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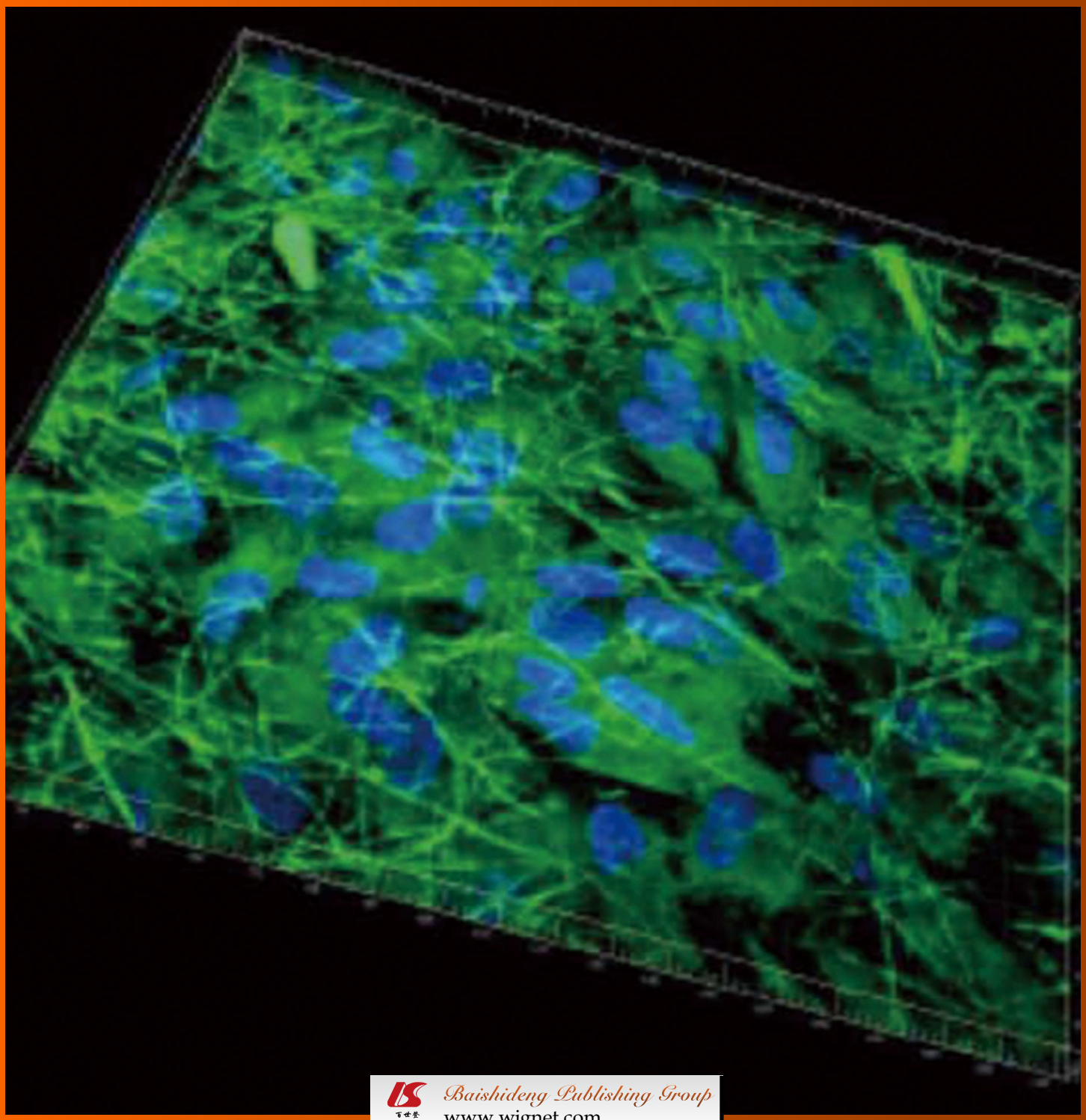

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FIELD OF VISION

Can we still learn from single center experience after PARTNER?

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Abstract

With the publication of the Placement of Aortic Transcatheter Valves (PARTNER) trial, transcatheter aortic valve replacement (TAVR) has undoubtedly become the gold standard for severe aortic stenosis in patients that are not suitable candidate for surgical aortic valve replacement (AVR). The PARTNER trial also showed that TAVR is non-inferior to AVR in high-risk patients. A recent publication by Ben-Dor *et al* evaluated the outcome of high-risk patients with severe aortic stenosis who were referred to their institution for participation to the PARTNER trial. Only a minority of patients made it in the trial and the majority of patient ended being treated medically. Some patients were also treated with AVR outside the trial. The outcomes of all these patients were stratified by the treatment they received (AVR, TAVR or medical therapy with or without balloon aortic valvuloplasty). The 3 groups were different in their baseline characteristics. Ben-Dor *et al* found that patients treated medically had greater mortality than patients treated with TAVR or AVR. The survival of patients treated with TAVR was similar to those treated with AVR. Independent predictors of mortality were also found from their analysis. In this commentary, we discuss the finding of this study and compare it with the current literature.

Key words: Aortic stenosis; Transcatheter aortic valve replacement; Aortic valve replacement; Balloon aortic valvuloplasty

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COMMENTARY ON HOT TOPICS

We have read with great interest the recent manuscript by Ben-Dor *et al*[1] evaluating the outcome of high-risk patients with severe aortic stenosis (AS) referred to their institution for a trial of transcatheter aortic valve replacement (TAVR) stratified by the treatment they received, and believe it is worth discussion. Symptomatic severe AS is a deadly and incapacitating disease when left untreated. For many decades, surgical aortic valve replacement (AVR) has been considered the treatment of choice because of its ability to improve survival and symptoms. It was however shown that approximately one third of patients with severe symptomatic AS do not beneficiate from AVR because of multiple of reasons[2]. Balloon aortic valvuloplasty (BAV), although less invasive than AVR is only palliative. More recently, TAVR has been shown to be superior to medical therapy (including BAV) in patients that are not candidate for AVR[3] and to be non-inferior to AVR in high-risk patients[4].

Ben-Dor *et al*[1] reviewed 900 patients who were referred for TAVR evaluation (PARTNER trial) between April 2007 and May 2011. These patients had severe AS defined by a mean gradient $\geqslant$ 40 mmHg or valvular area $<$ 1 cm[2]. Only 13% (n = 19) of AVR and 4.9% (n = 29)

of medially treated patients were enrolled in the PARTNER trial. The PARTNER trial as been described in details[3,4] but in summary consisted of two parallel studies. The cohort A consisted of patients at high-risk for AVR (risk of 30-d mortality ⩾ 15%) that were randomized to TAVR (from a trans-femoral or trans-apical approach) *vs* AVR. The cohort B included patients that were deemed non-operative based on an estimated risk of morality or major irreversible morbidity of ⩾ 50%; which were randomized to TAVR *vs* medical therapy (including possible BAV). Ben-Dor *et al*[1] evaluated the outcomes of patients treated in their institution stratified by the treatment they received. Medical treatment was adopted in 66.1% of patients (n = 595), among whom 345 patients also had BAV, 17.6% (n = 159) had TAVR and 16.3% (n = 146) had AVR. Groups were significantly different in their baseline characteristics with younger and healthier patients undergoing AVR and sicker patients with lower ejection fraction and higher BNP value in the medical treatment group. The STS score was significantly different across groups with values of 8.5%, 11.8% and 12.1% for AVR, TAVR and medical treatment respectively (P < 0.001). The transcatheter heart valve (THV) used for TAVR was the Edwards SAPIEN THV (Edwards Life Sciences, Irvine, CA, United States). A trans-femoral (TF) approach was used in 69.1% (n = 110) of cases and a trans-apical (TA) approach in 30.9% (n = 49).

In their study, Ben-Dor *et al* found a 1-year mortality of 21.2%, 21.3% and 36.4% for patients treated with TAVR, AVR and medical therapy respectively (P < 0.001). In the medical therapy group, patient who had a BAV performed had higher mortality (55% *vs* 34%, P < 0.01). Thirty-day mortality was 11.7%, 12.8% and 10.1% for TAVR, AVR and medical therapy respectively. The STS score predicted 30-d mortality was 11.8%, 8.4% and 12.3% while the logistic Euroscore predicted 41.2%, 25.6% and 43.1% for TAVR, AVR and medical therapy respectively. Patients with STS score ⩾ 15 had a significantly greater mortality (59.2%) compared with those with STS score < 15 (35.2%). In the entire cohort, atrial fibrillation and renal failure were found to be independent predictor of mortality. When stratified by the treatment received, independent predictor of mortality were STS score and renal failure for patients undergoing TAVR, renal failure and NYHA class Ⅳ for patients undergoing AVR and renal failure, pulmonary artery pressure and aortic systolic pressure for patient treated medically.

This is a retrospective, non-randomized single center study evaluating outcomes of patients referred for TAVR, stratified by the treatment received. Multiple limitations from the trial should be discussed. Because of the absence of randomization, the 3 groups compared in this study represent very different populations. The medical therapy group consisted mainly of patients that were not randomized in the PARTNER trial, most likely representing patients that are just too sick to beneficiate from TAVR (often referred has the cohort C patients). In fact, 30-d mortality was higher (10.1%) in these patients compared to the medically treated patients from the PARTNER trial (2.8%). What is surprising is that the 1-year mortality of medically treated patients in this study is lower (36.4%) then the 49.7% observed in PARTNER. These findings are hard to explain and should raise questions about the clinical follow-up of this study, which is not detailed in the manuscript. An alternative is that some patients received medical therapy because they had asymptomatic aortic stenosis, hence no indication for valve replacement. TAVR and AVR patients were also different. Non-operable patients received TAVR and lower risk patients that would not qualify for the PARTNER trial based on their risk were included in the AVR group. Despite these differences, the 1-year mortality was similar between both groups. Interestingly, the STS predicted 30-d mortality for AVR was lower than what was observed, a finding that is in contradiction with the observations from the PARTNER trial. TAVR patients, despite all being part of the PARTNER trial had a 30-d mortality (11.7%) that was worse than in the trial (5.0% for non operative and 3.4% for high-risk patients). Given the absence of randomization between the AVR and TAVR groups in this study, it would be unadvisable to conclude to the equivalence of these two approach solely based on this study. In a recent meta-analysis of 16 TAVR studies using VARC criteria and regrouping 3519 patients[5], the 1-year mortality was 22.1%, similar to what observed by Ben-Dor *et al*[1]. Significant outcomes such as vascular complications, stroke, acute kidney injury are absent from this present trial and could put some light on the early mortality.

Medically treated patients are driving the results of their multivariable analysis. Also, their multivariable analysis for TAVR and AVR patients are over fitted in relation to the number of events. Renal failure was found to be a predictor of mortality for all patients and is consistent with the current literature[6,7]. They however did not define was they considered as renal failure and did not report on acute kidney injury which has been described as an independent predictor of mortality after TAVR and AVR[8]. The proportion of patient on dialysis was also not reported in this study. The PARTNER trial excluded patients on chronic dialysis and patients with a serum creatinine ⩾ 3 mg/dL. It would have been interesting to know the proportion of these patients represented in the AVR and in the medically treated groups. No data on frailty was presented in this study. Frailty is known to be an independent predictor of mortality after open-heart surgeries[9], is often a cause of non-operability and has now been characterize in the VARC-2 consensus document[10]. Frailty could be an unmeasured confounder that could alter the results of this multivariable analysis.

In conclusion, this single center, non-randomized study is globally consistent with the PARTNER trial[3,4] and larger multicenter registries[11-13]. TAVR is already recognized as the gold standard therapy for non-operative patients that cannot beneficiate from aortic valve replacement. The biggest challenge remaining will be to identify

patients that are dying with severe AS and not from AS and that would not improve after TAVR. New trials (PARTNER 2, SURTAVI)[14] are already randomizing moderate-risk patients to AVR *vs* TAVR, searching for potential benefits of TAVR in these patients.

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FIELD OF VISION

Pulmonary endarterectomy in chronic thromboembolic pulmonary hypertension: How can patients be better selected?

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Abstract

Chronic thromboembolic pulmonary hypertension (CTEPH) comprises organizing thrombotic obstructions in the pulmonary arteries by nonresolving thromboemboli, formation of fibrosis and remodeling of pulmonary blood vessels. Surgical pulmonary endarterectomy (PEA) is the therapy of choice for patients with surgically accessible CTEPH, which leads to a profound improvement in hemodynamics, functional class and survival. Selecting the candidates that will benefit from surgery is still a challenging task. Criteria for surgical suitability have been described but the decision-making for or against surgical intervention remains still subjective. The optimal characterization of the reciprocal contribution of large vessel and small vessel disease in the elevation of pulmonary vascular resistance is crucial for the indication and outcome of PEA. Recently, Toshner *et al* intended to validate the partition resistance into small and large vessels compartments (upstream resistance: Rup) by the occlusion technique in the preoperative assessment of PEA. We discuss the advantages and disadvantages of Rup and compare it with other hemodynamic predictor to evaluate operative risk in CTEPH patients.

Key words: Pulmonary endarterectomy; Operability; Chronic thromboembolic pulmonary hypertension; Pulmonary artery occluded pressure; Pulmonary vascular resistance

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COMMENTARY ON HOT ARTICLES

We read with great interest the recent article by Toshner *et al*[1] describing the analysis of pressure decay curve after pulmonary arterial occlusion (between the moment of occlusion and the pulmonary artery occluded pressure, PAOP) to test if the occlusion technique distinguished small from large vessel disease in chronic thromboembolic pulmonary hypertension (CTEPH).

Pulmonary endarterectomy (PEA) of major, lobar, and segmental pulmonary arteries branches is the mainstay of therapy for patients with CTEPH. The best surgical results are achieved with complete endarterectomy and early postoperative reduction of pulmonary vascular resistance (PVR) to < 500 dyn.s.cm^{-5}[2,3]. The cause of residual pulmonary hypertension in most cases is from concomitant small vessel disease, with three possible scenarios: (1) predominant obstructions of small subsegmental elastic pulmonary arteries; (2) classic pulmonary

arteriopathy of small muscular arteries and arterioles distal to non-obstructed elastic pulmonary artery; and (3) pulmonary arteriopathy of small muscular arteries and arterioles distal to partially or totally obstructed elastic pulmonary artery[4,5].

The optimal characterization of the contribution of large vessel and small vessel disease to the elevation of afterload and its influence on the hemodynamic severity is crucial for the preoperative assessment and outcome of PEA[6-8].

An approach for the identification of distal vasculopathy in CTEPH is the analysis of pressure decay curve after pulmonary arterial occlusion (between the moment of occlusion and the pulmonary artery occluded pressure, PAOP)[9]. Such curves are made of a first fast component, which corresponds to the reduction of flow through arterial resistance, and a slower component, which corresponds to the emptying of compliant capillaries through a venous resistance. This biexponential fitting of the pressure decay curve allows identification of an inflection point (Poccl), from which one calculates an upstream resistance (Rup), essentially determined by the resistive properties of the large pulmonary arteries, and a downstream resistance determined by the cumulated resistance of small arterioles, capillaries and veinules. Rup is calculated as follows: Rup (%) = 100 × (mPAP-Poccl)/(mPAP-PAOP). In patients with small-vessel arteriopathy the Poccl pressure was higher (a longer time was required for the pressure to reach PAOP), and therefore the Rup was lower. Patients with CTEPH and Rup value < 60% appear to be at highest risk[9].

To test the hypothesis that the occlusion technique is able to discriminate large vessel organized thrombus from distal vasculopathy. Toshner *et al*[1] performed occlusion pressures on patients with operable CTEPH, distal inoperable CTEPH and post-PEA residual CTEPH. They also undertook measurements in patients with idiopathic or connective tissue associated pulmonary arterial hypertension (PAH), as additional controls, where more diffuse vasculopathy is traditionally accepted. They employed both, the standard flow-directed measurement and the wire-directed approach. The latter involved a wire being passed into an alternative segmental artery and subsequently being floated into a distal artery. The authors found that Rup as measured by the occlusion technique is increased in operable predominantly proximal CTEPH when compared with inoperable CTEPH and idiopathic PAH. However, they obtained a higher Rup cutoff value compared to Kim *et al*[9]: 79% (sensitivity 100%, specificity 57%) *vs* 60% (sensitivity and specificity 100%) and they did not explain the differences of the Rups values, including the values of the two operate patients who died (68% and 73%). They recognized that the occlusion technique would not interrogate the correct range of vessel caliber and would mislabel a significant portion of resistance in these small vessels as upstream. This can be supported by the fact that the idiopathic PAH and inoperable CTEPH cohorts had a much higher Rup than would be expected if resistance had been accurately partitioned into clinically relevant small and large vessels[1]. Finally, they proposed the multiple wire-directed measurements in conjunction with the flow-directed one, in order to provide additional information on disease heterogeneity in CTEPH, although they recognize their data does not support the clinical use of this technique in routine assessment.

Beyond the embolic or thrombotic hypothesis of pathogenesis of CTEPH, once vessel obliteration is sufficient to cause increase of pulmonary arterial pressure, a process of pulmonary vascular remodeling like idiopathic PAH lesions is started which self-perpetuates the progression of pulmonary hypertension[10,11]. The presence of large-vessel remodeling process of thrombus organization and small vessel disease might create a wide spectrum of dynamic (steady and pulsatile) afterload in CTEPH patients[12,13]. We proposed the study of Zup, a novel hemodynamic index. Zup is calculated by (mPAP-dPAP) × 100/(mPAP-PAOP), where mPAP and dPAP are mean and diastolic pulmonary arterial pressure, respectively[14]. mPAP is the time-averaged PAP throughout cardiac cycle length and it is accurately described by cardiac output, total PVR and right atrial pressure. Previous studies have established a link between the steady and pulsatile component of PA pressure by estimating mPAP from systolic PAP (sPAP) and dPAP ('two-pressure model')[15-17]. The geometric mean of sPAP and dPAP was the most precise estimate of mPAP (mPAP2 = sPAP × dPAP). sPAP and dPAP mainly depend on total PVR and pulmonary artery stiffness and wave reflection. Increasing total PVR results in both sPAP and dPAP increase while increasing pulmonary artery stiffness and wave reflection generate a wider pPAP without significant mPAP change[14,18,19]. A more proximal occlusive site by the fibrotic organized thromboembolic material incorporated into the native vascular intima causes a higher pulmonary artery stiffness. Stiffening of proximal pulmonary arteries could increase characteristic impedance and wave reflection (higher upstream afterload), increasing total PVR but with a lower dPAP, a faster pressure decay profile and Zup increase. Therefore, the balance between mPAP and dPAP provides a rapid tool to describe the functional afterload status of a CTEPH patient, since their absolute contributions on Zup value are higher than PAOP[14].

Unlike the partition method described by Kim *et al*[9] and used by Toshner *et al*[1], Zup index can be obtained directly from hemodynamic data without assumptions or fitting, and is affected by the extent and localization of anatomic obstruction, vascular remodeling and microvascular disease, setting a wide spectrum of dynamic afterload (steady and pulsatile components)[14]. According to the univariate analysis, we showed that low Zup value (cut-off point < 47%) predicted mortality after PEA with a sensitivity of 100% and a specificity of 78%. The latter increased to 86% when we analyzed the subgroup of 23 patients with higher preoperative PVR (> 9 wood units, median of the cohort), by contrast PVR lost its capacity to predict mortality in this group[14]. In contrast

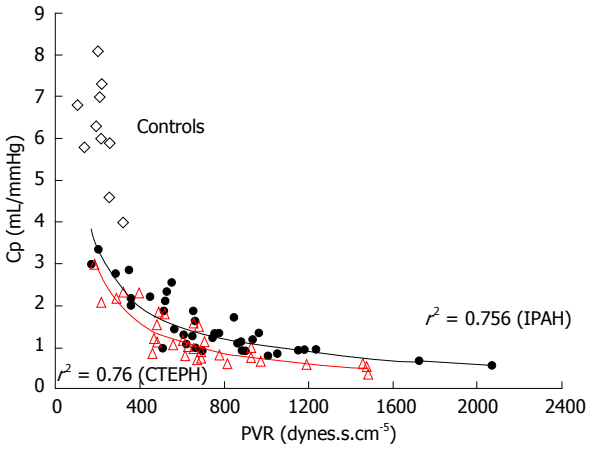

Figure 1 Inverse proportional relation between pulmonary vascular resistance and arterial compliance for operable chronic thromboembolic pulmonary hypertension and Idiopathic pulmonary arterial hypertension patients (Hollow diamonds represent ten normal subjects without pulmonary hypertension). CTEPH: Chronic thromboembolic pulmonary hypertension; IPAH: Idiopathic pulmonary arterial hypertension; PVR: Pulmonary vascular resistance.

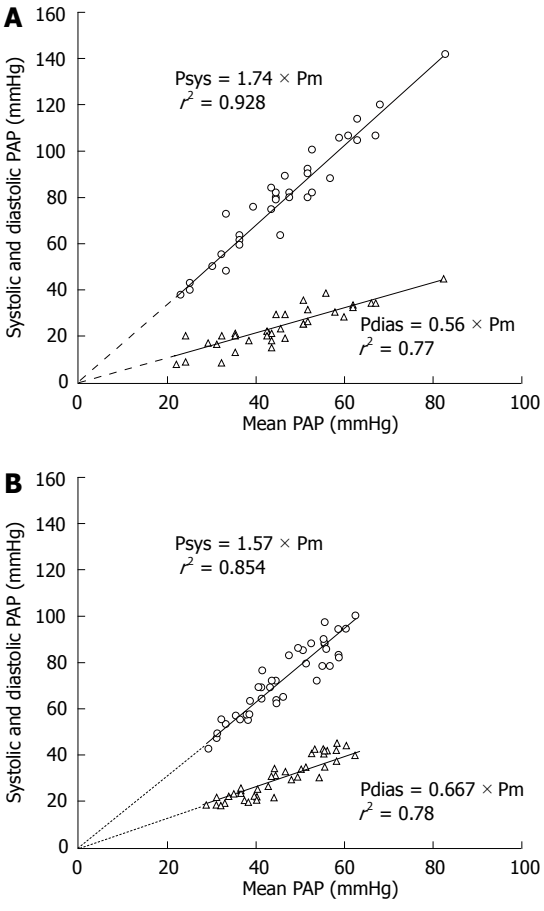

Figure 2 Interrelationship of pulmonary arterial pressures for operable chronic thromboembolic pulmonary hypertension and idiopathic pulmonary arterial hypertension patients with similar pulmonary vascular resistance. A: Chronic thromboembolic pulmonary hypertension; B: Idiopathic pulmonary arterial hypertension.

with Toshner *et al*[1], the Zup value in idiopathic PAH patients was significantly lower than in operable CTEPH patients (43% ± 15% *vs* 57% ± 15%)[20]. Preoperative operable CTEPH is characterized by a more predominant wave reflection, explaining the lower pulmonary vascular capacitance with a downward and leftward displacement of the PVR-Capacitance curve of the CTEPH patients and a disproportionate increase in sPAP and decrease of dPAP with respect to idiopathic PAH cohort (Figures 1 and 2)[20-22].

CTEPH has been recognized as a "dual" pulmonary vascular disorder consisting in a major vessel vascular remodeling process of thrombus organization combined with a small vessel vascular disease[23,24]. PEA is the therapy of choice for patients with surgically accessible CTEPH[25]. The optimal characterization of the reciprocal contribution of large vessel and small vessel disease in the elevation of PVR is crucial for the indication and outcome of PEA[26]. This determination requires the development of diagnostic techniques capable of more objectively partitioning the central surgically correctable component of the PVR from the peripheral component[26]. Although pulmonary arterial occlusion waveform analysis has emerged as a possible way of quantifying the degree of small-vessel disease, it only evaluates the steady component of the afterload and it is possible that this technique inaccurately partitioned resistance into clinically relevant small and large vessels. We proposed a novel hemodynamic index that considers both steady (PVR) and pulsatile (Capacitance) components of the right ventricular afterload simultaneously and could therefore be a complementary tool to improve the risk assessment for PEA in patients with CTEPH.

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TOPIC HIGHLIGHT

Treating blood pressure to prevent strokes: The age factor

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Abstract

The importance of systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP), on the incidence of coronary heart disease (CHD) and stroke are known. However, the importance of blood pressure (BP)-age shifts regarding the stroke incidence is not clearly known. The BP changes with the advancement of age from the predominance of DBP in the young to the predominance of SBP in the old. This change is due to the stiffening of the large arteries as a result of the aging process and the replacement of the elastic fibers with collagen fibers. This change results in the loss of compliance and the elastic recoil of these vessels leading to increase in pulse wave velocity, central SBP and widening of pulse pressure leading to an increased incidence of CHD and strokes. It has been demonstrated epidemiologically that the SBP rises linearly with age, whereas the DBP rises up to the age of 45-50 years, and then begins to decline after the age of 60 years leading to a progressive widening of PP. Several studies have shown an inverse relationship between DBP and CHD, whereas no such relationship has been demonstrated for stroke. However, a recent study showed an inverse relationship with DBP and stroke when it dropped below 71 mmHg in subjects 50 years of age or older. In contrast, there was a positive association between BP and stroke when both SBP and DBP were ⩾ 71 mmHg. These findings suggest that in treating systolic hypertension in the elderly to reduce stroke risk, attention should be paid on the potential harm of low DBP and the widening of PP regarding CHD and stroke. The implications of BP shifts with age and the potential risks of low DBP regarding the risk of stroke will be discussed in this concise review.

Key words: Age; Blood pressure; Pulse pressure; Stroke; Age blood pressure interaction

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INTRODUCTION

The treatment of hypertension has become quite complicated lately. In the old days the physician had to measure the blood pressure (BP) in the office using a mercury sphygmomanometer, and if the BP was ⩾ 140/90 mmHg, he initiated treatment. Today, the office BP is disputed as being representative of the person's actual BP, since new BP entities have been discovered, such as white coat hypertension (WCH), and masked hypertension (MH) by using ambulatory BP monitors (ABPM). These two entities have opposite meanings where WCH is the condition with elevated BP in the doctor's office or clinic and normal BP outside the doctor's office measured with either ABPM or a home BP monitor[1]. In contrast, MH is the condition with normal BP at the doctor's office and elevated BP outside the doctor's office measured by the same means[2]. Also, the use of the mercury sphygmomanometer, a gold standard for the diagnosis and treatment of hypertension has been deemphasized lately and soon will be extinct due to environmental reasons and the development of new instruments such as ABPMs and semi-automatic aneroid sphygmomanometers for home BP

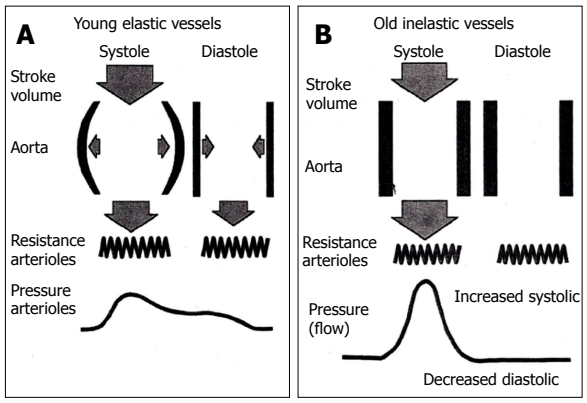

Figure 1 In a young person the elastic aorta expands during systole and absorbs part of the stroke volume. A: This figure depicts the function of the central aorta of a younger person during systole and diastole. During systole, the elastic aorta with each cardiac stroke volume (top filled arrow) is dilated and functions as a reservoir. As a result, not all stroke volume (SV) is transmitted distally. During diastole the elastic recoil of the aorta expels the remnant original SV to distal arteries and arterioles. This function results in a smooth contour of the arterial pulse wave and a narrow pulse pressure (PP) (bottom); B: In an older person, the aorta has lost most of its elasticity resulting in a reduction of its reservoir or capacitance function, resulting in the expulsion of almost the entire SV to the distal arteries with practically no diastolic blood flow (top filled arrow. This results in a distortion of the arterial pulse wave (bottom), an increase in systolic blood pressure, a decrease in diastolic blood pressure, and a widening of PP. Adapted with permission from Franklin *et al*[12].

monitoring. In addition, the emphasis on treating hypertension has now been shifted to systolic BP (SBP), since SBP is the most prevalent BP in older age[3], and some authors have gone into the extreme, stating that "systolic blood pressure is all that matters"[4]. This is a significant change from the early years where the focus was on treating the diastolic BP (DBP), because SBP was considered a normal development of the aging process. Even the reports of the Joint National Committees on the detection, evaluation, and treatment of high blood pressure did not emphasize the treatment of SBP till their 5th report in 1993[5]. Recently it has been suggested that in treating hypertension, the age of the subject should be considered since DBP is the predominant BP of the young and SBP is the predominant BP of the older person. The DBP rises from childhood till the age of 50 years and then begins to decline after the age of 60 years, whereas SBP rises continuously from adulthood to the old age. The significance of BP change with age was first pointed out by the Framingham Heart Study[6]. Previous studies used the BP in correlation with various age subgroups to determine its association with the risk of cardiovascular disease[7-9]. It has been suggested, that if age was used as a continuous variable, this could have offered a clearer picture at which age the relative importance of SBP begins to exceed DBP with respect to stroke incidence. This concept was tested in a recent study[10].

PATHOPHYSIOLOGY OF ARTERIOSCLEROSIS AND SYSTOLIC HYPERTENSION

The large arteries in young persons possess two functions, one to act as conduits transferring the blood to vital organs and tissues, and the other to act as cushions to smooth out the pulsatile blood flow produced by the intermittent contractions of the heart into a continuous and steady blood flow[11]. However, as the person ages these functions of the large arteries are modified by arteriosclerosis, which is a consequence of the aging of blood vessels. The primary cause of arteriosclerosis is the fragmentations of the elastic lamellae which become thinned, frayed, and are replaced with collagen tissue. The fracturing of the elastic fibers is the result of the fatiguing effect produced by the cycling stress of the pulsatile blood flow. In a young person the elastic aorta expands during systole and absorbs part of the stroke volume[12]. During diastole it recoils back and sends the retained blood volume distally, thus converting the intermittent blood flow into a continuous steady flow (Figure 1A). In an elderly person, the elasticity and compliance of the aorta is lost[12] and most of the stroke volume is transmitted distally during systole with practically no blood flow during diastole (Figure 1B). The direct result of this function is an increase in SBP, a decrease in DBP, and a widening of pulse pressure (PP). This process is accelerated in the presence of hypertension. These latter changes in the older person lead to acceleration of the pulse wave velocity, which is the main diagnostic characteristic of arteriosclerosis. In addition, the morphology of the pulse wave changes (Figure 2). The pressure wave is a composite of the incident (forward) wave generated by the contraction of the heart and the reflected (backward) wave generated by the small muscular arteries and arterioles. In young persons the reflected wave travels slower and reaches the central aorta in early diastole leading to augmentation of the DBP, which is useful for the perfusion of coronary arteries. In older persons the reflected wave travels a lot faster and reaches the central aorta at the end of systole thus augmenting the central aortic SBP, which increases the pressure load on the left ventricle and leads to the development of left ventricular hypertrophy. In addition, the increased central SBP is associated with a higher incidence of cardiovascular disease (CVD) and stroke complications[13].

TREATMENT OF SBP: THE AGE FACTOR

The brain is protected against stroke through wide fluctuations of BP by the autoregulation of cerebral circulation. Cerebral autoregulation (CA) is the intrinsic capacity of the cerebral vessels to maintain constant cerebral blood flow (CBF) for the metabolic needs of the brain[14]. The CBF is also regulated, besides BP, by the arterial CO_2 level of the brain as well. The CA consists of two components, the static and the dynamic component. The static CA regulates CBF during gradual and progressive increases in BP[15], whereas the dynamic CA regulates the CBF during rapid changes in BP[16]. It has been demonstrated that the CBF remains constant through wide changes in mean arterial pressure (MAP) ranging from 60 to 150 mmHg (Figure 3) or from 40 to 125 mmHg from

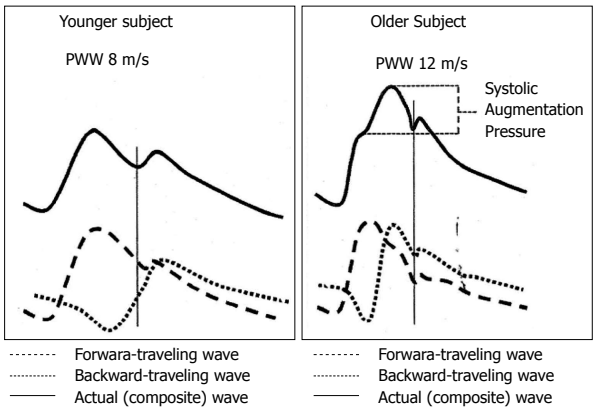

Figure 2 This figure depicts the configuration of the arterial waveforms in the younger person (left) and the older person (right). The arterial waveforms are composite waves (top heavy line), composed of a forward traveling wave (dashed line) and a backward traveling reflective wave (dotted line). The vertical line represents the closure of the aortic valve. The top solid line indicates the peak systolic blood pressure (SBP) in the younger person (left) and the older person (right) together with the augmentation pressure. The reflected wave in the younger person (left), returns to the aortic root early in diastole augmenting the diastolic blood pressure and improving the coronary circulation. In the older person (right), the reflected wave returns to the aortic root late in systole, thus augmenting the SBP and increasing the left ventricular outflow pressure leading to left ventricular hypertrophy. Due to the arterial stiffness in the older person, the pulse wave velocity is increased (12 m/s) compared to the younger person (8 m/s). Adapted with permission from Franklin *et al*[12].

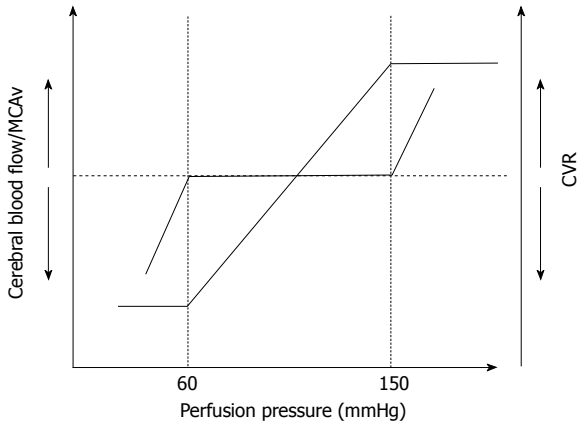

Figure 3 This figure depicts the cerebral blood flow autoregulation and the range of perfusion pressure. An autoregulatory plateau is seen between 60 to 150 mmHg of mean arterial pressure (MAP). This autoregulatory plateau is maintained through changes in cerebral vascular resistance (CVR). Once the limits of autoregulation are reached, CVR cannot correct for further changes in pressure as demonstrated by the MAP limits of < 60 mmHg (lower limit) and > 150 mmHg (upper limit). Adapted with permission from Lucas *et al*[14].

a recent study using transcranial Doppler[14]. These studies show that the CBF is not seriously affected even with very low DBP, and this could, perhaps, explain the lack of a J curve effect for stroke incidence with low DBP in contrast to the heart which is susceptible to a J curve effect with low DBP[17]. However, a recent study showed that there might be a J curve effect with DBP < 71 mmHg in older persons[10]. This study demonstrated the impact of age on the importance of SBP and DBP for stroke risk. In this study, 68 551 subjects 19 years to 78 years old from several European countries free of CVD and not taking antihypertensive drugs at entry of study, were followed for 13.2 years. The subjects were divided in 4 age groups, 19-39 years, 40-49 years, 50-59 years, and 60-78 years. When the SBP and DBP were considered separately, both pressures ⩾ 71 mmHg were significantly associated with a higher stroke risk across the 4 age groups ($P < 0.0001$). In contrast, when the SBP and DBP were considered together, the SBP became no significant in the 19-39 year olds, and the DBP became no significant for stroke risk in the 50-59 and 60-78 year olds. However, for DBP < 71 mmHg there was an inverse relationship between DBP and stroke incidence, which became significant in the 60-78 year olds. Regarding the association of MAP and stroke risk, this was strongest in the younger ages, since MAP represents mostly the DBP, and it declined with advancing age, becoming no significant after the age of 69 years for men and the age of 73 years for women. In addition, there was a significant association between PP and stroke risk, which was independent of age and remained significant after multivariate adjustments. In this study the BP was measured at the doctor's office and might have missed subjects with WCH, or MH. However, the significance of WCH as a cardiovascular risk is debatable, because the pressure load on the heart is minimal, since WCH is elevated only during the visit at the doctor's office, and medical treatment is not associated with further lowering BP and may lead to hypotension[1]. On the contrary, MH is associated with increased cardiovascular complications, since the pressure load on the heart is prolonged. Its discovery is difficult, since the hypertension is diagnosed by office BP measurement and the BP in MH is normal at the doctor' s office. Therefore, its discovery is difficult and is, usually, identified by home BP measurements or by ABPM. Treatment of MH is absolutely necessary[2].

DISCUSSION

New evidence suggests that there is an age factor on the importance of SBP and DBP regarding the incidence of fatal and nonfatal strokes[10]. In this study, participants with SBP and DBP ⩾ 71 mmHg, had a higher risk for stroke until the age of 62 years, after which, only the SBP remained significant. In addition to age, there was also a sex effect between the MAP and stroke risk up to the age of 69 years for men and 73 years for women. Similar findings in shifts of BP with age have been reported from the Framingham study for coronary heart disease (CHD), but not stroke[18]. In the study by Vishram *et al*[10], in persons < 50 years of age, the DBP was the strongest predictor for stroke risk, whereas in persons ⩾ 60 years of age the SBP was the strongest predictor. In persons 50-59 years of age, both pressures were equally important. Another significant finding of this study was the J curve effect of DBP with stroke risk for participants with a DBP < 71 mmHg. In this group there was an increase in stroke risk and this became significant after the age

of 60 years. Such an association is not commonly seen with strokes in contrast to CHD[17,19-23], although it has been reported by some investigators[24]. This is important when treating elevated SBP in the elderly. Kannel *et al*[25] showed that the incidence of cardiovascular events increased with a decrease in DBP < 80 mmHg, when the SBP remained ⩾ 140 mmHg. Similarly Fagard *et al*[24] suggest that the antihypertensive treatment in subjects with systolic hypertension should be stopped when the DBP reaches the level of 55 mmHg to prevent further widening of PP and the higher risk for cardiovascular complications. In the study by Kannel *et al*[25], the 10-year risk ratio of cardiovascular events for men and women was 1.22 (95%CI: 0.97-1.50) with PP 46-55 mmHg, and 1.66 (95%CI: 1.32-2.07) with PP 55.5-136 mmHg. The significance of PP as a stroke risk in elderly subjects has been demonstrated besides Vishram *et al*[10], by other investigators as well[7,18,26-28]. The higher cardiovascular risk with wide PP has been attributed to the increased pulsatile burden on the heart and blood vessels produced by the wide PP[27]. In this report from the Framingham study, the age and sex of 4993 participants were tracked for 28 years and demonstrated that the SBP and PP became higher with older age, and were higher in older women compared to men of similar age[27]. In a large meta-analysis of older subjects with systolic hypertension it was shown that the PP was more important in inducing cardiovascular complications than the MAP[28]. Given that both PP and chronological age are positively associated with cardiovascular risk and strokes, PP may be regarded as an index of arterial aging. This could suggest that the biology of aging differs between men and women, and has been suggested that the chronological age as determined by calendar time, is distinct from the biologic age, which is a progressive and irreversible process of deterioration of the vitality of organ systems[26]. In addition, an inverse association was found between PP and telomere length suggesting that the biologic age of persons with wide PP is more advanced than their chronological age would indicate[26]. With respect to age-BP interrelationship regarding the risk of stroke, it appears that both SBP and DBP are important up to the age of 50 years after which, the significance of SBP supersedes that of DBP. In addition, in treating the SBP in older persons attention should be paid to the level of DBP not to be lower than 71 mmHg, although this finding was observed in a small number of subjects. Based on other studies, the risk of cardiovascular events increased when the DBP dropped to 55 mmHg[24], or to 80 mmHg, if the SBP was ⩾ 140 mmHg[25]. The current National and International guidelines recommend reducing BP to < 130/80 mmHg in high risk subjects regardless of age[29,30]. However, some investigators suggest that the SBP and DBP not to be lower than 130-139 mmHg and 80-90 mmHg, respectively[31], whereas others propose to test the safety of SBP in the range of 130-150 mmHg[21]. Regarding drug selection for the treatment of hypertension in the elderly, drugs that block the rennin-angiotensin-aldosterone system (RAAS) and calcium channel blockers (CCB) either alone or in combination are preferable as first line treatment, since these drugs have been shown to be more effective in lowering the central SBP and PP than b-blockers (atenolol) and thiazide diuretics[32]. Also, a recent Japanese study showed that the combination of RAAS blockers with CCBs was more effective in reducing the BP and cardiovascular complications than high dose RAAS blockers in high risk elderly hypertensive patients with or without renal disease[33]. Older b-blockers like atenolol are not as effective in lowering central aortic SBP and preventing strokes[34]. However, it would be useful, if the BP besides the doctor's office, is also measured by ABPM to diagnose the presence of WCH, where antihypertensive treatment is, usually, not necessary[1], and especially to diagnose MH, where treatment is necessary, since MH is associated with increased cardiovascular complications and death[2].

In summary, this concise review has demonstrated that the SBP increases linearly with the advancement of age and becomes the dominant factor for stoke risk after the age of 60 years. In contrast, the DBP is more dominant in younger persons and its rise with age levels off at the age of 50 years and begins to decline after the age of 60 years. In addition, new evidence suggests a J-curve effect for stroke risk with DBP < 71 mmHg or lower and the importance of wide PP as a risk factor for cardiovascular events. Finally, in treating the SBP in the elderly, drugs that block the RAAS in combination with CCBs is the best regimen in lowering the SBP. Hwever, care should be taken not to lower the DBP below 55 mmHg, because the risk for stroke and cardiovascular complications increases significantly.

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ORIGINAL ARTICLE

Cardiogenic differentiation of mesenchymal stem cells on elastomeric poly (glycerol sebacate)/collagen core/shell fibers

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Abstract

AIM: To facilitate engineering of suitable biomaterials to meet the challenges associated with myocardial infarction.

METHODS: Poly (glycerol sebacate)/collagen (PGS/collagen) core/shell fibers were fabricated by core/shell electrospinning technique, with core as PGS and shell as collagen polymer; and the scaffolds were characterized by scanning electron microscope (SEM), fourier transform infrared spectroscopy (FTIR), contact angle and tensile testing for cardiac tissue engineering. Collagen nanofibers were also fabricated by electrospinning for comparison with core/shell fibers. Studies on cell-scaffold interaction were carried out using cardiac cells and mesenchymal stem cells (MSCs) co-culture system with cardiac cells and MSCs separately serving as positive and negative controls respectively. The co-culture system was characterized for cell proliferation and differentiation of MSCs into cardiomyogenic lineage in the co-culture environment using dual immunocytochemistry. The co-culture cells were stained with cardiac specific marker proteins like actinin and troponin and MSC specific marker protein CD 105 for proving the cardiogenic differentiation of MSCs. Further the morphology of cells was analyzed using SEM.

RESULTS: PGS/collagen core/shell fibers, core is PGS polymer having an elastic modulus related to that of cardiac fibers and shell as collagen, providing natural environment for cellular activities like cell adhesion, proliferation and differentiation. SEM micrographs of electrospun fibrous scaffolds revealed porous, beadless, uniform fibers with a fiber diameter in the range of 380 ± 77 nm and 1192 ± 277 nm for collagen fibers and PGS/collagen core/shell fibers respectively. The obtained PGS/collagen core/shell fibrous scaffolds were hydrophilic having a water contact angle of 17.9 ± 4.6° compared to collagen nanofibers which had a contact angle value of 30 ± 3.2°. The PGS/collagen core/shell fibers had mechanical properties comparable to that of native heart muscle with a young's modulus of 4.24 ± 0.7 MPa, while that of collagen nanofibers was comparatively higher around 30.11 ± 1.68 MPa. FTIR spectrum was performed to confirm the functional groups present in the electrospun scaffolds. Amide Ⅰ and amide Ⅱ of collagen were detected at 1638.95 cm^{-1} and 1551.64 cm^{-1} in the electrospun collagen fibers and at 1646.22 cm^{-1} and 1540.73 cm^{-1} for PGS/collagen core/shell fibers respectively. Cell culture studies performed using MSCs and cardiac cells co-culture environment, indicated that the cell

proliferation significantly increased on PGS/collagen core/shell scaffolds compared to collagen fibers and the cardiac marker proteins actinin and troponin were expressed more on PGS/collagen core/shell scaffolds compared to collagen fibers alone. Dual immunofluorescent staining was performed to further confirm the cardiogenic differentiation of MSCs by employing MSC specific marker protein, CD 105 and cardiac specific marker protein, actinin. SEM observations of cardiac cells showed normal morphology on PGS/collagen fibers and providing adequate tensile strength for the regeneration of myocardial infarction.

CONCLUSION: Combination of PGS/collagen fibers and cardiac cells/MSCs co-culture system providing natural microenvironments to improve cell survival and differentiation, could bring cardiac tissue engineering to clinical application.

Key words: Mesenchymal stem cells; Cardiac cells; Co-culture; Cardiac patch; Poly (glycerol sebacate); Core/shell fibers.

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INTRODUCTION

Myocardial infarction (MI), known as "heart attack" leads to loss of cardiomyocytes and the injured heart tissue is replaced by scar tissue[1]. When the scar is large enough to interfere with the hearts normal rhythm, heart failure occurs. Nearly 8 million Americans each year experience myocardial infarction[2] and must cope with the consequences of compromised heart muscle function. The myocardial tissue lacks significant intrinsic regenerative capacity to replace the lost cells[3]. Moreover, the relative shortage of organ donors to recipients, and the ineligibility of many heart patients for transplantation revamp the search for new strategies to repair the injured myocardium. The current methods employed include cardiac restraint devices like acorn corcap heart mesh (knitted polyester)[4], marlex mesh (polypropylene)[5], and Merselene mesh (knitted polyester)[6]. Cell transplantation therapies have shown promise for improving heart function after myocardial infarction[7]. However, the cell engraftment efficiency is low due to significant loss of cells from the site of injury following transplantation.

One promising approach is to prevent the increase of heart failure after myocardial infarction is the implantation of engineered cardiac patch at the site of infarction. In addition of having enough elasticity for mechanical support, an ideal cardiac patch material must provide an excellent milieu for cell survival. Furthermore, the ideal biomaterial should be capable of being safely replaced by newly formed tissue and also degrade appropriate time period without producing any toxic products[8]. Naturally derived materials used in experimental or clinical treatment of infarcts include tumor-derived basement membrane matrix gel (matrigel)[9], alginate[10], collagen[11], laminin[12], fibrin[13] and decellularized extracellular matrix (ECM)[14], all of which can enhance cell and tissue function in the myocardial region. They provide a natural substrate for cellular attachment, proliferation, and differentiation in its native state. For the above-mentioned reasons, natural polymers like collagen could be a favourable substrate for tissue engineering applications[15,16]. Furthermore, collagen is the most abundant protein in the human body, and imparts structural integrity and tensile strength to tissues.

Tissue disruption following injury requires collagen for the repair and restoration of structure and function. In addition, collagens have a low antigenicity, being only weakly immunogenic largely due to their homology across species and are biodegradable due to their proteinaceous nature[17]. Using matrigel, a collagen-based multicomponent mixture of ECM proteins and growth factors, Zimmermann *et al*[18] have established one of the most convincing models of three-dimensional cardiac cell cultures, where differentiation status and functional parameters are similar to that of native myocardium. However, there are concerns about Matrigel's safety because matrigel and basement membrane matrix are known to enhance tumorigenesis and tumor growth *in vivo*[19-22]. In 1997, Eschenhagen *et al*[23] reported for the first time, an artificial heart tissue, which was termed engineered heart tissue (EHT). The embryonic chick cardiomyocytes were mixed with collagen solution and allowed to form gel for EHT. By culturing the cardiomyocytes in the collagen matrix, they produced a spontaneously and coherently contracting 3D heart tissue construct *in vitro*. However, poor mechanical supportive ability of collagen gels was a major drawback associated with this approach. Hence a combination of poly (glycerol sebacate) (PGS) with collagen was suggested with an objective to overcome this drawback for cardiac tissue engineering (CTE). The elastomer PGS, recently developed for soft tissue engineering[24,25], represents a feasible substrate from the mechanical perspective; Collagen favours enhanced cell adhesion and prevents cells loss at the site of implantation. The conventional electrospinning technique is to dissolve the polymer in a solvent, which evaporates during the spinning process. However, this approach is not pragmatic with PGS. Although, there are solvents available for dissolving PGS[26], its low molecular weight results in such a low solution viscosity that even with a high concentration solution, the electrospinning of PGS fibers cannot occur. Hence, it was necessary to develop a core/shell electrospinning pro-

cess[27] to produce PGS fibers with a protective shell polymer. The combination of PGS/collagen core/shell fibers with a unique ECM like topography has been suggested to be a potential cardiac patch material for MI. Fabricated core/shell material; the core material is solely responsible for mechanical properties, whereas the shell material is responsible for extrinsic factors like cell adhesion and proliferation. The optimal cell source to create an engineered myocardial patch should be easy to harvest, proliferative, non-immunogenic and has the ability to differentiate into mature, functional cardiomyocytes. Studies have shown that after expansion, stem cells can be directed to differentiate into cardiomyogenic lineages[28,29]. Cardiomyocytes have natural contractile and electrophysiological properties, are difficult to obtain, to expand, and are allogenic. In contrast, bone marrow derived stem cells have the ability to differentiate into any desired cell type in the presence of cues and are non-immunogenic, making them an ideal cell source.

In this study, we hypothesize that a combinatorial approach of PGS/collagen core/shell fibrous patch material and stem cell therapy is of potential interest for the treatment of heart failure rather than either strategy alone. Our approach takes advantage of the ability of an elastomeric biomaterial sheet comprising of PGS/collagen fibers to act as a flexible patch; with this approach: (1) Cells would remain adhered to the nanofibrous patch preventing cell loss and providing a more site-directed repair mechanism. It is increasingly accepted that physical cues play a key role in cell growth and tissue assembly[30,31]. These signals are important in stem cells during self-renewal, proliferation, and differentiation; (2) A softer substrate and the ability to tune the mechanical properties within a given range could be advantageous as cell differentiation was shown to be affected by substrate stiffness[32]; (3) Additionally, it has been estimated that a cell number on the order of one billion would need to be replaced in patients with heart failure[33]. The present study proposed PGS/collagen core/shell fibrous scaffold similar to cardiac ECM like topography, which promotes in situ regeneration and homing of cells; thereby reducing the number of requisite cells, is desirable for cardiac tissue engineering.

MATERIALS AND METHODS

Fabrication of core/shell fibers

PGS was synthesized by the procedure described by Wang *et al*[34] a mixture of glycerol and sebacic acid in the ratio of 1:1 was reacted at 120 ℃ under nitrogen for 24 h. The pressure was then reduced to 40 m Torr and the reaction held at 120 ℃ for 48 h to synthesise PGS. Collagen type I (8%) was dissolved in 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFP) (Aldrich Chemical Company, Inc., St. Louis, United States) to form shell solution and PGS (15%) was dissolved in same solvent to form the core solution. The coaxial spinneret had an inner diameter of 1 mm and an outer diameter of 2.0 mm was designed such that the fluids were immiscible before exiting the nozzle. Fluid was loaded to the nozzle by two syringe pumps (KD Scientific Inc., MA, United States) that provide a constant-volume flow rate of 0.3 mL/h for core solution and 1.2 mL/h for shell solution. A high voltage electric field (DC high voltage power supply from Gamma High Voltage Research, FL, United States) of 15 kV was applied at the tip of the spinneret. A collector plate was placed at a distance of 15 cm from the tip of the spinneret to collect core/shell fibers. Collagen nanofibers were also fabricated using 8% w/v solution in HFP separately. The electrospinning conditions used were 1.2 mL/h flow rate, 12 cm distance between the needle tip and collector plate and 12 kV voltage supply. The fibers produced were subsequently vacuum dried to remove the residual solvents. The fibers were then cross-linked using 50% glutaraldehyde (Sigma) vapour for 24 h in order to improve its mechanical stability.

Material characterization

The surface morphology of electrospun nanofibrous scaffolds was studied under scanning electron microscope (JEOL JSM-5600LV) at an accelerating voltage of 10 kV, after gold coating (JEOL JFC-1200 fine coater, Japan). For calculating the fiber diameter of the nanofibers from the SEM images, n = 10 fibers were chosen randomly on each of the scaffolds. For each scaffold material n = 5 samples were chosen for measuring the fiber diameter. The average fiber diameter along with SD was then analyzed from the SEM images using image analysis software (Image J, National Institutes of Health, United States). Functional groups present in the scaffolds were analyzed using Fourier Transform Infrared (FTIR) spectroscopic analysis on avatar 380, (Thermo Nicolet Waltham, MA, United States) over a range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. The hydrophobic or hydrophilic nature of electrospun fibers was measured by sessile drop water contact angle measurement using VCA optima surface analysis system (AST products, Billerica, MA, United States). Tensile properties of electrospun fibrous scaffolds were determined using a tabletop tensile tester (Instron 3345, United States) at 10 mol/L load capacity under dry testing conditions. Rectangular specimens of dimensions 10 mm × 20 mm were used for testing at a rate of 10 mm/min. The data's were recorded at room conditions 25 ℃ and 34% humidity. In order to avoid uncertainty of data, only the results of those samples which have failed in the centre; giving a dog bone like appearance have been used for the stress-strain curves calibration. The data's of those samples which have failed at the grips, where the load was being applied, were not employed for the calculation. The tensile stress-strain curve was drawn using excel sheet.

Cell culture

The electrospun fibers collected on round glass cover slips of 15 mm in diameter, were placed in a 24-well plate with stainless steel rings to prevent lifting on the cover slips.

The fibers were sterilized under ultraviolet (UV) light for 2 h, washed thrice with phosphate buffered saline (PBS) for 15 min each, in order to remove any residual solvent and prevent cytotoxicity from glutaraldehyde. The fibers were subsequently immersed in Dulbecco's modified Eagle's medium (DMEM) overnight before cell seeding. The rabbit cardiac cells were isolated using collagenase treatment. The rabbit heart was fragmented into tiny pieces and washed thoroughly with 2× and 3× antibiotic solutions made in PBS, thrice for 30 min. Subsequently, it was followed by treatment with 1% collagenase in PBS at 37 ℃ for 30 min. The fragmented tissues were cultured in DMEM media supplemented with 10% FBS (GIBCO Invitrogen, United States) and 1% antibiotic and antimycotic solutions (Invitrogen Corp, United States) in a 75 cm^2 cell culture flask to isolate cardiomyocytes. The culture medium was changed once in every 2 d. MSCs (PT-2501, Lonza, United States) were cultured in low glucose DMEM media supplemented with 10% FBS (GIBCO Invitrogen, United States) and 1% antibiotic and antimycotic solutions (Invitrogen Corp, United States) in a 75 cm^2 cell culture flask. Cells were incubated in CO_2 incubator at 37 ℃ at 5% CO_2. Before seeding the cells were detached by adding 1 mL of 0.25% trypsin containing 0.1% EDTA. Detached cells were centrifuged, counted by trypan blue assay using a hemocytometer and seeded on the scaffolds. The scaffolds were separated into three groups-the control tissue culture plate (TCP), collagen nanofibrous scaffolds and the PGS/collagen core/shell fibers. These were further segregated into (1) co-culture scaffolds, onto which both MSCs and cardiac cells were seeded in the ratio of 1:1 at a seeding density of 10 000 cells per well (5000 MSCs:5000 cardiac cells); (2) positive control scaffolds, onto which cardiac cells were seeded at the same seeding density of 10 000 cells per well and (3) The final batch comprised of the negative control scaffolds, onto which MSCs were seeded at the same seeding density of 10 000 cells per well.

Cell proliferation

The cell proliferation on different scaffolds was analyzed using MTS assay (CellTiter 96 Aqueous One solution reagent, purchased from Promega, Madison, WI, United States). The rationale behind the MTS assay involves the reduction of yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium] in MTS to form purple formazan crystals by the dehydrogenase enzymes secreted by mitochondria of metabolically active cells. The formazan dye shows absorbance at 490 nm and the amount of formazan crystals formed is directly proportional to the number of cells. After 5 d of cell seeding, the media was removed from the well plates and the scaffolds were washed in PBS. The scaffolds were then incubated in a 1:5 ratio mixture of MTS assay and serum free DMEM medium for 3 h at 37 ℃ in a 5% CO_2 incubator. After the incubation period, the samples were pipette out into 96 well plates. The absorbance reading was then taken at 490 nm using a microplate reader (Fluostar Optima, BMG Lab Technologies, Germany). The same procedure was repeated for day 10 and day 15 samples.

Cell morphology

The cell morphology was analyzed using SEM. After 15 d of seeding cells on the scaffolds, the media was removed from the wells and the samples were fixed with 3% glutaraldehyde in PBS for 3 h. The scaffolds were then rinsed with distilled water for 15 min and then dehydrated with a series of ethanol gradients starting from 30% to 50%, 75%, 90% and 100% (v/v). Subsequently the samples were treated with Hexamethyldisilazane (HMDS) solution (Sigma) and allowed to air-dry at room temperature in the fume hood. The samples were then gold coated and the cells morphology was analyzed using SEM.

Expression of cardiac marker protein

To observe whether the MSCs co-cultured with cardiac cells have undergone cardiogenic differentiation, immunofluorescent staining of the selected proteins of cardiomyocytes was performed. For confocal analysis, the cells were fixed with 100% ice cold methanol for 15 min. The samples were then washed with PBS once for 15 min and incubated in 0.5% Triton-X solution for 5 min to permeabilize the cell membrane. Non-specific sites were blocked by incubating the cells in 3% BSA (Sigma) for 1 h. Following which primary antibodies α-actinin and troponin-T (Sigma) were added into separate wells, at the dilution of 1:100 and incubated for 90 min at room temperature. This was followed by washing the samples thrice with PBS for 15 min, to remove the excess unbound primary antibodies; followed by incubation for 60 min with Alexa Fluor 488 secondary antibodies (Invitrogen) in the dilution of 1:250 at room temperature. The samples were again washed thrice with PBS for 15 min. Negative controls were also employed in each analysis to delete the disturbance of the primary or secondary antibody. The cell nuclei were stained using 1:5000 dilution of 4,6-diamidino-2-phenylindole hydrochloride (DAPI; Sigma) for 30 min at room temperature. The cells were again washed with PBS thrice to remove any excess staining. The samples were then removed and mounted over glass slides using H-1000 vectashield mounting medium (Vector Laboratories, United States). The edges of the coverslips were sealed using fluoromount. The samples were then viewed using fluorescence microscopy for the cardiac marker protein expression (Olympus FV 1000).

Double immunofluorescent staining was further performed on the co-culture scaffolds to confirm the differentiation of MSCs into cardiomyocytes. The MSC-cardiac cells co-culture cells cultured on TCP, collagen nanofibers and PGS/collagen core/shell fibers were stained with MSC specific marker CD 105 (abcam, United States) in the dilution 1:100 for 90 min. at room temperature; prior to which the non-specific sites were blocked with 3% BSA. This was followed by the addi-

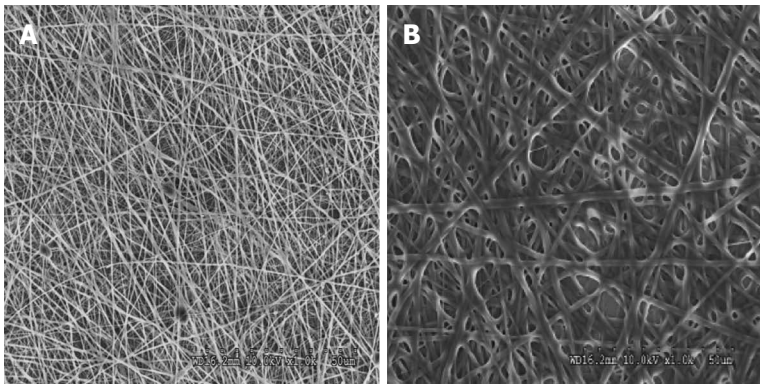

Figure 1 Scanning electron microscope image showing the fiber morphology of (A) collagen fibers with a fiber diameter of 380 ± 77 nm (B) poly (glycerol sebacate)/collagen core/shell fibers with a fiber diameter of 1192 ± 277 nm at 1000 × magnification.

Table 1 Tensile properties of collagen and poly (glycerol sebacate)/collagen fibrous membranes

Membrane	Tensile stress (MPa)	Tensile strain at maximum load	Tensile strain at break	Young's modulus (MPa)	Water contact angle mean ± SD
Collagen	2.79	13.59%	47.92%	30.11	30 ± 3.2
PGS/collagen	2.06	57.87%	83.65%	4.24	17.9 ± 4.6

PGS: Poly (glycerol sebacate).

tion of secondary antibody Alexa Fluor 488 (green) in the dilution 1:250 for 60 min. at room temperature. The samples were washed with PBS thrice to remove the excess staining. The samples were then treated with cardiac specific marker protein actinin in the dilution 1:100 for 90 min at room temperature. This was followed by the addition of the secondary antibody Alexa Fluor 594 (red) (Invitrogen) in the dilution 1:250 for 60 min. at room temperature. The samples were washed with PBS thrice to remove the excess staining. The samples were then incubated with DAPI in the dilution 1:5000 for 30 min at room temperature. The samples were then removed and mounted over a glass slide using vectashield mounting agent and examined under the fluorescent microscope (Olympus FV 1000).

Statistical analysis

For each experiment $n = 5$ samples were tested and all the data presented are expressed as mean ± SD and were analyzed using Student's *t*-test for the calculation of statistical significance. Differences were considered statistically significant at $P \leqslant 0.05$ and $P \leqslant 0.01$.

RESULTS

Material characterization

Electrospinning is a versatile technique for bio-mimicking the natural environment similar to that of ECM for better cell adhesion and tissue growth[35]. It has been estimated that a cell number on the order of one billion would need to be replaced in patients with heart failure[33]. The proposed scaffold with ECM like topography is desirable as it has been suggested that the ECM scaffold itself contains components that are chemo-attractants to endogeneous stem cells[36]. Collagen lacks mechanical integrity upon hydration in an electrospun form for tissue engineering. Without cross-linking electrospun structure it does not have sufficient mechanical strength. Previously established methods of cross-linking with glutaraldehyde, was successful in increasing the strength of electrospun structures[37]. Moreover, the existence of collagen on the surface provides uninterrupted cell recognition signals with polymer degradation, which is essential for cell function and development[38]. SEM micrographs (Figure 1) of electrospun fibrous scaffolds revealed porous, beadless, uniform fibers with a diameter in the range of 380 ± 77 nm and 1192 ± 277 nm for collagen fibers and PGS/collagen core/shell fibers respectively. The PGS/collagen core/shell fibers have a contact angle of 17.9 ± 4.6° compared to collagen 30 ± 3.2°. FTIR spectrum was carried out to confirm the functional groups present in electrospun scaffolds. Amide I and amide Ⅱ of collagen were detected at 1638.95 cm^{-1} and 1551.64 cm^{-1} in the electrospun collagen fibers and at 1646.22 cm^{-1} and 1540.73 cm^{-1} for PGS/collagen core/shell fibers respectively as shown in Figure 2. The characteristic peak of PGS at 1740 cm^{-1} is absent in the PGS/collagen core/shell FTIR spectrum because PGS has been incorporated as the core material in the core/shell system.

For any tissue-engineered construct to be functional, it is imperative to determine its material properties and compare against the native heart muscle. A comparison of tensile properties of nanofibers with respect to tensile stress and strain is illustrated in Table 1. We found that the Young's modulus of collagen decreases further in the presence of PGS from 30.11 ± 1.68 MPa to 4.24 ± 0.7 MPa. The principal structural protein of adult myocardium is collagen and of the total collagen in the myocardium 85% is type Ⅰ and 11% is type Ⅲ[39]. The roles of collagens Ⅰ and Ⅲ are different: collagen Ⅰ is thicker and provides stiffness and structural support while collagen Ⅲ is thinner and provides flexibility and elastic recovery[40]. The two types of collagens jointly support and maintain the myocyte alignment, whereas their

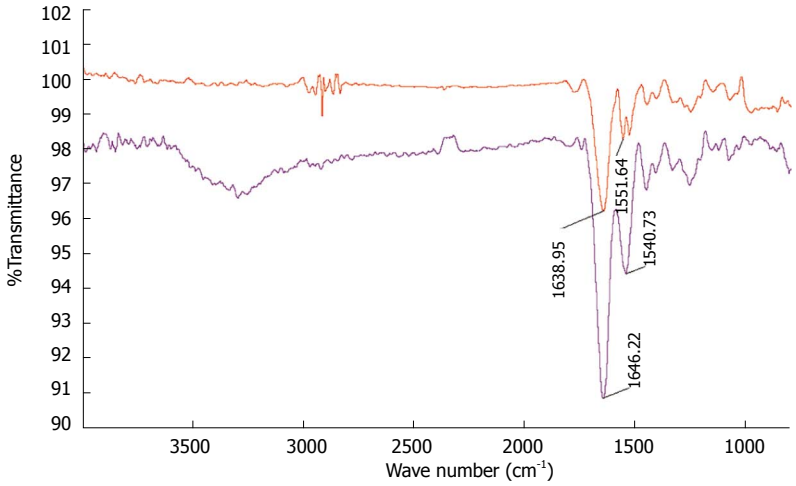

Figure 2 Fourier transform infrared spectroscopy images of red curve-collagen fibers showing characteristic amide peaks at 1638.95 $cm^{-1}$and 1551.64 cm^{-1} blue curve-poly (glycerol sebacate)/collagen core/shell fibers showing characteristic amide peaks at 1646.22 cm^{-1} and 1540.73 cm^{-1}.

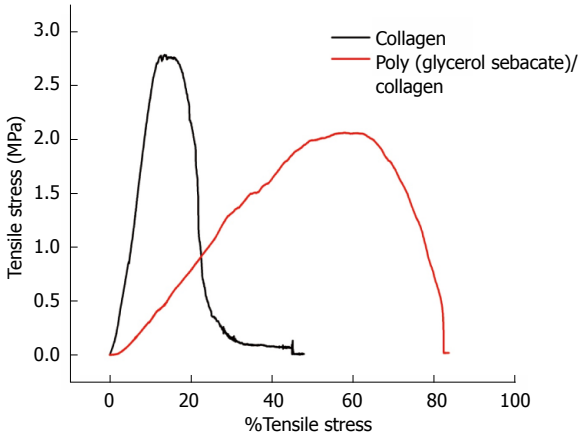

Figure 3 Tensile stress-strain curves of collagen fibers and poly (glycerol sebacate)/collagen core/shell fibers.

tensile strength and resilience resist the deformation, thereby contributing to the passive and active stiffness of the myocardium[41]. Radisic *et al*[42] applied electrical signals onto ultrafoam collagen sponges using matrigel seeded with rat cardiomyocytes to mimic the native heart for cardiac tissue engineering. However, poor mechanical properties, the lack of stability and large degree of swelling (approximately 30%) immediately following hydration in culture medium[43], hampered its clinical applications. Figure 3, we observed that the elastic modulus of PGS/collagen core/shell fibers is 4.24 ± 0.7 MPa, which is nearing to native myocardium, while that of collagen nanofibers was comparatively higher around 30.11 ± 1.68 MPa. Hence, PGS/collagen core/shell fibers are a novel concept for improving the mechanical stability as well as not compromising on the biocompatibility of the patch material. Additionally, PGS has other desirable properties including control of its mechanical properties, which can match those of native myocardium[44], and support of adhesion and phenotypic protein expression for a variety of primary cell types *in vitro*[45].

DISCUSSION

The scaffold properties play an important role in influencing the cell responses for cardiac tissue engineering. Cell behaviour such as adhesion and proliferation represent the initial phase of cell-scaffold communication that subsequently influences the cell differentiation[46]. The cell proliferation results as shown in Figure 4, we observed that the MSC and co-culture cells adhesion and proliferation were significantly ($P \leqslant 0.05$) higher on day 10 and day 15 on PGS/collagen scaffold compared to the TCP. This was because of the nanofibrous scaffold which resembles the ECM and thereby provides necessary cues, promoting cell proliferation and differentiation as discussed in our previous studies[46-49]. Moreover, studies have reported that cardiomyocyte adhesion and organization into a contractile tissue have been far superior on natural scaffolds compared to synthetic scaffolds[50]. The results observed that the proliferation of co-culture cells was significantly ($P \leqslant 0.01$) higher on PGS/collagen core/shell fibers compared to collagen nanofibers on day 10 and day 15 (Figure 4). Additionally, the MSCs proliferation was also significantly higher on day 10 and day 15 on PGS/collagen scaffolds compared to collagen fibers. The percentage increase in the rate of proliferation of co-culture cells from day 5 to day 15 has been calculated to be 87.45% and 118.91% on collagen nanofibers and PGS/collagen core/shell fibers respectively. Enhanced cell population on the co-culture group maybe due to the synergistic effect of cardiac cells and MSCs. The MSCs provide the necessary paracrine signals to prevent apoptosis of the cardiac cells. Recent studies suggest that the transplanted MSCs interact with local tissues in the heart, releasing paracrine factors that support the regenerative process. Thus the beneficial effects of MSCs transplantation are probably mediated primarily through the preservation of cardiac myocytes within the infarction[51]. Additionally, large amount of protective cytokines secreted by MSCs, functioned as the limitation of inflammation, inhibition of apoptosis, and stimulating myoangiogenic differentiation[52]. Stem cell/cardiomyocyte interactions regulate not only cardiac development[53] but also cardiomyocyte function in the adult heart[54]. In MSC-cardiomyocyte co-culture environment, stem cells may promote cardiomyocyte survival[55]. Given the rapid loss

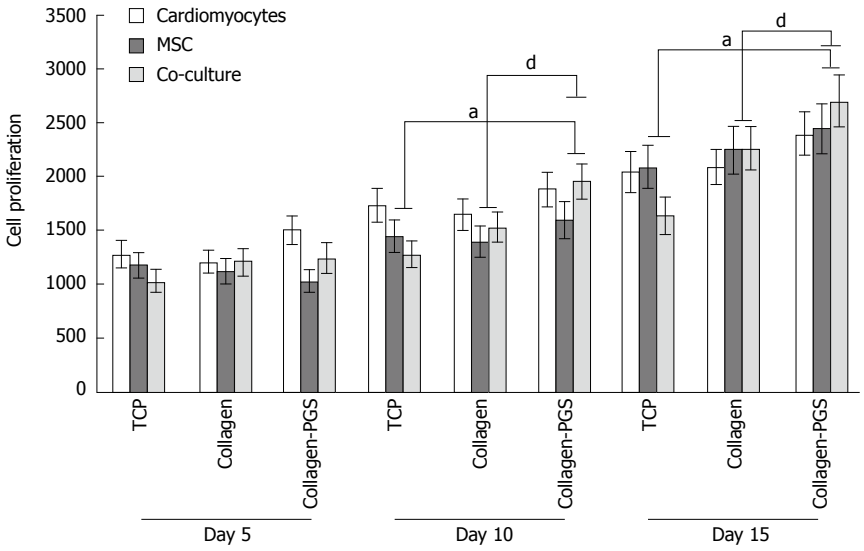

Figure 4 Cell proliferation study for days 5, 10 and 15 on tissue culture plate, poly (glycerol sebacate)/collagen core/shell fibers and collagen nanofibers using cardiomyocytes, mesenchymal stem cells and cardiomyocytes-mesenchymal stem cells co-culture. Denotes statistical significant difference MSC $^{a}P \leqslant 0.05$ *vs* co-culture cells cultured on TCP and PGS/collagen core/shell fibers; Denotes statistical significant difference MSC $^{d}P \leqslant 0.01$ *vs* co-culture cells cultured on collagen and PGS/collagen core/shell fibers. TCP: Tissue culture plate; PGS: Poly (glycerol sebacate); MSCs: Mesenchymal stem cells.

of cardiomyocytes after ischemic injury, promoting cardiomyocyte survival is an efficient strategy for preserving viable myocardium[56].

To observe the cellular responses of the patches to the myocardiogenic differentiations of MSCs, the immunofluorescence stains of specific proteins of cardiomyocytes like troponin T and α-actinin were selected[57-59]. Troponin-T is important for effective cardiomyocytes which contain contractile proteins, as it regulates the force and velocity of myocardial contraction[57], and actinin is an important constituent of the contractile apparatus. Many investigators agree that hMSCs also exhibit some cardiogenic potential but the frequency at which cardiac differentiation occurs without any added induction factors is small[60]. Figure 5D-F and Figure 6D-F, demonstrated that the cardiogenic choice can be enhanced *in vitro* by co-culturing MSCs with cardiac cells. The biological cues secreted by cardiac cells would drive the differentiation of MSCs into cardiac lineage. It has been reported that, in the presence of certain physical and chemical cues, MSCs differentiate into cells that resemble cardiac myocytes and may be applicable for cardiac regeneration[46]. Figure 5 observed that the expression of cardiac proteins actinin (Figure 5D-F) and troponin (Figure 6D-F) are higher in co-culture environment compared to individual cell culture systems of MSCs (Figure 5G-I and Figure 6G-I and cardiac cells (Figure 5A-C and Figure 6A-C). As shown in Figure 5G-I and Figure 6G-I, the MSCs did not express the marker proteins actinin and troponin-T in their undifferentiated state. Hence they express DAPI alone which stains the nucleus of MSCs. It is increasingly accepted that physical cues play a role in cell growth and tissue assembly[30,31]. These signals are important in stem cells (SCs) during self-renewal, proliferation and differentiation. Hence the presence of cardiac cells in the stem cell-cardiac cells co-culture provides the necessary cues to trigger the cardiogenic differentiation of MSCs, in the absence of any other soluble differentiating factors. Furthermore, the cardiac marker expression was higher on the PGS/collagen scaffold compared to the collagen nanofibers. This increase in protein expression is because of the difference in the mechanical property between PGS/collagen and collagen substrates. It has been shown that a softer substrate and the ability to tune the mechanical properties within a given range could be advantageous as cell differentiation was shown to be affected by substrate stiffness[32]. Owing to the favourable mechanical properties of PGS, the cell differentiation was more on PGS/collagen scaffolds compared to collagen scaffolds. There was no protein expression in MSC culture group (Figure 5G-I and Figure 6G-I) proving that in the absence of cues, the cardiogenic differentiation of MSCs may not occur. However in the co-culture environment we found that more cells express the cardiac specific marker proteins, indicating that the MSCs have differentiated into cardiac cells and therefore express troponin T and actinin markers. This cardiogenic differentiation of MSCs was further confirmed by dual immunostaining. Figure 7A, D, G shows the expression of MSC specific marker protein CD 105 by the MSCs cultured in the co-culture environment on TCP, collagen and PGS/collagen core/shell fibers. Figure 7B, E, H shows the expression of cardiac marker protein actinin. The MSCs which have undergone cardiogenic differentiation, express both CD 105 and the cardiac specific marker protein actinin. This result in dual expression of both CD 105 and actinin by the MSCs which have undergone cardiogenic differentiation, as shown in Figure 7C, F, I. We observed that PGS/collagen core/shell fibers (Figure 7I) express higher level of actinin expression in differentiated MSCs compared to collagen scaffolds (Figure 7F). However, the differentiated MSCs did not exhibit any contraction. A similar

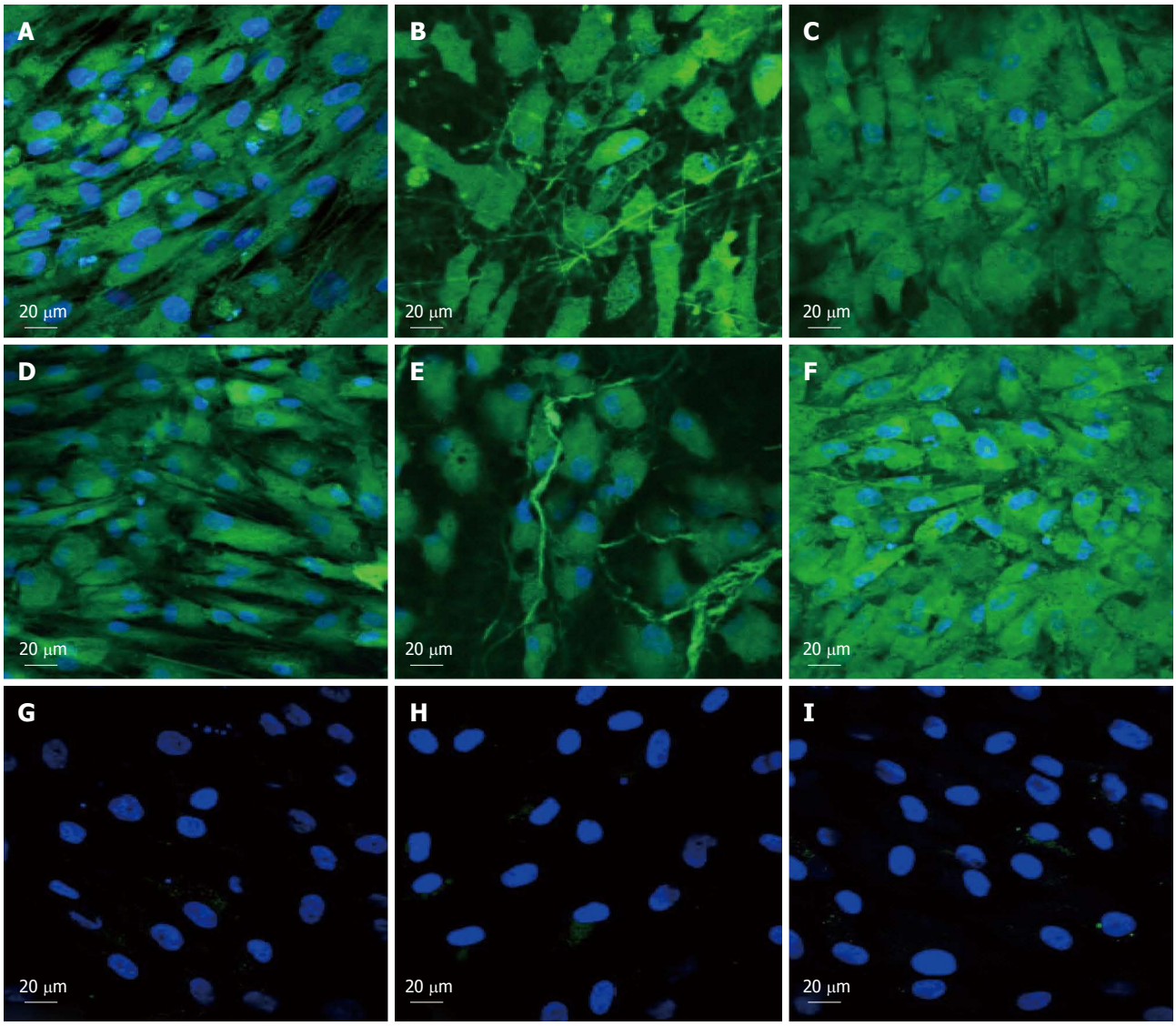

Figure 5 Immunocytochemical analysis for the expression of cardiac marker protein actinin at 60 × magnification on the tissue culture plate (A, D, G), collagen nanofibers (B, E, H) and poly (glycerol sebacate)/collagen core/shell fibers (C, F, I) comprising of cardiomyocytes (A-C), mesenchymal stem cells-cardiomyocytes co-culture group (D-F) and mesenchymal stem cells (G-I). Nucleus stained with 4,6-diamidino-2-phenylindole hydrochloride.

study was reported[61], where stem cells were delivered to the canine RV on an ECM patch, and tracked with quantum dots, some of these cells were demonstrated to differentiate to mature myocytes. Similar to our study, these cells were considered "cardiogenic", since they express cardiac markers like troponin T, but do not contract *in vitro* and have not fully differentiated into functional cardiac myocytes. Moreover, the study also suggested that since the cardiogenic cells did not exhibit the complete cardiac phenotype *in vitro*, it remained possible that the 'committed' cells retained the capacity to proliferate *in vitro*. Ideally, it has been reported that a full cardiogenic differentiation of stem cells should not occur until the cells are transplanted into the heart, since different regions of the heart have unique ion currents and the expression of these currents may be affected by the local environment[62].

The ability of seeded cells to adhere, survive and migrate within a scaffold is crucial when trying to regenerate a tissue *in vivo*. Previous reports of cardiac tissue engineering noted that even if the surface layers of the construct were filled with cells, its interior was frequently devoid of tissue regeneration[63]. Since the fibrous scaffolds are highly porous, it favours the cells to penetrate deep within the scaffold. This was further confirmed by 3D confocal images, as shown in Figure 8, processed using Imaris software. It was observed that the cells penetrated more profoundly within the PGS/collagen fibers (542 nm) when compared to the collagen scaffolds (475 nm). This is because of the favourable mechanical property and flexibility of PGS, which favours easier cell migration, causing the cells to crawl inside, towards the interior of the scaffold.

Quantification of the number of cells present on nanofibrous scaffolds and cellular behaviour is also another pivotal indicator to determine the potential appli-

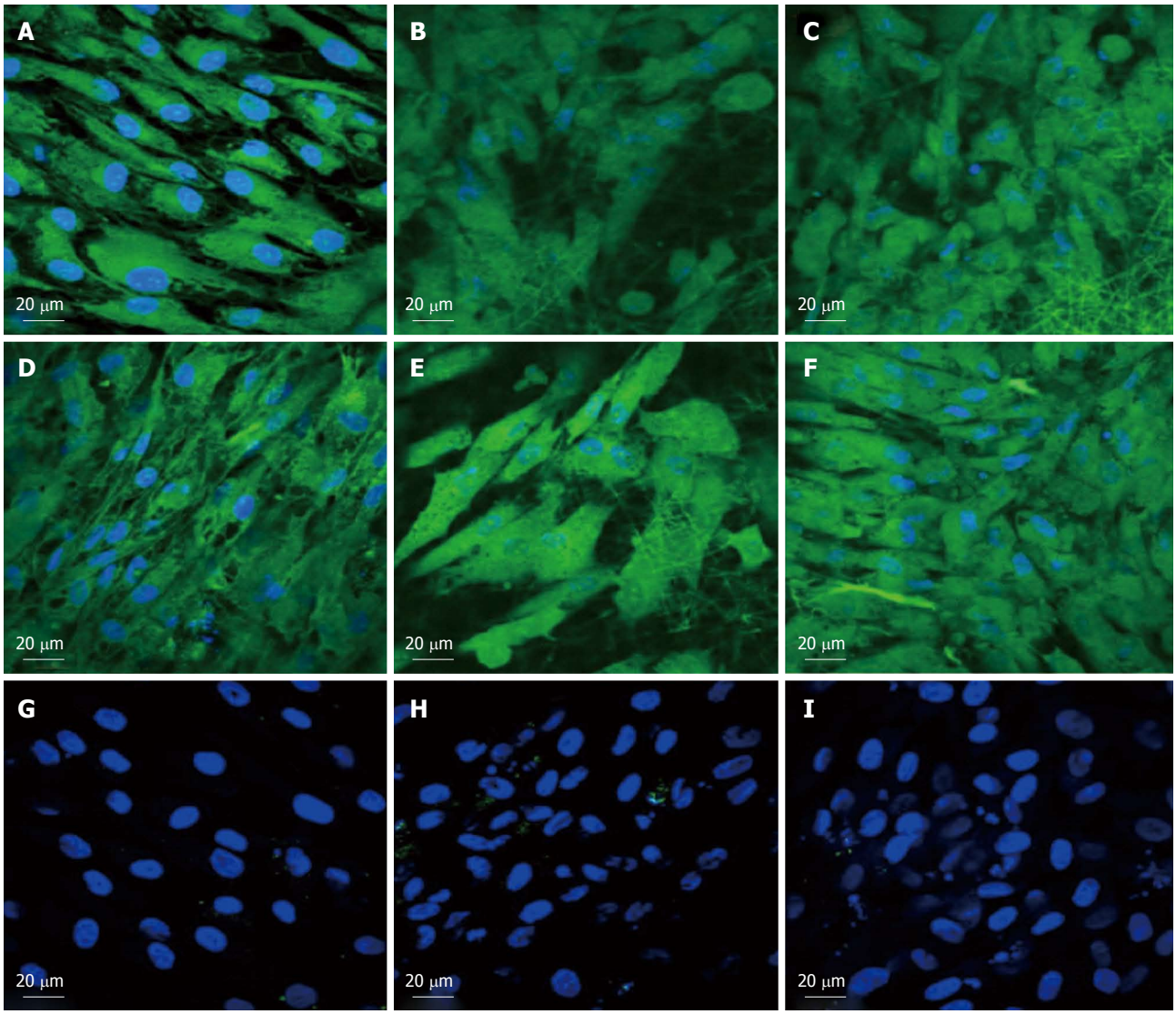

Figure 6 Immunocytochemical analysis for the expression of the cardiac marker protein Troponin at 60 × magnification on the tissue culture plate (A, D, G), collagen nanofibers (B, E, H) and poly (glycerol sebacate)/collagen core/shell fibers (C, F, I) comprising of cardiomyocytes (A-C), mesenchymal stem cells-cardiomyocytes co-culture group (D-F) and mesenchymal stem cells (G-I). Nucleus stained with 4,6-diamidino-2-phenylindole hydrochloride.

cation of a material construct for any tissue engineering application. The cell morphology was studied using SEM images as shown in Figure 9. It showed that the nanofiber contour allowed the cardiac cells to make extensive use of available cues for isotropic or anisotropic growth, and to some degree even to crawl inside and pull the fibers, as evidenced in Figure 9A-C. The results showed that the cell-to-cell interaction between the MSC and cardiac cells group, leading to cell fusion, was observed in co-culture group (Figure 9D-F). This cell-to-cell interaction favoured the MSCs to undergo cardiogenic differentiation. The differentiated MSCs in the co-culture system acquired the cardiomyocyte phenotype as revealed in Figure 9D-F. MSCs also showed favourable growth on the nanofibers with the greater cell-to-cell contact and extension of filopodia as evidenced in Figure 9G-I.

We have employed the use of co-culture system of stem cells with scaffold microenvironments engineered to improve tissue survival and enhance differentiation. Transplanted MSCs may differentiate *in situ* into cells of cardiomyogenic lineage, promoted by the local microenvironment. These in turn could integrate into the myocardium and help to regenerate damaged tissue. We have reported that direct cell-to-cell contact between MSC and adult cardiac cells is necessary for the differentiation of MSC into cardiac cells. This integrative approach of PGS/collagen core/shell nanofibrous cardiac patch material, to prevent cell loss, and stem cell therapy, would maximize the capacity of myocardial tissue regeneration.

In conclusion, even though the implanted stem cells survived and regenerated the infarcted myocardium, the site of MI is a poor environment for cell growth. To increase cell viability, some factors to improve such an infertile environment are desirable. We aimed at improving the quality of the local microenvironment by trapping the cells within the nanofiber mesh and then transplanting to the infarcted site, which may in turn improve survival of the cells and facilitate the biological behaviour

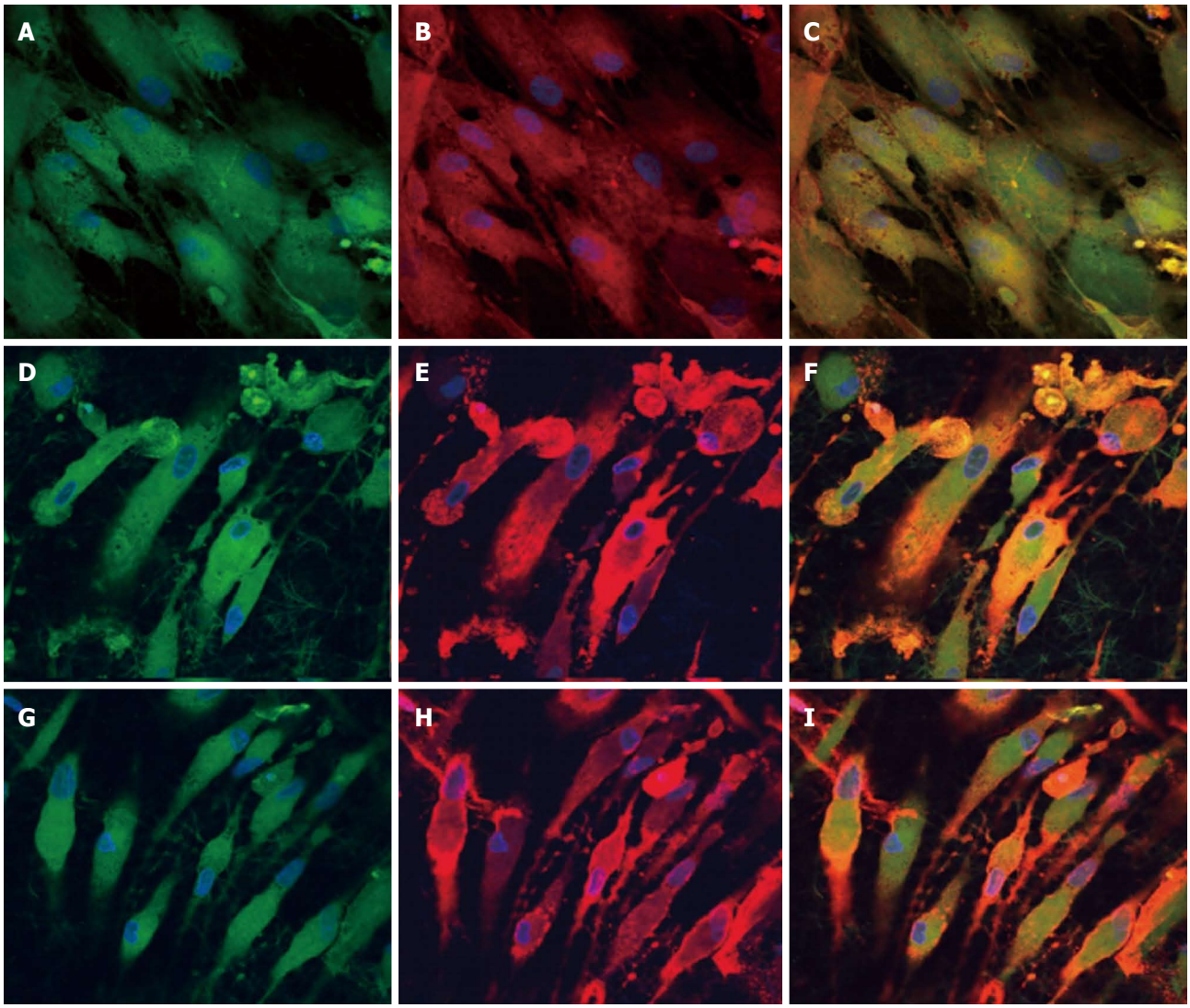

Figure 7 Dual immunocytochemical analysis for the expression of mesenchymal stem cells marker protein CD 105 (A, D, G) and cardiac marker protein Actinin (B, E, H) in the co-culture samples and the merged image showing the dual expression of both CD 105 and Actinin (C, F, I); on the tissue culture plate (A, B, C), collagen nanofibers (D, E, F) and poly (glycerol sebacate)/collagen core/shell fibers (G, H, I) at 60 × magnification. Nucleus stained with 4,6-diamidino-2-phenylindole hydrochloride.

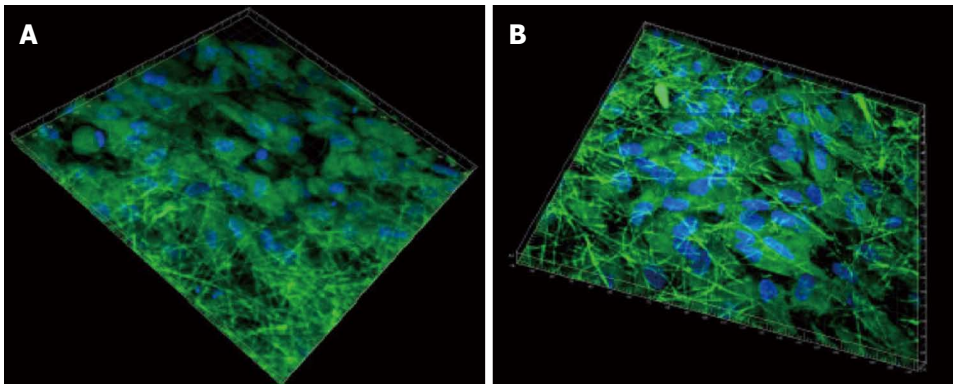

Figure 8 3D image using Imaris software of cardiomyocytes-mesenchymal stem cells co-culture group stained with cardiac specific marker protein troponin at 60 × magnification on (A) collagen fibers (B) poly (glycerol sebacate)/collagen core/shell fibers. Nucleus stained with 4,6-diamidino-2-phenylindole hydrochloride.

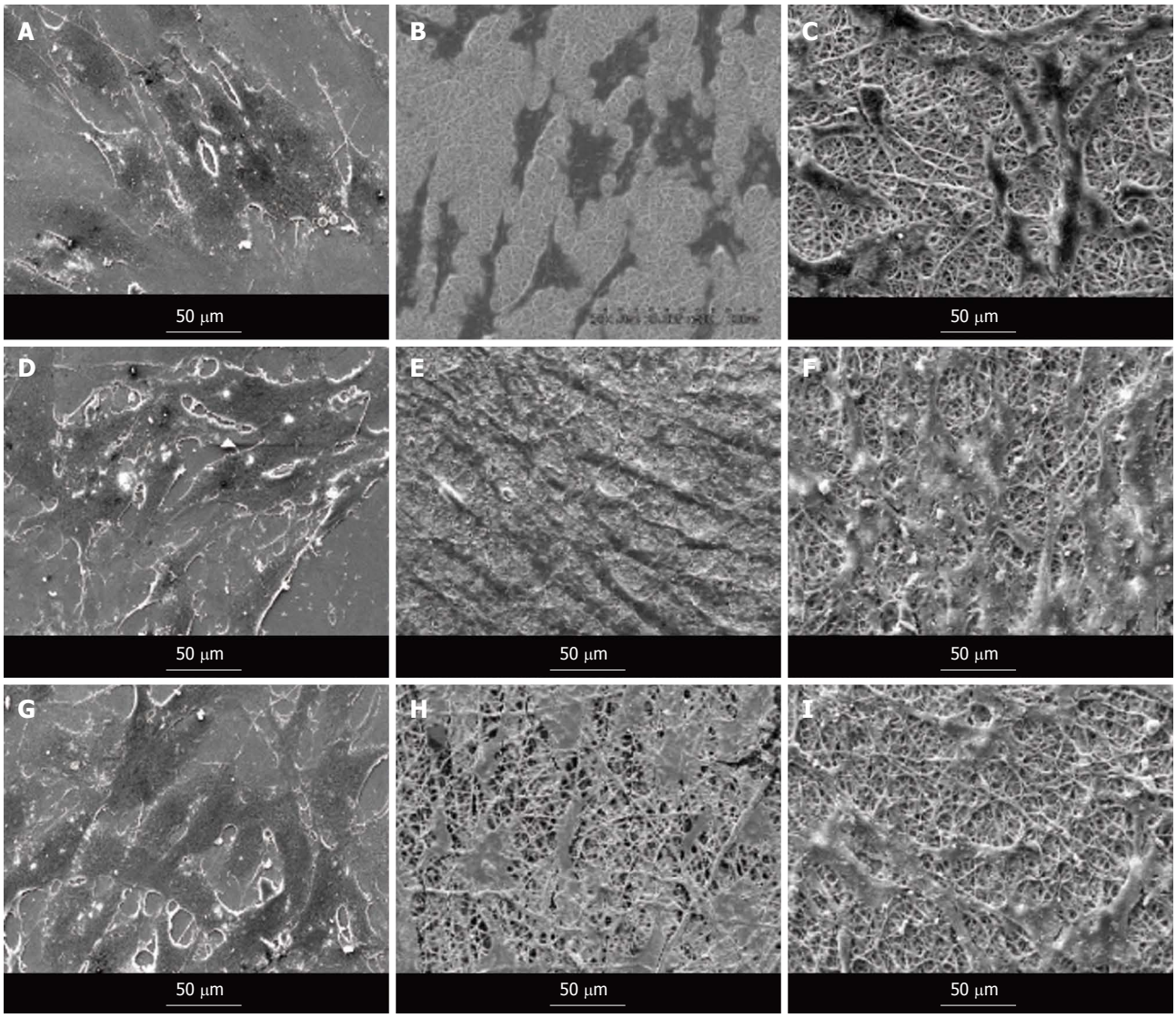

Figure 9 Scanning electron microscope images showing the cell morphology of cardiomyocytes (A-C), cardiomyocytes-mesenchymal stem cells co-culture cells (D-F) and mesenchymal stem cells (G-I) grown on tissue culture plate (A, D, G), collagen nanofibers (B, E, H) and poly (glycerol sebacate)/collagen core/shell fibers (C, F, I) on day 15 at 500 × magnification.

of implanted cells. Electrospun fibrous scaffolds provide both flexibility and guidance for cardiac cells growth and MSC differentiation into cardiac lineage and thus can be successfully applied to obtain structurally and functionally competent cardiac tissue constructs. There have been several studies of different scaffolds for cardiac tissue engineering, but there has been few research focused on elastomeric scaffolds like PGS for CTE. In fact, no research has been done so far on the PGS/collagen scaffold material for MI. New solutions including the recruitment, *in vitro* proliferation and homing of the patients own stem cells, combined with biocompatible and biomimicking materials will open new doors in the field of cardiac tissue engineering for MI. The biomaterial employed should be able to interact on the molecular level, with the cells in a precise and controlled manner, similar to the natural interactions existing between cells and the native ECM. We have shown that the direct cell-to-cell contact between MSCs and adult cardiac cells, governed the differentiation of MSCs into cardiac cells. This novel combinatorial paradigm of a PGS/collagen mechanical construct with MSC-cardiac cells co-culture environment might ultimately bring cardiac tissue engineering into clinical application.

COMMENTS

Background

Myocardial infarction leads to weakening of collagen extracellular matrix leading to loss of ventricular function and the injured heart tissue ultimately becomes scar tissue. When the scar is large enough to interfere with the hearts normal rhythm, heart failure occurs. One promising approach is to prevent the increase of heart failure after myocardial infarction is the implantation of engineered cardiac patch at the site of infarction.

Research frontiers

Poly (glycerol sebacate) (PGS) is an elastomeric material recently applied in the area of soft tissue engineering. In the area of cardiac tissue engineering, the research hotspot is how to fabricate PGS nanofibers by electrospinning technique to engineer a cardiac patch with mechanical properties similar to that of the heart tissue.

Innovations and breakthroughs

The conventional electrospinning technique is to dissolve the polymer in a solvent, which evaporates during the spinning process. However, this approach is not pragmatic with PGS, due to its low molecular weight which results in such a low solution viscosity that even with a high concentration solution, the electrospinning of PGS fibers cannot occur. Hence, the authors developed a core/shell electrospinning process to produce PGS fibers with a protective shell polymer.

Applications

The combination of PGS/collagen core/shell fibers with a unique extracellular matrix like topography has been suggested to be a potential cardiac patch material for myocardial infarction.

Terminology

Myocardial Infarction, known as "heart attack" leads to weakening of collagen extracellular matrix leading to the loss of left ventricular function and the injured heart tissue ultimately becomes scar tissue. When the scar is large enough to interfere with the hearts normal rhythm, heart failure occurs.

Peer review

The article by Ravichandran *et al* is an interesting article that addresses the important issue of optimizing biomaterials as scaffolding for stem cells. The methodology and data is well presented.

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BRIEF ARTICLE

Ultrasound-assessed non-culprit and culprit coronary vessels differ by age and gender

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Abstract

AIM: To investigate age- and gender-related differences in non-culprit versus culprit coronary vessels assessed with virtual histology intravascular ultrasound (VH-IVUS).

METHODS: In 390 patients referred for coronary angiography to a single center (Luzerner Kantonsspital, Switzerland) between May 2007 and January 2011, 691 proximal vessel segments in left anterior descending, circumflex and/or right coronary arteries were imaged by VH-IVUS. Plaque burden and plaque composition (fibrous, fibro-fatty, necrotic core and dense calcium volumes) were analyzed in 3 age tertiles, according to gender and separated for vessels containing non-culprit or culprit lesions. To classify as vessel containing a culprit lesion, the patient had to present with an acute coronary syndrome, and the VH-IVUS had to be performed in a vessel segment containing the culprit lesion according to conventional coronary angiography.

RESULTS: In non-culprit vessels the plaque burden increased significantly with aging (in men from 37% ± 12% in the lowest to 46% ± 10% in the highest age tertile, $P < 0.001$; in women from 30% ± 9% to 40% ± 11%, $P < 0.001$); men had higher plaque burden than women at any age ($P < 0.001$ for each of the 3 age tertiles). In culprit vessels of the lowest age tertile, plaque burden was significantly higher than that in non-culprit vessels (in men 48% ± 6%, $P < 0.001$ as compared to non-culprit vessels; in women 44% ± 18%, $P = 0.004$ as compared to non-culprit vessels). Plaque burden of culprit vessels did not significantly change during aging (plaque burden in men of the highest age tertile 51% ± 9%, $P = 0.523$ as compared to lowest age tertile; in women of the highest age tertile 49% ± 8%, $P = 0.449$ as compared to lowest age tertile). In men, plaque morphology of culprit vessels became increasingly rupture-prone during aging (increasing percentages of necrotic core and dense calcium), whereas plaque morphology in non-culprit vessels was less rupture-prone and remained constant during aging. In women, necrotic core in non-culprit vessels was very low at young age, but increased during aging resulting in a plaque morphology that was very similar to men. Plaque morphology in culprit vessels of young women and men was similar.

CONCLUSION: This study provides evidence that age- and gender-related differences in plaque burden and plaque composition significantly depend on whether the vessel contained a non-culprit or culprit lesion.

Key words: Coronary vessels; Anatomy and histology; Coronary artery; Ultrasonography; Coronary artery disease; Atherosclerosis; Etiology; Age factors

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INTRODUCTION

Age and gender have well-established relations to the prevalence of coronary artery disease (CAD) and the incidence of coronary events[1-5]. A few pathologic studies evaluated age- and gender-related differences in coronary plaques and found significant differences[6-9]. However, these studies were restricted to patients who died from a coronary event. Only two studies so far characterized age- and gender-related differences in coronary plaques *in vivo* with the use of virtual histology (VH) intravascular ultrasound (IVUS)[10,11]. Both studies reported that plaque burden as well as necrotic core and dense calcium increased with increasing patient age, that at any age men had a more rupture-prone plaque morphology than women and that gender differences diminished with increasing age. However, in these studies the compositional characteristics of non-culprit and culprit lesions were not distinguished. The present study therefore examines age- and gender-related differences in non-culprit versus culprit coronary vessels assessed with VH-IVUS.

MATERIALS AND METHODS

Study population

Consecutive patients referred for coronary angiography during daytime to a single center (Luzerner Kantonsspital, Switzerland) between May 2007 and January 2011 were evaluated for this study. Patients presented with stable angina or acute coronary syndromes (ACS) (*i.e.*, unstable angina, non-ST-elevation myocardial infarction or ST-elevation myocardial infarction). The decision to perform coronary angiography was taken according to the local guidelines. Patients in cardiogenic shock and hemodynamically unstable patients depending on inotropes were excluded. Patients with total or subtotal stenosis of the proximal left anterior descending (LAD) or circumflex (CX) artery as well as high-grade left main coronary artery stenosis with potential for hemodynamic instability during the IVUS procedure were also excluded. Finally, 390 patients qualified for inclusion in the study and were willing to participate. All patients provided written informed consent. The institutional ethical committee approved the study, which was conducted in accordance with the declaration of Helsinki.

Measurements

In all 390 patients, clinical characteristics (age, sex, cardiovascular risk factors, clinical presentation) were assessed at baseline. Hypertension was defined as a repeatedly elevated blood pressure > 140/90 mmHg according to current guidelines[12-14]. Dyslipidemia was defined as total cholesterol > 234 mg/dL (6.0 mmol/L) or low-density lipoprotein cholesterol > 117 mg/dL (3.0 mmol/L)[15]. Diagnosis of diabetes mellitus was made if fasting plasma glucose was ⩾ 7 mmol/L on at least two different days or if postprandial plasma glucose was ⩾ 11.1 mmol/L[16]. Patients were considered as smokers, if they currently smoked ⩾ 1 cigarette per week. Patients who previously stopped smoking were considered non-smokers[17]. A positive family history of CAD was defined as evidence of CAD in a parent or sibling before 60 years of age[18]. ACS was defined according to guidelines of the European Society of Cardiology and the American College of Cardiology/American Heart Association[19,20].

IVUS procedure and outcome

In all 390 participating patients, IVUS was performed in the proximal 4 cm of LAD, CX and right coronary artery (RCA). If the coronary anatomy was unsuitable for the IVUS procedure (*e.g.*, small or tortuous vessels), the IVUS procedure was not performed in the corresponding vessel. IVUS was acquired using an Eagle Eye® Gold Catheter (20 MHz) and an automatic continuous pullback device (Volcano Corp., Rancho Cordova, CA, United States)[21-24]. Pullback velocity was 1 mm/s. Frames were acquired ECG-gated/R-wave triggered. The vessels in which the IVUS was performed were classified into either vessels containing a culprit lesion or vessels containing non-culprit lesions. To classify a vessel as vessel containing the culprit lesion, the following criteria had to be fulfilled: (1) the patient had an ACS; and (2) the IVUS was performed in the vessel segment containing the culprit lesion according to conventional coronary angiography. All other vessels were classified as vessels containing non-culprit lesions.

Analysis of the IVUS procedure was performed offline by a specially trained single investigator who was blinded to the clinical and angiographic data. The analysis embraced the whole vessel segment in which the IVUS was performed. Contours of lumen and media-adventitia interface were detected semi-automatically. For every cross-sectional area, vessel and lumen diameters as well as the area for the different plaque components were calculated using a special software package (pcVH2.2, Volcano Corp., Rancho Cordova, CA, United States). Spectral analysis of IVUS radiofrequency signals provided a histology of the plaque identifying 4 major plaque components, namely fibrous, fibro-fatty, necrotic core and dense calcium[25]. After manual detection, the software calculated the absolute volumes of each of the 4 plaque components as well as their relative amounts expressed as percentages of the total volume of the 4 components within the 4 cm segment. Total plaque volume was calculated as the sum of the volumes of all 4 plaque components and of the media-adventitia. Plaque burden was defined as the ratio between plaque volume and the sum

Table 1 Baseline characteristics (n = 130) n (%)

Characteristic	Patients in the lowest age tertile	Patients in the middle age tertile	Patients in the highest age tertile	*P* value[1]
Age, mean ± SD (range), yr	47.6 ± 6.8 (20.5-55.5)	59.6 ± 2.4 (55.5-64.2)	70.9 ± 4.8 (64.3-83.7)	< 0.001
Female sex	29 (22.3)	36 (27.7)	43 (33.1)	0.053
Body mass index, mean ± SD, kg/m^2	26.9 ± 5.1	28.0 ± 4.9	27.4 ± 3.6	0.408
Obesity[2]	25 (19.2)	37 (28.5)	30 (23.1)	0.466
Systolic BP, mean ± SD, mmHg	128 ± 19	134 ± 19	133 ± 20	0.017
Diastolic BP, mean ± SD, mmHg	77 ± 12	77 ± 11	73 ± 11	0.007
Cardiovascular risk factors				
Hypertension	58 (44.6)	85 (65.4)	95 (73.1)	< 0.001
Dyslipidemia	77 (59.2)	101 (77.7)	90 (69.2)	0.082
Current smoker	47 (36.2)	30 (23.1)	16 (12.3)	< 0.001
Diabetes	11 (8.5)	23 (17.7)	30 (23.1)	0.001
Positive family history	47 (36.2)	48 (36.9)	51 (39.2)	0.609
Manifest atherosclerosis				
CAD	78 (60.0)	101 (77.7)	112 (86.2)	< 0.001
ACS	35 (26.9)	32 (24.6)	33 (25.4)	0.777
Previous stroke	1 (0.8)	5 (3.9)	7 (5.4)	0.038
Peripheral artery disease	2 (1.5)	11 (8.5)	6 (4.6)	0.250
Medication[3]				
ACE inhibitor	29 (22.3)	46 (35.4)	52 (40.0)	0.002
Betablocker	47 (36.2)	60 (46.2)	67 (51.5)	0.013
Statin	36 (27.7)	57 (43.9)	62 (47.7)	0.001
Other measurements				
LDL, mean ± SD, mmol/L	2.8 ± 1.0	2.7 ± 1.2	2.6 ± 1.0	0.263
LVEF, mean ± SD, %	70 ± 11	68 ± 13	70 ± 12	0.875

[1]*P* value for trend across age groups was calculated using linear regression; [2]Defined as body mass index ⩾ 30 kg/m^2; [3]Long-term medication in the two weeks before intravascular ultrasound. ACE: Angiotensin converting enzyme; ACS: Acute coronary syndrome; BP: Blood pressure; CAD: Coronary artery disease; LDL: Low-density lipoprotein cholesterol.

of plaque and lumen volumes.

Statistical analysis

Data were analyzed using Stata software (Stata 11.2, StataCorp LP, College Station, TX, United States). For two-group comparisons, Student's *t* test was used after checking for normal distribution; Mann-Whitney rank-sum test was used for non-normally distributed continuous variables. Distributional differences between categorical variables were assessed by a χ^2 test and Fisher's exact test. The *P* value for a trend across age groups was calculated using linear regression. In the regression model, age was analyzed as tertile. For all statistical comparisons, a *P* value < 0.05 was considered significant.

RESULTS

Baseline characteristic are shown in Table 1. Mean age was 59.4 ± 10.7 years with a range from 20.5 to 83.7 years. Hypertension, dyslipidemia and diabetes were increasingly prevalent with increasing patient age. Fewer patients in the highest age tertile were current smokers as compared to younger patients. Prevalence of CAD increased with increasing patient age, whereas patients with ACS were similarly prevalent in all age tertiles (Table 1).

Overall, 691 vessel segments in LAD, CX and/or RCA were imaged by IVUS in the 390 participating patients. The LAD was imaged in 319 patients, the CX in 214 patients, and the RCA in 158 patients. All three vessels were depicted in 84 patients (21.5%), two vessels in 133 patients (34.1%), and only one vessel in 173 patients (44.4%). 618 vessels (89.4%) contained non-culprit lesions and 73 vessels (10.6%) contained culprit lesions of patients with ACS. Age- and gender-related differences of plaque components in vessels containing non-culprit or culprit lesions are summarized in Table 2 and in Figure 1.

Age- and gender-related differences in non-culprit vessels

In non-culprit vessels plaque burden as well as fibrous and necrotic core volumes were higher in young men than in young women (Table 2). The plaque burden in men increased significantly with age; however, the plaque component characteristics of young age were preserved until old age. Women also had a significant increase in plaque burden during aging, but plaque burden remained generally lower than in men. In contrast to men, plaque composition of non-culprit vessels in women altered during aging with increasing volumes of fibrous and necrotic tissue, so that differences in plaque composition between women and men disappeared in the elderly. Old women had a plaque composition that was similar to men, even compared to young men. Interestingly, the most important change of plaque composition in women occurred between the lowest and middle age tertile (*i.e.*, after menopause).

Age- and gender-related differences in culprit vessels

Table 2 summarizes plaque burden and composition of culprit vessels. The plaque composition of culprit vessels in young men and women presenting with ACS was very similar. In contrast to women, plaque composition

Table 2 Age- and gender-related differences of plaque burden and plaque components in vessels containing non-culprit or culprit lesions (mean ± SD)

Plaque component	Patients in the lowest age tertile			Patients in the middle age tertile			Patients in the highest age tertile			*P* value[1]	*P* value[2]
	Male	Female	*P* value	Male	Female	*P* value	Male	Female	*P* value		
Non-culprit vessels											
Segments available for analysis	*n* = 154	*n* = 44		*n* = 159	*n* = 54		*n* = 140	*n* = 67			
Plaque burden, %	37 ± 12	30 ± 9	< 0.001	42 ± 11	35 ± 12	< 0.001	46 ± 10	40 ± 11	< 0.001	< 0.001	< 0.001
Fibrous volume, %	53 ± 12	44 ± 20	< 0.001	51 ± 12	54 ± 13	0.161	52 ± 11	53 ± 12	0.947	0.949	0.005
Fibro-fatty volume, %	13 ± 11	17 ± 19	0.966	11 ± 9	17 ± 15	0.053	13 ± 9	12 ± 10	0.365	0.992	0.060
NC volume, %	18 ± 8	15 ± 10	0.033	21 ± 9	16 ± 10	< 0.001	20 ± 8	19 ± 9	0.646	0.13	0.017
DC volume, %	17 ± 14	24 ± 22	0.324	17 ± 12	13 ± 14	0.003	15 ± 10	16 ± 12	0.814	0.323	0.028
Lumen volume, mm^3	398 ± 182	342 ± 115	0.053	348 ± 133	308 ± 130	0.057	310 ± 133	332 ± 127	0.267	< 0.001	0.815
Culprit vessels											
Segments available for analysis	*n* = 22	*n* = 5		*n* = 19	*n* = 3		*n* = 16	*n* = 8			
Plaque burden, %	48 ± 6	44 ± 18	0.376	43 ± 11	47 ± 14	0.578	51 ± 9	49 ± 8	0.724	0.523	0.449
Fibrous volume, %	57 ± 9	58 ± 8	0.795	51 ± 10	67 ± 12	0.026	44 ± 12	57 ± 11	0.024	< 0.001	0.727
Fibro-fatty volume, %	13 ± 9	13 ± 6	0.683	9 ± 5	8 ± 2	0.562	7 ± 5	11 ± 7	0.069	0.006	0.561
NC volume, %	19 ± 8	19 ± 7	0.930	23 ± 9	17 ± 8	0.285	28 ± 7	20 ± 7	0.018	0.003	0.697
DC volume, %	11 ± 8	10 ± 8	0.851	17 ± 8	8 ± 7	0.113	22 ± 12	13 ± 12	0.040	0.001	0.655
Lumen volume, mm^3	340 ± 144	398 ± 340	0.541	385 ± 171	236 ± 133	0.168	271 ± 82	307 ± 116	0.394	0.201	0.505

[1]*P* value for trend across age groups in men. [1]*P* value was calculated using linear regression; [2]*P* value for trend across age groups in women. *P* value was calculated using linear regression. DC: Dense calcium; NC: Necrotic core.

in culprit vessels of men changed significantly during aging with increasing volumes of necrotic core and dense calcium (*i.e.*, old men need a rupture-prone plaque morphology to present as ACS). Plaque composition in old women was similar to that in young women, therefore leading to significant differences in plaque composition between men and women in the highest age tertile. Old women also presented as ACS with less necrotic core and dense calcium than men. Plaque burden remained similar between men and women and also between the three age groups.

Differences between non-culprit and culprit vessels

In the lowest and middle age tertiles, differences between non-culprit and culprit vessels were similar for men and women. In the lowest age tertile, plaque burden in culprit vessels was significantly higher than that in non-culprit vessels ($P < 0.001$ for men and $P = 0.004$ for women), but no significant differences in plaque composition were found between non-culprit and culprit vessels. In the middle age tertile of men and women, there were no significant differences of plaque burden and composition between non-culprit and culprit vessels. In men of the highest age tertile, plaque composition in non-culprit and culprit vessels differed significantly with more necrotic core ($P < 0.001$), more dense calcium ($P = 0.012$), less fibrous ($P = 0.003$) and less fibro-fatty ($P = 0.004$) volume in the culprit vessel, whereas plaque burden was not significantly ($P = 0.110$) different. In women in the highest age tertile, plaque burden in culprit vessels was significantly higher than in non-culprit vessels ($P = 0.017$), whereas no significant differences in plaque composition were found.

Analysis of combined non-culprit and culprit vessels

If non-culprit and culprit vessels are combined for analysis, plaque burden increased significantly in men ($P < 0.001$) and women ($P < 0.001$). Men had more plaque burden than women in every age tertile ($P < 0.001$ for lowest, middle or highest age tertile). Percentages of necrotic core increased with increasing patient age in both men ($P = 0.019$) and women ($P = 0.016$). Plaque composition significantly differed between men and women in the lowest and middle age tertile, whereas in the highest age tertile no significant differences were found.

DISCUSSION

This study provides evidence that age- and gender-related differences in plaque burden and plaque composition significantly depend upon whether the vessel contained a non-culprit or culprit lesion. Effects of pathophysiologic processes during aging are presumably better observable in non-culprit lesions where acute processes play a minor role. In non-culprit vessels of men, plaque burden increases with increasing age, but plaque composition remains constant during aging. Compared with men, young women have a significantly lower plaque burden and lower amount of necrotic core which may reflect protective effects of female hormones. After menopause the plaque composition in non-culprit vessels of women approximates that of men. The plaque burden of women increases during aging like men, but it remains lower than in men until old age. In culprit vessels the situation is different. Culprit lesions reflect an acute stage of disease and influencing factors other than the long-term pathophysiologic processes which occur during aging presumably come to the fore. Thus, culprit vessels of young women and men exhibit similar rupture-prone plaque morphology with a high percentage of necrotic core and a relatively high plaque burden. In men, a significant increase of necrotic core and dense calcium is required to result in a rupture-prone morphology at old age. In old women, morphology similar to young women and men is

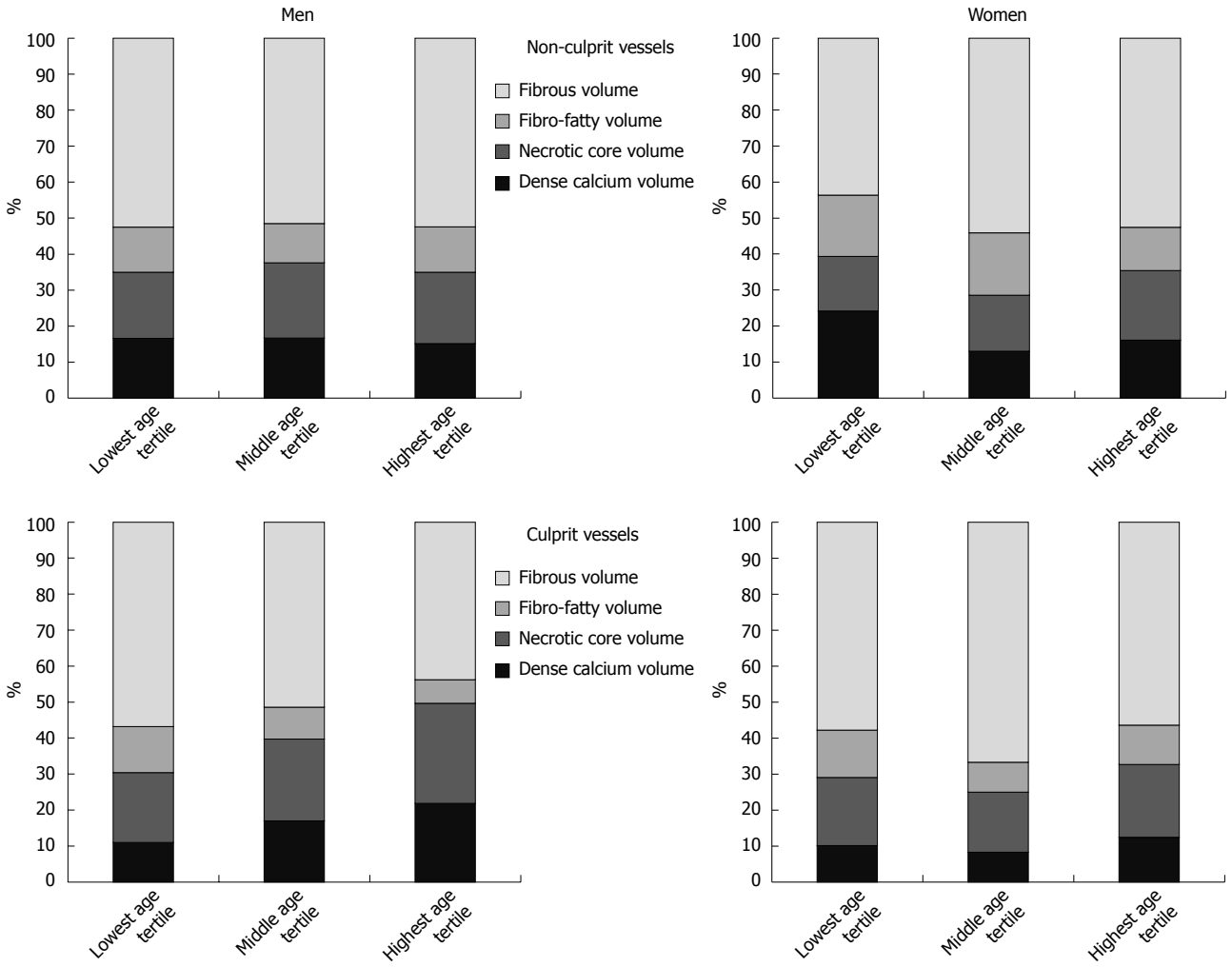

Figure 1 Plaque composition in men and women during aging in non-culprit and culprit vessels.

sufficient to result in a rupture-prone culprit lesion.

Qian *et al*[10] reported that both women and men had an increase in plaque with increasing age, that at any age, men had more plaque than women, that percentages of dense calcium and necrotic core increased with age in both men and women, and that gender differences were lowest in the oldest tertile. Our study confirms these findings, if non-culprit and culprit lesions are analyzed together. However, if non-culprit and culprit lesions are analyzed separately, then age- and gender-related differences are more complex.

Our study is in accordance with previous pathologic studies although comparability of IVUS and pathologic studies is limited due to several reasons[6-9]. Dollar *et al*[6] reported that young women had more fibrous and fibro-fatty tissue and less necrotic core than old men. Our study confirms this finding. Burke *et al*[8] reported that plaque erosion, the major substrate for thrombosis in premenopausal women, does not appear to be inhibited by estrogen. Presumably this could explain why we did not find any differences in the plaque composition of culprit vessels between young men and women.

The present study has limitations. First, study participants underwent coronary angiography for stable angina or ACS. This might restrict extrapolation of our findings to a more healthy general population. Second, this was not a lesion-specific analysis; rather, LAD, CX and/or RCA were imaged in a proximal 4 cm segment. However, it has been shown that vessel-based measurements correlate well with lesion-specific analysis[26]. Third, the number of culprit vessels available for analysis in women was too low to exclude type II error. Fourth, in culprit vessels it may be difficult to differentiate plaque from thrombi by 20 MHz IVUS. Therefore, plaque composition in culprit vessels may be affected by thrombi. Fifth, hormone replacement therapy was not assessed in women.

In conclusion, the present study has revealed that age- and gender-related differences in plaque burden and plaque composition significantly depend upon whether the vessel contained a non-culprit or culprit lesion. More research is needed to understand the pathophysiology of plaque morphology changes during aging in women and men.

COMMENTS

Background

Only few studies so far characterized age- and gender-related differences in coronary plaques *in vivo* with the use of virtual histology (VH) intravascular

ultrasound (IVUS). In these studies the compositional differences of non-culprit and culprit vessels were not distinguished. The present study therefore examines age- and gender-related differences in non-culprit versus culprit coronary artery vessels assessed with VH-IVUS.

Research frontiers

Differences in the plaque composition of non-culprit and culprit vessels according to age and gender have not been described so far.

Innovations and breakthroughs

The present study shows that age- and gender-related differences in plaque burden and plaque composition significantly depend on whether the vessel contained a non-culprit or culprit lesion. More research is needed to understand the pathophysiology of plaque morphology changes during aging in women and men.

Applications

The present study has research implications. More research is needed to understand the pathophysiology of plaque morphology changes during aging in women and men.

Terminology

Vessel containing a culprit lesion: To classify as vessel containing a culprit lesion, the patient had to present with an acute coronary syndrome, and the VH-IVUS had to be performed in a vessel segment containing the culprit lesion according to conventional coronary angiography.

Peer review

The authors showed the characteristics of coronary disease according to gender and age and different patterns of plaque composition between culprit and non-culprit vessels. However, several issues should be considered and clarified more (*e.g.*, lesion inclusion criteria and the definition of culprit/non-culprit vessels should be clarified).

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BRIEF ARTICLE

Air pollution and heart failure: Relationship with the ejection fraction

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Abstract

AIM: To study whether the concentrations of particulate matter in ambient air are associated with hospital admission due to heart failure in patients with heart failure with preserved ejection fraction and reduced ejection fraction.

METHODS: We studied 353 consecutive patients admitted into a tertiary care hospital with a diagnosis of heart failure. Patients with ejection fraction of $\geqslant$ 45% were classified as having heart failure with preserved ejection fraction and those with an ejection fraction of < 45% were classified as having heart failure with reduced ejection fraction. We determined the average concentrations of different sizes of particulate matter (< 10, < 2.5, and < 1 μm) and the concentrations of gaseous pollutants (carbon monoxide, sulphur dioxide, nitrogen dioxide and ozone) from 1 d up to 7 d prior to admission.

RESULTS: The heart failure with preserved ejection fraction population was exposed to higher nitrogen dioxide concentrations compared to the heart failure with reduced ejection fraction population (12.95 ± 8.22 μg/m^3 *vs* 4.50 ± 2.34 μg/m^3, $P < 0.0001$). Multivariate analysis showed that nitrogen dioxide was a significant predictor of heart failure with preserved ejection fraction (odds ratio ranging from (1.403, 95%CI: 1.003-2.007, $P = 0.04$) to (1.669, 95%CI: 1.043-2.671, $P = 0.03$).

CONCLUSION: This study demonstrates that short-term nitrogen dioxide exposure is independently associated with admission in the heart failure with preserved ejection fraction population.

Key words: Air pollution; Heart failure; Preserved ejection fraction; Reduced ejection fraction; Nitrogen dioxide

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INTRODUCTION

Ambient air pollution is a recognized risk factor for cardiovascular morbidity and mortality[1-3]. Nitrogen dioxide (NO_2) is a strong respiratory irritant gas originating from high-temperature combustion. Main outdoor sources of NO_2 include vehicle exhausts (particularly those equipped with diesel engines) and fossil-fuel power plants, whereas the most important indoor sources are gas heaters, stoves, and environmental tobacco smoke[4].

Large meta-analyses of studies on the short-term health effects of NO_2 have been carried out in Europe[5,6], the United States[7,8], and Canada[9]. The results indicate a positive association between daily increases of NO_2, cardiovascular and respiratory mortality. Several studies using administrative databases have shown a positive association between short-term increases in respirable or fine particles and the risk of hospitalization for congestive heart failure (HF)[10-12].

The aim of this investigation was to study whether the concentrations of particulate matter in ambient air are associated with hospital admission due to HF in patients with HF with preserved ejection fraction (HF-PEF) and reduced ejection fraction (HF-REF).

MATERIALS AND METHODS

Study population

We prospectively enrolled 458 consecutive patients admitted into a tertiary care hospital with a diagnosis of HF. The diagnosis of HF had to be established according to the clinical Framingham criteria[13]. We did not include patients with severe primary valve heart disease (n = 13), chronic obstructive pulmonary disease (n = 30), airway hyperresponsiveness (n = 25), asthma (n = 16) and presence of respiratory infection 15 d before admission (n = 21). Hence, 353 patients were included in the study. Patients with ejection fraction of $\geqslant$ 45% were classified as having HF-PEF and those with an ejection fraction of < 45% were classified as having HF-REF[14].

The study was planned according to the Declaration of Helsinki and approved by the local ethics committee, and all patients provided signed informed consent. Clinical data, including age, sex, arterial hypertension (> 140/90 mmHg), hypercholesterolemia (> 5.17 mmol/L), smokers, diabetes and left ventricular ejection fraction, were analyzed as baseline variables on admission. The left ventricular ejection fraction was measured using the modified Simpson's rule[15].

Air pollution measurements

The atmospheric pollutants were measured in an urban background monitoring station using reference methods (Directive 2008/50/EC). Concentrations of particulate matter (PM) smaller than 10, 2.5 and 1 μm (PM10, PM2.5 and PM1 respectively) were measured with automatic analyzer and the gravimetric method[16].

The concentrations of gaseous pollutants were measured using different methods: (1) sulphur dioxide was measured using ultraviolet fluorescence (Thermo Electron Corporation™, model 43C); (2) NO_2 was measured using chemiluminescence (Thermo Electron Corporation™, model 42C); (3) ozone was measured using ultraviolet absorption (Thermo Electron Corporation™, model 49C); and (4) carbon monoxide was measured using the technique NDIR-Gas Correlation Filter Analyser (Thermo Electron Corporation™, model 48C). The analyzers were calibrated every 3 mo and they always had a high linearity (r^2 = 0.99)[17]. Meteorological variables (temperature, relative humidity and wind speed) were measured using standard techniques. These variables were measured with 1 min resolution. Then, 24 h averages from the previous day up to 7 d prior to admission were calculated.

Statistical analysis

Results for normally distributed continuous variables are expressed as mean ± SD. Continuous variables with non-normal distribution are presented as median values and interquartile intervals; categorical data are expressed as percentages. Analysis of normality of the continuous variables was performed with the Kolmogorov-Smirnov test. Differences between groups were assessed by unpaired 2-tailed t test and the Mann-Whitney U test for continuous variables, as appropriate. Categorical data and proportions were analyzed by use of χ^2 or Fisher's exact test when required. In our study, all of the pollutants were expressed as the 24 h average concentrations from the previous day up to 7 d prior to admission.

A multivariate analysis was carried out using a binary logistic regression model to estimate the risk of admission for HF-PEF compared to admission for HF-REF, according to sizes of particulate matter and concentrations of gaseous pollutants during 7 d prior to admission. All of the variables with a value of $P < 0.05$ in the univariate analysis were included in the model. Differences were considered statistically significant if the null hypothesis could be rejected with > 95% confidence. All probability values are 2 tailed. The SPSS 15 statistical software package (SPSS Inc, Chicago, IL, United States) was used for all calculations.

RESULTS

According to the pre-established criteria, 124 patients were classified as HF-PEF. The baseline characteristics of the patients with HF-PEF and HF-REF are listed in Table 1. The HF-PEF population was significantly older and included a larger proportion of women. There were no significant differences between groups regarding presence of conventional coronary risk factors for coronary

Table 1 Clinical variables of 353 consecutive patients with heart failure: Comparison between patients with heart failure and preserved ejection fraction and patients with heart failure and reduced ejection fraction *n* (%)

Variables	HF-PEF (*n* = 124)	HF-REF (*n* = 229)	*P* value
Age (yr)	69 ± 8	66 ± 12	0.01
Male gender	56 (45.2)	154 (67.2)	< 0.001
Hypertension	75 (60.5)	84 (36.7)	< 0.001
Hypercholesterolemia	35 (28.2)	52 (22.7)	0.25
Smokers	14 (11.3)	40 (17.5)	0.12
Diabetes	45 (36.3)	104 (45.4)	0.09
LVEF (%)	55 ± 9	33 ± 6	< 0.001

Data are expressed as mean ± SD, and *n* (%) for categorical variables. HF-PEF: Heart failure with preserved ejection fraction; HF-REF: Heart failure with reduced ejection fraction; LVEF: Left ventricular ejection fraction.

Table 2 Data on atmospheric pollution in ambient air and meteorological variables between the previous day and the 7 d prior to admission for both of the study group

	HF-PEF (*n* = 124)	HF-REF (*n* = 229)	*P* value
Meteorological variables			
Wind speedy (m/s)	2.72 ± 0.68	2.62 ± 0.78	0.21
Temperature (℃)	19.76 ± 2.52	20.08 ± 2.82	0.32
Relative humidity (%)	67.68 ± 6.10	66.85 ± 7.02	0.29
Gaseous pollutants (mg/m^3)			
CO	172.44 ± 23.89	177 ± 27.10	0.11
SO_2	8.1 ± 4.40	7.33 ± 3.46	0.06
NO_2	12.95 ± 8.22	4.50 ± 2.34	< 0.0001
O_3	59.80 ± 12.52	61.25 ± 11	0.26
Atmospheric particles (mg/m^3)			
PM10	21 (13-30)	25 (17.5-32)	0.02
PM2.5	13.5 (9-21)	16.5 (11-21)	0.12
PM1	8 (6-16)	9.5 (7-13)	0.42

All of the pollutants are expressed as the average concentration of the pollutant. Data are expressed as mean ± SD, and median values (interquartile intervals). HF-PEF: Heart failure with preserved ejection fraction; PM: Particulare material with an aerodynamic diameter; PM10: PM < 10 μm; PM2.5: PM < 2.5 μm; PM1: PM < 1 μm.

artery disease, with the exception of hypertension, which were higher in the patients with HF-PEF. Left ventricular ejection fraction was significantly reduced in the patients with HF-REF.

No statistically significant differences were found in the meteorological variables between both groups. Regarding gaseous pollutants, we found no statistically significant differences, except that there were higher concentrations of NO_2 exposure in patients with HF-PEF. When comparing, exposure to concentrations of sizes of particulate matter, between patients with HF-PEF and HF-REF, the first group tended to have lower values of PM10 (Table 2). We carried out partial multivariable binary logistic regression analyses, using a stepwise selection model. This analysis showed that exposure to NO_2 was a significant predictor of HF-PEF [odds ratio ranging from (1.403, 95%CI: 1.003-2.007, P = 0.04) to (1.669, 95%CI: 1.043-2.671, P =0.03); Table 3].

Table 3 Multivariate binary logistic regression analysis including nitrogen dioxide as the main independent variable

	OR	95%CI	*P* value
Model 1 (unadjusted)			
NO_2	1.428	1.001-2.055	0.04
Model 2			
NO_2	1.669	1.043-2.671	0.03
Age	1.278	0.912-1.618	0.33
Model 3			
NO_2	1.429	0.992-2.058	0.05
Gender	0.948	0.111-8.067	0.96
Model 4			
NO_2	1.403	1.003-2.007	0.04
Hypertension	0.36	0.037-3.482	0.37
Model 5			
NO_2	1.516	1.005-2.397	0.01
LVEF	1.024	0.791-1.324	0.85
Model 6			
NO_2	1.489	1.009-2.453	0.01
PM10	1.124	0.997-1.974	0.94

LVEF: Left ventricular ejection fraction; NO_2: Nitrogen dioxide; PM: Particulare material with an aerodynamic diameter; PM10: PM < 10 μm.

DISCUSSION

Short-term exposure to air pollution is associated with acute cardiovascular events[18-20]. Our results show that HF-PEF is common and accounts for a significant proportion of admissions in patients with HF, 35% of our patients. This rate of patients is similar to that reported in previous studies[14,21]. This group of patients had different characteristics from those of patients with HF-REF, including older population, higher proportion of women, and more frequent history of hypertension. In the present study, we demonstrated that short-term exposure to raised NO_2 levels are an independent risk factor for admission to hospital for HF-PEF population, even superior to classical described predictors, such as age, sex, hypertension and left ventricular ejection fraction[21,22].

Despite the large body of evidence linking NO_2 with daily mortality, few studies have addressed the issue of susceptibility to NO_2 by performing analyses by age, sex, and chronic morbidity[6]. Recents epidemiology studies have focused on cardiopulmonary dysregulation, including the role of air pollutant exposure in provoking decompensated congestive HF[3,10,23]. A number of mechanisms have been proposed to explain the cardiovascular effects of air pollutant. At the cellular level, these various mechanisms involve free radical production, oxidative stress, cytokine release, inflammation, endotoxin-mediated damage, stimulation of capsaicin receptors, autonomic nervous system activity and covalent modification of key cellular molecules[24-27].

Ongoing investigation suggests that, although diastolic abnormalities may be present in many patients with HF-PEF, other aspects of pathophysiology likely also contribute to symptoms. Previous studies have concluded that inflammation contributes to diastolic abnormalities in HF-PEF[28]. In our study, we discovered that NO_2 may

be a precipitating factor for admission for HF-PEF rather than the cause of this condition. The short-term elevations of NO_2 could play an important pathophysiologic role in HF-PEF population, perhaps through of the activation of molecular inflammatory pathways that could be transduced systemically even in the absence of obvious alveolitis or interstitial pneumonitis[29]. In this way, Briet et al demonstrated that exposure to urban gaseous pollutant (including NO_2) affect artery endothelial function in patients as well as healthy control subjects[30].

Our study has some limitations. We did not use time series analysis in our study to examine the short-term relationship between the variations in atmospheric pollution and HF. This was because daily variations in the pollutants during the 7 d prior to admission were small enough to allow us to exclude the time series analysis[29]. Moreover, the sample size could be small, but the association between NO_2 and HF-PEF was highly significant.

This is the first study that demonstrates that short-term exposure to NO_2 is independently associated with the HF-PEF population, when compared to the HF-REF population.

COMMENTS

Background

Several studies using administrative databases have shown a positive association between short-term increases in respirable or fine particles and the risk of hospitalization for congestive heart failure.

Research frontiers

Heart failure is of growing incidence and prevalence and is now the main cause for hospital admission among the elderly and increasing expenditure in medicine. Ambient air pollution is a recognized risk factor for cardiovascular morbidity and mortality. Despite the large body of evidence linking nitrogen dioxide with daily mortality, few studies have addressed the issue of susceptibility to nitrogen dioxide by performing analyses by age, sex, and risk of admission for heart failure with preserved ejection fraction and reduced ejection fraction.

Innovations and breakthroughs

Nitrogen dioxide is a strong respiratory irritant gas originating from high-temperature combustion. Main outdoor sources of nitrogen dioxide include motor vehicles (particularly those equipped with diesel engines) and fossil-fuel power plants, whereas the most important indoor sources are gas heaters, stoves, and environmental tobacco smoke. In this study, authors found statistically significant association between nitrogen dioxide and admission in the heart failure with preserved ejection fraction population.

Applications

Several precautionary recommendations can be made for healthcare providers who interact with individuals who are at risk for cardiovascular diseases. Although they have not been clinically tested or proven to reduce mortality, they are practical and feasible measures that may help to reduce exposures to air pollution and therefore potentially lower the associated cardiovascular risk. These recommendations can be: (1) all patients with cardiovascular disease should be educated about the cardiovascular risks posed by air pollution; (2) part of patient education should include the provision of information regarding the available sources (local and national newspapers) that provide a daily air quality index; and (3) on the basis of the forecast air quality index, prudent recommendations for reducing exposure and limiting activity should be provided based on the patient's level of risk.

Terminology

Heart failure is a condition that is usually caused by a reduction of the contractile function of the ventricular chambers or an impairment of the relaxation properties of the cardiac chambers. Air pollution is the introduction into the atmosphere of chemicals, particulate matter, or biological materials that cause discomfort, disease, or death to humans.

Peer review

This is a good descriptive study in which the authors analyze effect of short-term exposure of nitrogen dioxide in patients with the clinical syndrome of heart failure with preserved and depressed left ventricular ejection fraction. The results are interesting and suggest that other aspects, as the exposure of nitrogen dioxide can contribute to pathophysiology of the heart failure with preserved ejection fraction. These data are of public health importance.

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BRIEF ARTICLE

Myocardial perfusion imaging in patients with a recent, normal exercise test

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Abstract

AIM: To investigate the added value of myocardial perfusion scintigraphy imaging (MPI) in consecutive patients with suspected coronary artery disease (CAD) and a recent, normal exercise electrocardiography (ECG).

METHODS: This study was a retrospective analysis of consecutive patients referred for MPI during a 2-year period from 2006-2007 at one clinic. All eligible patients were suspected of suffering from CAD, and had performed a satisfactory bicycle exercise test (*i.e.*, peak heart rate > 85% of the expected, age-predicted maximum) within 6 mo of referral, their exercise ECG was had no signs of ischemia, there was no exercise-limiting angina, and no cardiac events occurred between the exercise test and referral. The patients subsequently underwent a standard 2-d, stress-rest exercise MPI. Ischemia was defined based on visual scoring supported by quantitative segmental analysis (*i.e.*, sum of stress score > 3). The results of cardiac catheterization were analyzed, and clinical follow up was performed by review of electronic medical files.

RESULTS: A total of 56 patients fulfilled the eligibility criteria. Most patients had a low or intermediate ATPⅢ pre-test risk of CAD (6 patients had a high pre-test risk). The referral exercise test showed a mean Duke score of 5 (range: 2 to 11), which translated to a low post-exercise risk in 66% and intermediate risk in 34%. A total of seven patients were reported with ischemia by MPI. Three of these patients had high ATPⅢ pre-test risk scores. Six of these seven patients underwent cardiac catheterization, which showed significant stenosis in one patient with a high pre-test risk of CAD, and indeterminate lesions in three patients (two of whom had high pre-test risk scores). With MPI as a gate keeper for catheterization, no significant, epicardial stenosis was observed in any of the 50 patients (0%, 95% confidence interval 0.0 to 7.1) with low to intermediate pre-test risk of CAD and a negative exercise test. No cardiac events occurred in any patients within a median follow up period of > 1200 d.

CONCLUSION: The added diagnostic value of MPI in patients with low or intermediate risk of CAD and a recent, normal exercise test is marginal.

Key words: Single photon emission tomography; Ischemic heart disease; Myocardial perfusion imaging; Pre-test risk; Post-test risk; Added value; Exercise electrocardiography

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INTRODUCTION

Treadmill or bicycle exercise electrocardiography (ECG) has been the test of choice for many years for the diagnosis of coronary artery disease (CAD) for reasons of diagnostic performance, cost, and availability. According to the American guidelines, exercise testing remains the test of choice among symptomatic patients with low or intermediate pre-test risk of CAD, provided the patient is able to exercise and the ECG is analyzable for ischemia[1]. A normal exercise test is consistent with a good prognosis with regard to cardiac events and cardiovascular and overall mortality[2].

In patients with intermediate or high pre-test risk of CAD, non-invasive imaging methods and invasive coronary catheterization are preferred[3-6]. Myocardial perfusion scintigraphy imaging (MPI) is one of the most frequently used non-invasive methods for the assessment of the extent and severity of ischemia in patients with intermediate risk of CAD[6]. Several studies have shown that exercise or pharmacological MPI is superior to exercise ECG for the identification of ischemic heart disease in these patients[7-9]. Still, the use of MPI is considered inappropriate in patients with a low risk of CAD and the ability to exercise with an analyzable ECG[1]. Thus, the diagnostic performance of MPI in low-risk patients and its added value to a normal exercise ECG remain unclear. There are contradictory recommendations in the international guidelines on the management of patients with a normal exercise test but continued suspicion of CAD[1,10]. Apparently, no trials have directly addressed this issue. The purpose of this study was to evaluate the diagnostic outcome of MPI in patients with a recent history of a normal bicycle exercise ECG.

MATERIALS AND METHODS

Patients

Retrospective data were extracted from consecutive patients who performed a bicycle exercise MPI from January 1, 2006 through December 31, 2007 in a single nuclear medicine center at a regional hospital. The inclusion criteria included the following: (1) the patient was referred for MPI due to suspicion of CAD; (2) the patient had performed a bicycle exercise ECG within six months of referral; (3) the symptoms were unchanged from the time of the exercise ECG to MPI, and no cardiovascular events had occurred; (4) the maximum heart rate obtained at the referral exercise ECG test was at least 85% of the expected age-related maximum; (5) the exercise test was not terminated due to exercise-limiting angina; (6) the baseline ECG was suitable for the assessment of exercise-induced ischemia; and (7) the exercise ECG was classified as negative for ischemia according to the European guidelines for exercise ECG[10]. Patients with known CAD were excluded. Pre-test risk of CAD was calculated per the ATP Ⅲ classification.

Referral exercise ECG

All original exercise ECGs were evaluated and reported by a trained cardiologist at the time of testing. Additionally, all exercise tests were retrospectively reviewed by another board-certified cardiologist. In the case of discrepancy between the initial reading and the second opinion, a third cardiologist read the test, and a decision was made based on majority voting. The post-test risk of cardiovascular events was calculated with DanStress® software (Svendborg, Denmark) using the algorithm provided by Mark *et al*[2].

Myocardial perfusion scintigraphy

All MPIs were performed as a two-day, stress-rest standard protocol with two days between the stress and the rest tests, as previously described[11]. No attenuation correction was used. Patients with a normal stress MPI did not have a rest MPI. All MPIs were initially reported as positive or negative for ischemia by subjective analysis only. Therefore, all MPIs were retrospectively reviewed in a blinded fashion by a board-certified nuclear medicine physician without any clinical information. Manual segmental scoring was performed using a 17-segment model with a score from 0 to 4 for each segment, from which the sum of stress score (SSS), the sum of rest score (SRS), and the sum of difference score between stress and rest images (SDS) were calculated[12]. In addition, SSS, SRS and SDS were automatically calculated with dedicated software. A SSS score of 0 to 3 was considered to be normal, and SSS > 3 to be abnormal[13]. In the case of discrepancy in disease classification (*i.e.*, normal or abnormal) between the automatic and manual score, a second nuclear medicine physician performed an additional manual segmental score, and the decision was determined by majority voting. Further in this manuscript, ischemia was present only if confirmed by subjective visual interpretation as well a SSS > 3.

Coronary catheterization

Cardiac catheterization was performed in accordance with the standard institutional practice and current guidelines[10]. A significant stenosis required any luminal narrowing of 70% or more of the diameter of a major epicardial vessel or 50% or more of the diameter of the left main coronary artery. Any investigation with no more than 20% luminal narrowing of any vessels was classified as normal. Results other than stenosis or normal were reported as indeterminate. All findings by angiography were assessed by a board of cardiologists, and the report represented their consensus.

Clinical follow up

Clinical follow up was performed to assess the cardiovascular event rates in the study population. The institutional electronic patient file system was reviewed for hospital admissions and outpatient contacts for the study population, and any cardiovascular events (cardiac and non-

Table 1 Patient demographics and clinical variables *n* (%)

Men/women (*n*)	32/24
ATPⅢ 10-year CAD risk	
Low (< 10%)	33 (59)
Intermediate (10% to 20%)	17 (30)
High (20% and above)	6 (11)
Individual CAD risk factors	
Hypertension	22 (39)
Diabetes	1 (2)
Hypercholesterolemia	21 (38)
Body mass index > 30 kg/m^2	8 (14)
Family history of CAD	25 (47)
Smoking (current or former)	31 (55)
Current medication	
Beta blockers	3 (5)
ACE inhibitor or Angiotensin Ⅱ receptor antagonists	4 (7)
Diuretics	4 (7)
Calcium channel blockers	5 (9)
Aspirin	33 (59)
Clodipogrel	1 (2)
Statins	10 (18)
Slow release nitrates	1 (2)
Prior non cardiac vascular conditions	
Stroke or TIA	2 (4)
PAD	0 (0)

CAD: Coronary artery disease; PAD: Peripheral arterial occlusive disease; TIA: Transient ischemic attack.

cardiac) were recorded.

Ethical approval

The study was approved by the Danish Data Protection Agency. Retrospective informed consent to obtain data from patient files was obtained through an approval by the Danish Board of Health. Due to the retrospective design, no approval by an Ethical Committee was required.

Statistical analysis

Descriptive statics comprised means and standard deviations of the mean (SD) and proportions (%). Exact confidence limits of proportions were read from Geigy Scientific Tables (volume 2, 1998; CIBA-GEIGY Ltd, Basel, Switzerland). No analytical statistics were used.

RESULTS

Patient population

Of 179 patients who underwent exercise MPI during the observation period, 56 patients fulfilled the eligibility criteria. The main reasons for exclusion were no prior exercise test (n = 97), known CAD (n = 5), and criteria related to the referral exercise test such as ECG compatible with ischemia (n = 15), insufficient heart rate response (n = 4), and lack of access to the original exercise test data (n = 2). The mean time from exercise ECG to MPI was 82 d (range: 8-179 d). Patient demographics and clinical variables are shown in Table 1. A large proportion of patients had hypertention and hypercholesterolemia so mild that is it was not considered therapy-requiring by the referring physicians. A total of 35 patients were asymptomatic at baseline. Functional grading of angina showed Canadian Cardiovascular Society (CCS) grades 1-2 in 11 patients and grades CCS 3-4 in 8 patients; data was missing in two patients. No patients had known heart failure.

Referral exercise ECG

All patients terminated the exercise test because of exhaustion or muscle fatigue. No patients had exercise-limiting angina. The mean peak heart rate was 164 beats per minute, which corresponded to 102% (range 87% to 128%) of the predicted, age-adjusted peak heart rate. The mean workload was 155 W (96%, range 60% to 160%). Twelve patients (21%) reported non-specific chest pain, and the remainder of the patients (79%) were asymptomatic during the exercise test. The mean post-test Duke score was 5 (range -2 to 11), which translated into a low post-test risk in 37 patients (66%) and a moderate risk in 19 patients (34%).

MPI

A total of 53 of 56 patients completed the exercise MPI with a heart rate of least 85% of the age-predicted peak heart rate (mean 99.4%). The mean workload was 166 W. One patient was stopped at 84% of the peak heart rate due to exercise-limiting angina (see later). Two patients failed to reach their target heart rate, and they underwent an adenosine stress test with 25 W of bicycle exercise[11]. Stress-only MPI was performed in 14 (25%) of the patients. The criteria for accepting a stress-only test were as previously described[14].

A total of 7 patients were reported as suffering from ischemia and presented also with quantitative, documented ischemia (SSS > 3) with reversible defects in most patients (Table 2).

Three patients were reported as normal in the original MPI report (two patients with low risk and one patient with intermediate risk) but showed SSS > 3 by segmental score as performed as part of this retrospective analysis. None of these patients had reversible defects. MPI was performed without attenuation correction, and segmentation may be falsely high. Based on minor fixed perfusion defects, and normal wall motion pattern in the affected regions, such patients are mostly reported as normal. None of these patients underwent catheterization or experienced cardiac events during the follow up period.

Coronary catheterization

All patients but one in Table 2 with reported ischemia as well as SSS > 3 underwent cardiac catheterization. MPI served as a gatekeeper for catheterization. Thus, the remainder 49 patients with a visual normal MPI (as well as a normal exercise test) were not routinely referred for cardiac catheterization. However, 3 of these 49 patients underwent catheterization during the follow up and none showed significant stenosis.

One patient with a positive MPI and SSS > 3 was diagnosed with significant CAD (patient 4, Table 2). This

Table 2 Clinical and imaging data for patients classified with coronary artery disease by myocardial perfusion imaging

Patient No	Gender	Age (yr)	ATPⅢ pre test risk	Duke post test risk	SSS > 3	SDS > 3	Cardiac catheterization
1	Male	79	High	Intermediate	+	+	Indeterminate
2	Male	52	Intermediate	Intermediate	+	+	Normal
3	Male	71	Intermediate	Low	+		Normal
4	Male	43	High	Intermediate	+	+	Significant stenosis
5	Female	65	Low	Low	+	+	Not done
6	Male	63	Intermediate	Low	+		Indeterminate
7	Male	48	High	Low	+	+	Indeterminate

SSS: Sum of stress score; SDS: Sum of difference score.

patient with significant two-vessel stenosis had a high pre-test risk of CAD, completed the referral exercise test with non-specific chest pain, reached 104% of peak heart rate, presented an exercise capacity of 200 W, showed no ECG changes, and had an intermediate Duke post-test risk score. He experienced no cardiac events or aggravation of symptoms from referral exercise test to MPI. During MPI exercise testing 4.5 mo later, he experienced exercise-limiting angina and received the radiotracer at 84% of his predicted peak heart rate. There were no ECG changes; however, the MPI showed significant ischemia.

Among the 50 patients with a low or intermediate pre-test risk of CAD, the final diagnostic work up, with the clinical MPI report as a gatekeeper for cardiac catheterization, showed no significant anatomical stenosis in any of these patients (0/50; 0%, 95%CI: 0.0-7.1). One of these patients had an indeterminate lesion (patient 6 in Table 2).

Clinical follow-up

The median follow up time was 1277 d (range 917-1566 d). No patients had any documented cardiac events, such as non-fatal myocardial infarction, cardiac interventions (percutaneous coronary interventions or bypass surgery), or sudden cardiac death (0/56; 0% 95%CI: 0.00-6.38). All patients were alive at follow up. One patient experienced a non-fatal stroke (patient 7 in Table 2).

DISCUSSION

In this study, we investigated the diagnostic value of MPI in patients with a recent, normal exercise ECG. To the best of our knowledge, this study is the first of its kind. Among 56 patients one patient had a significant stenosis as shown by cardiac catheterization. This patient had a high pre-test risk of CAD and should, according to current guideline recommendations, be referred directly for coronary catheterization. By contrast, an exercise MPI did not reveal any significant anatomical stenosis in any of 50 patients with low to intermediate pre-test risk of CAD and a recent, normal exercise ECG test. The majority of these patients had a negative MPI (and thus no subsequent catheterization) or a positive MPI with either normal vessels or insignificant anatomical stenosis).

MPI has solid documentation for the diagnosis and risk stratification of patients with CAD[6]. Several groups have documented that the imaging results from MPI have higher sensitivity and specificity compared to exercise data obtained from the same exercise MPI[7-9]. However, results from a recent, large study showed that the perfusion imaging component of an exercise MPI did not add diagnostic value in patients who were able to perform an adequate workload[15]. Most studies with exercise MPI include patients with intermediate risk of CAD, *i.e.*, the target population for MPI. The difference in diagnostic performance between exercise ECG and MPI in low-risk patients with a low prevalence of CAD remains unknown. Despite guideline recommendations against the use of MPI in low-risk patients, MPI is used widely for such patients[1,16]. The European Society of Cardiology (ESC) gives a class I recommendation for MPI in patients with an inconclusive exercise ECG but reasonable exercise tolerance and a low to intermediate risk of CAD in whom the diagnosis is still in doubt[10]. The clinical documentation for this recommendation is mainly based on patients with established CAD, including patients with prior coronary artery bypass grafting[17]. The ESC guidelines also give a class I recommendation for exercise ECG in patients with intermediate risk of CAD and a class Ⅱb recommendation for low-risk patients[10], which is in direct contrast to US guidelines, which recommend exercise ECG for low-risk patients and MPI for intermediate risk patients[1]. The discrepancy in recommendations across guidelines has been the subject of several recent systematic reviews[18,19]. There is a need for evidence-based guidelines for cardiovascular imaging[20].

In patients with low or intermediate risk of CAD and the ability to exercise, exercise ECG may still provide a sufficient diagnostic test and be a valid gate-keeper modality for additional anatomical and/or functional investigations. This is in accordance with US guidelines[1]. Our findings are also consistent with a recent, large study showing that the perfusion imaging component of an exercise MPI did not add diagnostic value in patients who were able to perform an adequate workload[15].

There are several limitations to this study. First, we recognize that the size of this study population is limited. However, we included well-characterized patients with a technically successful but negative exercise ECG referred for MPI for further diagnostic work up. To the best of our knowledge, no prior studies have described such pa-

tients. Second, cardiac catheterization was not performed in all of the patients, and this may influence the diagnostic accuracy. However, it would not be appropriate to do cardiac catheterization in low-risk patients with a normal exercise and MPI. Even with a positive MPI, catheterization should be optional, depending on the symptoms and extent of functional ischemia. This situation reflects the emerging scenario where only patients with notable ischemia are candidates for revascularization. Recent studies have confirmed clinical benefit to intervention beyond optimal medical therapy in cases of severe ischemia only[21,22]. The extent of symptoms and co-morbidities may have influenced the decision among cardiologists not to perform coronary catheterization. Nevertheless, our study has sufficient power with regards to the negative predictive value of a normal exercise test in patients with low or intermediate risk. None of 50 patients with low or intermediate risk by exercise test were found to have significant stenosis with MPI as the gatekeeper for catheterization (one patient had a non-significant stenosis).

In recent years, a number of new non-invasive tests have been introduced for the diagnosis of CAD, with computerized tomography angiography as one of the most promising techniques[3]. In centers where non-invasive imaging methods are available, such methods will eventually be used as a first-line option for the non-invasive diagnosis of CAD. In other situations, exercise ECG may persist as a gatekeeper modality for further diagnostic work up. Recent studies have shown that coronary computerized tomography angiography surpasses exercise-ECG in cost only, but not in diagnostic performance[23]. Further information is expected from large, ongoing trials comparing different types of anatomical and functional testing methods in patients with low to intermediate risk of CAD.

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COMMENTS

Background

Diagnosis of coronary artery disease (CAD) is important to initiate appropriate interventions and to prevent future major cardiovascular events. Depending of the risk of disease, different diagnostic tests are recommended. The choice of test reflects the diagnostic characteristics of the test (*e.g.*, positive and negative predictive diagnostic values and prognostic value), availability, cost, and risk of procedure. Generally, tests at each level serve as a gate keeper for next-level tests.

Research frontiers

Myocardial perfusion scintigraphy imaging (MPI) is generally indicated for patient with intermediate risk of CAD. International guidelines display diverging recommendations for additional imaging in low risk patients with normal exercise tests.

Innovations and breakthroughs

Prior studies have examined the importance of exercise data with imaging results in exercise MPI. The independent values of these data are relevant for intermediate risk patients referred directly for MPI. This is the first study examining directly the added value of MPI in low to intermediate risk patients who recently performed a normal exercise test.

Applications

By studying the diagnostic value MPI, used as a gate keeper for subsequent cardiac catheterization, for identification of significant stenosis, it was revealed that the diagnostic value of additional imaging was marginal in patients with low and intermediate risk of CAD. The data on high risk patients are not powered to provide firm conclusions.

Terminology

MPI is a functional test for myocardial ischemia. The relation of functional assessment of ischemia and anatomical stenosis is much debated.

Peer review

The authors demonstrated that myocardial perfusion imaging following normal exercise electrocardiography (ECG) did not add diagnostic value in patients with a low or intermediate risk of coronary artery disease, if they could be able to perform an adequate work. No significant conclusions could be made in the subgroup of patients with high-risk. These results support the current and logical clinical management in CAD-suspected patients with normal maximal ECG-stress tests. The study results are in line with previous studies.

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BRIEF ARTICLE

Electrocardiographic features of patients with earthquake related posttraumatic stress disorder

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Abstract

AIM: To analyze electrocardiographic features of patients diagnosed with posttraumatic stress disorder (PTSD) after the Van-Erciş earthquake, with a shock measuring 7.2 on the Richter scale that took place in Turkey in October 2011.

METHODS: Surface electrocardiograms of 12 patients with PTSD admitted to Van Erciş State Hospital (Van, Turkey) from February 2012 to May 2012 were examined. Psychiatric interviews of the sex and age matched control subjects, who had experienced the earthquake, confirmed the absence of any known diagnosable psychiatric conditions in the control group.

RESULTS: A wide range of electrocardiogram (ECG) parameters, such as P-wave dispersion, QT dispersion, QT interval, Tpeak to Tend interval, intrinsicoid deflection durations and other traditional parameters were similar in both groups. There was no one with an abnormal P wave axis, short or long PR interval, long or short QT interval, negative T wave in lateral leads, abnormal T wave axis, abnormal left or right intrinsicoid deflection duration, low voltage, left bundle branch block, right bundle branch block, left posterior hemiblock, left or right axis deviation, left ventricular hypertrophy, right or left atrial enlargement and pathological q(Q) wave in either group.

CONCLUSION: The study showed no direct effect of earthquake related PTSD on surface ECG in young patients. So, we propose that PTSD has no direct effect on surface ECG but may cause electrocardiographic changes indirectly by triggering atherosclerosis and/or contributing to the ongoing atherosclerotic process.

Key words: Earthquake; Posttraumatic stress disorder; Cardiovascular disease; Electrocardiogram

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a psychiatric disease that is characterized by recurrent symptoms of stress and anxiety that develop after exposure to an extreme psychological trauma, such as earthquake, war or accidents[1]. Although acute cardiovascular events, such as sudden death, myocardial infarction and Takotsubo cardiomyopathy, are frequently reported, especially during the early phases of these mental stressors[2-5], cardiovascular effects of PTSD are not well known. While there are a few studies examining electrocardiographic features of veterans with PTSD[6,7], we did not encounter any study examining electrocardiographic features of patients with PTSD re-

lated to an earthquake. In this study, we aimed to analyze electrocardiographic features of patients diagnosed with PTSD after the Van-Erciş earthquake, with a shock measuring 7.2 on the Richter scale that took place in Turkey in October 2011.

MATERIALS AND METHODS

Patients

Twelve patients with PTSD admitted to Van Erciş State Hospital (Van, Turkey) from February 2012 to May 2012 were included in the study. Anyone in the study population with a rhythm other than sinus rhythm, a history of cardiovascular or other chronic medical disorders, or using beta-blockers, tricyclic antidepressants or other medications that affect autonomic function and electrocardiogram (ECG) patterns was excluded from the study. Transthoracic echocardiography was performed in all subjects of the study and subjects with any chamber enlargement, systolic or diastolic dysfunction, valvular heart disease, pulmonary hypertension or any other detectable heart disease were excluded from the study. Psychiatric interviews of the sex and age matched control subjects who had experienced the earthquake confirmed the absence of any known diagnosable psychiatric conditions.

Analysis of electrocardiograms and definitions

Recording of a 12-lead ECG was performed after 10 min of supine rest at standard sensitivity (10 mm = 1 mV) and a paper speed of 50 mm/s. ECG was obtained at the same time of the day (between 09:00 AM and 11:00 AM) for all participants and in a quiet room to minimize external noise. ECGs were scanned to digital media in 300 dpi. Then they were transferred to high-resolution computer screens and evaluated by two of the investigators who were blind to clinical and patient information. Three consecutive beats were used for analysis.

PR interval, R wave amplitude and QRS duration were calculated in lead V5. A PR interval longer than 200 ms was defined as long PR and shorter than 120 ms was defined as short PR. A QRS duration longer than 120 ms was defined as long QRS. The presence and site of pathological Q waves were recorded. A Q wave in any leads longer than 40 ms was defined as pathological q(Q) wave. Abnormal P wave axis was defined as P axis < 0 degrees or > 75 degrees[8]. Intrinsicoid deflection is the duration of the earliest appearing Q or R wave to the peak of the R wave. It was calculated in V2 for the right ventricle and V5 for the left ventricle. Abnormal intrinsicoid deflection was defined > 35 ms and > 45 ms for right and left ventricle respectively.

P wave duration was defined as the time measured from the onset to the end of the P wave deflection. The onset of the P wave was considered as the junction between the isoelectric line and first visible upward or downward slope of the trace. The return of the trace to the isoelectric line was considered to be the end of the P wave. P wave dispersion (Pd) was defined as the difference between maximum and minimum P wave durations (Pmax and Pmin, respectively) occurring in any of the 12 leads[9]. QT interval was defined as the interval from the beginning of the QRS complex to the end of the T wave. The end of the T wave was defined as intersection of the terminal limb of the T wave with the isoelectric baseline[10]. The longest and shortest QT intervals across 12 leads were defined as the maximum QT (QTmax) and the minimum QT (QTmin) intervals, respectively. They were corrected according to heart rate by using the Bazett formula and were defined as corrected QTmax (cQTmax) and corrected QTmin (cQTmin), respectively. cQT dispersion (cQTd) was defined as the difference between cQTmax and cQTmin. For the Tpeak to Tend interval (TpTe) measurement, time interval between the peak of T wave, *i.e.*, the time point in which T wave had highest amplitude and end of the T wave which also was defined as the crossing point of the T wave and isoelectric line, was noted as a function of time. TpTe was also corrected according to heart rate and referred to as cTpTe. Abnormal ECG recordings with ambiguous T-waves, distorted, flat or with high noise levels by any means were excluded.

Left atrial enlargement was defined as a P wave with a broad and negative (> 1 mm) terminal part in lead V1 and/or P wave duration ⩾ 120 ms in leads I or II. Right atrial enlargement was defined as P wave amplitude > 0.2 mV in leads II and aVF and/or > 0.1 mV in lead V1 and V2. Supraventricular or ventricular ectopic beats were defined as one or more supraventricular or ventricular extrasystoles in 10 s. A QRS duration ⩾ 120 ms was defined as prolonged QRS. Ventricular conduction abnormalities were classified as right bundle branch block (RBBB), left bundle branch block (LBBB), left anterior hemiblock (LAH) or left posterior hemiblock (LPHB). Deviation of the QRS or T wave axis to the left (-30 <) or to the right (> 90) was defined as an abnormal QRS axis. The QT interval was corrected using the Bazett formula ($QTc = QT/\sqrt{RR}$). Left ventricular hypertrophy was defined by the Sokolow-Lyon criterion (S in V1 + R in V5 or V6 ⩾ 3.5 mV). Low voltage was diagnosed when the amplitude of the QRS complex in each of the three limb leads (I, II, III) was < 5 mm. Repolarization abnormalities included ST segment elevation, ST segment depression and T-wave inversion.

Statistical analysis

Statistical analysis was performed with SPSS 16.0 (IBM Inc., New Orchard Road, Armonk, NY, United States). Continuous variables were given as mean ± SD and categorical variables were given as percentages. Due to small sample size, comparisons between groups were performed with nonparametric tests. For continuous parameters, Mann-Whitney *U* test was used, while χ^2 or Fisher's exact test was used as appropriate for categorical variables. All statistical comparisons were made within 95%CI. A *P* value of less than 0.05 was accepted as statistically significant.

RESULTS

Except for diastolic blood pressure, demographic fea-

Table 1 Demographic and clinical features of the groups (mean ± SD)

	PTSD (n = 12)	Controls (n = 12)	P value
Age (yr)	28.4 ± 7.5	28.5 ± 7.2	NS
Gender (%female)	91.6	83.30	NS
Body mass index (kg/m^2)	23.8 ± 4.3	24.3 ± 3.1	NS
Creatinine Clearance (mL/min)	99.4 ± 26	99.8 ± 16	NS
Current smoker (%)	16.6	16.6	NS
Family history of coronary artery disease (%)	0	8.3	NS
Systolic blood pressure (mmHg)	103.5 ± 8.8	110.4 ± 11.4	NS
Diastolic blood pressure (mmHg)	64.5 ± 6	71.9 ± 7	0.03
Heart rate (beat/min)	78.7 ± 18.6	78.8 ± 13.5	NS

PTSD: Posttraumatic stress disorder; NS: Not significant.

tures of the groups were comparable (Table 1). There was no one with a history of hypertension, hyperlipidemia or diabetes mellitus in either group. Transthoracic echocardiography was available in all patients and control subjects. Basic echocardiographic measurements of the groups were also similar (Table 2).

Electrocardiographic features of the groups are presented in Table 3. There was no difference between ECG parameters of the groups. There was no one with abnormal P wave axis, short or long PR interval, long or short QT interval, negative T wave in lateral leads, abnormal T wave axis, abnormal left or right intrinsicoid deflection duration, low voltage, left bundle branch block, right bundle branch block, left posterior hemiblock, left or right axis deviation, left ventricular hypertrophy, right or left atrial enlargement and pathological q(Q) wave in either group.

DISCUSSION

Unanticipated catastrophic events resulting in acute psychological stress have been extensively reported as a cause of cardiovascular events and mortality[1]. Kim *et al*[4] reported the relationship between severe emotional stress and vasospastic angina in patients without organic coronary heart disease. Meisel *et al*[11] documented an increase in the incidence of acute MI and sudden death in the Tel Aviv area during the initial phases of the Gulf War in 1991. An increase in hospital admissions for acute MI in England on the day of the 1998 World Cup match against Argentina has also been reported[12]. There is also extensive literature demonstrating increased cardiovascular events and mortality after earthquakes which are good examples of unique unpredictable disasters resulting in severe mental stress. Tsuchida *et al*[13] demonstrated that severe earthquakes result in an increased incidence of acute coronary syndromes and cerebral hemorrhage. Increased cerebrovascular events were also reported after the Hanshin-Awaji earthquake[14,15]. Although the precise pathophysiological mechanism of the cardiovascular consequences of acute mental stressors are not well known, some physiological responses have been proposed to be potential triggers of myocardial supply-demand and by atherosclerotic plaque disruption, thrombus formation and eventually ischemia-arrhythmia[16,17]. Increase in heart

Table 2 Transthoracic echocardiographic features of the groups (mean ± SD)

	PTSD (n = 12)	Controls (n = 12)	P value
Left ventricular end diastolic diameter (mm)	42.8 ± 2.6	43.1 ± 3.4	NS
Left ventricular end systolic diameter (mm)	23.3 ± 1.7	24.9 ± 3.8	NS
Left ventricular ejection fraction (%)	63.3 ± 2.3	63.7 ± 1.8	NS
Interventricular septum thickness (mm)	8 ± 0.6	8.3 ± 0.7	NS
Left ventricular posterior wall thickness (mm)	7.8 ± 0.4	8.2 ± 0.6	NS
Left atrial anteroposterior diameter (mm)	31.5 ± 4.8	29.2 ± 4.1	NS

PTSD: Posttraumatic stress disorder; NS: Not significant.

Table 3 Electrocardiographic features of the groups (mean ± SD)

	PTSD (n = 12)	Control (n = 12)	P value
P wave parameters			
Pmax (ms)	96 ± 15	96.6 ± 11.5	NS
Pmin (ms)	67.7 ± 14.4	72.5 ± 8.6	NS
Pd (ms)	27.7 ± 11.3	24.2 ± 11.6	NS
QT parameters			
QT V5 (ms)	361 ± 41.3	350 ± 37.7	NS
cQT V5 (ms)	403.4 ± 35.1	401.2 ± 20.2	NS
Qtmax (ms)	373.3 ± 44.6	358.3 ± 32.4	NS
Qtmin (ms)	340 ± 36.2	325.8 ± 36.3	NS
QTd (ms)	33.3 ± 26.1	32.5 ± 17.6	NS
cQTmax (ms)	415.5 ± 27.5	411.5 ± 23.2	NS
cQTmin (ms)	380 ± 37.5	373.8 ± 30.3	NS
cQTd (ms)	35.5 ± 26.8	37.7 ± 19.5	NS
T wave parameters			
TpTe V5 (ms)	80.4 ± 17.4	83.3 ± 12.3	NS
cTpTe V5 (ms)	89.3 ± 15.2	95.4 ± 10.9	NS
Presence of negative T wave (anterior leads) (%)	66.6	58.3	NS
Presence of negative T wave (inferior leads) (%)	16.6	0	NS
Presence of U wave (%)	8.3	8.3	NS
PR interval (ms)	145.83 ± 23.53	143.33 ± 16.70	NS
QRS duration (ms)	73.3 ± 9.6	82.5 ± 11.4	NS
Right ventricle intrinsicoid deflection (ms)	26.6 ± 6.8	28.9 ± 4.9	NS
Left ventricle intrinsicoid deflection (ms)	34.5 ± 4	34.4 ± 5.1	NS
R wave amplitude	0.9 ± 0.3	1.2 ± 0.5	NS
Infra HIS conduction abnormalities			
Left anterior hemiblock (%)	0	8.3	NS
Left axis deviation (%)	0	8.3	NS
ST segment elevation (%)	0	16.6	NS

PTSD: Posttraumatic stress disorder; NS: Not significant; Pmax: Maximum P wave duration; Pmin: Minimum P wave duration; Pd: P wave dispersion; cQT: Corrected QT interval; QTd: QT dispersion; cQTd: Corrected QT dispersion; QTmax: Maximum QT interval; cQTmax: Corrected maximum QT interval; QTmin: Minimum QT interval; cQTmin: Corrected minimum QT interval; TpTe: Tpeak to Tend interval; cTpTe: Corrected Tpeak to Tend interval.

rate and blood pressure[18], rising sympathetic activation, decreased parasympathetic tone[1] and sudden catecholamine discharge[19] are some of the blamed psychobiological triggers of cardiovascular events during these catastrophes. All these factors along with vasoconstriction may result in increased shear stress on the vasculature, causing endothelial damage with the potential to disrupt vulnerable plaque[16]. On the other hand, activation of the inflammatory process seems to be most important contributor. Steptoe *et al*[17] showed increased interleukin-6 and tumor necrosis factor alpha following emotional stress, which are stimulators of macrophages/T-lymphocytes, leading to matrix metalloproteinases secretion and atherosclerotic fibrous cap degradation[1,20]. Diminished fibrinolytic activity and increased fibrinogen, von Willebrand factor, Factors VII and VIII could lead to a prothrombotic imbalance during acute mental stress[21,22]. Platelet activation was also shown to be increased during emotional stress secondary to sympathetic activity in plasma, caused by platelet-derived growth factors[23,24]. In addition, in some animal models, phenylephrine or electric shock and noise induced stress gave rise to increased blood pressure, heart rate, ejection fraction, maximal systolic flow velocity, norepinephrine and fibrinogen levels and eventually plaque rupture[25,26].

However, short or long term impact of repetitive mental stress on cardiovascular system in patients with PTSD is much less known. Weiss *et al*[27] found an association between increased rates of metabolic syndrome and PTSD. In another study, PTSD patients were found to have diminished levels of high-density lipoprotein cholesterol and elevated levels of serum cholesterol, triglycerides and low-density lipoprotein cholesterol[28]. One of the few studies examining the long-term mortality risk of patients with PTSD has been published recently[29]. In that study, PTSD was found to be an independent predictor of mortality in multivariate analysis for their study population, 891 military veterans (HR: 1.79, 95%CI: 1.15-2.79, $P = 0.001$). In addition, patients with PTSD ($n = 91$, 98% male) had a trend toward worse survival on Kaplan-Meier analysis ($P = 0.057$). Heart failure, increased end-systolic left ventricular diameter, left ventricular systolic dysfunction and arrhythmia were more frequent in patients with PTSD. In another study, PTSD was prospectively associated with heart disease mortality among veterans free of cardiac disease at baseline[30].

There are few studies comparing ECGs of patients with PTSD and healthy controls[6,7]. Boscarino *et al*[6] showed increased signs of atrioventricular conduction abnormalities and myocardial infarction in male veterans with PTSD. In the other study, Kazaie *et al*[7] examined ECGs of patients with post-war PTSD and detected more ECG abnormalities (abnormal QT interval, inverted T waves, ST segment depression, low voltage QRS complex, sinus tachycardia) in PTSD patients than in controls. However, the patients were older, mostly male and had the disease much longer in those studies than in ours. Therefore, ECG abnormalities found in those studies seem to be a consequence of ischemic heart disease caused by traditional risk factors with a probable contribution of PTSD. On the contrary, in our study, which is the first one comparing the ECGs of patients with earthquake related PTSD and healthy subjects, we could not find any electrocardiographic difference between groups.

In conclusion, our study showed no direct effect of earthquake related PTSD on surface ECG, at least not in short term follow up. Although long term follow up may disclose some ECG changes, most probably these changes will be due to atherosclerotic coronary artery disease. Therefore, we propose that PTSD has no direct effect on surface ECG but may cause electrocardiographic changes indirectly by triggering and/or contributing to the ongoing atherosclerotic process.

COMMENTS

Background

Acute mental stress is a well known trigger of myocardial infarction and anxiety has been recently found to be an independent risk factor for incident coronary heart disease. However, the effects of repetitive anxiety on electrocardiography are not well known.

Research frontiers

This study aimed to analyze electrocardiographic features of patients diagnosed with posttraumatic stress disorder, a psychiatric disease that is characterized by recurrent symptoms of stress and anxiety.

Innovations and breakthroughs

That study is the first one comparing the electrocardiograms (ECGs) of patients with earthquake related posttraumatic stress disorder (PTSD) and healthy subjects in an early period after the disaster.

Applications

The study's results showed no direct effect of earthquake related PTSD on surface ECG in young patients.

Peer review

The article is short, concise and the authors found no ECG abnormalities (such as P-wave dispersion, QT dispersion, QT interval, Tpeak to Tend interval, intrinsicoid deflection durations and other traditional parameters) in victims of an earthquake who developed PTSD compared to control subjects exposed to the same trauma (earthquake) but who did not develop PTSD.

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CASE REPORT

Pacemaker implantation in a patient with brugada and sick sinus syndrome

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Abstract

Brugada syndrome (BrS) is a rare and inherited primary arrhythmic syndrome characterized by ST-segment elevations in the right precordial leads (V_1-V_3) with an increased risk of sudden cardiac death (SCD). Arrhythmias in BrS are often nocturne, and bradyarrhythmias are often seen in patients with loss-of-function mutations in *SCN5A*. In this case-report we present a 75-year old woman referred to our outpatient clinic for inherited cardiac diseases for a familial clinical work-up. Since childhood she had suffered from dizziness, absence seizures, and countless Syncope's. In 2004 sick sinus syndrome was suspected and she was treated with implantation of a pacemaker (PM) at another institution. An inherited cardiac disease was one day suddenly suspected, as the patient had a 61-year old brother who was diagnosed with symptomatic BrS, and treated with an implantable cardioverter defibrillator (ICD) after aborted SCD. A mutation screening revealed a *SCN5A* [*S231CfsX251* (*c.692-693delCA*)] loss-of-function mutation not previously reported, and as a part of the cascade screening in relatives she was therefore referred to our clinic. In the 7 year period after PM implantation she had experienced no cardiac symptoms, although her electrocardiogram changes now were consistent with a BrS type 1 pattern. A genetic test confirmed that she had the same mutation in *SCN5A* as her brother. In this case-report we present a loss-of function mutation in *SCN5A* not previously associated with BrS nor presented in healthy controls. Sinus node dysfunction has previously been documented in patients with symptomatic BrS, which suggests it is not a rare concomitant. The only accepted treatment of BrS is today implantation of an ICD. In the future studies should evaluate if PM in some cases of symptomatic BrS can be used instead of ICDs in patients with a loss-of-function *SCN5A* mutations

Key words: Brugada syndrome; Pacemaker; Arrhythmias; Sudden cardiac death

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INTRODUCTION

Brugada syndrome (BrS) is a rare and inherited primary

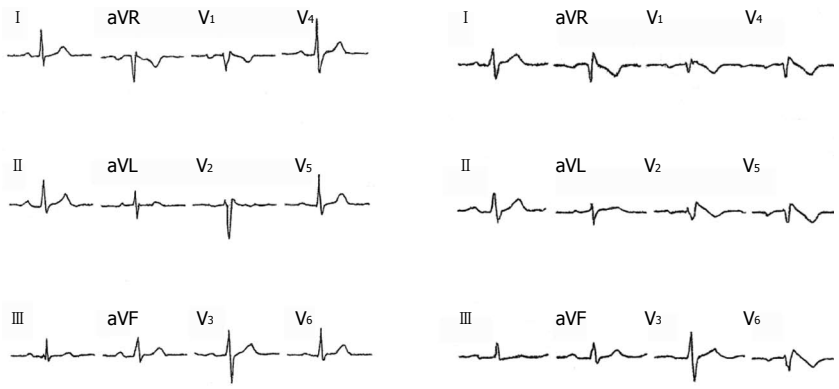

Figure 1 Baseline and Brugada type 1 electrocardiogram. A: Baseline electrocardiogram (ECG) recording; B: Brugada type 1 ECG under 150 mg Flecanide over 10 min intravenously. In V_{4-6} in the recording were placed as elevated electrode position in IC2 at V_1 at sternum and V_2, respectively.

arrhythmic syndrome characterized by ST-segment elevations in the right precordial leads (V_1-V_3) with an increased risk of sudden cardiac death (SCD) due to malignant ventricular arrhythmias in the absence of a structural heart disease[1]. Arrhythmias in BrS are often nocturne, and brady-arrhythmias are often seen in patients with loss-of-function mutations in the *Sodium channel gene* (*SCN5A*)[2]. *SCN5A* encodes the alpha subunit ($Na_v1.5$) of the cardiac sodium channel complex and it is the only recommended gene for targeted screening in BrS[3]. Mutations in *SCN5A* have been reported to be associated with several other types of disease entities such as lone atrial fibrillation and the Long QT Syndrome[4]. It is suggested that the loss-of-function mutations in *SCN5A* create the substrate for a re-entry circuit in the ventricular myocardium, but may also increase vagal activity, thus facilitating development of arrhythmias[1].

CASE REPORT

A 75-year old woman was in 2011 referred to our outpatient clinic for inherited cardiac diseases for a familial clinical work-up. Since childhood she had suffered from dizziness, absence seizures, and countless syncope's. In 2004 sick sinus syndrome was suspected as a 24 h Holter monitoring revealed 67 sinus arrests [electrocardiogram (ECG) not available] of more than 2.5 s with the longest being 6.85 s. The asystole were followed by dizziness but no syncope's appeared under surveillance. The SSS diagnosis seemed obvious, and she was treated with implantation of a pacemaker (PM) at another institution.

An inherited cardiac disease was one day suddenly suspected, as the patient had a 61-year old brother who was diagnosed with symptomatic BrS, and treated with an implantable cardioverter defibrillator (ICD) after aborted SCD. A mutation screening revealed a *SCN5A* [*S231CfsX251* (*c.692-693delCA*)] loss-of-function mutation not previously reported, and as a part of the cascade screening in relatives she was therefore referred to our clinic.

In the 7 year period after PM implantation she had experienced no cardiac symptoms. An ECG and elevated electrode placement ECG (EEP-ECG) showed no BrS pattern. However, BrS was suspected due to the family history and a Flecanide test (150 mg intravenously) was performed. After infusion, 1 and 2 mm ST elevations appeared in V_1 and V_2, respectively (Figure 1). Changes were consistent with a BrS type 1 ECG pattern and a genetic test confirmed that she had the same mutation in *SCN5A* as her brother. As the patient had been free of symptoms after PM implantation, it was decided not to upgrade to an ICD unless syncope would re-appear.

DISCUSSION

In this case-report we present a loss-of function mutation in *SCN5A* not previously associated with BrS nor presented in healthy controls[5]. It has been suggested that the loss-of-function mutations in *SCN5A* create the substrate for a re-entry circuit in the ventricular myocardium, but may also increase vagal activity, thus facilitating development of arrhythmias in BrS[1]. The mutation, that we report, was initially found in a brother diagnosed with BrS after aborted SCD and hereafter in our patient, initially diagnosed with SSS. Sinus node dysfunction has previously been documented in patients with symptomatic BrS, which suggests it is not a rare concomitant[6,7]. However, PM implant in this case kept the patient free of symptoms for several years, and this support the theory that increased vagal tonus may cause bradycardia-related arrhythmias which in other isolated cases have been treated successfully with rapid pacing as well[1]. The only accepted treatment of BrS is today implantation of an ICD. In the future studies should evaluate if PM in some cases of symptomatic BrS can be used instead of ICDs in patients with ICDs in patients with *SCN5A* loss-of-function mutations.

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Issue with no volume

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Statistical data
Write as mean ± SD or mean ± SE.

Statistical expression
Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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