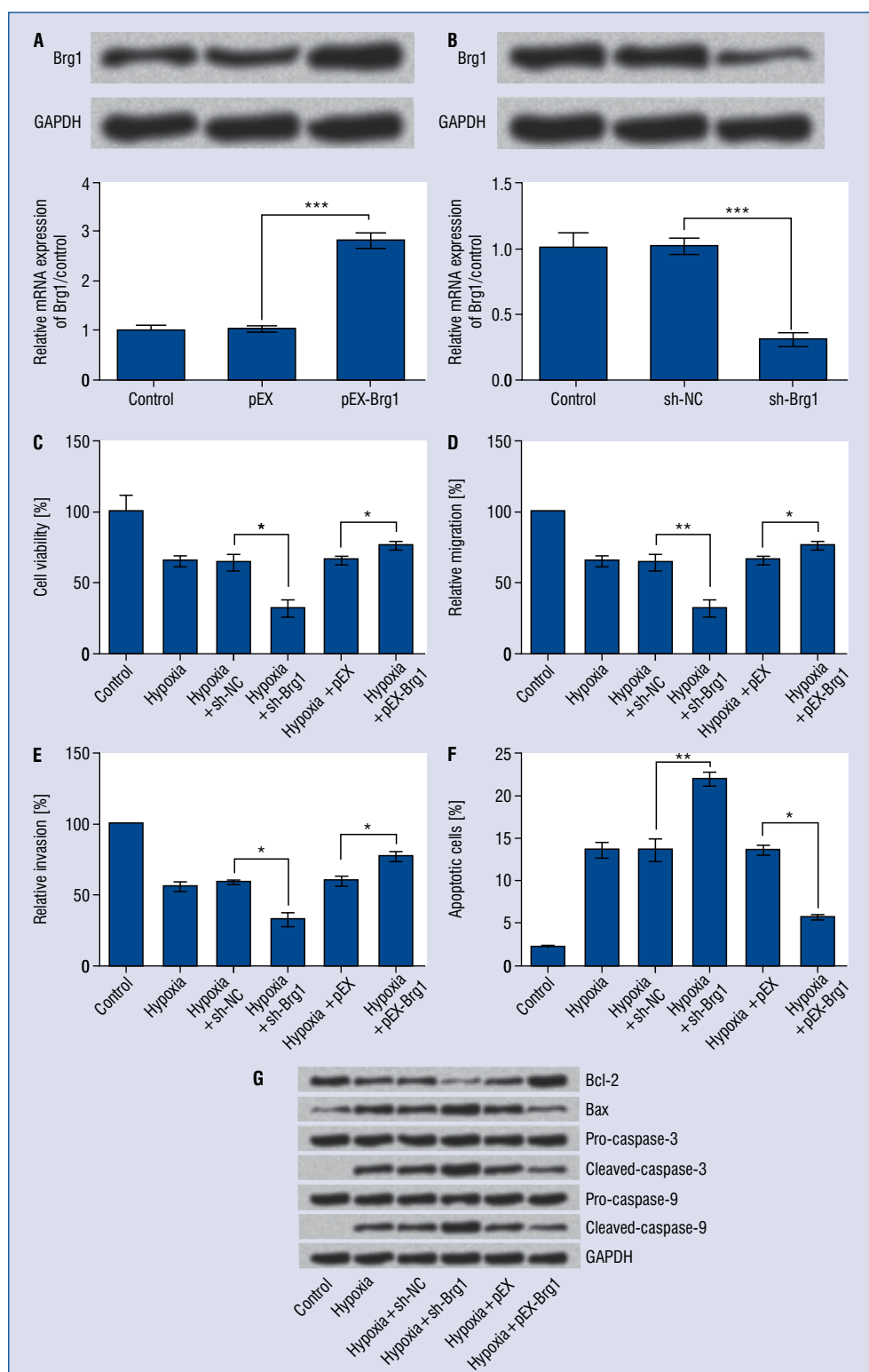
Figure 6. Brahma-related gene 1 (Brg1) was involved in hypoxia-induced cell injuries in H9C2 cells. H9C2 cells were transfected with pEX-Brg1, or sh-Brg1 or their corresponding controls, i.e., pEX and sh-NC and then were incubated in hypoxic incubator for 4 h to simulate hypoxia. **A, B.** The mRNA and protein expression of Brg1 were detected by quantitative real time polymerase chain reaction (qRT-PCR) and Western blotting. Cell viability (**C**), relative migration (**D**) and invasion (**E**), apoptotic cells rate (**F**), and the expression of apoptosis-related factors (**G**) were respectively assessed by trypan blue exclusion assay, transwell analysis, flow cytometry, and Western blotting; *p < 0.05; **p < 0.01; ***p < 0.001.
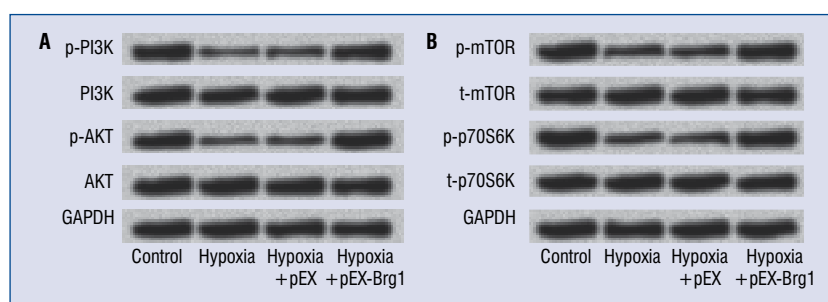
Figure 7. Brahma-related gene 1 (Brg1) regulated PI3K/AKT and mTOR signaling pathways. H9C2 cells were transfected with pEX-Brg1 or pEX and then were incubated in hypoxic incubator for 4 h to simulate hypoxia. The protein expressions of main factors related with PI3K/AKT (**A**) and mTOR (**B**) signaling pathways were detected by Western blotting.

and neuronal development [30]. In embryos, Brg1 promotes cardiomyocyte proliferation by maintaining Bmp10 and suppressing p57^{kip2} expression. However, in adults, Brg1 is turned off in cardiomyocytes and it is reactivated by cardiac stresses, and preventing Brg1 re-expression decreases hypertrophy [31]. Zebrafish experiment also showed that Brg1 promotes heart regeneration by repressing cyclin-dependent kinase inhibitors partly through Dnmt3ab-dependent DNA methylation [30]. Results herein showed that Brg1 was positively regulated by miR-99a in H9C2 cells, and overexpression of Brg1 attenuated hypoxia-induced cell injuries. It was supposed that THRIL may sponge with miR-99a and that miR-99a further regulate Brg1, thus regulating hypoxia-induced injury in H9C2 cells. However, further study is needed to confirm this hypothesis. In addition, it was also found that overexpression of Brg1 activated PI3K/AKT and mTOR signaling pathways. The PI3K/AKT and mTOR signaling pathways are important signal transduction pathways that control cardiomyocyte survival and functions [32, 33]. The activation of PI3K/AKT and mTOR pathways may contribute to inhibiting myocardial cells apoptosis and promoting cell survival in a damaged heart [34, 35]. It has been reported that Brg1 promotes osteogenic differentiation of mesenchymal stem cells (MSCs), and the mechanism might be by regulating Runx2-mediated downstream Wnt and PI3K/AKT pathways. Brg1 overexpression statistically increased the expression of PI3K/AKT pathways key proteins [36]. Present results are consistent with previous reports about regulation of Brg1 on PI3K/AKT and mTOR pathway, and these data suggest that miR-99a overexpression might activate the PI3K/AKT and mTOR signaling pathways through up-regulated Brg1.

Conclusions

In conclusion, the present study demonstrated that THRIL knockdown attenuated hypoxia-induced injuries of H9C2 cells through directly up-regulated expression of miR-99a. Interestingly, miR-99a positively regulated Brg1 expression, and Brg1 overexpression exerted similarly protective effects to THRIL inhibition. According to available research, this is the first study to demonstrate that THRIL is associated with hypoxia-induced injuries of H9C2 cells, which direct sponging with miR-99a. It was also revealed that Brg1 was positively regulated by miR-99a in H9C2 cells for the first time. It may allow a better understanding of the pathological process of MI and ultimately contribute to the development of THRIL-directed diagnostics and therapeutics against myocardial infarction.

Conflict of interest: None declared

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ORIGINAL ARTICLE

Effect of LCZ696, a dual angiotensin receptor neprilysin inhibitor, on isoproterenol-induced cardiac hypertrophy, fibrosis, and hemodynamic change in rats

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Abstract

Background: *Recent clinical studies have shown that treatment with LCZ696, a complex containing the angiotensin receptor blocker valsartan and neprilysin inhibitor sacubitril, improves the prognosis of heart failure patients with a reduced ejection fraction. This study evaluated whether LCZ696 affects left ventricular hypertrophy, fibrosis, and hemodynamics in isoproterenol (ISO)-treated rats compared with valsartan alone.*

Methods: *Male Wistar rats received subcutaneous saline (n = 10), subcutaneous ISO (2.4 mg/kg/day; n = 10), subcutaneous ISO + oral LCZ696 (60 mg/kg/day; n = 20) (ISO-LCZ), or subcutaneous ISO + oral valsartan (30 mg/kg/day; n = 20) (ISO-VAL) for 7 days.*

Results: *LCZ696 and valsartan did not significantly reduce the increased heart weight/body weight ratio in rats treated with ISO. Echocardiography showed that the deceleration time shortened by ISO was restored by LCZ696 but not valsartan alone (p = 0.01 vs. the ISO group). Histological analysis showed that cardiac interstitial fibrosis increased by ISO was decreased significantly by LCZ696 but not valsartan alone (control: 0.10 ± 0.14%; ISO: 0.41 ± 0.32%; ISO-LCZ: 0.19 ± 0.23% [p < 0.01 vs. the ISO group]; ISO-VAL: 0.34 ± 0.23% [p = 0.34 vs. the ISO group]). Quantitative polymerase chain reaction showed that mRNA expression of Tgfb1, Col1a1, Ccl2, and Anp increased by ISO was significantly attenuated by LCZ696 but not valsartan alone (p < 0.05 vs. the ISO group).*

Conclusions: *LCZ696 improves cardiac fibrosis, but not hypertrophy, caused by continuous exposure to ISO in rats.* (Cardiol J 2019; 26, 5: 575–583)

Key words: cardiac hypertrophy, neprilysin, angiotensin receptor blocker, fibrosis

Introduction

Left ventricular (LV) hypertrophy (LVH) is reported to be involved in heart failure with a preserved ejection fraction (HFpEF) [1]. HFpEF is associated with considerable morbidity and mortality, and the risk for adverse outcomes increases with the severity of diastolic dysfunction [2]. Treatment with anti-hypertensive drugs may be effective to prevent HFpEF. However, randomized controlled trials of antihypertensive drugs, including angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs), have been generally disappointing with no convincing dem-

onstration of mortality or morbidity reductions [3–5]. Accordingly, therapies for HFpEF are of great clinical interest.

Inhibition of neprilysin is an attractive strategy to increase natriuretic peptide levels. Natriuretic peptides stimulate diuresis and vasodilation, improve myocardial relaxation, and reduce LVH [6]. Recently, a first-in class dual angiotensin receptor and neprilysin inhibitor (ARNI), which contains equimolar amounts of valsartan and sacubitril, has been developed [7]. In the PARAMOUNT study (prospective comparison of ARNI with ARBs for management of HFpEF), LCZ696 reduced the serum level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) more than valsartan at 12 weeks and was well tolerated when used for HFpEF [8]. In the PARADIGM-HF study (prospective comparison of ARNI with ACEI to determine impact on global mortality and morbidity in heart failure), LCZ696 was also effective in heart failure patients with a reduced ejection fraction (HFrEF) [9]. However, the mechanism underlying the effectiveness of LCZ696 is poorly understood and little is known about its effects in HFpEF patients with LVH.

It was previously reported that continuous infusion of isoproterenol (ISO) induces cardiac hypertrophy and diastolic dysfunction in rats [10, 11]. The current study investigated whether LCZ696 affects LVH, fibrosis, and hemodynamics in ISO-treated rats.

Methods

Ethical approval

All procedures were performed in accordance with the institutional guidelines for animal research and approved by the Animal Care and Use Committee of Okayama University (OKU-2012297).

Experimental animals and drugs

Male Wistar rats weighing 187–211 g were purchased from Japan SLC (Shizuoka, Japan). Rats were housed at an animal facility in a 12-h light-dark cycle and were provided with standard rat chow and water ad libitum. LCZ696 and valsartan were kindly supplied by Novartis Pharma AG (Basel, Switzerland). LCZ696 includes molecular moieties of valsartan and sacubitril at a 1:1 ratio. L-ISO was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Study protocol

The detailed protocol is shown in Figure 1 and a previous study [11]. Delivery of ISO or saline was
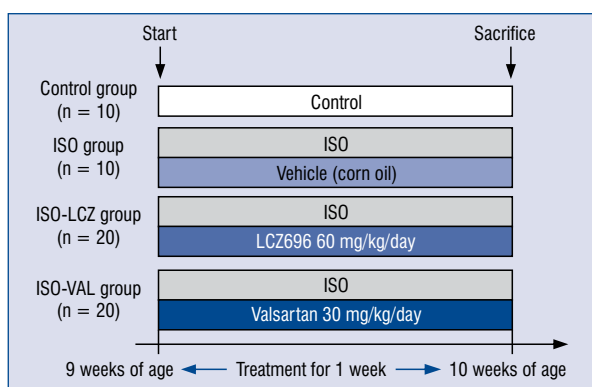
Figure 1. Schematic of the treatments; ISO — subcutaneous isoproterenol (2.4 mg/kg/day); LCZ — LCZ2696; VAL — valsartan.

performed by subcutaneously implanting an osmotic minipump (Alzet, model 2001; 1.0 μL/h) into the neck under 2% isoflurane anesthesia. LCZ696 and valsartan were dissolved in corn oil and administered by gastric gavage. Rats were divided into four groups and treated for 7 days: control group (subcutaneous HCl [pH 4] in saline, n = 10); ISO group (subcutaneous ISO [2.4 mg/kg/day] and oral corn oil, n = 10); ISO-LCZ group (subcutaneous ISO [2.4 mg/kg/day] and oral LCZ696 [60 mg/kg//day], n = 20); and ISO-VAL group (subcutaneous ISO [2.4 mg/kg/day] and oral valsartan [30 mg//kg/day], n = 20). Systolic blood pressure (SBP) was measured in rats using a tail cuff attached to a MK-2000ST Blood Pressure Monitor for Rats (Muromachi, Tokyo, Japan).

Hemodynamic measurements

Seven days after infusion, rats were anesthetized with 2% isoflurane, and a micro-tip pressure transducer (Millar Instruments Inc., Houston, TX, USA) was inserted into the right carotid artery. The catheter was advanced into the LV cavity. After a 5 min period of stabilization, heart rate, LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and developed LV pressure (dLVP = = LVSP − LVEDP) were measured. For indices of contractility and relaxation, the maximal rates of increase and decrease in LVP dp/dt maximum and dp/dt minimum were determined. Tau was calculated according to a previous report [12].

Echocardiography

Seven days after infusion, transthoracic echocardiography was performed using a 10-MHz phased array transducer (Aplio ver. 6.0; Toshiba, Tokyo, Japan) under 2% isoflurane, as described previously [11]. M-mode echocardiography was

Table 1. Effects of LCZ696 on heart weight and systolic blood pressure.

	Control	ISO	ISO-LCZ	ISO-VAL
Number of rats	10	10	20	20
Body weight [g]	242 ± 15	238 ± 8	236 ± 7	232 ± 11
Heart weight [g]	0.81 ± 0.08	1.05 ± 0.11*	0.99 ± 0.10*	0.99 ± 0.08*
Heart weight/body weight × 10^3	3.35 ± 0.27	4.47 ± 0.39*	4.14 ± 0.30*	4.30 ± 0.30*

*$p < 0.05$ vs. control. Values represent the mean ± standard deviation; ISO — isoproterenol; LCZ — LCZ696; VAL — valsartan

performed using the parasternal short-axis view at the level of papillary muscles. The LV posterior wall thickness (PWT) and interventricular septal diastolic wall thickness (IVST) were measured during diastole (d) and systole (s), as were the LV internal diameter at end-diastole (LVDd) and LV internal diameter at end-systole (LVDs). Fractional shortening (FS) was then calculated according to the formula: FS = [(LVDd – LVDs) / LVDd] × 100. The apical 4-chamber view was used to assess early and late transmitral peak diastolic flow velocities (E and A waves, respectively).

Histology

The LV was fixed with 4% paraformaldehyde in phosphate buffered saline, embedded in paraffin, and cut into 5 μm-thick sections. The sections were stained with Masson's trichrome to detect fibrosis and examined by light microscopy. In sections stained with Masson's trichrome, interstitial fibrosis was measured using computer-assisted image analysis, and the percentage of fibrosis was calculated [13, 14]. The widths of 30 individual cardiomyocytes were measured in each sample. The percentage of the fibrotic area in the LV was analyzed using Image J software (ver. 1.47).

Quantitative PCR

Total RNA was extracted from heart tissue using Trizol (Invitrogen, Carlsbad, CA, USA). Total RNA (2 μg) was reverse transcribed using ReverTra Ace (Toyobo, Osaka, Japan). cDNAs were diluted 5-fold before conventional reverse transcription-polymerase chain reaction (RT-PCR) amplification or 50-fold before quantitative PCR analysis. Real-time PCR assays were performed to assess gene expression of rat Tgfbeta1, Col1a1, Ccl2, Anp, and Gapdh using the corresponding primer pairs (Rn00572010_m1, Rn01523309_m, 1Rn00664637_g1, Rn00580555_m1, and Rn01775763_g1, respectively; Applied Biosystems) using the StepOnePlus Real-Time PCR System (Applied Biosystems). Glyceraldehyde-3-posphate dehydrogenase (GAPDH) served as

an internal control. The $\Delta\Delta$Ct method was used to analyze expression level of each gene.

Measurements of NT-proBNP

Serum NT-proBNP was measured using an enzyme-linked immunosorbent assay (MBS704791, MyBioSource, San Diego, CA, USA).

Statistical analysis

Data are expressed as the mean ± standard deviation. For comparisons of each parameter among groups, statistical analysis was performed by analysis of variance with Bonferroni tests. A p-value of less than 0.05 was considered as statistically significant. Data were analyzed using SPSS 17.0 for Windows (ver. 24; SPSS Inc., Chicago, IL, USA).

Results

Heart weights and SBP

Body weight, heart weight, and the heart-to-body weight ratio are shown in Table 1. No difference was observed in body weights among the three groups at day 7 post-treatment with ISO, ISO-LCZ, and ISO-VAL. The increases in heart weight and heart-to-body weight ratio observed in the ISO group were not significantly suppressed in ISO-LCZ and ISO-VAL groups (p = 0.33). There was no difference in heart-to-body weight ratios between ISO-LCZ and ISO-VAL groups. Table 2 shows the changes in SBP derived from the tail cuff. SBP at day 7 in ISO-LCZ and ISO-VAL groups was significantly suppressed compared to that in control and ISO groups. However, there was no difference in SBP at day 7 between ISO-LCZ and ISO-VAL groups (p = 0.11).

LV hemodynamics

Cardiac catheterization and echocardiography were performed at day 7 (Table 3). During cardiac catheterization, increases in the heart rate, LVSP, LVEDP, and dP/dt maximum and a decrease in minimum dp/dt were observed in the ISO group compared with the control group. LCZ696 or val-

Table 2. Change in systolic blood pressure.

	Control	ISO	ISO-LCZ	ISO-VAL
Baseline	119 ± 6	117 ± 8	120 ± 6	118 ± 5
Day 7	116 ± 3	119 ± 5	112 ± 4*†	110 ± 4*†

*p < 0.05 vs. control; †p < 0.05 vs. ISO. Values represent the mean ± standard deviation; ISO — isoproterenol; LCZ — LCZ696; VAL — valsartan

Table 3. Effects of LCZ696 on left ventricular hemodynamics and echocardiographic data.

	Control (n = 10)	ISO (n = 10)	ISO-LCZ (n = 20)	ISO-VAL (n = 20)
Cardiac catheterization				
Heart rate [bpm]	408 ± 30	498 ± 36*	477 ± 54*	459 ± 30*#
LVSP [mmHg[	101 ± 16	115 ± 10*	113 ± 9*	110 ± 14*
LVEDP [mmHg]	2.3 ± 1.3	3.5 ± 1.1*	2.5 ± 1.6	2.4 ± 1.5
dP/dtmax [mmHg/s]	8104 ± 1705	12798 ± 4227*	8450 ± 1122†	7838 ± 1246†
dP/dtmin [mmHg/s]	–7107 ± 1077	–8598 ± 1837*	–6941 ± 969†	–6468 ± 982†
Tau [ms]	10 ± 1	10 ± 2	9 ± 2	10 ± 3
Echocardiography				
LVDd [mm]	6.1 ± 0.5	6.1 ± 0.4	6.2 ± 0.4	6.1 ± 0.5
LVDs [mm]	3.5 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.4 ± 0.3
FS [%]	44 ± 5	43 ± 7	41 ± 3	42 ± 2
IVST [mm]	1.0 ± 0.1	1.3 ± 0.1*	1.3 ± 0.1*	1.3 ± 0.1*
PWT [mm]	1.1 ± 0.1	1.4 ± 0.2*	1.4 ± 0.1*	1.3 ± 0.1*
E/A	1.5 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.4 ± 0.2
Deceleration time [ms]	36.3 ± 4.2	34.2 ± 6.7*	38.5 ± 4.7†‡	33.8 ± 5.5*

*p < 0.05 vs. control, †p < 0.05 vs. ISO and ‡p < 0.05 vs. ISO-VAL. Values represent the mean ± standard deviation; ISO — isoproterenol; LCZ — LCZ696; VAL — valsartan; LVSP — left ventricular systolic pressure; LVEDP — left ventricular end-diastolic pressure; LVDd — left ventricular end-diastolic diameter; LVDs — left ventricular end-systolic diameter; FS — fractional shortening; IVST — interventricular septal thickness; PWT — posterior wall thickness; E — early transmitral peak diastolic flow velocity; A — late transmitral peak diastolic flow velocity

sartan alone did not suppress the increased heart rate and LVSP. However, LCZ696 and valsartan alone suppressed the increases in LVEDP and dP/ /dt maximum significantly in ISO-LCZ and ISO-VAL groups compared with the control group. However, there were no differences in the cardiac catheterization parameters between ISO-LCZ and ISO-VAL groups. During echocardiography, increases in heart rate, IVST, and PWT and a decrease in the deceleration time were observed in the ISO group compared with the control group. LCZ696 or valsartan alone did not suppress the IVST or PWT. The deceleration time shortened by ISO was restored by LCZ696 but not valsartan alone. There was significant difference in the deceleration times between ISO-LCZ and ISO-VAL groups (p = 0.01).

Histology

Masson's trichrome staining revealed that areas of cardiac fibrosis were significantly smaller in ISO-LCZ group, but not in ISO-VAL group,

compared with ISO group (area of fibrosis: control, 0.10 ± 0.14%; ISO, 0.41 ± 0.32%; ISO-LCZ, 0.19 ± ± 0.23%; ISO-VAL, 0.34 ± 0.23) (Fig. 2). The reduction of cardiac fibrosis by LCZ696 was significantly greater than that by valsartan alone (p = 0.01).

Cardiac gene expression

Figure 3 shows mRNA expression levels in the heart after treatments. The gene expression of Tgfb1 and Clo1a1, which are involved in cardiac fibrosis, was significantly increased in the control group. LCZ696, but not valsartan, significantly reduced Tgfb1 and Col1a1 mRNAs (Fig. 3A, B). The decreases in Tgfb1 and Col1a1 expression in the ISO-LCZ group were significantly greater than those in the ISO-VAL group (p = 0.02 and p = 0.03, respectively). The increased expression of Ccl2, which is an inflammatory marker, was not significantly reduced by LCZ696 or valsartan (Fig. 3B). In addition, gene expression of Anp, which is a marker of a failing heart, was not decreased
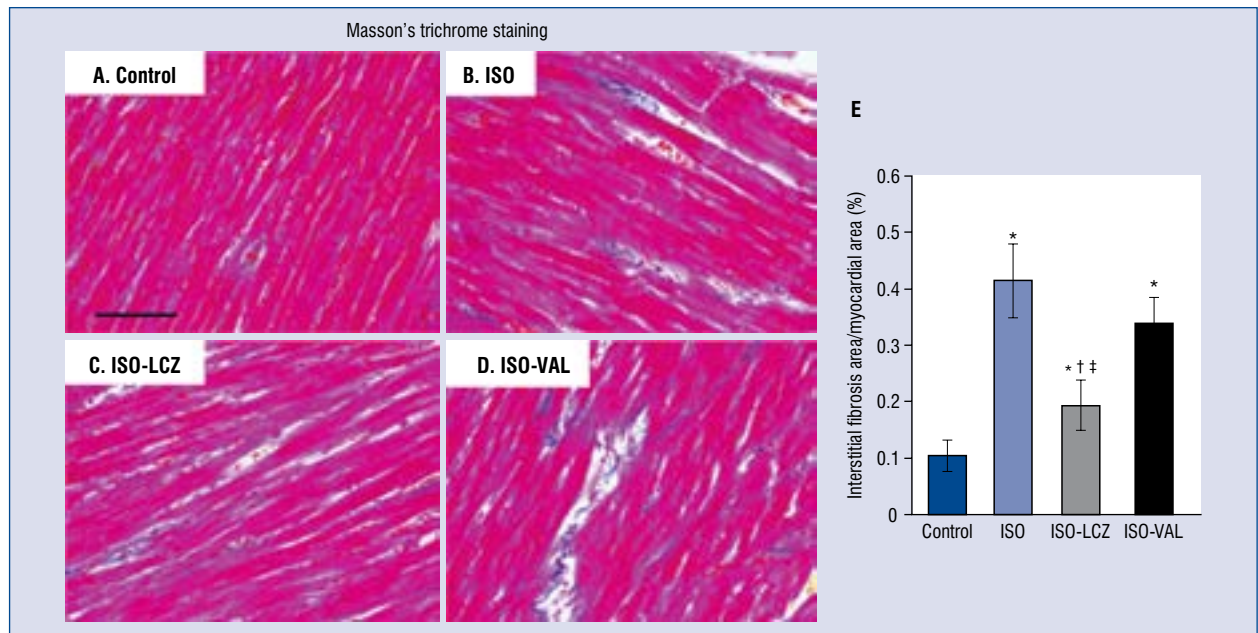
Figure 2. Masson's trichrome staining of hearts. Myocardial fibrosis is evident as blue staining. **A.** Heart sections from a control rat; **B.** Isoproterenol (ISO)-treated rat; **C.** ISO-treated rat administered LCZ696 (ISO-LCZ); **D.** ISO-treated rat administered valsartan (ISO-VAL). Scale bar = 100 μm. **E.** Bar graph shows the area of fibrosis (%). Values represent the mean ± standard deviation; n = 10 in control and ISO groups; n = 20 in ISO and ISO-VAL groups; *p < 0.05 vs. control, †p < 0.05 vs. ISO; ‡p < 0.05 vs. ISO-VAL.

by LCZ696 or valsartan (Fig. 3C). There were no differences in Ccl2 or Anp expression between ISO-LCZ and ISO-VAL groups.

Measurements of NT-proBNP

Figure 4 shows the serum NT-proBNP level after 7 days of treatments. The increase in the NT-proBNP level in the ISO group was significantly suppressed in the ISO-LCZ group but not the ISO-VAL group (control, 40.1 ± 6.2 pg/mL; ISO, 62.4 ± 10.9 pg/mL; ISO-LCZ, 41.2 ± 9.8 pg//mL, p = 0.04 vs. ISO; ISO-VAL, 54.2 ± 9.5 pg/mL; p = 0.48 vs. ISO).

Discussion

In this study, it was found that short term treatment with LCZ696 significantly prevented LV fibrosis, but not LVH, caused by continuous infusion of ISO compared with valsartan alone. Additionally, significantly greater reductions in Tgfb1 and Col1a1 mRNA expression were induced by LCZ696 than valsartan alone. Echocardiography showed that LCZ696 significantly improved the decreased deceleration time, a parameter of LV diastolic functions, compared with valsartan alone. These results suggest more favorable effects of

LCZ696 on LV fibrosis and hemodynamics in ISO--treated rats compared with valsartan alone.

von Lueder et al. [15] reported that LCZ696 reduces cardiac remodeling and dysfunction after myocardial infarction by reducing cardiac fibrosis and hypertrophy in rats. Suematsu et al. [16] reported that LCZ696 improves HFrEF in mice with streptozotocin-induced diabetes. These studies used a model of HFrEF, which differs from the current study involving evaluation of a model without a reduced ejection fraction. As a model without a reduced ejection fraction, other studies evaluated LVH in rats with spontaneous hypertension, which was suppressed by valsartan [17, 18]. A model of chronic beta-adrenergic stimulation in rats has also been reported. ISO treatment for 2 weeks induced LVH and fibrosis that were prevented by an angiotensin II type I receptor blocker such as losartan and valsartan [19, 20]. However, a hemodynamic study was not performed in these models of LVH. According to available research, this is the first animal study performed to investigate the hemodynamic effect of LCZ696 on LV functions in the rat hypertrophied heart with a preserved ejection fraction. Improvement in cardiac fibrosis by LCZ696 was consistently observed in this study as well as previous studies. However, in contrast
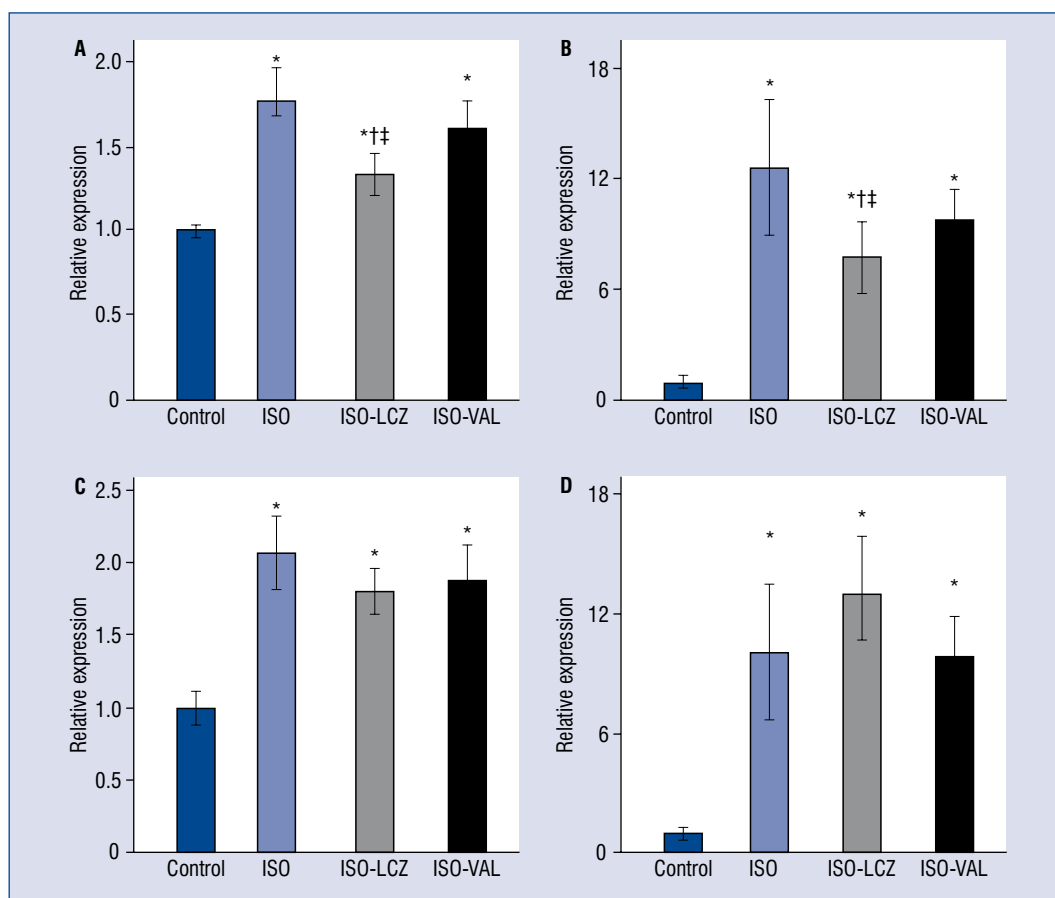
Figure 3. Cardiac gene expression in hearts. Total RNA was extracted from cardiac tissues and analyzed for mRNA expression of Tgfb1 (**A**), Col1a1 (**B**), Ccl2 (**C**), and Anp (**D**) by real-time RT-PCR analysis. ISO — isoproterenol-treated rats; ISO-LCZ — ISO-treated rats administered LCZ696; ISO-VAL — ISO-treated rats administered valsartan. Values represent the mean ± standard deviation; n = 10 in control and ISO groups; n = 20 in ISO and ISO-VAL groups; *p < 0.05 vs. control, †p < 0.05 vs. ISO; ‡p < 0.05 vs. ISO-VAL.

to the results obtained using rats with spontaneous hypertension [17], the current study did not reveal a protective effect on LVH by LCZ696 or valsartan alone. An explanation is the difference in mechanisms of cardiac hypertrophy and fibrosis induction between ISO-treated rats and spontaneously hypertensive rats. ISO directly affects cardiac hypertrophy and fibrosis in addition to elevated SBP. Another possibility is that the duration of administration in the present study was relatively short compared with that in previous studies. Therefore, a lower dose of ISO and long-term treatment may address these issues.

In this study, 60 mg/kg LCZ and 30 mg/kg valsartan was selected because several experimental studies using rat models employed these doses of valsartan and LCZ696 [16, 17, 21, 22]. The previous studies showed greater decreases in SBP induced by LCZ696 than that by valsartan alone. In contrast,

the present study showed that SBP at day 7 did not statistically differ between the two groups. This finding suggests that a greater suppression of LV fibrosis by LCZ696 compared with that by valsartan alone is independent of blood pressure. To confirm this effect independently of blood pressure, future experiments should employ other models such as spontaneous hypertension rats in which blood pressure is matched between LCZ696 and valsartan groups by adjusting their doses.

There are several potential mechanisms underlying the protective effect of LCZ696 in preventing ISO-induced cardiac fibrosis. In experimental HFrEF in streptozotocin-induced diabetic mice, the mRNA level of transforming growth factor beta (TGF-β) in the LV was reported to be suppressed in LCZ696 group compared with that of control and valsartan groups [16]. These results were in line with the present study showing that LCZ696
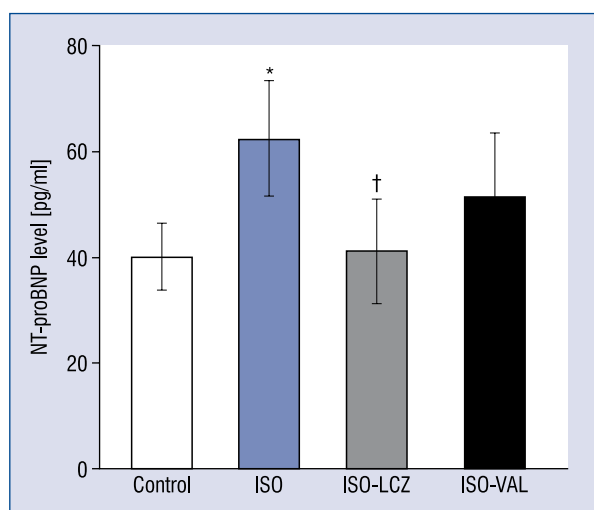
Figure 4. Serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured for 7 days after treatments. ISO — isoproterenol-treated rats; ISO-LCZ — ISO-treated rats administered LCZ696; ISO-VAL — ISO-treated rats administered valsartan. Values represent the mean ± standard deviation; n = 10 in control and ISO groups; n = 20 in ISO and ISO-VAL groups; *p < 0.05 vs. control; †p < 0.05 vs. ISO.

significantly limited the increase in TFG-β upon ISO treatment. One mechanism underlying the suppression of TGF-α caused by specific inhibition of neprilysin may be the involvement of natriuretic peptides. First, the inhibition of neprilysin by sacubitril prevents breakdown of endogenous natriuretic peptides, which inhibits cardiac fibrosis [6]. Second, it has been reported that circulating angiotensin II levels increase after treatment with LCZ696 in Wistar Kyoto rats [23]. After blockade of angiotensin type I receptor, stimulation of angiotensin type II receptor via circulating angiotensin II would reduce cardiac fibrosis. Thus, the combination of angiotensin type I receptor and neprilysin inhibitors may augment their favorable effects through the angiotensin type II pathway. Finally, another mechanism may involve bradykinin. Bradykinin, which is a substrate of neprilysin, has been reported to suppress myocardial fibrosis [24], and its effect may be enhanced by angiotensin receptor-blocking therapy [25]. It is therefore possible that neprilysin inhibitor treatment contributes to cardioprotection by inhibiting bradykinin metabolism. Seki et al. [26] reported that inhibition of neprilysin with LCZ696 does not appear to have an additional benefit over valsartan alone for the endothelial functions of arteries in spontaneously hypertensive rats. Their study

suggests that vasoactive peptides, such as C-type natriuretic peptide, bradykinin, and substance P, which can accumulate because of neprilysin inhibition, may not play a crucial role in the improvement of endothelial functions. The difference in target organs between their study and this one may have contributed to the favorable effects of LCZ696 compared with valsartan alone in the present study. Seki et al. [26] focused on endothelial functions, whereas here the study focused on cardiac tissue including cardiomyocytes and fibroblasts. Another explanation is the difference in animal model. This rat model was simulated with ISO, and the elevation of blood pressure was less than that in a spontaneously hypertensive rat. Further basic and clinical studies are warranted to clarify the underlying mechanism of these differences.

In the current study, LCZ696 significantly improved cardiac hemodynamics derived from catheterization in the ISO group. LVEDP in ISO-LCZ and ISO-VAL groups did not increase compared with the control group without a significant change in SBP. Notably, the echocardiographic findings suggested a significantly greater improvement in the deceleration time, a parameter of LV diastolic functions, by LCZ696 compared with valsartan alone. It was previously reported that cardiomyocyte stiffness, which is associated with diastolic heart failure, increases in ISO-induced hypertrophied hearts [10]. Although cardiomyocyte stiffness was not evaluated directly in this study, suppression of cardiac fibrosis may contribute to improvement of diastolic dysfunction in the hypertrophied heart.

The current study demonstrated that increased NT-proBNP levels in ISO group were significantly reduced by LCZ696 but not valsartan alone. Because BNP is degraded by neprilysin, LCZ696 may affect the BNP level. NT-proBNP, which is cleaved from proBNP along with BNP, is not a substrate of neprilysin degradation. Thus, a change in the NT-proBNP level reflects LV wall stress even under the use of LCZ696. The PARA-MOUNT study showed that LCZ696 reduced the NT-proBNP level more than valsartan alone at 12 weeks of therapy in patients with HFpEF [8]. Considering both these findings and those of the current study, LCZ696 might have a favorable effect on the prognosis of HFpEF patients, and a further large scale study evaluating cardiovascular events is thus warranted.

Limitations of the study

There are several limitations in this study. First, different doses of LCZ696 and valsartan

were not tested. Therefore, dose dependencies of LCZ696 and valsartan in the present model are unknown. Second, Gu et al. [7] reported that plasma concentration-time profiles of valsartan are similar between administration of a single oral dose of 400 mg LCZ696 and 320 mg valsartan in healthy human participants. 60 mg/kg LCZ696 and 30 mg/ /kg valsartan was chosen, which are similar to those in previous studies of valsartan and LCZ696 in rat models [16, 17, 21, 22], because pharmacodynamic data for LCZ696 could not be found in the ISO-treated rat model. Therefore, it cannot be denied that the pharmacodynamics of these drugs may have influencedthe present results. Third, the duration of LCZ696 or valsartan treatments in the ISO-treated model was short. Long-term treatment may facilitate understanding the effect of LCZ696 on LVH, fibrosis, and hemodynamics in ISO-treated rats.

Conclusions

LCZ696 improved LV fibrosis caused by continuous exposure to ISO in this population of rats. The results suggest the possibility of using LCZ696 to therapeutically target LVH and diastolic LV dysfunction.

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VIA MEDICA

ORIGINAL ARTICLE

Proteomics study of serum exosomes in Kawasaki disease patients with coronary artery aneurysms

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Abstract

Background: *To study the protein profile of the serum exosomes of patients with coronary artery aneurysms (CAA) caused by Kawasaki disease (KD).*

Methods: *Two-dimensional electrophoresis (2-DE) was used to identify proteins from the exosomes of serum obtained from children with CAA caused by KD, as well as healthy controls. Differentially expressed proteins were identified using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF MS) analysis.*

Results: *Thirty two differentially expressed proteins were identified (18 up-regulated and 14 down-regulated) from serum exosomes of children with CAA and were compared to healthy controls. The expression levels of 4 proteins (TN, RBP4, LRG1, and APOA4) were validated using Western blotting. Classification analysis and protein–protein network analysis showed that they are associated with multiple functional groups, including host immune response, inflammation, apoptotic process, developmental process, and biological adhesion process.*

Conclusions: *These findings establish a comprehensive proteomic profile of serum exosomes from children with CAA caused by KD, and provide additional insights into the mechanisms of CAA caused by KD.* (Cardiol J 2019; 26, 5: 584–593)

Key words: Kawasaki disease, coronary aneurysm, exosome, proteomics

Introduction

Kawasaki disease (KD) is the most prevalent inflammatory coronary artery disease with an unknown etiology [1]. Eighty percent of patients are younger than 5 years old [2]. It is characterized by the development of coronary artery aneurysms (CAAs) [3]. CAAs may cause ruptures, thrombosis, stenosis, or myocardial ischemia if there is a failure or delay in treatment. It is therefore important to

make an accurate diagnosis and choose the appropriate therapy during early stages of the disease.

Several scoring systems have been established to identify the risk factors for CAA [4, 5]. Duration of fever has been reported as a potent risk factor. Male sex, younger patient age, and delayed diagnosis and treatment have also been found to be correlated with the development of CAA. Laboratory-detected indexes such as thrombocytopenia, leukocytosis, reduced hemoglobin, and reduced serum albumin are also prominent risk factors [6]. However, no specific laboratory test exists for CAA, and it is hard to establish a diagnosis, especially in early stages. The methods for diagnosing CAA are primarily imaging systems such as echocardiography [7], computed tomography (CT), and magnetic resonance imaging (MRI) [3]. However, these methods have a relatively low sensitivity. The incidence of CAA has decreased as a result of treatments with intravenous immunoglobulin (IVIG); however, up to 5% of treated patients still develop CAA compared to up to 25% of untreated patients [2]. Therefore, it is crucial to understand the molecular pathogenesis of CAA to improve diagnosis and therapy of CAA in patients with KD.

Exosomes, 30–100 nm membrane vesicles of endocytic origin that are secreted by most cell types, are important intercellular communicators [8, 9]. As exosomes can transmit many molecules intercellularly, they are considered a potential source of new biomarkers. Exosomes may act as mediators for the activation of signaling mechanisms in the target cell, intercellular communication, transfer of proteins and miRNAs, or the disposal of cellular components [10]. Thus, exosomes carry physiological and pathological biomarker information, and changes in serum exosome protein composition may reflect the pathological processes of systemic diseases. Proteomics analysis is currently considered to be a powerful tool for the global evaluation of protein expression and has been widely applied in studying biomarkers and the molecular pathogenesis of diseases [11].

In the current study, we employed two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF MS) to compare the proteomes of exosomes from serum samples collected from healthy children and from children with CAA caused by KD. Western blotting was used to confirm the results of the 2-DE analysis. According to available research, this is the first study to perform a proteomic analysis in the context of pathogenesis of CAA caused by

KD, providing a comprehensive atlas of the serum exosome proteome, and providing clues for further diagnostic and therapeutic strategies for CAA caused by KD.

Methods

Preparation of serum samples

This study was approved by the Ethical Committee of Guangzhou Women and Children's Medical Center (permit [2013]077); the legal guardians of all children enrolled in the study provided written informed consent. Blood samples from 5 patients with CAA caused by KD were randomly recruited according to the criteria of the American Heart Association and the Japanese Ministry of Health and Welfare [12]. The diagnoses of all patients were validated by more than 2 pediatric cardiologists, and all other possible diseases were ruled out. Blood samples from 5 healthy children were used as a control group. Blood samples were separated by centrifugation at $1,000 \times g$ for 10 min. Serum aliquots were collected and stored at $-80°C$. The serum obtained was further processed for exosome isolation.

Precipitation of serum exosomes

Exosomes were separated from the sera of all participants using ExoQuick precipitation (System Biosciences Inc., Palo Alto, CA, USA) according to the manufacturer instructions [13, 14].

Exosome characterization

Serum exosomes were precipitated using the exosome extraction reagent. The extracts were centrifuged at $1,500 \times g$ for 10 min at 4°C. The exosome pellet was suspended in phosphate-buffered saline (PBS) in $4 \times$ the volume of serum. A copper mesh was placed on a wax plate, then 100 μL exosome suspension was added for 4 min. The copper mesh was removed and placed in 2% phosphotungstic acid for 5 min. The mesh was then laid on filter paper for drying. The morphological features of the exosomes were observed using transmission electron microscopy (TEM).

Exosome characterization using Western blot analysis

The exosome pellet was dissolved in protein lysis buffer, and a Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to determine protein concentration. Samples were separated by one-dimensional (1-D) SDS-PAGE, then transferred to a PVDF membrane. The

membrane was incubated in anti-CD63 (1:1000), -HSP90α (1:1000), and -Flotillin (1:1000) antibodies at 4°C overnight, followed by incubation with secondary antibodies at room temperature for 1 h. Specific protein bands were visualized using the SuperSignal enhanced chemiluminescence (ECL) system (Thermo Fisher Scientific, Waltham, MA, USA) and imaged on x-ray film.

Protein extraction of serum exosome

The exosome samples were grouped into two pooled samples consisting of equal amounts of the 5 experimental samples from each group (CAA and control groups) before proteomic analysis. The Bradford protein assay kit was subsequently used to determine the final protein concentration of the exosomes according to the manufacturer instructions.

2-DE

According to a protocol published previously [15], 2-DE analysis was performed with slight modifications. In brief, the prepared pooled protein samples (120 mg protein on analytical gels, or 600 mg protein on preparative gels) were mixed with rehydration buffer to a volume of 450 μL. IPG strips (pH 4–7, 24 cm, GE Healthcare, Little Chalfont, UK) were used for the first dimension to isolate the altered proteins, and the following parameters were used: 20°C, 300 V for 30 min, 700 V for 30 min, 1,500 V for 1.5 h, 9,900 V for 3 h, 9,900 V for 6.5 h, 600 V for 20 h, 8,000 V constant for a total of 56 000 Vh. After completion of the isoelectric focusing (IEF) program, the strips were equilibrated in two steps: 15 min in an IPG equilibration buffer (6 M urea, 2% SDS, 30% glycerol, 0.375M Tris, 20 mg/mL DTT, and a trace of bromophenol blue), and then alkylated for 15 min. Subsequently, a 12.5% 2-D SDS-PAGE was performed. Electrophoresis was carried out at 20 mA per gel for 40 min and then at 30 mA per gel until the dye front reached the bottom. The protein spots were visualized by either Coomassie Brilliant Blue G-250 staining or silver staining.

Image analysis

The gels were analyzed using the Image-Master 2D Platinum software (GE Healthcare). The normalized protein amount for each spot was calculated as the ratio of the volume on each spot to the total spot volume on the gel. Protein spots with significant differences in abundance (increase or decrease of > 1.5-fold) were selected for further analysis.

In-gel digestion

Differentially expressed protein spots were manually excised from the 2-DE gels. Each gel piece was washed twice in deionized water, destained with 50% methanol for 30 min at 37°C, and dehydrated in 100 μL acetonitrile (ACN) for 20 min at room temperature. Next, the samples were swollen in 50 μL 100 mM NH_4HCO_3, dehydrated a second time, and incubated in 1 μg/50 μL trypsin (Promega, Madison, WI, USA) for 30 min at 4°C. Then, coverage solution (50 mM NH_4HCO_3, 10% ACN, deionized water) was added to the samples and incubated for 16 h at 37°C. After removing the coverage solution by aspiration, the peptide mixtures were extracted with 2.5% trifluoroacetic acid (TFA)/90% ACN for 30 min at room temperature and vacuum dried.

Protein identification

The material was dissolved in 1.5 μL solution containing deionized water, 0.1% TFA, and 50% ACN after vacuum drying. Then, 0.8 μL of the mixture was loaded onto a MALDI sample target plate using 0.5 μL HCCA (5 mg/mL a-Cyano-4-hydroxycinnamic acid) matrix, dried at room temperature and analyzed using the ABI MALDI-TOF/TOF Proteomics Analyzer mass spectrometer (Applied Biosystems, USA). The UV laser was operated at a 200 Hz repetition rate at a wavelength of 355 nm and an accelerated voltage of 20 kV.

Database searching

The experimental MS data were matched to a corresponding virtual peptide mass database derived from GPS Explorer v 3.6, Mascot, and the UniProt database under the taxonomy of *Homo sapiens*. Protein identification was carried out by peptide mass fingerprint (PMF) with Mascot software (http://www.matrixscience.com). The search parameters used in PMF were as follows: species: *Homo sapiens*; enzyme: trypsin; fixed modifications: carbamidomethylation; variable modifications: oxidation (M). The gene name, function, and Gene Ontology (GO) category of each protein was determined with the Mascot v 2.1 protein database search engine and the UniProt *Homo sapiens* protein database.

Western blot analysis

Protein extracts from the serum exosomes of 5 KD patients with CAA and 5 healthy children were separated by SDS-PAGE (11–15% acrylamide). After being transferred to PVDF membranes and blocked overnight, primary antibody
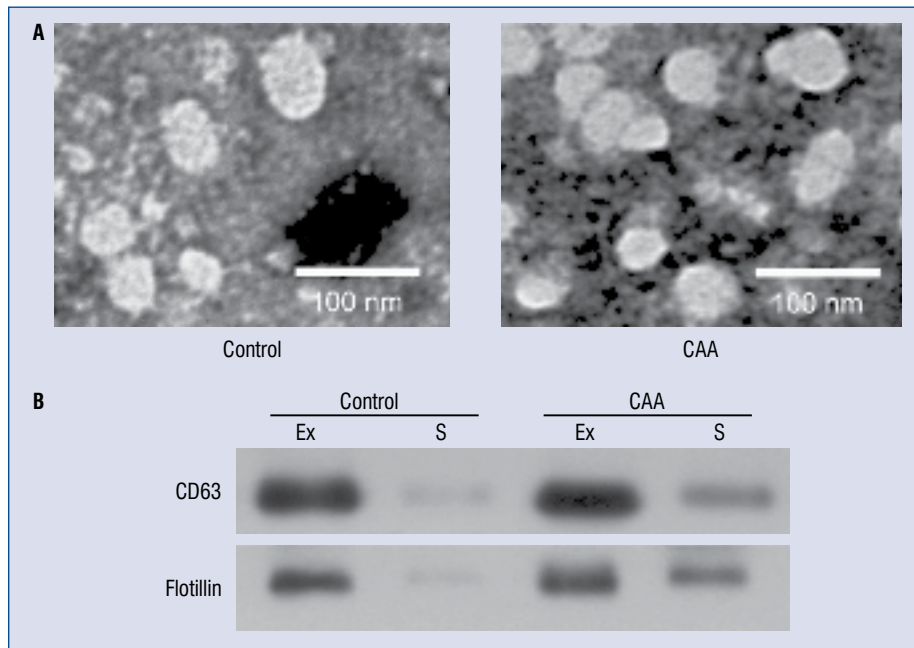
Figure 1. Characterization of exosomes in serum samples from patients with coronary artery aneurysm (CAA) caused by Kawasaki disease and healthy children. **A.** Morphological characterization of exosomes by transmission electron microscopy; **B.** Molecular characterization of exosomes by Western blotting. Protein extracts prepared from sera (S) or exosomes (Ex) were examined using antibodies against exosomal protein markers (CD63, HSP90α, and flotillin).

was added for 1 h, followed by washing in PBS, application of a secondary HRP-conjugated antibody, and development using an ECL system (Thermo Fisher Scientific). Anti-tetranectin (TN) (1:1000), anti-Leucine-rich alpha-2-glycoprotein (LRG1) (1:1000), anti-Retinol-binding protein 4 (RBP4) (1:1000), and anti-Apolipoprotein A-IV (APOA4) (1:1000) were used as primary antibodies.

Protein categorization

Differentially expressed proteins were classified using Protein Analysis Through Evolutionary Relationships (PANTHER; http://www.pantherdb.org). PANTHER ontology is a highly controlled vocabulary categorized according to molecular function, biological process, and protein class.

Protein network construction

An interaction map of the differentially expressed proteins was established using Pathway Studio 5.0 (Ariadne Genomics, Rockville, MD, USA), a text-mining tool that can construct protein interaction networks and pathways. It includes pathway components, protein–protein interactions, functional classes, proteins, and their cellular processes. In this study, the shortest path analysis was selected.

Results

Exosome isolation and validation

Microvesicles isolated from the sera of children with CAA caused by KD and healthy controls were examined by TEM and Western blotting. Spherical structures 30–100 nm in diameter were observed by TEM (Fig. 1A). It was validated that the microvesicles were exosomes through Western blot analysis using antibodies against 3 exosomal markers: HSP90α, CD63, and Flotillin. The expression levels of these 3 markers were markedly higher in the microvesicle fraction than in the serum fraction (Fig. 1B). These results validated that the isolated microvesicles were exosomes.

Identification of differential proteins between CAA patients and healthy controls by proteomic analysis

Pooled serum exosome proteins from 5 KD patients with CAA and 5 healthy controls were separated by 2-DE (Fig. 2A–B). After visual review, 39 differential protein spots with a minimum of 1.5-fold difference in expression between the two groups were selected for MALDI-TOF/TOF MS analysis. Differentially expressed proteins were picked from gels and identified using MALDI-
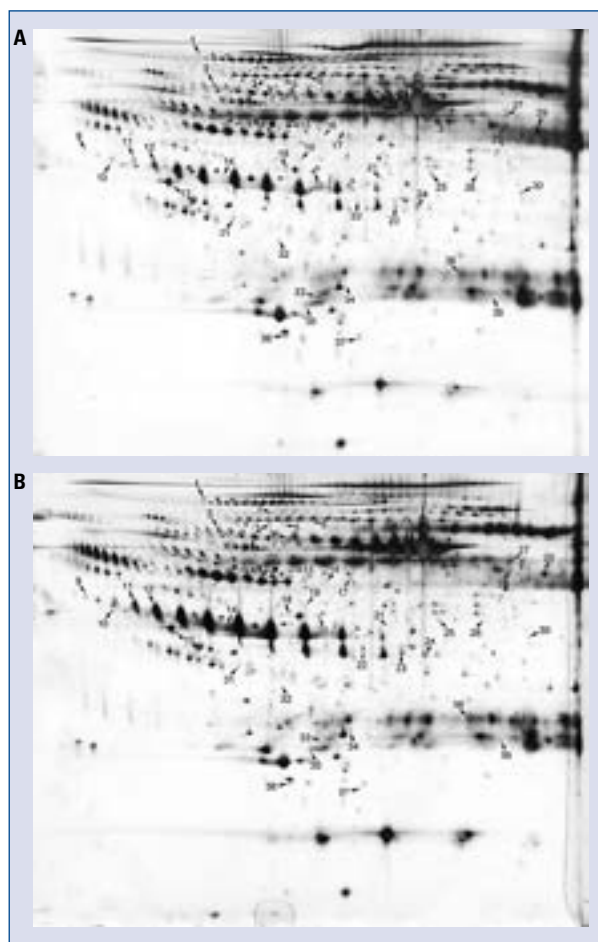
Figure 2. Two-dimensional electrophoresis analysis of the proteome of serum exosomes. Gels were visualized by silver staining. Arrows indicate differentially expressed proteins in Kawasaki disease (KD) patients with coronary artery aneurysm (CAA) and healthy controls. Spot numbers correspond to the proteins in Table 1. **A.** Healthy children; **B.** KD patients with CAA.

TOF/TOF MS. In total, 32 differentially expressed proteins (18 up-regulated and 14 down-regulated) were successfully identified (Table 1). Some proteins appeared as multiple spots in 2-DE, likely due to their isoforms and/or post-translational modifications.

Validation of differential proteins by Western blotting

To further confirm the proteomic analysis results, Western blotting was performed to examine the expression levels of APOA4, LRG1, RBP4, and TN in protein extracts from the serum exosomes of 5 patients with CAA caused by KD and 5 healthy controls. The expression levels of APOA4 and LRG1 were significantly higher in the CAA group than in the control group (Fig. 3). The expression levels of RBP4 and TN were significantly lower in the CAA group than in the control group (Fig. 3). This result was consistent with the results of proteomic analysis.

Classification analysis of identified proteins

According to GO cellular functions and processes, the proteins were distributed into categories based on molecular function, biological processes, and protein classes using the PANTHER classification system (Fig. 4). Most of the identified proteins played roles in the metabolic process (18.0%) and immune system process (14.0%). Other biological processes that the proteins were involved in included response to stimulus (13.0%), biology regulation (10.0%), and localization (9.0%). In terms of protein class, most of the proteins were classified as defense/immunity protein (17.0%), enzyme modulator (13.0%), or transfer/carrier protein (11.0%); others belonged to receptor (11%), hydrolase (9.0), signaling molecule (8.0%), and others. As for molecular function, the most dominant function that the identified proteins were involved in was catalytic activity (37.0%), followed by enzyme regulator activity (18.0%), binding (18.0%), receptor activity (16.0%), transporter activity (8.0%), and structural molecule activity (3.0%).

Network modeling of differentially expressed proteins

To explore the associations between differentially expressed proteins, a protein network was constructed with Pathway Studio 5.0. Complex interactions between mitochondrial, nuclear, cytoplasmic, and extracellular proteins were present in the prediction network (Fig. 5). Further research is needed to explore the role of these proteins and their interaction pathways in the pathogenesis of CAA due to KD.

Discussion

Cardiovascular complications are a major cause of morbidity in KD; the primary concern is CAA, which develops in 15–25% of untreated children [16]. Importantly, there is no specific modality to establish a diagnosis, especially in early stages. Treatment with a high dose of IVIG is proven to be effective in reducing the prevalence of CAA to less than 5%. However, about 10% of patients do not respond to this treatment, which represents an important clinical dilemma owing to the increased risk of developing CAA [17]. Therefore, it is criti-
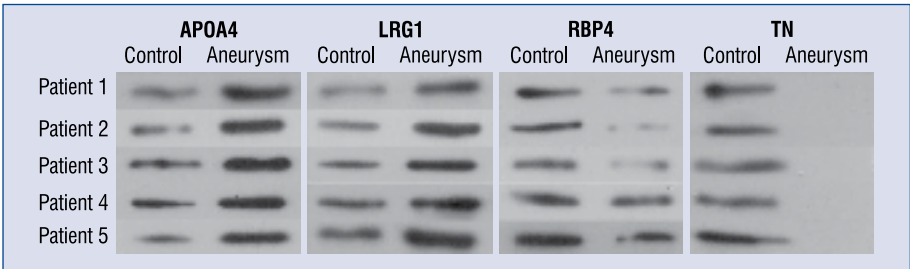
Table 1. Differentially expressed proteins between Kawasaki disease patients with coronary artery aneurysm and healthy children.

Spot No.*	Protein	Accession no.	Protein MW (Da)	Protein pI	CAA/C	Sequence coverage	Protein score
1	Alpha-1-antitrypsin	P01009	44280	5.37	1.95588	32%	319
2	Complement C4-B	P0C0L5	194216	6.89	1.67713	13%	255
3	Inter-alpha-trypsin inhibitor heavy chain H4	Q14624	103536	6.64	2.05879	18%	251
4	Afamin	P43652	70963	5.64	1.90293	23%	287
5	Inter-alpha-trypsin inhibitor heavy chain H4	Q59FS1	76971	5.72	2.36148	23%	183
6	Complement C4-A	P0C0L4	84758	5.33	−1.96522	26%	229
7	Insulin-like growth factor-binding protein complex acid labile subunit	Q8TAY0	66735	6.33	1000000	26%	325
8	Alpha-1B-glycoprotein	P04217	52479	5.65	3.7226	26%	185
9	Leucine-rich alpha-2-glycoprotein	P02750	38382	6.45	2.34081	31%	464
10	Complement C4-B	P0C0L5	40795	5.19	1.50231	26%	137
11	Haptoglobin	P00738	38868	6.26	8.13111	29%	269
12	Haptoglobin	P00738	38868	6.26	3.62451	20%	141
13	Complement C3	P01024	40204	4.79	1.71764	48%	278
14	Apolipoprotein A-IV	P06727	45371	5.28	2.06449	27%	158
15	Antithrombin-III	P01008	49350	5.95	−1.65022	40%	476
16	CFI protein	Q8WW88	44285	8.49	2.18167	7%	74
17	CFI protein	Q8WW88	44285	8.49	2.9125	9%	128
18	CD5 antigen-like	O43866	39603	5.28	−1.86353	43%	246
19	Adipocyte plasma membrane--associated protein	Q9HDC9	46622	5.82	1.63727	26%	230
20	Sex hormone-binding globulin	P04278	28876	5.32	−1.75668	46%	392
21	Sex hormone-binding globulin	P04278	28876	5.32	−1.57588	35%	88
22	Haptoglobin-related protein	P00739	39300	6.67	2.302	20%	156
23	Haptoglobin	P00738	39496	6.42	2.93725	28%	76
24	Complement factor H	P08603	36506	5.97	1.72789	39%	152
25	Complement factor H-related protein 1	Q03591	38766	7.38	−2.13194	29%	130
26	Ig mu chain C region	P01871	50117	6.4	−1.7426	21%	136
27	Alpha-2-macroglobulin	P01023	71321	5.47	1.87957	7%	115
28	Serum albumin	P02768	58513	5.96	−1.80408	40%	244
29	Beta-2-glycoprotein 1	D9IWP9	37485	8.37	−2.1629	28%	221
30	Serotransferrin	P02787	37241	6.49	−1.72825	39%	291
31	Clusterin	P10909	36997	5.74	−1.7058	14%	162
32	Transthyretin	P02766	12789	5.26	−1000000	68%	155
33	Ig kappa chain V-I region Rei	P01607	23779	8.75	−1000000	44%	120
34	Ig lambda chain V-IV region Hil	P01717	23020	6.69	1000000	32%	103
35	Ig kappa chain C region	P01834	23523	5.72	1.59839	42%	159
36	Retinol-binding protein 4	Q5VY30	21287	5.27	−2.64472	33%	130
37	Tetranectin	P05452	22951	5.52	−1000000	42%	295
38	Immunoglobulin lambda-like polypeptide 5	B9A064	22819	5.79	1.71135	42%	85
39	Ig kappa chain C region	P01834	24352	6.99	1000000	39%	118

*Spot numbers correspond to those in Figure 2. MW — molecular weight; pI — isoelectric point; CAA — coronary artery aneurysm; C — control

Figure 3. Western blot analysis showing the expression of TN, APOA4, RBP4, and LRG1 in 5 Kawasaki disease patients with coronary artery aneurysm and 5 healthy children.
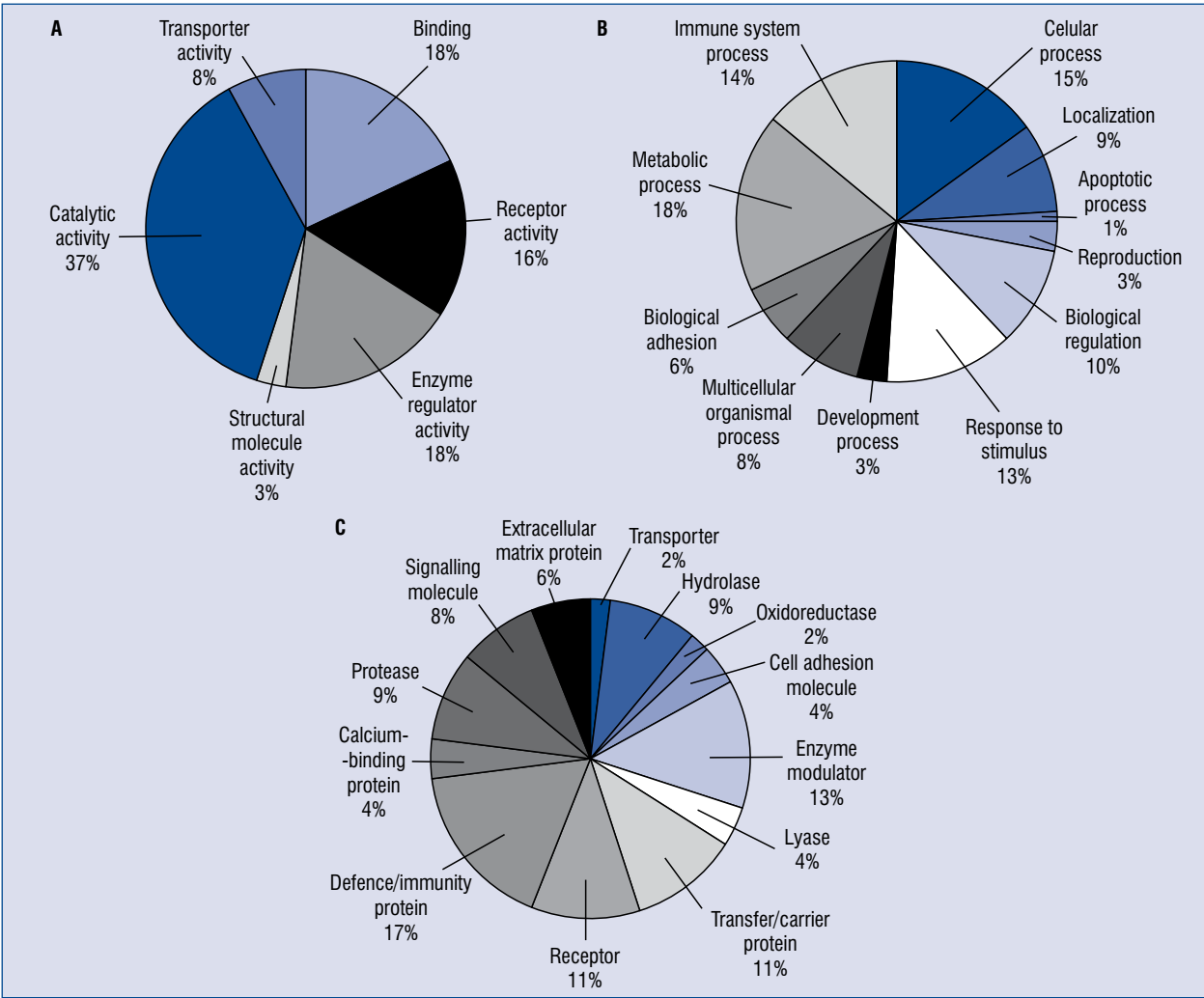
Figure 4. Classification analysis of differentially expressed proteins identified between Kawasaki disease patients with coronary artery aneurysm (CAA) and healthy controls. Categorization was based on information that was obtained from the online Protein Analysis Through Evolutionary Relationships (PANTHER) classification system in terms of (**A**) molecular function, (**B**) biological process, and (**C**) protein class.

cal to understand the molecular pathogenesis of CAA in order to improve the effectiveness of diagnosis and therapy for CAA caused by KD.

Protein profiles from patients at specific stages can be more accurate in reflecting the status of disease progression.
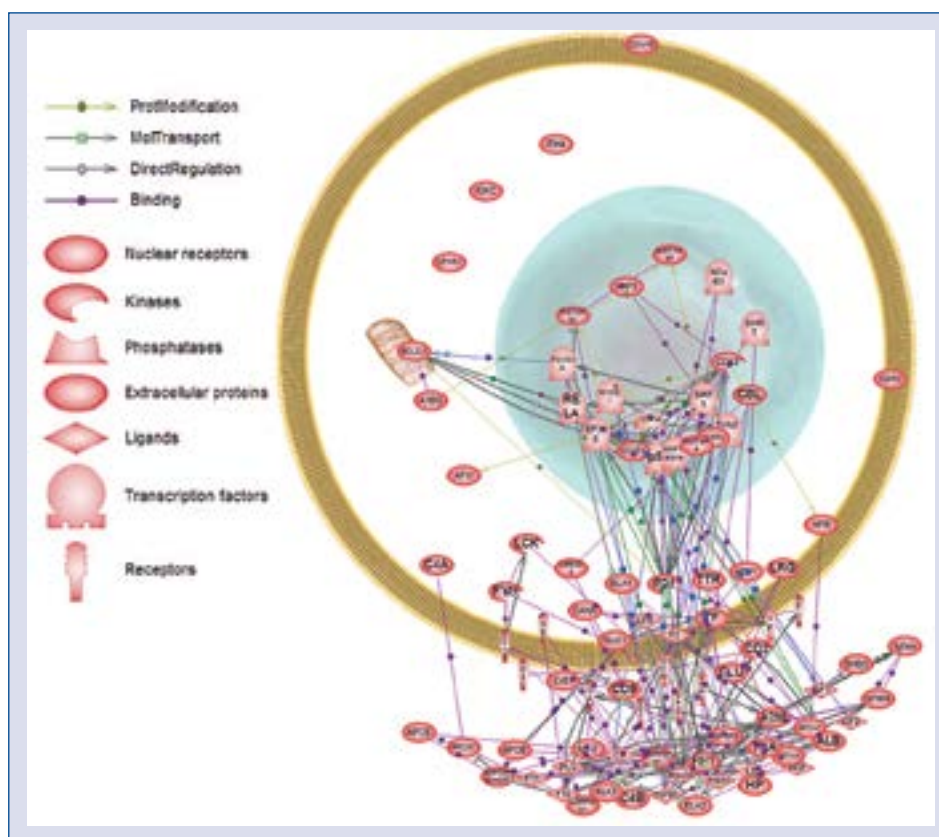
Figure 5. Protein network of the identified proteins constructed using Pathway Studio 5.0. The differentially expressed proteins in Table 1 were imported into Pathway Assist, and an interaction model was generated using the shortest path algorithm. The legend of the interaction network is summarized on the right of the figure. Each node represents either a protein entity or a control mechanism of the interaction.

In this study, 2-DE and MALDI-TOF/TOF MS were used to analyze proteomic changes in the serum exosomes of patients with CAA caused by KD. 32 differentially expressed proteins were identified in serum exosome samples, offering a spectrum of the proteomic changes caused by CAA. The differential expression of 4 proteins (TN, LRG1, RBP4, and APOA4) was then validated by Western blotting. Finally, these differentially expressed proteins were analyzed using a bioinformatics approach. The majority of differentially expressed proteins were involved in the host immune response, inflammation, apoptotic process, developmental process, and biological adhesion process. This largely favors molecular diagnosis and therapy for patients with CAA caused by KD.

Tetranectin, a plasminogen kringle 4 binding C-type lectin [18], is a plasma protein secreted by monocytes, neutrophils, and macrophages. The biological function of TN has not been fully elucidated, though it is thought to play an important role in the regulation of proteolytic and fibrinolysis processes by binding to plasminogen. It is also believed to play a critical role in mineralization during osteogenesis and in myogenesis during embryonic development [19]. Furthermore, TN has been implicated as a potential biomarker in conditions ranging from cancer to Parkinson's disease [20]. The present results, detected by 2-DE and confirmed by Western blotting, showed that the expression of TN in the CAA samples was significantly lower compared to healthy controls. Although validation with a larger sample size is necessary, these preliminary results have provided the first indication, according to available research, that TN potentially participates in the pathogenesis of CAA caused by KD, and that it is a potential CAA biomarker. The function and role of TN in CAA caused by KD still remains elusive and requires further investigation.

LRG1 is a protein from the leucine-rich repeat (LRR) family. LRG1 is expressed during granulocyte differentiation and might play an important role in the immune response when the host clears

infections. Previous research has shown that LRG1 is involved in important pathological and biological processes, such as cell adhesion, signal transduction, and protein–protein interactions [21]. Recently, LRG1 was found to be elevated in the serum of patients with lung cancer, liver cancer, and pancreatic adenocarcinoma [22, 23]. In the present study, it was found that LRG1 was up-regulated in CAA serum exosomes, suggesting that it may be involved in the development of CAA, and that the immune response to infection might contribute to the formation of CAA in KD.

RBP4 functions to deliver retinol to tissues [24]. RBP4 has been correlated with cardiometabolic risk in humans and is related to the degree of carotid intima–media thickness. Recent studies on aortic rings have shown the vasodilatory effect of RBP4 results from increased nitric oxide production in vascular endothelial cells. In addition, decreased levels of RBP4 have been found in men during the acute phase of myocardial infarction and in patients with familial hyper-cholesterolemia at high risk of an ischemic event; this suggests that decreased RBP4 levels lead to an increased susceptibility to ischemic events, and that RBP4 is a potential protective factor against cardiovascular events [25, 26]. In the present study, for the first time, it was shown that RBP4 was down-regulated in CAA serum exosomes compared to healthy controls. It seems that the down-regulation of RBP4 plays a role in the development of CAA due to KD.

APOA4, a 46-kDa glycoprotein, is produced primarily in the small intestine and is discharged into the mesenteric lymph [27]. It enters the plasma compartment as a structural protein of very low-density lipoproteins (VLDL), high-density lipoproteins (HDL), chylomicrons, or in the lipoprotein-free fraction [28]. Currently, the physiological function of APOA4 is not fully understood. Several studies have shown that it plays a role in reverse cholesterol transport. Some studies have reported that APOA4 has antioxidant and anti-atherogenic properties. A meaningful change in the serum APOA4 concentration has been found in patients with several disorders including renal disease, malabsorption syndrome, and chronic pancreatitis. In the present study, it was found that APOA4 was up-regulated in the serum exosome samples of patients with CAA. It seems that APOA4 may participate in the development of CAA due to KD.

Conclusions

In conclusion, a set of proteins were identified that were differentially expressed in CAA serum exosomes compared to healthy control samples. Of them, TN, RBP4, LRG1, and APOA4 were validated by Western blotting. Although none of the above-mentioned differentially expressed proteins is specifically correlated with CAA caused by KD, this preliminary study clearly demonstrated that the levels of these proteins were significantly changed in patients with CAA compared to healthy controls. Preliminary results suggest that these proteins may participate in the pathogenesis of CAA caused by KD. Future studies are necessary to elucidate the molecular role and mechanisms of these proteins in CAA caused by KD.

Acknowledgements

Conflict of interest: None declared

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STUDY PROTOCOL

VM
VIA MEDICA

The use of modern telemedicine technologies in an innovative optimal cardiac rehabilitation program for patients after myocardial revascularization: Concept and design of RESTORE, a randomized clinical trial

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Abstract

Despite proven efficacy of cardiac rehabilitation (CR) in reducing the all-cause mortality in patients after myocardial revascularization, the penetration of CR, due to patient-related factors and referral rates remains limited. To improve the outcomes, home-based tele-rehabilitation (TR) has been proposed recently. In theory TR enhances the effects of standard CR procedures due to implementation of an intelligent monitoring system designed to ensure optimal training through on-demand transmission of vital signs, aimed at motivating the patients through daily schedule reminders, setting daily goals and creating a platform for mutual feedback. Several meta-analyses assessing various studies comparing these two methods (CR and TR) have proven that they are at least equally effective, with some of the research showing superiority of TR. Although there was a small sample size, lack of long-term follow-up, reporting effects of TR itself, no integration with tools designed for coaching, motivating and promoting a healthy lifestyle constitutes an important limitation. The latter carries a hopeful prognosis for improvement when utilizing a broad-spectrum approach, especially with use of dedicated technological solutions exploiting the fact of a large and yet rapidly increasing penetration of smartphones, mobile PCs and tablets in the population. The above-mentioned findings worked as the basis and rationale for commencing the RESTORE project aimed at developing and delivering state-of-the-art, comprehensive TR for patients after myocardial revascularization and evaluating its molecular aspect in view of how it influences the atherosclerosis progression attenuation. This paper presents the current state and rationale behind the project based on up-to-date TR efficacy data. (Cardiol J 2019; 26, 5: 594–603)
Key words: tele-rehabilitation, myocardial revascularization, optimal cardiac rehabilitation, reduced rehospitalization rate

Introduction

The worldwide prevalence of cardiovascular diseases (CVD) has been increasing during the last few decades, and is the main cause of mortality in Europe with 3.9 million deaths each year according to European Cardiovascular Disease Statistics 2017, it also generates a staggering cost of €210 billion a year [1]. Coronary artery disease (CAD) accounts for 45% of all deaths from CVD and is the single most prominent cause of mortality [1]. With recent advances in diagnostics and therapy of CAD, including percutaneous coronary interventions (PCI) with new generation stent implantation, modern pharmacotherapy and minimally invasive surgical procedures, patient survival has improved, although long-term prognosis remains unsatisfactory. For instance, 1-year mortality following myocardial infarction (both ST-segment elevation myocardial infarction [STEMI] and non-ST-segment elevation myocardial infarction [NSTEMI]) in Poland, based on nationwide AMI-PL database was as high as 19.4% [2]. The increased risk of cardiac events is further associated with a high frequency of hospital re-admissions, resulting in a serious quality of life issue and negative economic impact. To reduce the risk and improve prognosis, the treatment strategy should not be limited to on-site intervention but encompass a full range of actions directed to ensure a complete recovery and long-term maintenance. Current European Society of Cardiology (ESC) guidelines recommend prompt implementation of extensive cardiac rehabilitation (CR) for all patients with ST-elevation acute myocardial infarction (Class I, Level B), NSTEMI (Class IIa, Level A) and stable coronary artery disease (Class I, Level A) [3]. The benefits of CR as a method of secondary prevention for patients with CAD are widely known and well documented. Numerous studies have demonstrated that CR is associated with significant reduction in all-cause mortality in patients after myocardial revascularization [4–7]. Ten-year follow-up analysis in patients who underwent percutaneous intervention due to acute coronary syndrome showed an almost 40% decrease in mortality in a group of CR patients in comparison to non-CR patients (14.7% vs. 23.5%) [8]. Further potential advantages include reduction in symptoms, improvement in exercise tolerance and physical work capacity, improvement in psychosocial well-being, quality of life and stress management, attenuation of the atherosclerotic processes, decreased risk of subsequent coronary events and revascularization, and a reduced frequency of hospital readmission. Despite undisputed benefits, due to patient-related factors and referral rates, CR penetration remains limited [9]. In European countries less than one-half of the coronary patients access cardiac prevention and rehabilitation programs [10]. Similar numbers occur in the population of the United States [11–13]. The most significant patient-related barriers encompass lack of resources, attitude problems, differences in geographic distribution of stationary rehabilitation centers among regions resulting in long distances, restrictions in access to appropriate services as well as reimbursement issues. Additionally, CR participation is further impaired by a failure to motivate patients to lifestyle changes, coupled with an inability to track progress and efficacy of rehabilitation programs. The major disadvantage of current CR programs is their relatively short duration without long-term follow-up, which discourages the development of sustainable changes in a patients' lifestyle. To improve outcomes, home-based tele-rehabilitation (TR) has been proposed recently. In theory TR enhances the effects of standard CR procedures due to the implementation of an intelligent monitoring system designed to ensure optimal training through on-demand transmission of vital signs such as electrocardiogram (ECG) and both the physical and mental state of patients. Moreover, it aims to motivate patients, reminding them of scheduled activities, setting daily goals, creating a platform for mutual feedback and thus promote shaping of new habits. Several meta-analyses assessing various studies comparing these two methods (CR and TR) have proven that they are at least equally effective, with some of the research showing a superiority of TR in terms of the frequency of adverse events, rehospitalization rate, physical activity levels, adherence to physical activity guidelines and both low-density lipoprotein and diastolic blood pressure levels (Table 1) [14–17]. However, significant limitations of a greater proportion of the studies were the relatively small sample size, lack of long-term follow-up and reporting effects of TR itself, lack of integration with tools designed for coaching, motivating the patient and promoting a healthy lifestyle. The latter carries a hopeful prognosis for improvement when utilizing a broad-spectrum approach, especially the use of dedicated technological solutions which exploit a large and yet rapidly increasing penetration of smartphones, mobile PCs and tablets in the population. Additionally, the lack of guidelines in this matter further highlights the need for large randomized studies

Table 1. Comparison between tele-rehabilitation and center-based rehabilitation programs (based on review analyses).

Author	Year	Type of study	No. of trials/ /patients	Primary endpoint	Additional observations
Frederix et al. [14]	2015	Systematic review	37 trials	Impact on adverse events and RR: Favors TR (1.30 [1.13–1.50])	Adherence to physical activity: Favors TR (0.56 [0.45–0.69])
Huang et al. [16]	2015	Systematic review	9 trials/1546 patients	No statistical difference in all-cause mortality	Comparable in exercise capacity, lipid profile, QoL, BP
Hwang et al. [17]	2015	Systematic review	11 trials	No difference in exercise capacity expressed as 6MWD and VO2 peak	Higher adherence rates of TR compared to CBR, no difference in QoL
Rawstorn et al. [15]	2016	Systematic review	11 trials/1189 patients	Physical activity level and exercise adherence: Both favors TR (0.42 [0.21–0.64]; §0.75(28))	TR and CBR were comparably effective for improving maximal aerobic exercise capacity and other modifiable CV risk factors

Statistical results presented as: odds ratio [95% confidence interval]. 6MWD — 6-minute walk distance; BP — blood pressure; CBR — center-based rehabilitation; CV — cardiovascular; QoL — quality of life; RR — rehospitalization rate; TR — tele-rehabilitation

to be conducted. The above-mentioned findings worked as a basis and rationale for commencing the RESTORE project aimed at developing and delivering state-of-the-art, comprehensive TR for patients after myocardial revascularization. The project is substantially funded by the Polish National Centre for Research and Development. The project obtained local bioethics committee approval (Bioethics Committee in Bielsko-Biala; approval no. 2016/02/11/05).

Clinical Trial Information

Utilization of Telemedicine in Optimal Cardiac Rehabilitation Program in Patients After Myocardial Revascularization (RESTORE); NCT03375944; https://clinicaltrials.gov/ct2/show/NCT03375944.

Restore project

Objectives

The aim of the RESTORE project was to determine an optimal cardiac rehabilitation (OCR) strategy using novel medical technologies and ensuring comprehensive CR through effective patient monitoring utilizing a remotely controlled CR program to decrease annual mortality and risk of cardiovascular events in CAD patients at 9 and 12-month follow-up (with an expected decrease to fall below 0.5%). Additionally, an intensive dietary

and educational program focused on lifestyle and risk factor modification to be implemented. As a novelty, the intravascular imaging with atherosclerotic plaque and intraarterial lipid characterization combined with a molecular aspect of OCR will also be evaluated in view of how it influences atherosclerosis progression attenuation. Primary endpoint will be maximum lipid core burden index (LCBI; 4 mm) and secondary endpoints (among others): change in the amount of LCBI and change in the angle of lipid core.

Combining all these components should help to establish new strategies translating into up-to-date CR and secondary prevention of cardiovascular events. To properly initiate and conduct this study the RESTORE Consortium has been created, consisting of prime Polish and European science and industry representatives led by American Heart of Poland SA.

Methodology

The project was split into key phases. The stepwise approach will ensure that any problems encountered during the study will be identified and resolved quickly, allowing timely execution of the project.

The first and crucial part of the project will be to design and build the platform of optimal home-based cardiac TR. The platform will consist of four critical and synchronized elements that enable fluent, efficient and safe operation of the
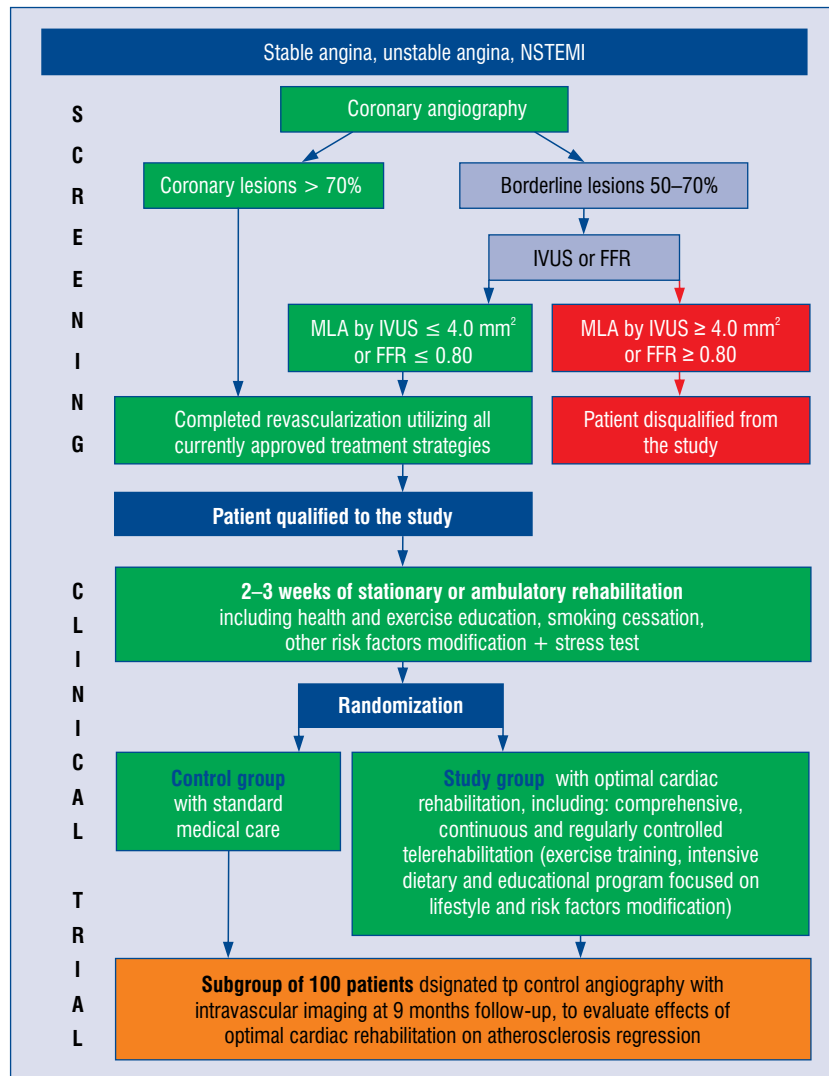
Figure 1. RESTORE randomized trial schematics; STEMI — ST-segment elevation myocardial infarction; IVUS — intravascular ultrasound; FFR — fractional flow reserve; MLA — minimal lumen area.

whole system. The elements include: (1) dedicated software for remote patient monitoring, which will be based on algorithms that allow automatic analysis of physiological signals; (2) peripheral medical devices that measure physiological signals including ECG, heart rate, and blood pressure; (3) a central system (coordinating center) that collects physiological parameters and to help medical staff and implemented algorithms allowing for rapid action in order to improve patient safety; (4) mobile applications which help patients manage their health status and allow for data transmission between medical devices and central system. Once all these devices are established and technological requirements met, the technical tests will be conducted. After positive testing, progression to the next phase and use of the system in a clinical setting will be enabled.

The next step is comprised of a prospective and randomized multi-center study including 5 cardiology units located in the Polish cities of Tychy, Bielsko-Biala, Dabrowa, Ustron, Chrzanow (Fig. 1). All patients enrolled in the study must be younger than 70 years old, presenting with stable or non-ST-elevation acute coronary syndrome (including NSTEMI and unstable angina) and must have completed coronary revascularization with any available method including coronary angioplasty, coronary artery bypass grafting or hybrid procedures. All lesions of ≥ 70% of diameter stenosis will be considered significant. If a lesion is assessed as 50–70% of diameter stenosis, additional imaging (intravascular ultrasound or optical coherence tomography) or functional (fractional flow reserve) tests will be performed at the physician's discretion in order to confirm the significance

Table 2. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Age over 18 and under 70	Ejection fraction < 40%
Completed revascularization in patients with stable, unstable angina or after NSTEMI	Acute myocardial infarction with ST segment elevation/new onset of LBBB
	Suboptimal (not completed) myocardial revascularization
Eligibility to participate in a program of early cardiac rehabilitation	Acute heart failure (Killip IV) at the time of admission to the hospital
	Dual antiplatelet therapy cannot be maintained for 1 year after PCI
The ability to use tele-rehabilitation system	Hemorrhagic stroke in the past
	Ischemic stroke or transient ischemia in previous 6 weeks
Signed informed consent form	Platelet count < 100,000/mm^3
	Chronic renal failure with creatinine clearance < 30 mL/min/1.73 m^2
	Planned surgery
	Pregnancy or planned pregnancies
	Expected life expectancy less than 3 years after enrollment

LBBB — left bundle branch block; NSTEMI — non-ST-segment elevation myocardial infarction; PCI — percutaneous coronary intervention

of stenosis. In patients following myocardial infarction with extensive scar tissue, the imaging tests were performed in order to assess viability before assigning any patient for the study. In addition, all patients must have ejection fraction equal or above 40%. The main inclusion and exclusion criteria are presented in Table 2. After revascularization was completed all patients will undergo 2 to 3 weeks of stationary (in-hospital) or outpatient (ambulatory) CR. This allows enough time for health and exercise education, smoking cessation and other risk factor modifications. At the end of this period all patients will undergo a stress test, which will be then repeated at 9-month follow-up. During stationary or ambulatory rehabilitation all patients will be randomized to one of the following groups: (A) control group with standard medical care — optimal medical therapy, regular check-ups (n = 500) or (B) study group with optimal, remotely controlled and intense CR (n = 500). Before randomization, all patients (n = 1000) will undergo technical training in order to get familiar with the TR program and medical devices provided. Patient number justification is based on the following assumption: at least 630 patients are required to have a 90% chance of detection, and be significant at the 5% level, a decrease in the primary outcome measure from 4.5% in the control group [18] to 0.5% in the experimental group. Taking into account previous reports on adherence rates to regular physical exercise it was decided to increase the number of patients to 500 per group.

Additionally, a subgroup of 100 patients (50 in the study group and 50 in the control arm) in whom, based on angiography, vessels with borderline lesion was left untreated, will undergo combined intravascular imaging with intravascular ultrasound (IVUS) and near-infrared spectroscopy (NIRS) using the commercially available TVC Imaging System™ (Infraredx, Inc., Burlington, MA, USA) immediately following the last procedure and at 9-month follow-up (Fig. 2). The imaging will track potential changes in atherosclerotic plaque volume and composition (including the amount of intraarterial lipids) after optimal CR. The quantitative and qualitative analysis will be performed in independent core laboratory. Furthermore, a group of 100 patients will be qualified to take part in an additional molecular sub-study in which selected particles and processes, that potentially could change following physical activity and the rehabilitation program will be evaluated. This includes, among other factors, the activity and level of antioxidative enzymes, signs of oxidative damage to proteins, the expression of gene encoding enzymes involved in the defense against oxidative stress and markers of blood-brain-barrier integrity. Polymorphism of genes related to endothelial and vascular function will be studied in order to find possible predictors indicating a positive effect of rehabilitation in cardiovascular system disorders. Molecular studies will be conducted immediately after the last procedure and at 9-month follow-up in the Laboratory of Molecular Studies at the Academy of Physical Education in Katowice.

Cardiac rehabilitation program

The essential part of the OCR program will be CR based on a protocol developed by an experienced cardiovascular team consisting of physicians and physiotherapists from American Heart of
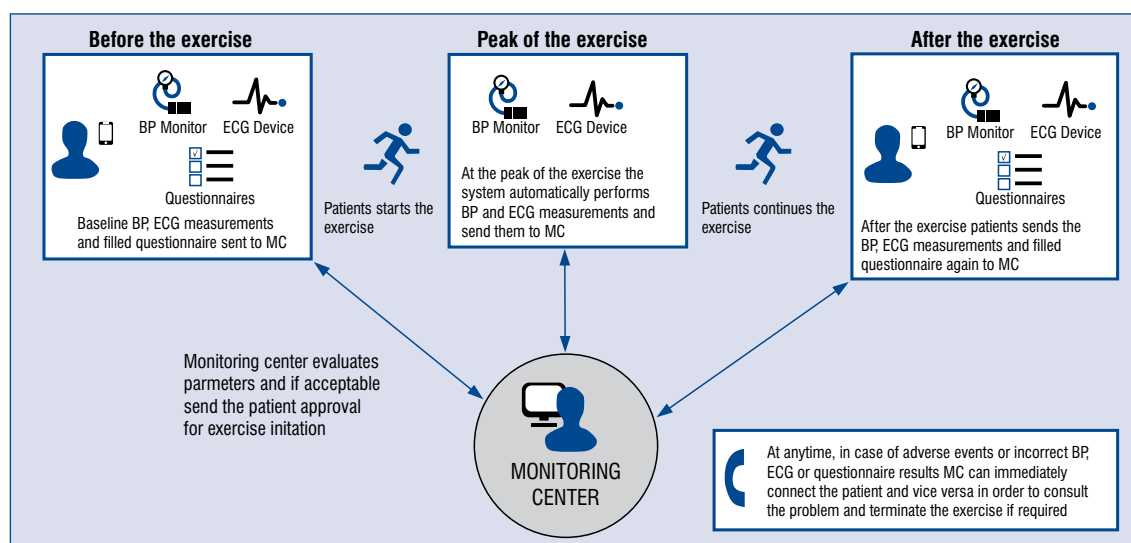
Figure 2. Concept and design of home training utilizing telemonitoring technology; BP — blood pressure; ECG — electrocardiogram; MC — Monitoring Center

Poland SA and the Academy of Physical Education in Katowice. The optimal protocol development will be multi-level. The main steps are presented below:

— **First level — qualification for the program of CR:** at the beginning of stationary or ambulatory rehabilitation the following data will be analyzed: medical history, resting ECG, baseline blood pressure and heart rate measurements, complete blood count including lipid profile, glucose and electrolyte levels, body weight and body composition measurements. In all patients, cardiac echo will be performed. In addition, all patients will fill out questionnaires to assess their quality of life, physical activity and depression level. The crucial part of this step will be to perform a stress test using a treadmill or bicycle in order to qualify the patient for an adequate rehabilitation model. Before stress test initiation, target heart rate will be calculated using the following formula: 85% of maximal heart rate (208-07 × age). In case of beta-blocker intake, heart rate will be adjusted (minus 20–30% heart rate sub-max). For the treadmill exercise, standard or modified Bruce protocols will be used. During the exercise ECG, blood pressure, heart rate and Borg scale will be used and monitored.

— **Second level of rehabilitation (stationary or outpatient CR; duration 2–3 weeks):** model A of CR will be designated for patients with the highest exercise tolerance, without angina or any other cardiovascular symp-

toms, and who have achieved the level of ≥ 7 MET or ≥ 100 W in the stress test. Intensity: 60–85% of heart rate reserve or 50–75 (80) % of maximal load. The exercise loads increase gradually through the whole period of rehabilitation. Model B and C of rehabilitation will be designated for patients with low exercise tolerance who achieved at least 6 MET or 75 W during the initial stress test. Similar to model A, the exercise loads increase gradually. For all patients and all models there will be a possibility for crossover (concerning patients unable to use the TR system). The following types of training have been scheduled: (1) endurance (aerobic), (2) general training to improve a patients' condition, (3) resistance training (aerobic, mixed into the last phase of the period). At the end of this phase all patients qualified to participate in the study group (OCR program) will receive a set of devices for cardiac TR, including a tablet with installed tele-rehabilitation software (designed and produced by the RESTORE Consortium), tele ECG with electrodes (ProPlus SA, Warsaw), blood pressure monitor and training bike (Kettler Polska Sp. z o.o., Poland). Patients will undergo detailed training in order to get familiar with this technology.

— **Third level of rehabilitation:** (frequency: a minimum of 3 times per week, optimum 5 times per week). Qualification of patients and exercise plan of rehabilitation will be similar to that described in the second level. Exercise

loads will be modified based on the Borg scale, heart rate (continuous monitoring during physical activity) and stress test performed every 3 months. There will be a possibility to cross over to the other model of rehabilitation. It will also be possible to include additional activities in order to improve patient motivation. These include: jogging, choreotherapy, spinning and other exercises. Furthermore, all patients will be encouraged to participate in periodic street races (including Nordic-walking) over a distance of 5 km. During this phase all patients enrolled in the study group will be obliged to use this novel system of TR according to the instructions and training obtained during the second phase of stationary rehabilitation. The concept and design of home training which utilizes telemonitoring technology is presented in Figure 2. The system allows medical staff to oversee patient parameters including ECG, blood pressure, extent of perceived exertion and quality of life. In addition, it allows monitoring of progress of exercise over time, remotely adjusting the exercise load (if required) and transferring information to the patient by displaying their progress in comparison to others.

Regardless of the intensity of their plan of exercise presented above, all patients will be educated during controlled ambulatory visits with regard to maintaining a proper diet, smoking cessation, risk factor modification and methods of excessive stress reduction. For all patients, echocardiography, stress test and blood analysis will be performed at 1 and 9 months. These tests will be accompanied by a physical examination and mental evaluation using questionnaires identical to the second phase.

## Current state of the project (as of August 2018)

The project commenced in March 2016 and its completion is planned for December 2019. To date, the first phase of the project has been completed. The comprehensive TR platform has been established and successfully tested confirming its efficacy of use, thus enabling progression to the next phase. Recently the enrollment process has begun and 144 patients have been recruited to the study and intravascular imaging with blood sampling for molecular sub-study, which has been performed with the first 10 patients (Fig. 3). Also, first patients enrolled in the study group have already started their TR program that is being monitored by Center for Heart Monitoring built within the structure of

the American Heart of Poland. Initial short-term results of the RESTORE project are expected at the end of 2018.

Discussion

The abovementioned CVD statistics highlight the need for comprehensive solutions in order to reduce the burden of CVD diseases both for affected individuals and the whole medical care system. The state-of-the-art therapy cannot be limited to on-site treatment but rather needs to be combined with a well-thought-out strategy aimed at bringing patients to full recovery, mobilizing them and, through a deep modification of their daily habits, establishing a highly effective preventive strategy for the future. The current guidelines of ESC recommend implementing CR from the very first moment after intervention to ensure accelerated patient convalescence [3]. The value and impact of CR have been thoroughly checked and confirmed in numerous studies, showing a significant decrease in 1-year mortality [4–6]. Sunamura et al. [8] reported an almost 40% reduction in overall mortality at 10-year follow-up in a group of CR patients in comparison to non-CR patients.

In most countries CR is delivered as a supervised center-based program. The necessity to attend scheduled trainings can be restricted by geographical and associated economic barriers, drastically reducing the number of attendees. Therefore, despite such encouraging results regarding CR efficacy, the general aptitude and patient compliance remains unsatisfactory. Studies show that merely 20% to 50% (up to 65% in several countries) of eligible individuals participate in CR in developed countries [19–23]. The causes differ, but decisive actions aiming to resolve this matter are undoubtedly required. Implementing home-based rehabilitation (HBR) could be seen as a partial solution due to improved accessibility. Metanalysis conducted by Buckingham et al. [24] on 17 studies with 2172 patients included, proved non-inferiority of HBR in comparison to center-based CR. What is more, the aspect of patient compliance as well as the probability of completing the whole course, HBR appears to be slightly superior. These findings were later confirmed with two other reviews by Anderson et al. [25] and Zwisler et al. [26], concluding that both forms of rehabilitation are similarly effective for the condition of a patient's health and improvement in quality of life, hinting at a positive impact of increased compliance in the HBR group. However, significant limitations of the
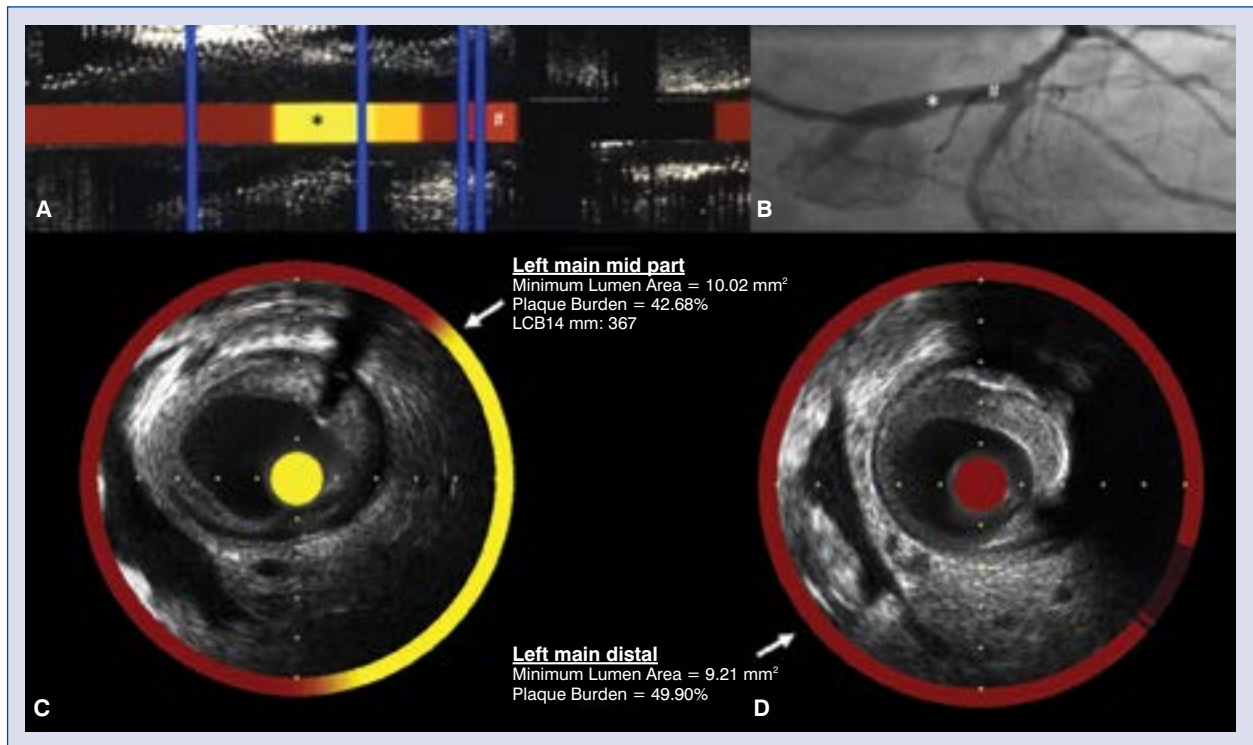
Figure 3. Greyscale intravascular ultrasound (IVUS) with near-infrared spectroscopy (NIRS) imaging of non-treated left main (LM) coronary artery with borderline atherosclerotic lesions performed in first patient enrolled to RESTORE imaging sub-study. **A.** Longitudinal view of LM with overlapping block chemogram presenting yellow segments with lipid-rich plaques (the asterisk shows mid part of LM) and red segments non-lipidic plaques (the hash shows distal part of LM); **B.** Angiographic view of LM; **C.** Cross sectional view of lipid-rich plaque in Segment 4 with maximum lipid core burden index (LCBI) equaled to 367; **D.** Cross sectional view of non-lipid plaque.

abovementioned studies include a short follow-up time, with only 3 papers exceeding 1-year observation, as well as the relatively small number of patients included in individual trials, thus requiring further examination.

Yet the question of how to make the impact of CR long-lasting still remains. Along with completing the CR program, usually spanning several weeks, the supervision is ceased, and the patient is left alone. According to a meta-analysis of 26 randomized clinical trials by ter Hoeve et al. [27], center-based CR is not sufficient to establish sustainable physical activity habits. Hence, better methods and solutions to provide feedback after completing the core CR are required.

The RESTORE project was created in 2014 to answer this challenge. The advantages of TR have been studied and its efficacy confirmed, but the low number of trials requires further examination [14––17]. The large number of patients to be included in the present study boosts the significance of this research. Additionally, the authors aim to conduct a long-term observation (obligatory 1-year follow-up with possibility to extend up to a 5 year follow-up) with periodical check-up not only to determine its impact on mortality and morbidity, but to determine whether via constant tele-monitoring, changes in one's lifestyle, physical activity and dietary habits, the positive impact of CR can be maintained. What is more, the imaging sub-study will be performed in order to assess progression, regression or stabilization of atherosclerotic plaques. In parallel a molecular sub-study will evaluate the levels of inflammatory and oxidative stress markers and endothelial function related genes with the aim of finding possible predictors of a positive effect of rehabilitation in cardiovascular system disorders. To enhance patient education and promote healthy living the RESTORE team has launched a program called "Active Heart" that will encourage cardiovascular patients to undertake regular, monitored physical activity. Their dedication will then be gratified by sponsors and all the funds raised in the program will be given to the charity.

Conclusions

This novel approach will enable outlining of possible flaws, gain a better understanding of the whole process and draw conclusions resulting in further improvement of optimal CR. Moreover, the underlying concept of the RESTORE project fulfils the expectations of the future role of CR being simultaneously easily accessible, comprehensive and cost-effective.

Consortium members of the RESTORE project

— Center for Cardiovascular Research and Development, American Heart of Poland SA;
— The Jerzy Kukuczka Academy of Physical Education in Katowice;
— AGH University of Science and Technology, Faculty of Electrical Engineering, Automatics, Computer Science and Biomedical Engineering;
— Silvermedia SA;
— Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.;
— Universitätsklinikum Carl Gustav Carus Dresden.

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BRIEF COMMUNICATION

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VIA MEDICA

Prevalence of United States adults with triglycerides ≥ 135 mg/dL: NHANES 2007–2014

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Hypertriglyceridemia is associated with increases in atherosclerotic cardiovascular disease (ASCVD) risk and remains prevalent among adults in the United States (US) due to an increasing prevalence of obesity, insulin resistance, diabetes mellitus, and other risk factors. Guidelines suggest target triglycerides (TG) should be < 150 mg/dL [1]. However, a number of studies have suggested that reduced cardiovascular risk is associated with lower TG [2–4]. Indeed, ASCVD risk remains even in patients with moderately elevated TG despite the control of low-density lipoprotein cholesterol (LDL-C) with statin therapy [2, 4–6]. The recently completed REDUCE-IT trial investigated the effects of icosapent ethyl 4 g/day in statin-treated patients with established cardiovascular disease, diabetes and other risk factors and TG 135–499 mg/dL. REDUCE-IT found a significant reduction in major adverse cardiovascular events when compared with a placebo (hazard ratio [HR] 0.75; 95% confidence interval [CI] 0.68–0.83; p < 0.001) over a median follow-up time of 4.9 years [4]. Subgroup analyses showed similar risk reduction both in persons with or without baseline TG ≥ 150 mg/dL [4]. Based on the findings of REDUCE-IT, the American Diabetes Association Standards of Care now includes a Level A recommendation that icosapent ethyl be considered for reducing cardiovascular risk in statin-treated patients with controlled LDL-C, elevated TG (135–499 mg/dL), diabetes, ASCVD or other cardiac risk factors [7].

The objective of this analysis was to examine the prevalence of TG ≥ 135 mg/dL in the overall US adult population and in those treated with statins, in accordance with the presence of ASCVD and//or diabetes.

This analysis included laboratory data, medical history, and prescription data from subjects aged 20 years and older who participated in the US National Health and Nutrition Examination Survey (NHANES; 2007–2014) and had morning fasting TG available. For the current report, the proportion and number (weighted in millions to the US population) of individuals with TG ≥ 135 mg/dL was estimated according to the following factors: statin use, LDL-C < 100 mg/dL, diabetes, ASCVD, and/or age ≥ 45 years, as well as the proportion and number of individuals with multiple risk factors. All analyses used the NHANES 8-year sample weighting to project the US population in millions. The general methodology of NHANES data collection was published previously [8].

Diabetes was defined as fasting glucose ≥ 126 mg/dL, non-fasting glucose ≥ 200 mg/dL, taking insulin or other medications to lower blood sugar, or diagnosed by a healthcare provider. LDL-C was calculated by the Friedewald equation.

The study sample included 40,617 individuals in the NHANES 2007–2014 survey. A total of 9593 subjects, projected to represent 219.9 million US adults, met the entry criteria and were included in the analysis. As shown in Table 1, the overall proportion of US adults with TG ≥ 135 mg/dL was 32.1% (representing 70.5 million individuals). Among statin-treated adults, the proportion with TG ≥ 135 mg/dL was 39.0% (15.2 million) and

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Table 1. Weighted prevalence of triglycerides (TG) ≥ 135 mg/dL among adults in the United States ≥ 20 years in NHANES 2007–2014.

	Prevalence	Patients with TG ≥ 135 mg/dL [millions*]	Total number of patients [millions*]
Overall	32.1%	70.5	219.9
Statin treated	39.0%	15.2	38.9
Statin treated and LDL-C < 100 mg/dL	35.0%	7.6	21.7
Statin treated and diabetes	47.6%	5.9	12.4
Statin treated and ASCVD	38.0%	3.8	10.1
Statin treated and diabetes or ASCVD	42.6%	7.9	18.6
Statin treated and diabetes or ASCVD and ≥ 45 years	42.6%	7.5	17.5
Statin treated and diabetes or ASCVD and ≥ 45 years and LDL-C < 100 mg/dL	38.8%	4.8	12.5

*Projected number of adults in the United States. Atherosclerotic cardiovascular disease (ASCVD) was defined as self-reported coronary heart disease, heart attack, or stroke; LDL-C — low-density lipoprotein cholesterol; NHANES — National Health and Nutrition Examination Survey

ranged from 35.0% to 47.6% for those who also had LDL-C controlled to < 100 mg/dL, diabetes, and/or ASCVD (Table 1).

Based on the present analysis, more than 30% of all adults in the US (70.5 million) have TG ≥ 135 mg/dL, including 39.0% (15.2 million) of those being treated with statins. In a recent study of this population, 56.9 million US adults were estimated to have TG ≥ 150 mg/dL [9], resulting in 13.6 million having TG ≥ 135 mg/dL and < 150 mg/dL based on that study and the present analysis.

Schwartz et al. [2] demonstrated a significant trend toward increased risk of both short- and long-term ASCVD following acute coronary syndrome, according to progressively higher tertiles or quintiles of TG concentrations (p = 0.03 and p < 0.001, respectively) [2]. They further reported that the adjusted risk of ASCVD increased by 1.8% for every 10 mg/dL increase in TG above 80 mg/dL. Another study reported a significantly elevated risk of myocardial infarction in patients with TG from 89 to 176 mg/dL, compared to those with TG < 89 mg/dL (HR 1.6; 95% CI 1.4–1.9), indicating substantially increased risk at higher TG levels [3]. In the placebo arm of REDUCE-IT, a primary composite endpoint event occurred in 22% of patients overall, and in 21% of the subset of patients with TG < 150 mg/dL, indicating substantial risk in such patients.

The current report estimates that 4.8 million US adults on statin therapy with either ASCVD or diabetes have TG ≥ 135 mg/dL but LDL-C < 100 mg/dL and may be possible candidates for icosapent ethyl therapy for the reduction of ASCVD risk based on results from REDUCE-IT. Better efforts are needed to identify and address remaining residual ASCVD risk in statin-treated individuals with TG ≥ 135 mg/dL, including lifestyle modification adherence measures and the use of evidence-based pharmacologic therapies shown to reduce ASCVD risk.

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Conflict of interest: Dr. Nathan D. Wong receives research support from Amarin Pharma Inc. and Amgen through his institution and has participated on advisory boards or speaker bureaus for Amarin Pharma Inc., Sanofi, and Novartis. Dr. Sephy Philip and Dr. Craig Granowitz are employees and stock shareholders of Amarin Pharma Inc. Dr. Peter P. Toth is a consultant and speaker for Amarin Pharma Inc., Amgen, Kowa, Novo Nordisk, Regeneron, and Sanofi. Dr. Wenjun Fan has no conflicts to report.

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VIA MEDICA

IMAGE IN CARDIOVASCULAR MEDICINE

Entrapment of guide wire by Chiari's network during pacemaker implantation

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A 35-year-old female patient was diagnosed with sick sinus syndrome and needed a pacemaker implantation. During the operation, the left sub-clavian vein was punctured when a J wire was attempting to be placed into the inferior vena cava to ensure entry to the venous system. In the process of adjusting position of the J wire, it was found that the J wire was entrapped in the junctional area of the lower right atrium and inferior vena cava. The J wire was rotated and was attempted to be pulled out, but it failed. Finally, the wire was forcefully pulled out, and it was found that the tip of J wire was wrapped completely around Chiari's network (Fig. 1).

Although it has never been reported that the wire was entrapped in Chiari's network was pulled out by force, it is not encouraged in consideration of the high risk of tearing the inferior vena cava. With the development of devices, new choices will be available to deal with this problem. It has been reported that an entrapped pacing lead was pulled out successfully with the use of a laser sheath and intracardiac echocardiogram. This method should be considered a good choice with less risk of injury. Above all, during cardiac intervention, rotating the J wire, lead or catheter in the junctional area of the lower right atrium and inferior vena cava should be avoided.

Conflict of interest: None declared
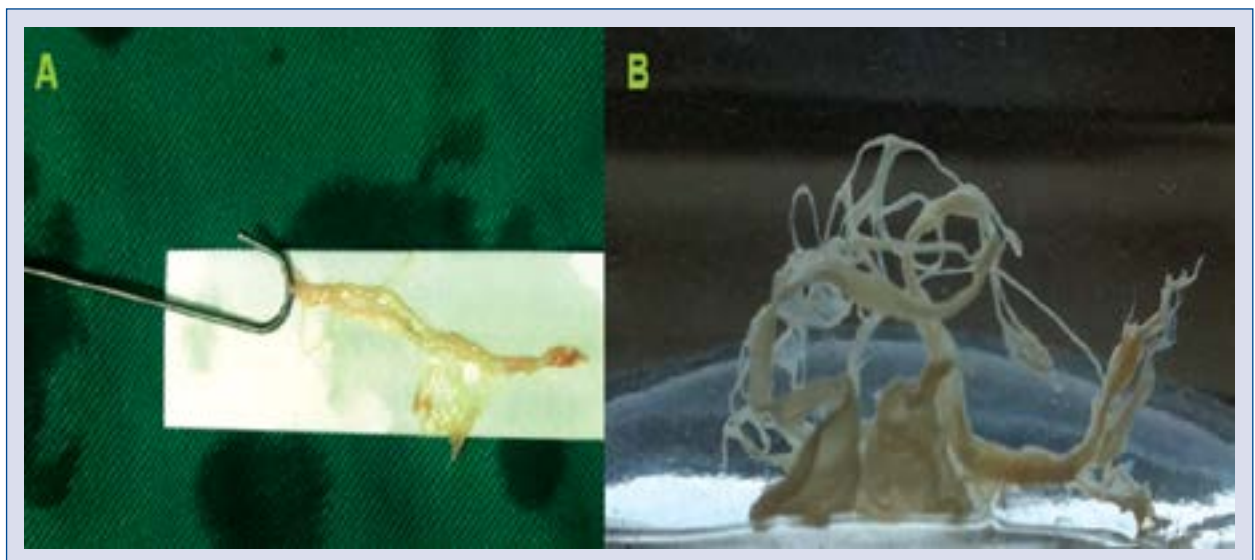
Figure 1. A. Chiari's network wrapped around the tip of J wire; B. Complete Chiari's network was shown in the water.

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IMAGE IN CARDIOVASCULAR MEDICINE

Intravascular lithotripsy for heavily calcified subtotal occlusion of right coronary artery

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A 64-year-old symptomatic man with Canadian Cardiovascular Society (CCS) class III angina was referred for percutaneous coronary intervention of subtotal occlusion of the ostial right coronary artery (RCA; Fig. 1A). Following intubation with a 7F AL 0.75 guiding catheter, and sequential high-pressure predilatation (1.2 mm semi-compliant balloon, and 2.0 mm to 3.0 mm non-compliant balloons), intravascular ultrasound (IVUS) revealed extensive three-to-four-quadrant (270° to 360°) calcification within proximal RCA along with persistent ostial stenosis of the vessel (Fig. 1D–F). To modify plaque within proximal RCA, a 4.0 × 12 mm intravascular lithotripsy (IVL) balloon was inflated to 4 atm, and 8 cycles of 10 pulses each were delivered, followed by further dilatation to nominal pressure (Fig. 1B). IVUS after IVL confirmed multiple calcium disruptions (Fig. 1D'–F') allowing for guideliner-facilitated delivery and deployment of 2 drug eluting stents (4.0 mm each), and further high-pressure postdilatation (at 22 atm) using a 4.5 non-compliant balloon. Optimal angiographic result (Fig. 1C) was subsequently verified with both IVUS (Fig. 1D''–F''; **Suppl. Video 1**) and instantaneous wave-free ratio.

Coronary IVL is a novel catheter-based technique that utilizes sonic pressure waves to disrupt calcified lesions. Herein we present a case of IVL for treatment of subtotal ostial coronary occlusion with severe calcification resulting in successful delivery and optimal expansion of coronary stents. Whether IVL may supplement available percutaneous techniques in coronary total occlusions is to be elucidated in future trials.

Conflict of interest: None declared

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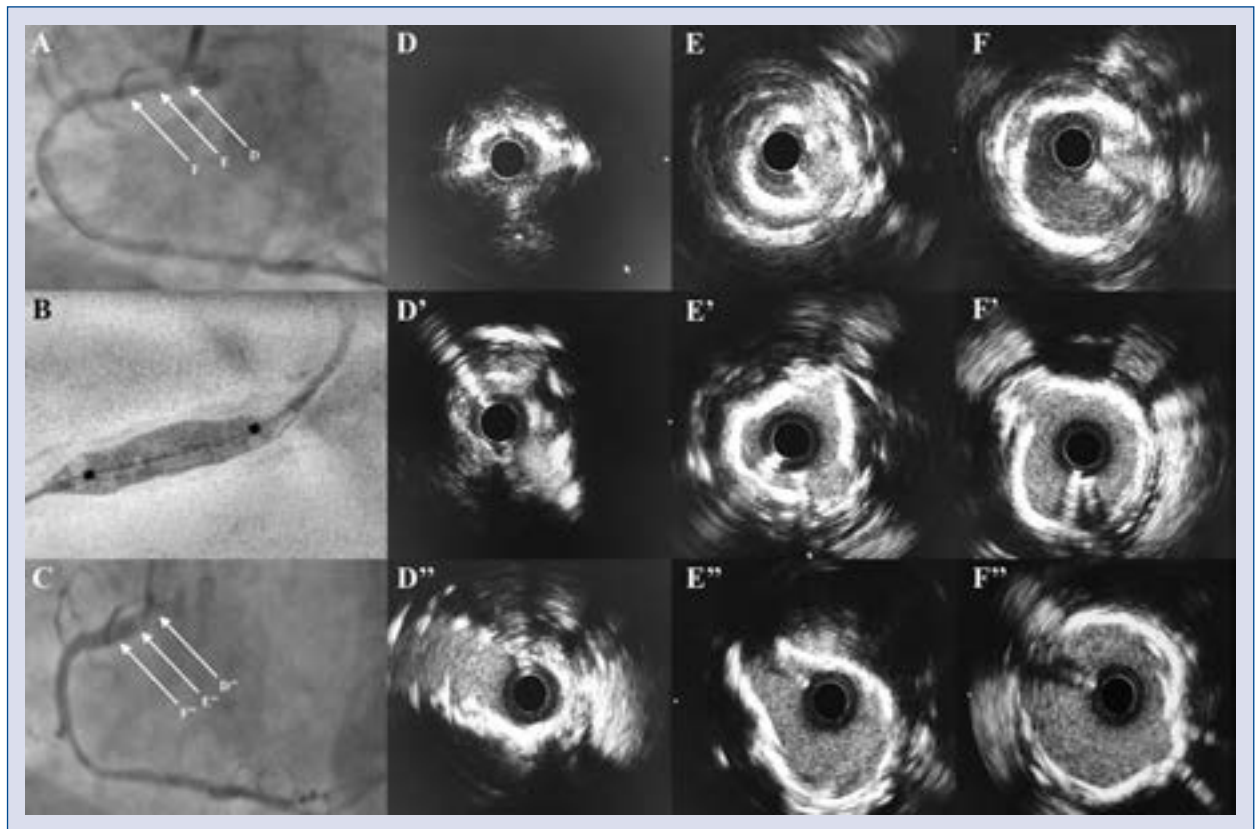
Figure 1. A. Subtotal occlusion of the ostial right coronary artery (RCA) with tortuous uptake from the aorta; **B.** Angiographic appearance of the Shockwave intravascular lithotripsy (IVL) balloon; **C.** Final angiographic result after stent implantation; **D–F.** Intravascular ultrasound (IVUS) before IVL revealing three-to-four-quadrant (270° to 360°) calcification of the proximal RCA along with severe ostial stenosis; **D'–F'.** IVUS after IVL demonstrating successful fracture of the calcified lesion within proximal RCA; **D''–F''.** Final IVUS showing optimal stent expansion.

IMAGE IN CARDIOVASCULAR MEDICINE

Percutaneous angioplasty at previous radial puncture site via distal radial access of anatomical snuffbox

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A 68-year-old woman was treated with percutaneous coronary intervention through the right radial artery because of exertional chest pain. Seven months later, the patient returned due to recurrent chest pain. A diagnostic coronary angiography was performed via distal radial access at anatomical snuffbox. The right radial artery pulsation was weak and the left radial artery was attempted. However, on the left side, the pulse was not palpable and the right distal radial artery was punctured with ultrasonography-guided. At the beginning of examination, the guide wire (JS angioguide wire, 0.035", A&A M.D. South Korea) was unable to pass through the previous puncture site of the right radial artery. Angiography of the radial artery via distal radial sheath revealed a significant stenosis and dissection at the previous puncture site of the radial artery (Fig. 1A, **Suppl. Video 1**). The operator changed the guide wire to a hydrophilic guidewire (Radifocus 0.035", TERUMO CORPORATION, Japan). In the documented hospital, coronary angiography was performed using 0.035" hydrophilic wire as the second option. The complication rate is low and success rate is high. However, the operator made a significant arterial dissection with the second wire and the radial artery was finally occluded. Another wire (Asahi SION BLUE), which was used in coronary intervention (0.014", ASHAHI INTECC, Japan) was introduced and finally passed through the lesion via true lumen. After a diagnostic coronary angiography, the operator decided to perform angioplasty at the radial artery. Because of her small radial artery (about 2–3 mm in diameter), a 0.014 system was chosen and the angioplasty was performed with a peripheral balloon (SLEEK® OTW0.014" percutaneous transluminal angioplasty catheter, 2.0 × 150 mm, Cordis, USA) without a guiding catheter (Fig. 1B). After percutaneous transluminal angioplasty, a final angiography revealed acceptable results (Fig. 1C). And then, right snuffbox puncture site sheath was removed and hemostasis was successful (Fig. 1D, E).

Repeated radial puncture increases the risk of arterial spasm and vascular stenosis. There is currently no established treatment guideline. This case may be presented as a method for treatment of infrabrachial stenosis.

Conflict of interest: None declared

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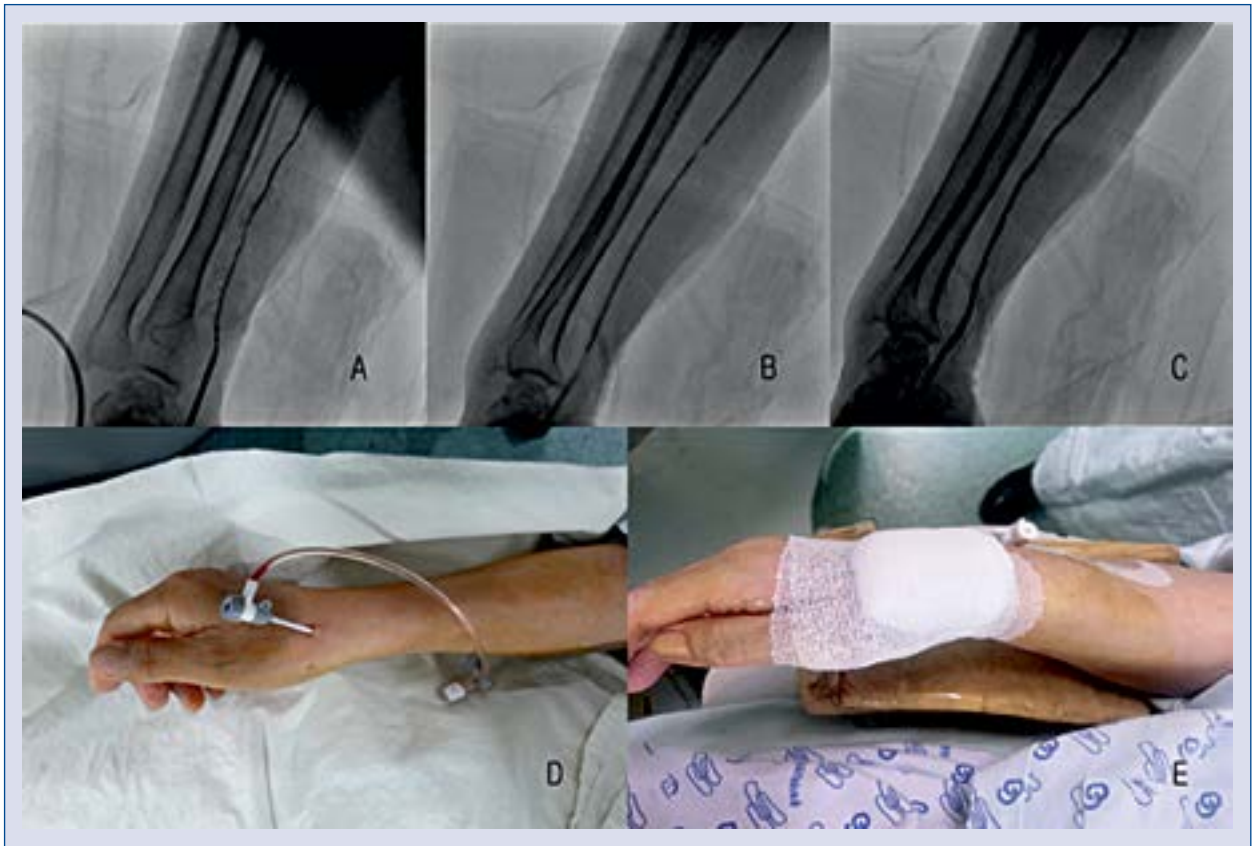
Figure 1. A. Severe stenosis of right radial artery, **B.** Balloon angioplasty of right radial artery; **C.** Final angiography of right radial artery; **D.** Right distal radial artery (snuffbox) puncture; **E.** Clear puncture site wound and hemostasis.

IMAGE IN CARDIOVASCULAR MEDICINE

# Intracoronary adenosine-induced torsades de pointes during fractional flow reserve measurement

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We report a 58-year-old woman with multiple cardiovascular risk factors presenting with stable angina. Echocardiography demonstrated normal systolic left ventricular function (ejection fraction 65%), with the absence of structural abnormalities. The patient was referred for coronary angiography, demonstrating angiographic intermediate stenosis in the proximal segment of the intermediate artery (IA) and left anterior descending artery (LAD) (Fig. 1).

Fractional flow reserve (FFR) measurement using adenosine (240 μg, volume 20 mL) was performed in the IA as well as LAD, both measures were negative, respectively 0.97 and 0.84 (Fig. 2). Following immediate intracoronary (IC) adenosine administration in the LAD the patient developed torsades de pointes (TdP) with circulatory collapse (Fig. 2). Intra-procedural electrocardiogram revealed supraventricular extra-systoles with short coupling
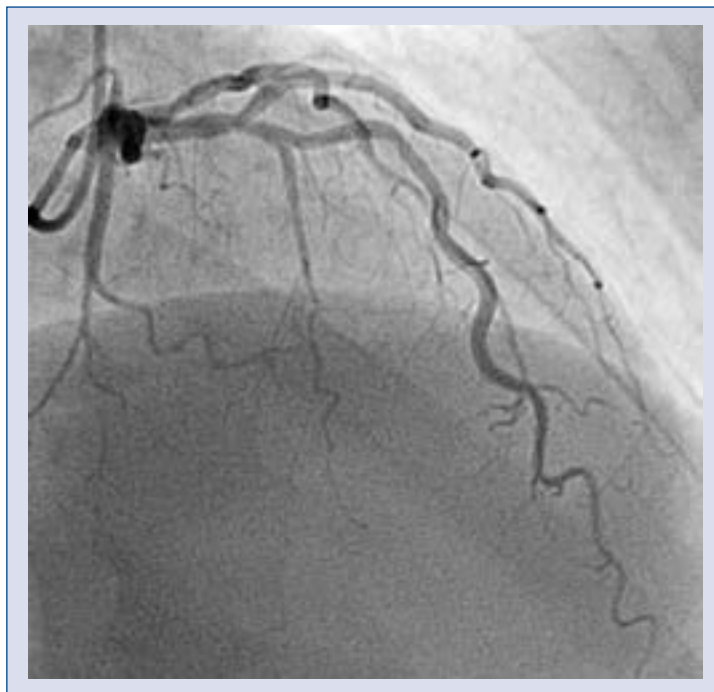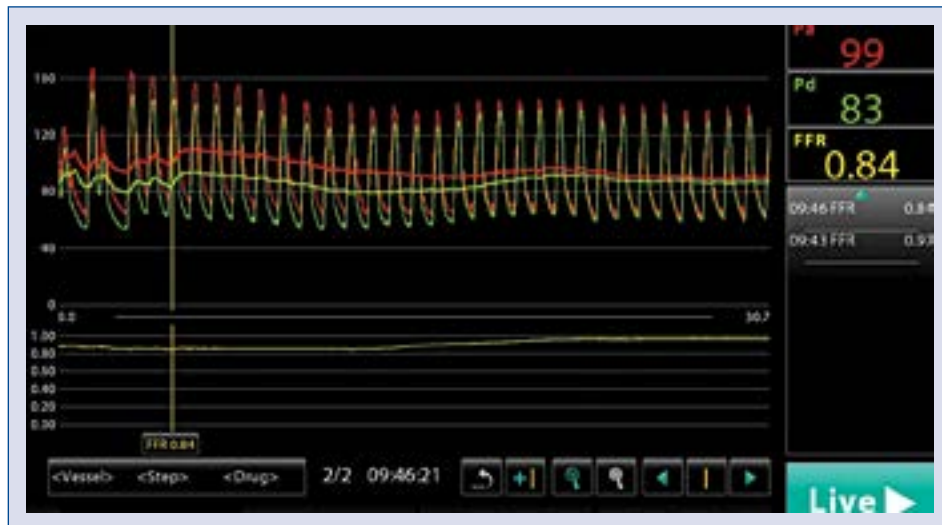

Figure 1. Coronary angiogram demonstrating angiographic intermediate stenosis of the proximal left anterior descending artery.

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Figure 2. Fractional flow reserve (FFR) console screenshot demonstrating FFR values measured in the intermediate artery (0.97) and left anterior descending artery (0.84).
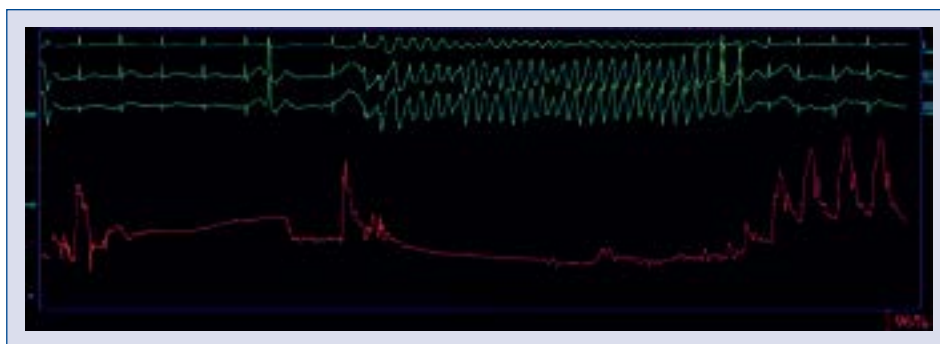
Figure 3. Electrocardiogram and arterial pressure following immediate intra-coronary adenosine administration for fractional flow reserve measurement of the left anterior descending artery, leading to torsades de pointes, with spontaneous self-termination.

at the time of adenosine injection, preceding the onset of TdP (Fig. 3).

Recognized adenosine-induced ventricular arrhythmias (VA) include (1) ventricular fibrillation (VF) in pre-excited atrial fibrillation, (2) polymorphic ventricular tachycardia (VT) in long Q-T syndromes, (3) degeneration of VT to VF and (4) non-sustained VT following termination of supraventricular tachycardia. VA induced by adenosine for FFR assessment is a rare complication described in 5 cases in available literature, occurring after adenosine administration in patients with FFR positive and negative lesions, suggesting a lack of causality between myocardial ischemia and the onset of VA. The mechanisms of VA induced by IC adenosine

administration might be related to adenosine dose and concentration, saline bolus injection volume, or pharmacological effect of adenosine (induces VF by "R on T" phenomenon with or without atrioventricular block). This is the first description of IC adenosine induced TdP with circulatory collapse. The mechanism seems to be at least partially linked to adenosine induced enhanced ventricular automaticity. FFR is currently considered the gold standard for the functional assessment of coronary stenosis; however, its measurement warrants the need of adenosine. The present case highlights that caution is of the essence when performing adenosine injection for an FFR measurement in a patient presenting extrasystoles with short coupling.

Conflict of interest: None declared

VIA MEDICA

IMAGE IN CARDIOVASCULAR MEDICINE

TAVI-in-TAVI — Is this the future?

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While an increasing number of younger patients with longer life-expectancy receive transcatheter aortic valve implantation (TAVI), one can assume that patients will outlive their bioprostheses. Thus, repeat interventions after TAVI are expected to rise dramatically. Presented herein are two cases of failed transcatheter heart valves (THV), treated effectively with transcatheter aortic valve-in-valve implantation (TAVI-in-TAVI).

The first, a 75-year-old woman that presented with clinical (exercise-induced dyspnea) and the echocardiographic (aortic valve area [AVA] was 0.75 cm^2, AVA index 0.43 cm^2/m^2, paravalvular regurgitation) symptoms of bioprosthetic valve failure (BVF), a Sapien XT 23 mm, which was implanted in 2013. Transfemoral TAVI-in-TAVI using the self-expandable Portico 23 mm, THV was performed resulting in a precise implantation (Fig. 1A). Post-operative echocardiography showed an excellent hemodynamic result (AVA 1.62 cm^2, AVA index 1.04 cm^2/m^2, without para-prosthetic leak), the patient reported symptom improvement and after 7 days was discharged.

The second, a 71-year-old man with clinical symptoms (exercise-induced dyspnea) of heart failure (NYHA III, ejection fraction 27%) and echocardiographically confirmed severe aortic regurgitation caused mostly by para-prosthetic leak (BVF, CoreValve 29 mm, implanted into the bicuspid valve in 2013). The Heart Team decided to proceed with transfemoral TAVI-in-TAVI using the balloon-expandable Sapien 3 29 mm bioprosthesis (Fig. 1B). Optimal implantation was achieved, postoperative echocardiography showed correct function of implanted bioprothesis; the para-prosthetic leak disappeared and gradient was < 10 mmHg. The patient was discharged after 6 days.

Taking into consideration the expanding recommendations and rapid growth of TAVI it can be expected that increasingly more BVFs and subsequent TAVI-in-TAVI procedures will occur. Hemodynamics with desired low trans-prosthetic gradient and possible future coronary access should be taken into consideration for optimal clinical effect. TAVI-in-TAVI procedures may also carry an elevated risk of debris embolizing to the brain, however, embolic protection devices can be a potential solution to decrease cerebral embolization and the associated neurological complications.

Conflict of interest: Szymon Jedrzejczyk, Piotr Scislo, Kajetan Grodecki and Bartosz Rymuza declare no conflict of interest. Janusz Kochman is proctor for Abbott and Zenon Huczek is proctor for Medtronic and Abbott.

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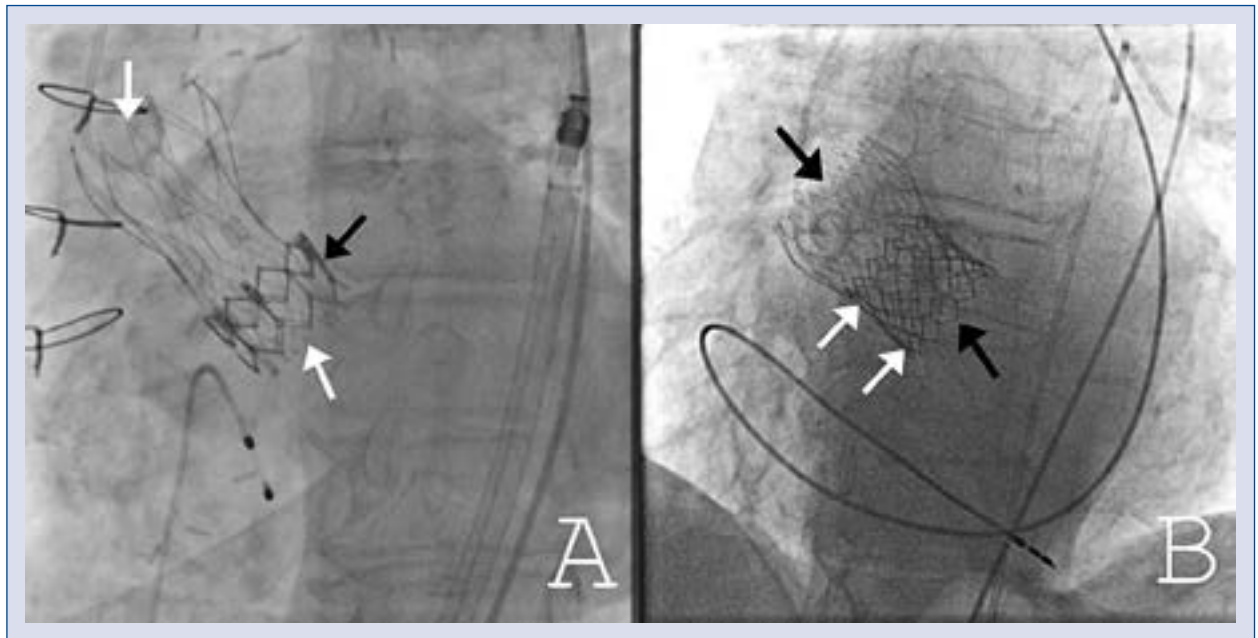
Figure 1. A. Portico 23 mm (upper and lower edge marked by white arrows) implanted into the failing Sapien XT 23 mm (marked by black arrow); **B.** Edwards Sapien 3 29 mm (marked by white arrows) implanted into CoreValve 29 mm (upper and lower edge marked by black arrows).

IMAGE IN CARDIOVASCULAR MEDICINE

VIA MEDICA

Extrapericardial cardiac tamponade due to massive retrosternal hematoma

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A 75-year-old female with a history of mechanical aortic and mitral valves, underwent aortic valve replacement due to dysfunction. During the fourth day after surgery, the patient developed sudden dyspnea at rest and chest discomfort. A physical examination exhibited jugular ingurgitation (Fig. 1A), tachycardia and hypotension. An emergent transthoracic echocardiography revealed a mass close to the right atrium (Fig. 1B, arrow). Because of the said finding, a computed tomography scan was performed which showed an 11 × × 9 cm retrosternal mass that was compressing the right cavities and posterior displacement of the heart (Fig. 1C, D, asterisk). She was brought back to surgery, where a massive retrosternal hematoma was discovered (Fig. 1E, F) with minimal active bleeding caused by three sternal suture points.

After evacuating the hematoma and controlling the bleeding, the patient had an uneventful recovery.

Retrosternal hematoma is a life-threatening condition, because it can result in an extrapericardial cause of cardiac tamponade, induced by heart compression. The most frequent etiologies are traumatic and post-surgical. Given the non-specificity of the signs and symptoms, a differential diagnosis between classic cardiac tamponade and this entity can be a challenge. Transthoracic echocardiography is the first imaging test for a differential diagnosis. A thoracic computed tomography scan can be helpful when the diagnosis is unclear and also allows rapid detection of active bleeding in some cases. Treatment consists of surgical removal of hematoma and also to control the bleeding, if present.

Conflict of interest: None declared

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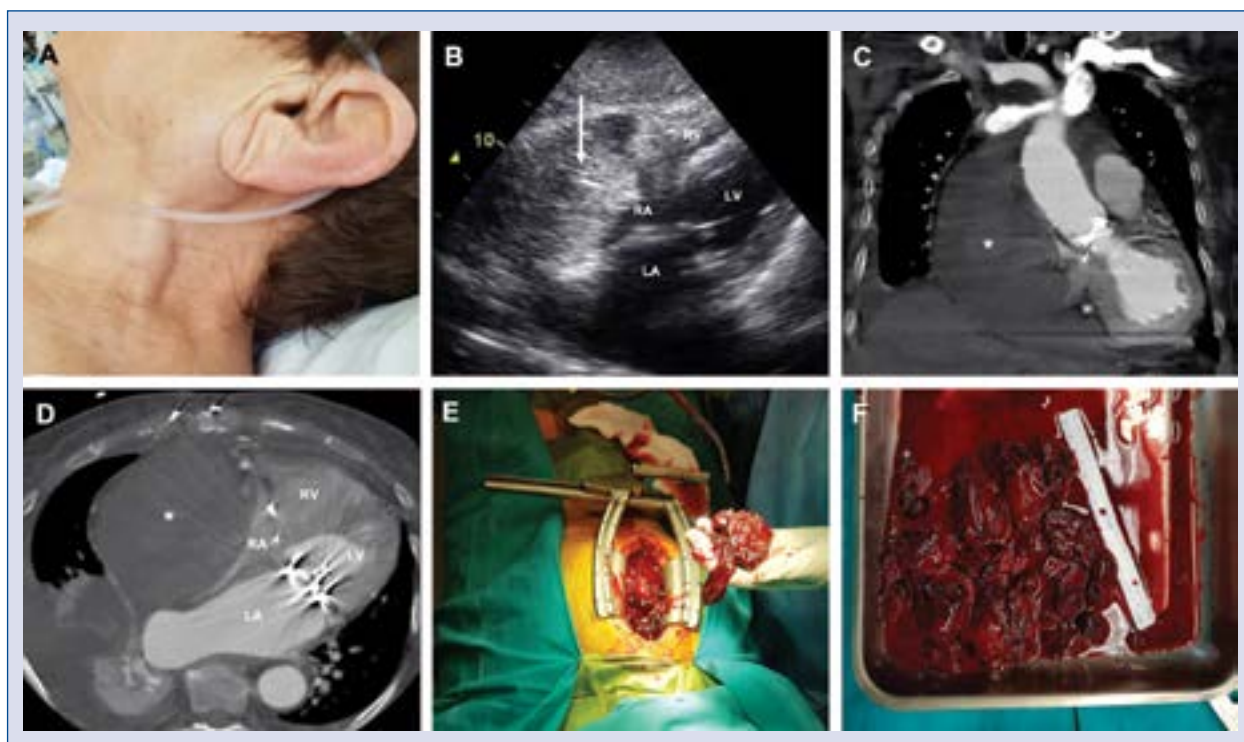
Figure 1. A. Jugular ingurgitation evidenced during physical examination; **B.** Transthoracic echocardiography, subcostal view. An echogenic mass close to the right atrium is observed (white arrow); LA — left atrium; LV — left ventricle; RA — right atrium; RV — right ventricle; **C.** Computed tomography scan, frontal axis, where a massive mass to the right of the left ventricle can be appreciated (white asterisk); **D.** Computed tomography scan, horizontal axis. The mass (white asterisk), compressing right cavities; **E.** The hematoma removed, in the hands of a surgeon; **F.** The hematoma removed.

IMAGE IN CARDIOVASCULAR MEDICINE

VIA MEDICA

Acquired double-chambered right ventricle

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The double chambered right ventricle is a rare complication following surgical closure of ventricular septal defect (VSD). Herein presented is a 53-year--old woman after surgical correction of VSD and the pulmonary stenosis in childhood. She also underwent pacemaker implantation due to third degree atrioventricular block. She was admitted to the documented clinic due to progressive exertional intolerance. Transthoracic echocardiography (TTE) discovered a large interventricular patch protruding into the right ventricle (RV) and causing a substantial or tight narrowing of the RV resulting in an intraventricular maximal pressure gradient of 110 mmHg. Also, insignificant left-to-right shunt (Fig. 1A, B) was detected.

The dimensions of the RV and the left ventricle were normal, and mild tricuspid regurgitation was demonstrated. The cardiac computed tomography confirmed a large protrusion of the patch closing VSD causing obstruction within the RV (Fig. 1C, D). The patient was referred to cardiac surgery. A large velour patch of 2.5 × 2 cm diameters was excised and subsequently the intraventricular septum was reconstructed by means of part of the patch. The postoperative period was uneventful. The follow-up TTE showed (Fig. 1E, F) normal dimensions and contractility of the RV; the pressure gradient within of the RV outflow was 12 mmHg. The patient was discharged in good condition.

Conflict of interest: None declared

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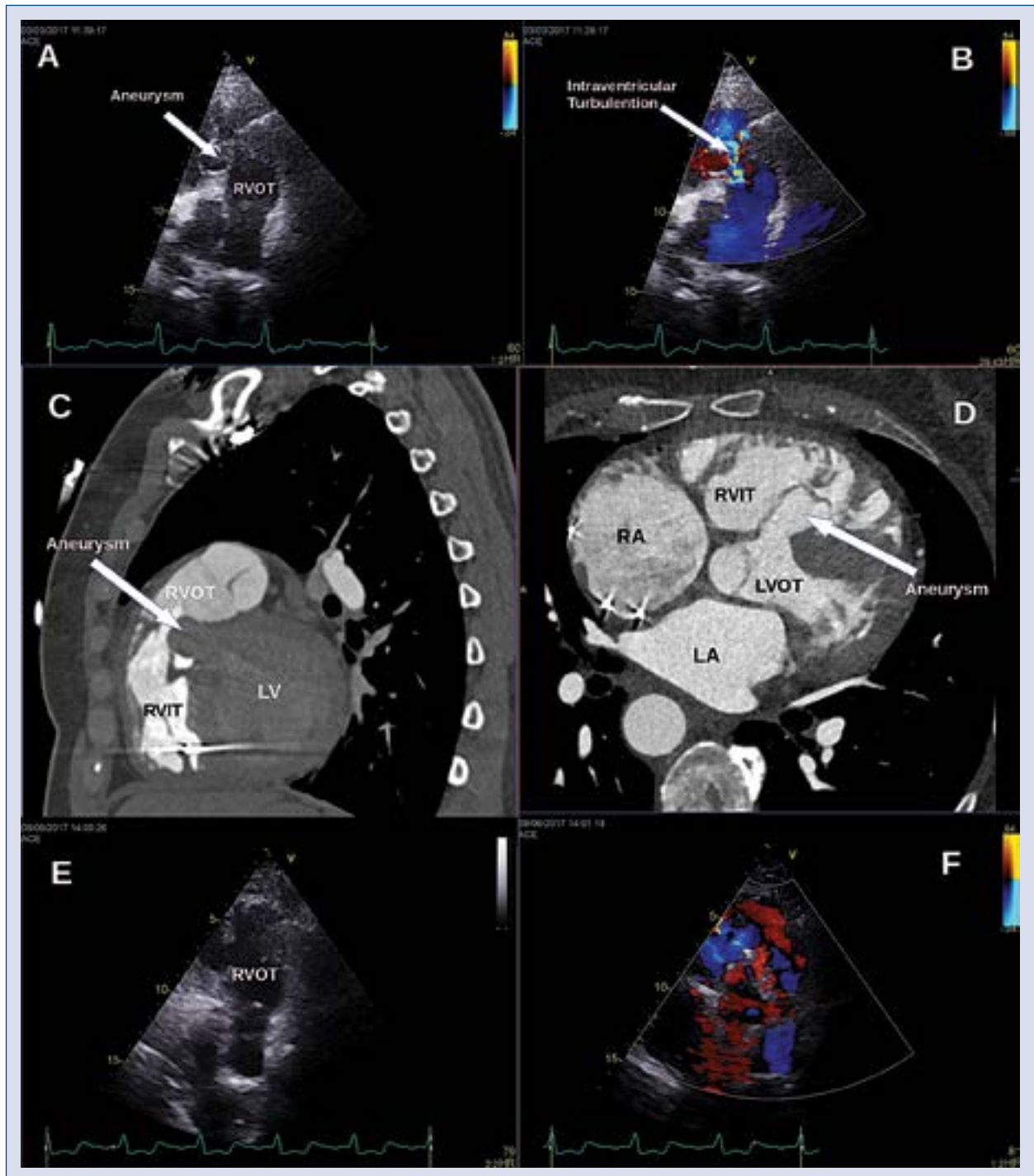
Figure 1. A, B. Transthoracic echocardiography (short axis) shows an aneurysm (arrow) and intraventricular turbulences; **C, D.** Cardiac computed tomography shows an aneurysm of a patch closing interventricular septal defect which is bulging into the right ventricle and causing significant stenosis of the right ventricle outflow; **E, F.** Transthoracic echocardiography (short axis) shows postsurgical right ventricular outflow tract (RVOT); LA — left atrium; LV — left ventricle RA — right atrium; RVIT — right ventricular inflow tract.

VM VIA MEDICA

IMAGE IN CARDIOVASCULAR MEDICINE

Myocardial ischemia with normal coronary angiography in a chronic kidney disease patient

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A 66-year-old male patient with end-stage chronic kidney disease (CKD), underwent renal transplant in 1997 that subsequently failed due to chronic allograft nephropathy, necessitating his return to hemodialysis. Since 2013 the patient was followed-up with myocardial perfusion imaging (MPI SPECT) because of atypical periodic chest discomfort but negative electrocardiogram and clinical examination. While the patient, being asymptomatic in successive MPI SPECT performed in the years 2013, 2014, 2016, and 2017 demonstrated progressive ischemia of the inferior/inferolateral cardiac wall and part of the myocardial apex, only partially reversible at rest. Bulls-eye displayed the perfusion abnormalities and assessed the affected myocardium. In the upper bulls-eye images (Fig. 1) a normal MPI SPECT is depicted in another individual for comparison, and in the lower images, the MPI SPECT of the present case demonstrated perfusion defects. The coronary arteries that perfuse each myocardial territory were also indicated. Coronary angiogram in 2017 showed no hemodynamically significant coronary abnormalities (Fig. 1), however myocardial blush was very low, indicating small vessels dysfunction. Due to a very small probability of significant epicardial stenosis no intravascular ultrasound or optical coherence tomography were performed.

Similar results were obtained by echocardiography which demonstrated a left ventricle with marginal systolic function, concentric hypertrophy and progressive hypokinesia of the mid-inferior septal and mid-inferior lateral regions and akinesia of basal inferior segments (**Suppl. Video S1**, 2012, 2016, 2017). Moreover, as part of the initial exam in 2013, a dipyridamole stress echocardiogram was performed in order to assess coronary flow reserve, which was relatively small in this patient (2.4).

The etiology of the discordant results in the present patient between MPI SPECT and angiography may be due to an irregular vasoconstrictor/vasodilatory coronary capability. Thus, episodes of increased coronary vasoconstriction may have resulted in progressive development of myocardial ischemia without obvious permanent coronary stenoses.

Conflict of interest: None declared

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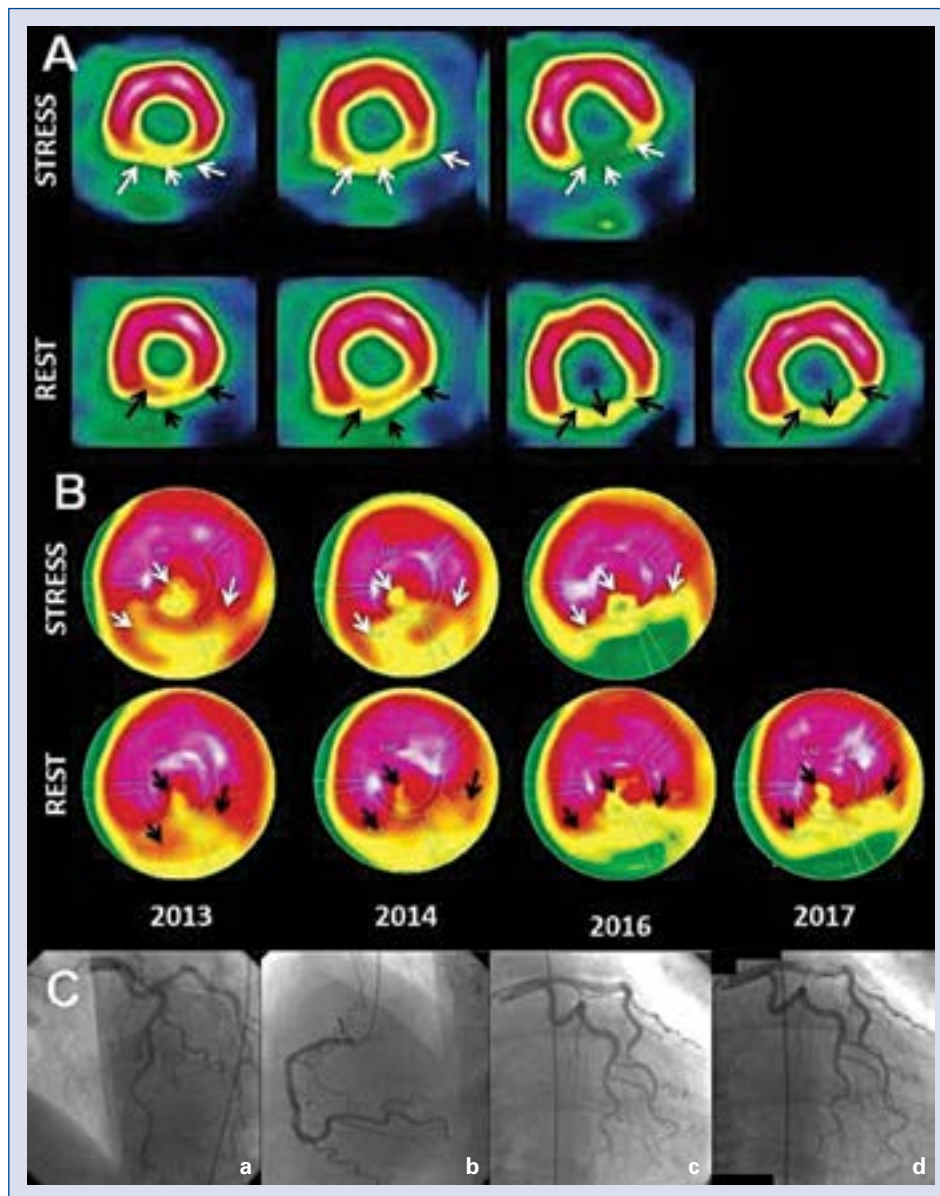
Figure 1. A. Shows the myocardium in the short axis; **B.** Bulls-eye myocardium perfusion images (MPI), depicting the entire myocardium. MPI scans were performed with a 1 day protocol using Technetium (^{99}mTc) Tetrofosmin in years, 2013, 2014, 2016, and 2017. Black arrows point to the hypoperfused areas during stress and the white arrows indicate at rest. The differences between the stress and at rest MPI demonstrate the progressive nature of the myocardial ischemia which appears to be less severe and reversible at rest in 2013 and more severe and partially reversible in 2016 and 2017. The parts of the ischemic myocardium predominantly involve the inferior, inferolateral and a segment of the apical wall (seen in the center of the Bulls-eye image); **C.** Coronary angiography (**a:** left coronary artery; **b:** right coronary artery; **c:** circumflex artery; **d:** circumflex artery with myocardial blush). There is no stenosis in any of the coronary arteries and with low myocardial blush.