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EDITORIAL

Peripheral reflex feedbacks in chronic heart failure: Is it time for a direct treatment?

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Abstract

Despite repeated attempts to develop a unifying hypothesis that explains the clinical syndrome of heart failure (HF), no single conceptual paradigm for HF has withstood the test of time. The last model that has been developed, the neurohormonal model, has the great virtue of highlighting the role of the heart as an endocrine organ, as well as to shed some light on the key role on HF progression of neurohormones and peripheral organs and tissues beyond the heart itself. However, while survival in clinical trials based on neurohormonal antagonist drugs has improved, HF currently remains a lethal condition. At the borders of the neurohormonal model of HF, a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems. There are several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to the evolution of the disease. Hence, on top of guideline-recommended medical therapy, mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional potential therapeutic targets.

Key words: Baroreflex; Chemoreflex; Ergoreflex; Heart failure; Sympathetic system; Neurohormones

Core tip: At the borders of the neurohormonal model of heart failure (HF), a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems. There are several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to the evolution of the disease. Hence, on top of guideline-recommended medical therapy mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional

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INTRODUCTION

Heart failure (HF), a pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the metabolizing tissues requirements, or can do so only with elevated filling pressures[1], is currently a real epidemic in western countries, affecting more than 20 million people in the world, with massive socio-sanitary costs[2].

Despite repeated attempts to develop a unifying hypothesis that explains the clinical syndrome of HF, no single conceptual paradigm for HF has withstood the test of time. The last model that has been developed, after the cardiorenal and the cardiocirculatory models focusing respectively on salt-water retention and low cardiac output/peripheral vasoconstriction, is the neurohormonal model[3]. This model has the great virtue of highlighting the role of the heart as an endocrine organ, as well as to shed some light on the key role on HF progression of neurohormones and peripheral organs and tissues beyond the heart itself. However, while survival in clinical trials based on neurohormonal antagonist drugs has improved, HF currently remains a lethal condition, with 50% mortality within 5 years of diagnosis and less than 15% survival after 10 years[2,4].

At the borders of the neurohormonal model, a partially unexplored path trough the maze of HF pathophysiology is represented by the feedback systems (Figure 1). There are indeed several evidences, from both animal studies and humans reports, that the deregulation of baro-, ergo- and chemo-reflexes in HF patients elicits autonomic imbalance associated with parasympathetic withdrawal and increased adrenergic drive to the heart, thus fundamentally contributing to worsening arrhythmias and haemodynamics. Hence, on top of guideline-recommended medical therapy mainly based on neurohormonal antagonisms, all visceral feedbacks have been recently considered in HF patients as additional therapeutic targets: baroreflex activation therapy for baroreceptors[5], physical training for muscle metaboreceptors[6], and carotid body (CB) denervation for chemoreceptors[7].

BAROREFLEX

The baroceptors are mechanoreceptors located in the sinus caroticus and in the aortic arch, where terminal nerve endings are endowed in the wall of these vessels and activated by blood pressure-induced wall stretch. Information deriving from these sites travel along a path constituted by the nerve of Hering, that merges with the fibres of the glossopharingeal nerve; those travelling from the aortic arch take the path of the afferent fibres of the vagus nerve. Inputs hence travel towards the principal centre of integration of information regarding the cardiovascular system, that is the nucleus tractus solitarii in the dorsal area of its medial and lateral divisions. Here signal are processed and integrated with information ascending from the periphery and descending from central nervous system and given back to the heart and peripheral arterial vessels *via* the vagus nerve[8]. The response is a vagally-mediated change in heart rate and a sympathetic modulation of vasomotion, in order to preserve blood pressure stability over time and avoid fluctuations[9]. Altered baroreflex sensitivity (BRS) has been demonstrated to independently contribute to worsen prognosis in HF, mainly by failing to counteract the adrenergic activation with consequent electrical instability and arrhythmic sequelae, in both the pre- and post-betablocker era[10,11].

The baroreflex has been the first neurovegetative feedback to be clinically targeted in HF. BRS activation was first indirectly attempted by vagal nerve stimulation (VNS). After the first safety and tolerability reports on VNS (side effects: Hoarseness, cough and sensation of electrical stimulation) some preliminary studies also showed amelioration of symptoms and indexes of left ventricular (LV) remodelling[12,13]. These observations led to a phase Ⅲ sham-controlled trial. The neural cardiac therapy for HF trial enrolled 87 patients with systolic HF [LV ejection fraction (LVEF) < 35%] who underwent device implantation and randomization to device in ON or OFF modality, but failed to demonstrate any effect of VNS on both primary (LV end systolic diameter) and secondary endpoints (LV end systolic volumes, LVEF, oxygen consumption and natriuretic peptide levels)[14].

Baroreceptor stimulation could also be achieved by directly stimulating carotid sinus by subcutaneously implanted device: This approach is known as baroreflex activation therapy (BAT). The first promising results obtained in an animal model of HF (dog with HF induced by microembolization) in terms of reverse remodelling, improved systolic function and amelioration of neurohormonal profile (reduced adrenergic activity), where secondarily confirmed also in a proof of concept study performed in humans, where an amelioration of symptoms was also observed[15]. Few on-going randomized studies are currently addressing the efficacy and therapeutic potential of baroreflex activation therapy in HF; in particular, the CVRx® Rheos® Diastolic Heart Failure Trial (clinicaltrial.org: NCT00718939) and the Rheos® HOPE4HF Trial (NCT00957073) will address the impact of BAT on diastolic HF (LVEF > 40%), whereas in systolic HF patients, the only ongoing randomized trial is the Barostim HOPE4HF (Hope for Heart Failure) study (NCT01720160).

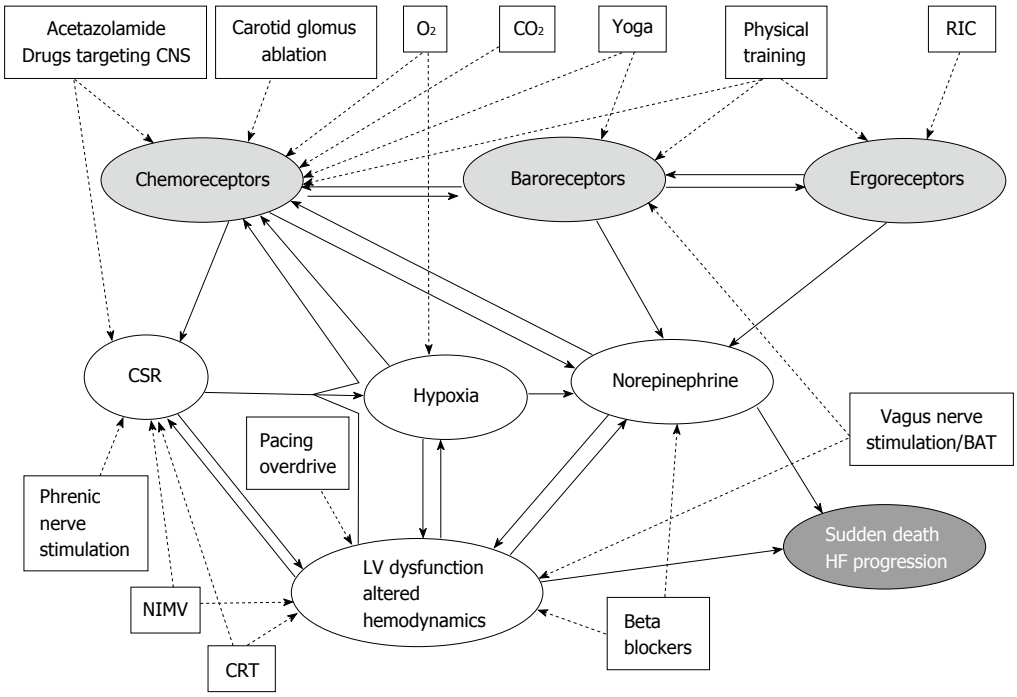

Figure 1 Schematic representation of the reflex feedbacks involved in heart failure. Arrows indicate direct effects/influences. Dotted arrows link established or potential therapeutic interventions with targets. CNS: Central nervous system; RIC: Remote ischemic conditioning; CSR: Cheyne-Stokes respiration; NIMV: Noninvasive mechanical ventilation; LV: Left ventricular; HF: Heart failure; BAT: Baroreceptor activation therapy; CRT: Cardiac resynchronization therapy.

CHEMOREFLEX

The chemoreflex is physiologically in charge of proportionally modulating ventilation in response to a change in the respiratory gases, namely oxygen (O_2) and carbon dioxide (CO_2), in order to keep pH constant for enzymatic processes. Classical physiology indicates two separate chemoreceptor groups: Peripheral chemoreceptors (PC) located in carotid-aortic bodies and sensitive both to hypoxia and hypercapnia/acidosis, and central chemoreceptors (CC) located in different regions of the brainstem, cerebellum, hypothalamus and glia and considered to be sensitive only to hypercapnia/acidosis.

Chemoreceptors seem to act as primary inputs in HF. Several studies indicate that both PC and CC are hyperactive in HF[16-19]. The increased activity of chemoreceptors is commonly considered the main determinants of Cheyne-Stokes respiration[16-19], a detrimental respiratory pattern (with prognostic significance) characterized by alternating cycles of hyperventilation and apneas, with unfavourable oxygen desaturation. Furthermore, PC/CC hypersensitivity also negatively impact on respiration kinetics during exercise with ventilatory inefficiency and dyspnoea on effort in HF patients[18,19]. The hyperactivity of PC/CC, both directly (baseline tonic activity and phasic stimulation during O_2/CO_2 changes)[20] and indirectly, *via* Cheyne-Stokes respiration (CSR) occurrence[21], is also responsible of increased adrenergic drive and arrhythmias in HF patients[17-19]. Finally, increased chemosensitivity to both hypoxia[16] and hypercapnia[19] was found to be an independent prognostic marker in HF.

A partial inhibitory effect on PC was shown in HF patients with both transient hyperoxia, and drugs, such as dihydrocodeine or acetazolamide. In HF patients, dihydrocodeine mediated PC inhibition was only associated with improved exercise performance[22]. In the same setting, acetazolamide[23] and hyperoxia[24] were instead associated with about 50% reduction of CSR severity, translating in the case of hyperoxia also with reduced sympathetic activity. Denervation of the PC chemoreceptors by CB ablation in animals with experimentally induced HF has recently emerged as a very promising option. CB ablation is indeed able to normalize the chemoreflex sensitivity in HF animals, with reduction of both adrenergic activity and disappearance of central apneas[7,25]. This was confirmed also by pharmacologic attenuation of CB activity with an inhibitor of hydrogen sulfide[26]. Interestingly, in a model of HF induced by coronary ligation in rats, CB also reduced the amount of myocardial fibrosis unrelated to myocardial infarction, with positive effect on left ventricular systolic function and, more importantly on short term survival[25]. A single report in a patient with HF has testified the feasibility in humans[27]. Differently from these still preliminary, but intriguing results on PC modulation, currently no studies have tested the possibility to directly act on CC, maybe due to the multiplicity of CC centers in the central nervous system, the complexity of their interlink, and the difficulty to directly and selectively act on these receptors.

ERGOREFLEX

The ergoreflex is the neural mechanism enabling to

modulate ventilation and sympathetic outflow according to the intensity of physical activity[28]. Its components are the metaboreflex, activated by the accumulation of metabolites in the exercising muscles, and the mechanoreflex, responsive to muscle tension during exercise[29-31].

HF patients frequently develop a skeletal myopathy ascribable to deconditioning, reduced perfusion of the muscles, inflammation, and a systemic catabolic state[29,30,32]. In 1994, a "muscle hypothesis" of HF was formulated, suggesting that ergoreceptor contribution to the autonomic, hemodynamic, and respiratory responses to exercise would be enhanced in CHF patients[33]. Two years later, ergoreflex overactivity was first found in HF patients compared with healthy subjects[6]. These results were corroborated by subsequent studies, which correlated increased ergoreceptor sensitivity to lower lean body mass, reduced exercise tolerance, decreased left ventricular function, and worse New York Heart Association functional class[30]. Interestingly, in HF patients with preserved exercise capacity, ergoreflex overactivity has been also associated with increased central and peripheral chemoreceptor sensitivity, and depressed baroreceptor sensitivity[30].

Currently the only acknowledged treatment for modulating ergoreflex overactivity is represented by exercise training. The effects of training on ergoreflex sensitivity have been evaluated mostly in animal models[34]. In humans, six weeks of forearm training were able to markedly reduce metaboreceptor sensitivity, while six weeks of detraining brought the situation back to baseline[29]. A positive effect on muscle structure and function has been after confirmed in other studies, still in HF patients[35,36]. It is reasonable to assume that the positive impact of exercise training on HF patients (in terms of increased exercise tolerance, quality of life, cardiac function, neuro-hormonal activation and overall prognosis)[37-40] partially relies upon reduced ergoreflex overactivity, as confirmed by a recent study[41].

CONCLUSION

The lessons learned from failures (*e.g.*, inotropic drugs) and the successes (*e.g.*, neurohormonal antagonist drugs) in treating HF indicate that the development of innovative treatments for HF should take into account the complex pathophysiology of the disease: In particular, new treatments should target the pathways involved in the evolution of the disease. As outlined above, peripheral reflexes are deeply involved in the pathophysiology of HF and represent a potential target of therapy. Although, some preliminary data in animals and humans are promising, more studies enrolling a large number of patients are clearly needed to reinforce the rationale of treating the peripheral reflex feedbacks and to disclose the prognostic value of these interventions.

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REVIEW

Do neutrophil extracellular traps contribute to the heightened risk of thrombosis in inflammatory diseases?

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Abstract

Thrombotic events, both arterial and venous, are a major health concern worldwide. Further, autoimmune diseases, such as systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, and antiphospholipid syndrome, predispose to thrombosis, and thereby push the risk for these morbid events even higher. In recent years, neutrophils have been identified as important players in both arterial and venous thrombosis. Specifically, chromatin-based structures called neutrophil extracellular traps (NETs) play a key role in activating the coagulation cascade, recruiting platelets, and serving as scaffolding upon which the thrombus can be assembled. At the same time, neutrophils and NETs are emerging as important mediators of pathogenic inflammation in the aforementioned autoimmune diseases. Here, we first review the general role of NETs in thrombosis. We then posit that exaggerated NET release contributes to the prothrombotic diatheses of systemic lupus erythematosus, ANCA-associated vasculitis, and antiphospholipid syndrome.

Key words: Thrombosis; Neutrophil extracellular traps; Lupus; Vasculitis; Antiphospholipid syndrome

Core tip: In order to capture and kill pathogens, neutrophils release webs of chromatin and antimicrobial proteins called neutrophil extracellular traps (NETs). These NETs are also emerging as important players in inflammatory and thrombotic disorders. In this review, we describe the mechanisms by which the various components of NETs promote thrombosis. Further, we highlight emerging evidence that NETs may play a particularly important role when thrombosis occurs in patients with systemic autoimmune diseases such as

lupus, vasculitis, and antiphospholipid syndrome.

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INTRODUCTION

Blood vessel occlusion attributable to thrombosis is a major health concern in both the United States and worldwide. Most United States studies have suggested annual incidence for venous thromboembolism (VTE) on the order of 1/1000. For example, a classic retrospective study reviewed medical records in Minnesota from 1966 through 1990, and found a VTE incidence of 117 per 100000[1]. A more recent community study addressed VTE incidence in Worcester, Massachusetts and found a similar incidence of 104 per 100000[2]. In Norway, incidence of first VTE is at a similar level, estimated at 1.43 per 1000 person years[3].

VTE morbidity is especially problematic in hospitals. For example, a multinational cross-sectional study of the acute inpatient setting noted that VTE, and specifically pulmonary embolism, accounted for 5%-10% of deaths in hospitalized patients[4]. It should also be noted that VTE carries a high risk of not just morbidity, but also death. In the aforementioned Worcester population study, acute all-cause mortality in patients with VTE was 6.6%[2]. Another United States community-based study, found 28-d mortality following VTE to be 11%, with that risk climbing to 25% in patients with cancer-associated thrombosis[5]. The aforementioned Norwegian study found the risk of death to be especially high following pulmonary embolism, specifically 2.1-fold higher than for deep vein thrombosis (DVT)[3].

Similar to VTE, cardiovascular disease (CVD), especially myocardial infarction and stroke, is a major cause of worldwide morbidity and mortality. CVD results from an inflammatory vasculopathy of arteries called atherosclerosis, which places patients at risk for acute arterial occlusions and downstream ischemia. Global data from the late 1990s suggest that on the order of one-third of all deaths worldwide are caused by CVD[6]. It has also been suggested that access to healthcare plays a critical role in the morbidity attributable to events like strokes, with countries in eastern Europe, north Asia, central Africa, and the south Pacific having particularly high levels of disability following such events[7].

While thrombotic events are clearly a major problem in the general population, the risk is further amplified in the setting of many systemic autoimmune diseases. For example, a meta-analysis of VTE risk in such diseases (excluding pregnant and postoperative patients) found an increased risk that was particularly striking in systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, with odds ratios of 7.29 and 7.97, respectively[8]. Another study of SLE patients found a 7.6% risk of thrombosis over approximately 10 years, which climbs as high as 20.1% in the presence of a particular class of autoantibodies referred to as antiphospholipid antibodies (discussed in more detail below)[9]. When an ANCA-associated vasculitis cohort was followed for six years, there was a 12% prevalence of VTE[10]; interestingly, the incidence was 1.8 per 100 person-years when disease was quiescent, and climbed to 6.71 per 100 during active disease[10].

Patients with systemic autoimmune diseases are also at high risk for CVD. For example, in a prospective cohort of SLE patients, 48% of deaths were attributable to CVD, with risk factors including smoking, endothelial activation, elevated C-reactive protein, and antiphospholipid antibodies[11]. SLE patients may be at particular risk for cerebrovascular events[12], with some studies suggesting that more than 20% of mortality may be attributable to stroke[13]. CVD has similarly been documented at increased levels in ANCA-associated vasculitis, with a rate of acute myocardial infarction that is at least 2.5-times higher than expected based on traditional cardiovascular risk factors[14].

NEUTROPHIL EXTRACELLULAR TRAPS

The neutrophil, as the most abundant leukocyte in circulating blood, plays a critical role in the innate immune system[15-20]. Formed in the bone marrow from myeloid precursors[21], neutrophils are then released into the bloodstream. From there, they can be recruited to sites of inflammation/infection in response to endogenous or pathogen-derived chemoattractants[17,20]. One strategy by which neutrophils target and kill microbes is phagocytosis[22]. Once pathogens are captured in intracellular vacuoles, they are destroyed by reactive oxygen species (oxidative burst)[23] and antimicrobial proteins (degranulation)[24]. Upon the completion of phagocytosis, neutrophils generally undergo apoptosis before being ingested by neighboring macrophages as inflammation resolves[25-27]. For decades, phagocytosis was considered to be the primary mechanism by which neutrophils targeted infections; however, that perception changed with the discovery of neutrophil extracellular traps (NETs) - one of the most interesting and intensively-studied aspects of neutrophil biology in recent years.

NETs target pathogens

NET release (or NETosis), as first described by Brinkmann *et al*[18] in 2004, is an active form of neutrophil death that releases a web of chromatin and antimicrobial proteins into the extracellular space. At the core of NETs are chromatin fibers (about 17 nm in diameter) composed of DNA and histones, positively-charged proteins that normally function in the nucleus to package DNA and regulate gene expression. These fibers are further

lined by granule-derived antimicrobial proteins such as neutrophil elastase, myeloperoxidase (MPO), cathepsin G, proteinase 3 (PR3), defensins, and cathelicidin LL-37. NETs target pathogens by a combination of sequestration (preventing their dissemination in the body) and highly-localized microbicidal activity[18]. Both Gram-negative (*Shigella flexneri*[18], *Klebsiella pneumoniae*[28]) and Gram-positive (*Streptococcus aureus*[29], *Listeria monocytogenes*[30]) bacteria can be targeted by NETs, as can fungi (*Candida albicans*[31], *Aspergillus nidulans*[32], *Aspergillus fumigatus*[33]). NETs have also been shown to be effective in killing particular protozoans and viruses[34-36]. Intriguing recent work has demonstrated that neutrophils are capable of sensing differences in microbe size such that NETs are preferentially released when the neutrophil is confronted by larger pathogens and microbial clusters that cannot be engulfed by phagocytosis[37].

It is also interesting to note that certain microbes have evolved mechanisms for evading NETs. For example, surface modification may dampen neutrophil activation and NET binding[38-40]. Also, pathogen-derived nucleases are well established as destabilizers of NETs[41-43]. That NETs form an important arm of antimicrobial innate immunity is exemplified by the fact that defects in NET generation, or experimental NET depletion, increase susceptibility to various kinds of infections in mice and humans[28,44-49].

Mechanisms of NET release

NET release can be triggered by a variety of stimuli including microbes, pharmacological agents (phorbol 12-myristate 13-acetate and calcium ionophore[50]), inflammatory cytokines (interleukin 8[51], tumor necrosis factor α[52]), growth factors (granulocyte colony-stimulating factor[53]), activated endothelial cells[54], activated platelets[55], and immune complexes[56]. Following this initial trigger, various pathways intersect to facilitate the extrusion of NETs. For example, some think of NETosis as a variant of autophagy since netting neutrophils display characteristics of autophagy including the formation of autophagosomes[57]. Indeed, inhibition of autophagy-associated signaling prevents NETosis in some contexts[58]. Generation of reactive oxygen species (ROS) by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex has also been considered by many as an absolute prerequisite to NET formation[48,59,60]. Mechanistically, protein kinase C activation[61] and RAF/MEK/ERK signaling[62] lead to phosphorylation of gp91^{phox}[63], p67^{phox}[64], and p47^{phox}[65], which results in assembly of the functional NADPH oxidase complex for ROS generation. However, recent evidence has also shown that activation of SK3 potassium channels, mediated by calcium influx, may lead to an alternative, NADPH oxidase-independent mechanism of NETosis[66].

Once activated, neutrophils preparing for NETosis flatten and adhere tightly to the substratum. ROS are generated and cytoplasmic granules disintegrate releasing their contents into the cytoplasm. Neutrophil elastase then migrates to the nucleus, where it degrades linker histone H1 and processes core histones, thereby promoting chromatin relaxation[28]. This is followed by the translocation to the nucleus of MPO, which also binds chromatin and promotes decondensation, albeit by an unknown mechanism[28]. In addition, the relaxation of chromatin is further promoted by post-translational modification of histone arginine residues to neutral citrullines by the enzyme peptidylarginine deiminase 4 (PAD4)[46,67-69]. Following dissolution of the nuclear membrane, the plasma membrane ruptures casting NETs into the extracellular space[48].

It should be noted that the above description is of what is sometimes called "suicidal" NETosis. However, NETs can also be released in more rapid fashion, in a manner that does not lead to neutrophil death. This concept of "vital" NETosis, which especially occurs in the context of the direct interaction between neutrophils and microorganisms, has been described in detail in a recent review article[70].

HOW DO NETS PROMOTE THROMBOSIS?

Thrombosis results from dysregulation of normally-protective hemostatic systems, with the end result being a clot in the vessel lumen and obstruction of blood flow. If the occlusion is not resolved, it can have marked consequences including infarction, embolization, and even death. Blood coagulation can be initiated by two classic pathways. The first, historically termed "extrinsic", starts with the release of thrombogenic tissue factor from endothelium and leukocytes, while the second "intrinsic" pathway is initiated by the activation of circulating clotting factors on negatively-charged surfaces. Both of these pathways converge at a common point (factor Ⅹ) with the subsequent activation of the protease factor Ⅱ (also called thrombin). Thrombin then converts fibrinogen into insoluble fibrin, which is indispensable for clot formation[71]. Platelet activation (associated with the release of procoagulant polyphosphates among other bioactive molecules) and platelet aggregation (to form a platelet plug) are also important processes in normal hemostasis, as well as pathologic thrombosis[72,73]. These pathways are further regulated by natural anticoagulants like tissue factor pathway inhibitor, antithrombin, thrombomodulin, and protein C, which act on various targets to limit thrombin generation[74].

NETs are now known to be an integral component of thrombi, and actually essential for thrombosis in many contexts (Figure 1). NETs serve as structural scaffolding for entrapment and aggregation of platelets and erythrocytes[75]. Additionally, negatively-charged NETs bind plasma proteins like fibrinogen, fibronectin, and von Willebrand factor (VWF), thereby stabilizing the clot[75]. In animal models, it has been shown that dismantling NETs by deoxyribonuclease (DNase) treatment or knocking out PAD4 (an enzyme essential for NET formation) diminishes thrombosis[76-79]. Mechanistically, interesting studies, using both *in vitro* and *in vivo* systems, have

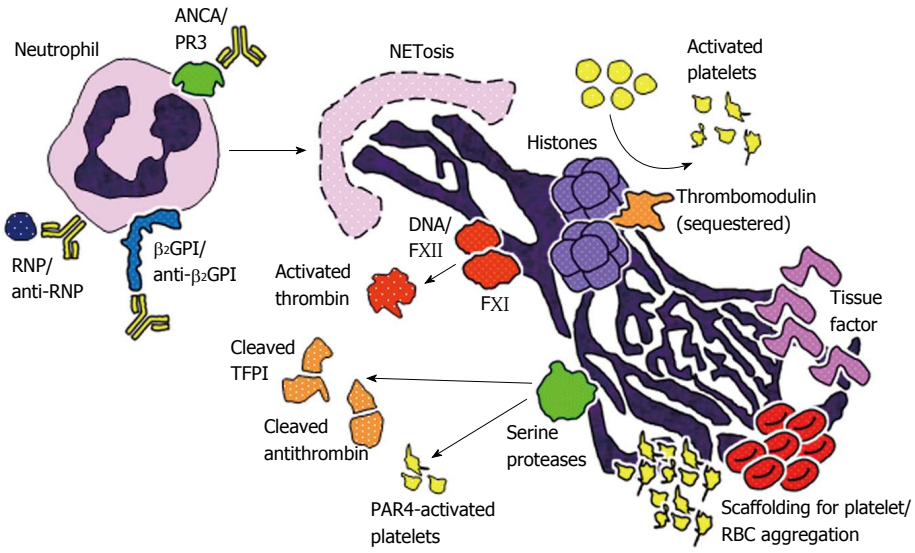

Figure 1 Schematic representation of potential mechanisms by which neutrophil extracellular traps may promote thrombosis in systemic autoimmune diseases. First, a number of stimuli may promote NETosis in systemic autoimmune diseases including ribonucleoprotein (RNP)/anti-RNP complexes in systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody (ANCA) engagement with surface proteinase 3 (PR3) in vasculitis, and the interaction of anti-beta-2 glycoprotein I (β_2GPI) with surface β_2GPI in antiphospholipid syndrome. The DNA component of NETs activates factor XII (FXII), initiating a cascade (along with factor XI) that ultimately leads to the formation of thrombin. Histones in NETs activate platelets and sequester certain anticoagulant molecules like thrombomodulin and protein C. Neutrophil serine proteases present in NETs, such as neutrophil elastase and cathespin G, cleave the anticoagulant molecules tissue factor pathway inhibitor (TFPI) and antithrombin, and also activate platelets through various pathways including protease-activated receptor 4. NETs also may present procoagulant tissue factor in some contexts. Finally, NETs serve as scaffolding for the assembly and aggregation of platelets and red blood cells (RBCs). NET: Neutrophil extracellular trap.

Table 1 Neutrophil extracellular trap-associated molecules that may play a role in promoting thrombosis

NET component	Role in thrombosis
DNA backbone	Negatively charged surface capable of activating factor XII[80]
	Coassembly of TFPI and serine proteases at thrombus[86]
Histones	Platelet activation[83,84]
	Prevent activation of natural anticoagulant, protein C[85]
Elastase	Cleavage of TFPI[86]
	Cleavage of antithrombin[87]
	Strips proteoglycan layer of arterial media to expose VWF[88]
Cathepsin G	Cleavage of TFPI[86]
	Platelet activation[89,90]
Tissue factor	Platelet activation[91,104]
	Thrombin generation by extrinsic pathway of coagulation[91,104]

TFPI: Tissue factor pathway inhibitor; VWF: Von Willebrand factor; NET: Neutrophil extracellular trap.

shown that several NET components are capable of contributing to coagulation and thrombus formation (Table 1).

DNA backbone

Coagulation factor XII, a plasma serine protease capable of activating factor XI and prekallikrein, is recognized as the traditional initiator of the intrinsic pathway. factor XII is well known to be activated by negatively-charged surfaces both *in vitro* and *in vivo*, and it turns out that the anionic backbone of NETs (*i.e.,* DNA) is a capable activator of factor XII[80]. It has consequently been shown that factor XIIa (the activated form) can contribute to thrombus formation by both factor XI-dependent and independent mechanisms[81,82].

Histones

As mentioned above, histones are positively-charged proteins that normally function to package DNA in the nucleus; they are also the most abundant proteins in NETs. Histones trigger platelet activation and thrombin generation in a dose-dependent manner[83,84]. Indeed, upon treatment with histones, platelets exhibit several activation-associated characteristics such as aggregation, exposure of phosphatidylserines, and surface expression of P-selectin[83,84]. The ability of histones to activate platelets seems to be at least partially dependent on signaling through platelet Toll-like receptor 2 (TLR2) and TLR4[83,84], with a further contribution from several notable intracellular pathways including ERK, Akt, p38, and nuclear factor-κB. Importantly, when histones complex with DNA (as is observed in NETs), their ability to promote platelet activation and thrombin generation is further amplified[84]. Intersecting with coagulation pathways, histone-activated platelets release polyphosphates, which potently promote thrombin activation[84]. Independent of platelets, it has also been suggested that histones contribute to the activation of thrombin by sequestering thrombomodulin and protein C (a natural anticoagulant), and thereby preventing thrombomodulin-dependent activation of protein C[85]. These varied experiments (primarily done with purified components *in vitro*) have been supported by work in animal models, where infusion of histones promotes DVT formation in mice in the context of inferior vena cava

flow restriction[76].

Serine proteases: Neutrophil elastase and cathepsin G

Granule-derived serine proteases, which are among the most abundant non-histone proteins in NETs, potentially engage with blood coagulation in a number of ways. For example, mice deficient in neutrophil elastase and cathepsin G exhibit defects in tissue factor activation, fibrin formation, and thrombus stabilization[86]; in this system, at least one function of the proteases is to degrade an antagonist of coagulation, tissue factor pathway inhibitor (TFPI). Interestingly, the DNA component of NETs is required for the coassembly of TFPI and the proteases, thereby inactivating TFPI at the point of thrombosis[86].

Other mechanisms have also been described. Neutrophil elastase promotes the proteolytic cleavage of the anticoagulant antithrombin[87]. Elastase (in cooperation with matrix metalloproteinase 9) also degrades the proteoglycan network of the arterial media, thereby exposing collagen for VWF binding and platelet adhesion[88]. Further, cathespin G can promote a procoagulant state by cleaving and activating platelet protease activated receptor 4 signaling, thereby enhancing thrombus formation and fibrin deposition under flow conditions[89,90].

Tissue factor

In 2012, von Brühl *et al*[80] showed that the combination of intravascular NET formation and tissue factor are essential for development of thrombi in a mouse model of DVT. The NETs were not only decorated with tissue factor, but also with protein disulfide isomerase, which can activate it. In this system, the tissue factor was felt to originate especially from monocytes, before migrating to, and activating on, the NETs[80]. However, in neutrophils isolated from patients with sepsis, neutrophils themselves seem to be the source of tissue factor, utilizing the machinery of autophagy to deliver tissue factor to the NETs[91]; indeed, in this context, tissue factor-bearing NETs can stimulate both thrombin generation and platelet activation *ex vivo*.

NETS AND THROMBOTIC EVENTS

Arterial and venous thrombotic events, despite certain common risk factors, are pathophysiologically-distinct processes[92,93]. For example, arterial thrombosis is particularly dependent on platelets since, under the high shear stress of arterial flow, platelets are effective at adhering to the vessel wall[94]. Rupture of atherosclerotic plaques (as in CVD) leads to marked platelet activation and aggregation, and ultimately to the development of platelet-rich "white" clots. In contrast, an important factor in venous thrombosis is a reduction in blood flow (stasis) with the development of red blood cell-rich "red" thrombi that result from the local accumulation and activation of circulating coagulation factors[95]. Interestingly, as the components of NETs are capable of activating both platelets and the coagulation cascade, NETs may be a unifying link/risk factor for the two processes. This notion has been validated in the animal models and clinical studies that are highlighted below.

Venous thrombosis

In one clinical study, 150 patients with symptomatic DVT were compared to controls who had clinical suspicion for DVT, but negative objective testing[96]. As compared to controls, patients with DVT had higher levels of both circulating nucleosomes and activated neutrophils, with elevated levels of either suggesting an approximately three-fold risk of DVT[96]. Another group obtained venous thromboembolism specimens from 11 patients and classified these into various stages of thrombus organization based on morphological characteristics[97]. Immunochemical staining suggested that NETs were especially present in organizing venous thrombi, indicating that they play an important role in thrombus maturation[97].

Experimentally, restriction (stenosis) of blood flow in the iliac vein of baboons[75] or the inferior vena cava of mice[76,80], results in elevation of plasma DNA levels and development of NET-containing venous thrombi. Further, in this model, infusion of histones increases both thrombus size and plasma levels of VWF, with the latter potentially contributing to platelet activation and recruitment[76]. Importantly, neutrophil depletion results in comparatively smaller thrombi[80], as does treatment with DNase[76]. Thrombus formation is also abrogated in PAD4-knockout mice, which are deficient in NET production[79]. In the PAD4 knockouts, thrombosis could be rescued by infusion of wild-type neutrophils[79], arguing that PAD4's role in thrombosis is at the level of neutrophils (and presumably NETosis).

Cardiovascular disease and arterial thrombosis

Correlation studies have hinted at a relationship between DNA, NETosis, and atherosclerotic/atherothrombotic disease[98]. In a cohort of 282 patients with well-characterized coronary artery disease, severity of disease was predicted by levels of circulating cell-free DNA as well as a number of NET markers (nucleosomes, citrullinated histone H4, and MPO-DNA complexes)[98]. Further, these markers also correlated with evidence of active coagulation (soluble CD163 and thrombin-antithrombin complexes)[98]. In mice, NETs can be detected in close association with plaques in the carotid lumen of atherosclerosis-prone ApoE(-/-) mice[99], while the PAD4 inhibitor Cl-amidine (which also blocks NETosis) prevents NET formation and decreases atherosclerotic lesion area in this model[77]. Mechanistically, the cathelicidin-derived proteins LL-37 (human) and CRAMP (mouse), which are abundant in NETs, seem to promote atherosclerosis[100,101]. For example, Döring *et al*[102] demonstrated that CRAMP-DNA complexes stimulate plasmacytoid dendritic cells (pDCs) to produce type I interferons that promote plaque growth, a phenotype that could be reversed by either CRAMP

deficiency or degradation of the DNA backbone of NETs.

Regarding arterial thrombosis, coronary thrombi can be rich in NETs as detected by immunochemical staining[103]; the authors of this study were particularly interested in the role of neutrophil interleukin-17A/F, and indeed both cytokines were present in not only neutrophils, but NETs themselves[103]. It has also been suggested that NETs present in the thrombi of acute myocardial infarctions expose tissue factor, which is functional in activating both thrombin generation and platelets when studied *ex vivo*[104]. However, that functionality was lost with digestion of the DNA backbone of NETs[104]. Finally, in a mouse model of arterial wall injury by ferric chloride, NET nucleosomes, as well as neutrophil serine proteases (elastase and cathepsin G), are essential for thrombus formation[86].

SLE

SLE is a systemic autoimmune disease that preferentially affects women. While the etiology of SLE is not fully understood, it is widely accepted that a hallmark of SLE is the near universal detection of an "antinuclear" autoimmune response. In particular, autoantibodies form to double-stranded DNA and to ribonucleoprotein (RNP) complexes. These autoantibodies participate in immune complex formation, with subsequent deposition in organs such as the kidneys (where they cause glomerulonephritis). Given the key roles of both autoantibodies and immune complexes in SLE pathogenesis, the majority of research over the years has understandably focused on abnormalities in the adaptive immune system, with particular attention paid to B cells, T cells, and antigen-presenting cells. However, in recent years, increasing attention has been paid to mediators of the innate immune response, especially neutrophils, which release NETs[105], and pDCs, which manufacture large quantities of type I interferons[106].

Regarding NETs, some patients with SLE have a deficiency in circulating DNase function, and therefore an impaired ability to degrade NETs in plasma[107,108]. This DNase defect fluctuates, and has been shown to correlate with both glomerulonephritis and hypocomplementemia[109]. Not surprisingly, the levels of circulating NETs themselves have also been shown to correlate with nephritis[110].

While impaired degradation surely plays a role in the increased levels of circulating NETs[110], the situation is further exacerbated by the increased propensity of SLE neutrophils to undergo NETosis[111-113]. In some cases this is likely a result of stimulation by circulating autoantibodies, such as anti-RNP and anti-LL-37, which are common in SLE patients[111,112,114]. In other cases, enhanced NETosis may be attributable to environmental factors, like low vitamin D levels[115], or increased susceptibility to infection resulting from treatment with immunosuppressive drugs. Accelerated NETosis may also stem from inherent differences in SLE neutrophils, as evidenced by their lower density (sometimes referred to as low-density granulocytes) and their proinflammatory phenotype[113]. Further, SLE NETs may be especially potent stimulators of the immune system. For example, they contain LL-37, which stimulates both pDCs and macrophages[112,116]. The immunostimulatory potential of SLE NETs may also be further amplified by acetylated histones and demethylated DNA[117,118].

As is discussed above, the risk of thrombotic events, both arterial and venous, is significant in SLE patients[119,120]. From an arterial perspective, the relative risk for myocardial infarction and stroke are both increased (10- and 7-fold, respectively) relative to that seen in the general population[121]. Similarly, the risk of DVT and pulmonary embolism is increased at least 10-fold in SLE[122]. Other venous complications, such as retinal vein occlusion[123], also stand out as more common. Further, it should be noted that with improved treatment of organ-threatening SLE manifestations such as kidney disease, 50% of SLE patients now die of some type of cardiovascular disease[11].

NETs, endothelial damage, and thrombosis in SLE

An important intersection between NETs and the vasculature involves the ability of SLE NETs to engage TLRs and thereby promote the formation of type Ⅰ interferons by pDCs[111-113]. Type Ⅰ interferons then play a multifaceted role in endothelial dysfunction, accelerating foam cell formation and impairing endothelial progenitor numbers and function[124,125]. Further, given the abundance of neutrophils in circulation (especially relative to rare cells like pDCs), it is noteworthy that netting neutrophils may themselves be a source of type Ⅰ interferons in SLE[113,126].

In addition to promoting the production of potentially anti-vascular cytokines like type Ⅰ interferons, NETs may also play a direct role in endothelial damage in SLE[113]. For example, SLE NETs contain matrix metalloproteinase-9, which activates endothelial matrix metalloproteinase-2, and thereby triggers endothelial cell death[127]. Endothelial damage may be further compounded in SLE by the NET- and MPO-mediated oxidation of high-density lipoprotein (HDL), which causes HDL to lose its normally vasculoprotective properties[128].

The best evidence for a role of NETs in not just the vascular damage, but also the prothrombotic diathesis of SLE, comes from mouse models of the disease. Indeed, NETs play an important role in pathogenesis of some[78,129], but not all[130], SLE models. In the NZM2328 model, NET release can be prevented by treatment with an inhibitor of PAD4 that prevents histone citrullination and consequently NETosis[78]. Over time, PAD inhibition protects against endothelial damage as measured by an acetylcholine-dependent vascular relaxation assay[78]. NZM2328 mice are also prothrombotic at baseline, rapidly forming carotid thrombi after photochemical injury of the endothelium. These carotid thrombi are rich in neutrophils and NETs, and can be prevented by treatment with either DNase or a PAD inhibitor[125]. These findings are reminiscent of work in models of

atherosclerosis, where NETs are important in not just vascular damage[100,102], but also thrombosis[77]. Whether prevention of NETosis can protect against thrombotic disease in patients with SLE remains to be determined, although it is noteworthy that antimalarial drugs like chloroquine both block NETosis[128], and track with a reduced risk of thrombosis in patients[131].

ANCA-ASSOCIATED VASCULITIS

ANCA-associated vasculitis describes a group of closely-related relapsing-remitting diseases, characterized by (1) small-vessel inflammation that especially targets the lungs and kidneys; and (2) autoantibodies against the neutrophil granule proteins MPO and PR3. The two best characterized syndromes are microscopic polyangiitis, in which patients are typically positive for anti-MPO, and granulomatosis with polyangiitis (Wegener's), which classically has anti-PR3 positivity. Neutrophils/NETs and ANCA likely interact in two important ways: (1) NETs contain both MPO and PR3 and may thereby stimulate the autoimmune response to these antigens; and (2) ANCA can interact with neutrophils to promote NET release, with NETs then contributing to vascular and organ damage.

Consistent with NETs playing a role in ANCA induction, netting neutrophils are more efficient than apoptotic neutrophils in loading murine myeloid dendritic cells with MPO and PR3[132]. This efficiency is dependent on the DNA backbone of NETs, as it can be almost completely abrogated with DNase[132]. NET-loaded dendritic cells induce glomerulonephritis in mice[132], while myeloid dendritic cells can be detected interacting with netting neutrophils in skin samples from patients with microscopic polyangiitis[132]. The ability of NETs to induce ANCA has also been observed anecdotally in patients, for example, in the setting of infectious endocarditis apparently driving both anti-PR3 formation and glomerulonephritis[133].

Mechanisms of ANCA-mediated NET release

Mechanistically, ANCA likely promote NETosis by engaging granule proteins that have migrated to the cell surface in primed neutrophils[134]. Indeed, one study found that ANCA are more potent than SLE IgG in this regard, and further that ANCA-associated NETosis correlates well with vasculitic disease activity[135]. The mechanism of NET induction by a nontraditional ANCA, anti-lysosomal membrane protein-2 (LAMP-2), has recently been investigated in detail. It appears that anti-LAMP-2 directs neutrophils away from apoptosis and toward NETosis by activating the vacuolization typically seen in autophagy[136]. Whether autophagy machinery is also required for NETosis mediated by traditional ANCA (anti-PR3 and anti-MPO) remains to be elucidated.

When NETs form in ANCA patients, they are relatively resistant to degradation by plasma DNase, an effect that is not explained by a direct effect of ANCA on DNase itself[135]. Along similar lines, the anti-thyroid drug propylthiouracil (PTU) is a recognized inducer of ANCA production in humans; in an animal model, PTU leads to the formation of NETs that are particularly resistant to DNase-mediated degradation, thereby exacerbating both pulmonary capillaritis and glomerulonephritis[137]. It was recently shown that ANCA-induced NETs appear to be relatively potent activators of the alternative complement cascade[138], and can also promote both platelet activation and conversion of pentameric C-reactive protein (CRP) into prothrombotic monomeric CRP[139].

ANCA-mediated NETs and thrombosis

NETs have been found in close proximity to inflamed glomeruli in vasculitic kidneys[134], as well as in vasculitic skin lesions[140], arguing that NETs play a role in tissue toxicity. It has also been suggested that NETs play a particular role in ANCA-associated thrombotic events, especially venous. For example, thrombi obtained from ANCA vasculitis patients are particularly rich in both NETs[141] and histone citrullination[142].

An intriguing mechanistic role has also been suggested for tissue factor[143]. Specifically, Kambas *et al*[143] demonstrated tissue factor-positive NETs in sera, bronchoalveolar lavage fluids, and renal biopsies of ANCA vasculitis patients. Further, tissue factor-positive NETs and microparticles correlated with higher disease activity (similar to thrombosis), and could be induced when control neutrophils were treated with ANCA *in vitro*[143]. How unique these phenotypes are to ANCA-associated NETs, as compared to NETs that form in other infectious and inflammatory diseases, remains to be determined.

ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS), an autoimmune disease of unknown etiology, is among the most common acquired causes of both thrombosis and pregnancy loss in the United States. About half of APS cases are diagnosed in patients with lupus, and the remainder as a standalone syndrome called primary APS[144]. Primary APS manifests not just with thrombosis and pregnancy loss, but also with additional features including livedo reticularis, thrombocytopenia, chorea, leg ulcers, cognitive dysfunction, seizures, alveolar hemorrhage, and nephropathy[145]. This heterogeneity of manifestations clearly points to APS as a truly systemic autoimmune disease on the spectrum of lupus, rheumatoid arthritis, and small-vessel vasculitis.

Pathophysiology of APS

Despite the name of the syndrome (anti-phospholipid), the best understood antigen in APS is not a phospholipid, but rather a lipid-binding protein that circulates at high levels in blood (100-200 μg/mL) called beta-2 glycoprotein I (β_2GPI). Autoantibodies to β_2GPI activate various types of cells *in vitro*[146-149], and promote both thrombosis and pregnancy loss when injected into mice[150,151]. Currently, three assays are used to diagnose APS clinically. These include tests for (1) anti-cardiolipin

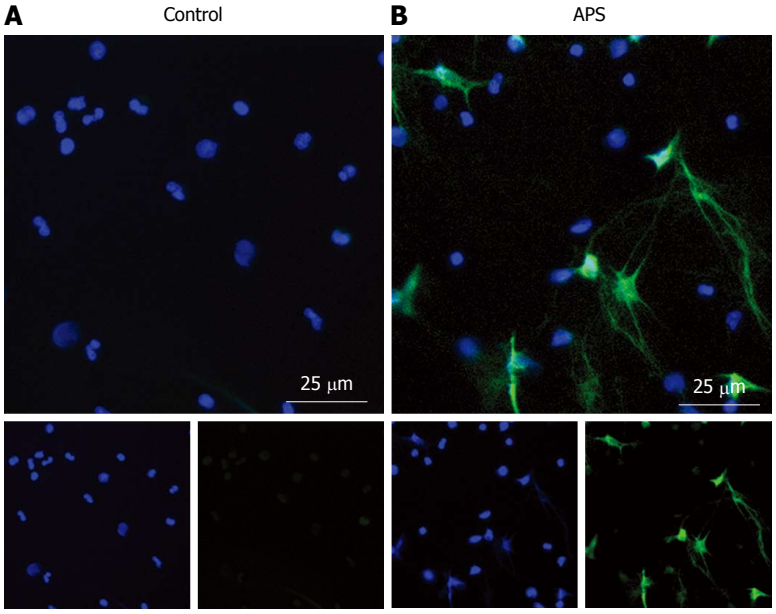

Figure 2 Antiphospholipid syndrome neutrophils are prone to "spontaneous" neutrophil extracellular trap release. Freshly-isolated neutrophils from a healthy control (A) or antiphospholipid syndrome (APS) patient (B) were seeded onto poly-lysine-coated coverslips and incubated in serum-free media for 2 h. Samples were then fixed with paraformaldehyde and stained with Hoechst 33342 (DNA = blue) and anti-neutrophil elastase (Abcam, green). Cells were not specifically permeabilized and neutrophil elastase staining is therefore primarily extracellular. These representative micrographs show more neutrophil extracellular trap release in the APS neutrophils, as determined by overlapping DNA and neutrophil elastase staining.

antibodies; (2) anti-β2GPI antibodies; and (3) a group of coagulation assays collectively referred to as "lupus anticoagulant" - functional testing that takes advantage of the fact that antiphospholipid antibodies paradoxically prolong phospholipid-dependent clotting assays *in vitro*. It is interesting to note that ELISAs for anti-cardiolipin are often actually detecting anti-β2GPI, with the reactivity to cardiolipin mediated by β2GPI protein present in the patient's serum. Antibodies to thrombin may also sometimes cause APS, although testing has not been standardized, and anti-thrombin is therefore not routinely assessed in clinical practice. In summary, this group of antibodies is (despite the inaccuracy) referred to as antiphospholipid antibodies, with anti-β2GPI being the best characterized and the most likely to be pathogenic.

While antiphospholipid antibodies are recognized to be pathogenic, the origin of these antibodies, and the reason that lupus patients are especially at risk for their development, are not well understood. Further, there are currently no targeted treatments for APS. Instead, therapy focuses on masking the prothrombotic effects of antiphospholipid antibodies with anticoagulant medications like warfarin and heparin. These drugs often need to be taken for life, and at the same time predispose to catastrophic bleeding complications[152]. While anticoagulants are somewhat effective in preventing APS-associated blood clotting, they often have no bearing on the neurologic and renal complications of APS, which can progress to organ failure[145].

Heightened NET release in APS

Our group has recently made a number of important observations about APS neutrophils[153]. First, NETs circulate at high levels in the plasma of APS patients, even between thrombotic episodes[153]. Indeed, freshly isolated neutrophils from APS patients are primed to undergo spontaneous NETosis when cultured *ex vivo* (Figure 2). Mechanistically, anti-β2GPI IgG promotes NETosis by engaging β2GPI protein on the neutrophil surface; this process is independent of the Fc receptor, but does require ROS production and TLR4 signaling[153]. Further, and pointing to disease relevance, anti-β2GPI-stimulated NETs promote thrombin generation *in vitro*[153]. In addition to our work, Leffler *et al*[154] have shown that some patients with APS have a defect in DNase-mediated NET degradation. This potentially sets up a vicious prothrombotic cycle, in which the threshold for NETosis is reduced in APS neutrophils, followed by the exaggerated persistence of the NETs that do form. A final interesting point is that antiphospholipid antibodies seem to engage not just neutrophils[153], but NETs themselves[154]. This observation deserves further exploration as to its potential role in APS pathogenesis.

CONCLUSION

While NETs have yet to be assigned a clear function in normal hemostasis, their roles in venous thrombosis, atherosclerosis, and arterial occlusions continue to be defined. It is notable that many systemic autoimmune diseases are not only associated with increased NETosis and decreased NET clearance, but also demonstrate an increased risk of both arterial and venous events. We therefore find it quite plausible that NETs contribute to the prothrombotic nature of diseases like SLE, ANCA-associated vasculitis, and APS. As is detailed above, there are also hints that these sterile inflammatory NETs

may differ structurally from NETs released during infection (for example, by being enriched in tissue factor or being more resistant to degradation), although further study in this area is clearly needed. More work in disease-specific experimental models will also be required before clinical interventions can be considered. In summary, there is a need to continue to explore the association between thrombosis and inflammatory disease-associated NETosis, in order to better understand whether treatment algorithms can be developed that will allow us to prevent, rather than simply treat, life-threatening thrombotic episodes in these at-risk patients.

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REVIEW

Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction

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Abstract

microRNAs (miRNAs) are powerful regulators of post-transcriptional gene expression and play an important role in pathophysiological processes. Circulating miRNAs can be quantified in body liquids and are promising biomarkers in numerous diseases. In cardiovascular disease miRNAs have been proven to be reliable diagnostic biomarkers for different disease entities. In cardiac fibrosis (CF) and heart failure (HF) dysregulated circulating miRNAs have been identified, indicating their promising applicability as diagnostic biomarkers. Some miRNAs were successfully tested in risk stratification of HF implementing their potential use as prognostic biomarkers. In this respect miRNAs might soon be implemented in diagnostic clinical routine. In the young field of miRNA based research advances have been made in identifying miRNAs as potential targets for the treatment of experimental CF and HF. Promising study results suggest their potential future application as therapeutic agents in treatment of cardiovascular disease. This article summarizes the current state of the various aspects of miRNA research in the field of CF and HF with reduced ejection fraction as well as preserved ejection fraction. The review provides an overview of the application of circulating miRNAs as biomarkers in CF and HF and current approaches to therapeutically utilize miRNAs in this field of cardiovascular disease.

Key words: MicroRNA; Heart failure; Cardiac fibrosis; Biomarker; Diagnostic; Prognostic; Heart failure with reduced ejection fraction; Heart failure with preserved ejection fraction

Core tip: Recent study results suggest microRNAs (miRNAs) as promising biomarkers in the diagnosis of heart failure (HF) with reduced ejection fraction (HFrEF) and with preserve ejection fraction (HFpEF). The therapeutic application of antagomirs and mirmimics in

heart failure is still in its infancy but promising experimental results are reported. This review provides an overview of miRNAs as diagnostic and prognostic biomarkers in HF and gives details on the utilization of miRNAs in the differentiated diagnosis of HFpEF and HFrEF. The manuscript evaluates the therapeutic applicability of miRNAs in HF and thus provides valuable information for researchers dealing with miRNAs in HF.

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INTRODUCTION

The term "microRNA" (miRNA) was established in 1993 when researchers started to study the function of small RNAs[1]. Lin-4 was the first miRNA described[2] and after its discovery scientists began to recognize miRNAs' importance as regulators in gene expression. Ever since miRNAs have not only been assessed for their promising regulatory role in various diseases, but also their diagnostic potential in risk prediction as well as their use as circulating biomarkers[3-5]. Furthermore, promising data have depicted miRNAs as gene specific therapeutic targets in disease modeling[6].

Numerous studies have analyzed miRNAs with respect to their utilization as disease-specific biomarkers. In cardiovascular disease, miRNAs have successfully been proven to be quantitatively modified in particular disease entities such as myocardial fibrosis and heart failure (HF)[7-12].

In this article we will review the value of miRNAs in cardiac fibrosis (CF) and HF. We will discuss the current knowledge about their role in two different entities of the disease - HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF). In particular, we will provide an overview about the use of miRNAs as diagnostic and prognostic biomarkers in CF and HF as well as potential therapeutic agents.

miRNA: GENERALLY SPEAKING

miRNAs are non-coding RNAs with a length of 19-25 nucleotides[13,14]. By binding to the 3'-untranslated region of target messenger RNAs (mRNAs), miRNA either initiate translational repression or degradation of mRNAs thereby regulating gene expression at the post-translational stage[15,16] (Figure 1). Every single miRNA has target sites in hundreds of different genes[17]. At the same time computational prediction of target mRNAs suggests that more than 60% of all mammalian protein-coding genes are conserved targets of miRNAs[18]. At the time of writing this review 2588 mature homo sapiens miRNAs were listed in "miRBase" (mirbase.org).

miRNA quantification showed organ- and cell-specific expression patterns of certain miRNAs[19] while quantification measures have shown concentration-dependent effects in pathologically altered organs[20]. *In-vitro* findings suggest groups of miRNAs being specifically up and down-regulated and polymorphisms in the miRNA regulatory pathway - so called miRSNPs - have been found to be associated with different types of disease[21-24]. miRNAs fulfill several criteria of an ideal biomarker: stability in the circulation, tissue- and pathology-specific regulation as well as high sensitivity and specificity. These characteristics predestine miRNAs as biomarkers. In fact, there is evidence that miRNAs' applicability as circulating biomarkers for certain diseases might even exceed that of protein-based biomarkers[25,26]. The field of miRNA research has paved the way for the development of new means of biomarker-based risk stratification for cardiovascular events. In this regard promising data have been collected in large-scale prospective clinical studies[27].

HF

The discovery of miRNAs as promising new biomarkers in cardiovascular disease has ignited great expectations and especially in the field of HF, the last years have witnessed great success. The syndrome of HF ranges among the leading causes of death and morbidity worldwide[28] with mortality rates of up to 50% in patients with new onset HF[29]. HF can be classified by the contraction of the left ventricle into HFrEF and HFpEF. While HFrEF is defined by a reduced left ventricular ejection fraction (LVEF), HFpEF describes HF patients with normal or only mildly reduced LVEF (over 50%)[30]. Approximately half of all HF patients present with preserved LVEF[31,32] illustrating its clinical importance, while morbidity and mortality are suggested to be equally distributed.

HF, CARDIAC HYPERTROPHY AND FIBROSIS

There are three major causes of HF: hypertensive heart disease, ischemic heart disease and idiopathic dilated cardiomyopathy. Hypertension initiates molecular pathways that lead to increased cardiomyocyte size and protein synthesis as well as augmented sarcomer organization[33,34]. Persisting hypertrophy is associated with an unfavorable outcome and can result in HF and sudden death[35,36]. Independently from the underlying pathology failing hearts remodel in regard to extracellular matrix and myocyte size. This leads to augmented hypertrophy and death of cardiomyocytes followed by tissue fibrosis and scarring[37]. CF results in increased myocardial stiffness affecting systolic as well as diastolic left ventricular function[37,38]. The initial molecular steps in the development of HF can hardly

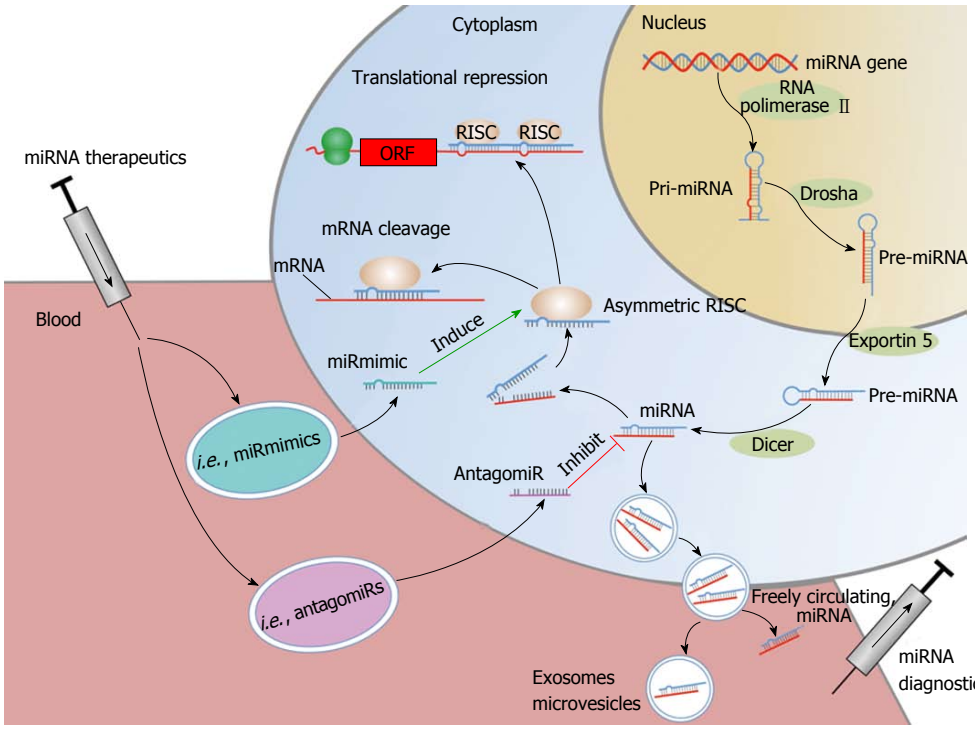

Figure 1 microRNA synthesis and mode of function. Pri-miRNAs are generated in the nucleus by RNA polymerase Ⅱ. The endonuclease "Drosha" catalyzes the transformation of pri-miRNA into pre-miRNA, which is transported into the cytoplasm by Exportin 5. Subsequently, the mature miRNA is generated by the endonuclease "Dicer". The mature miRNA is incorporated into the RISC complex; in this form leading to degradation of target mRNAs and/or inhibition of translation. Mature miRNAs can be released into the circulation, incorporated into vesicles such as MVB, exosomes, microvesicles or as freely circulating miRNAs. miRNA therapeutics such as miRmimics and antagomirs can be administered directly into the blood flow or applied by oral uptake. Antagomirs specifically bind to and silence endogenous miRNAs, leading to reduced RISC activation and mRNA degradation. miRmimics specifically bind to target mRNAs to increase their degradation. mRNA: Messenger RNA; miRNA: microRNA; ORF: Open reading frame; RISC: RNA-induced silencing complex; MVB: Multivescular bodies.

be analyzed using imaging techniques and protein biomarkers may be involved at later stages only[39]. In this respect the diagnosis of HF is most often been made at an advanced stage of the disease when symptoms and physical confinement have already developed. The fact that so-called early HFpEF usually is clinically apparent merely under exercise conditions complicates an early diagnosis[40-42]. This determines the clinical need for markers identifying the disease at the earliest possible stage.

miRNAS IN CARDIAC HYPERTROPHY AND FIBROSIS

Given that cardiac remodeling and fibrosis are significant factors in the development of ventricular wall stiffness with compromised ventricular contractility and compliance, the expression of miRNAs is directly linked to the development of HF - with preserved and with reduced ejection fraction. miRNAs were identified to play a major role in the transcriptional and translational changes in gene expression with respect to cardiac hypertrophy and fibrosis[36,43]. The regulatory involvement of miRNAs in the development of cardiac hypertrophy and fibrosis ultimately suggests their causal roll in HF.

In a mouse model of aortic constriction induced cardiac hypertrophy van Rooij *et al*[36] described altered levels of several miRNAs in murine cardiomyocytes while Sayed *et al*[44] found a set of more than 50 miRNAs dysregulated in a similar setting with induced hypertrophy. Especially miR-1 was identified as significantly down-regulated compared to sham operated controls, probably mediated *via* an inhibition of the translation of calmodulin-encoding mRNAs[45]. Similar results were reported by Carè *et al*[46]. They reported down-regulated levels of miR-1 and miR-133 in cardiomyocytes of hypertrophic murine as well as human hearts[46]. Furthermore, in an *in-vitro* model the authors found a causal relationship between adeno-virus induced elevation of miR-1 and miR-133 levels and an inhibition of cardiac hypertrophy. Supporting data were reported from an *in-vitro* study involving neonatal rat cardiomyocytes[47]. Recently miR-150 has been described as a regulator in cardiac hypertrophy[48]. In a mouse model the authors induced cardiac hypertrophy by aortic banding and found miR-150 levels down-regulated compared to sham operated animals[48]. Several more *in-vitro* and mouse model studies reported altered miRNA levels in cardiac hypertrophy on the one hand and induction of cardiac hypertrophy by artificial alterations of specific miRNAs on the other[49-52]. Especially miR-1[44,53], miR-21[54,55], miR-133[53,54], miR-195[36,56], miR-208[57-59] were proven to be involved in the regulation of cardiac hypertrophy. Alterations of miRNA levels were also

reported in induced hypertrophy of cardiomyocytes in engineered heart tissue[60].

The heart's initial hypertrophic response to volume overload or increased afterload as well as pathological conditions after myocardial infarction (MI) is followed by the process of remodeling which leads to CF. A major regulatory roll of miRNAs in the process of cardiac remodeling and fibrosis was suggested when a key enzymatic step towards miRNA activation catalyzed by the enzyme Dicer was blocked in knock out mice[61]. The authors found biventricular enlargement, myocyte hypertrophy and pronounced CF[61]. miRNAs involved in regulatory pathways of hypertrophy such as miR-208 have also been found in cardiac remodeling[57]. van Rooij *et al*[57] found mutant mice overexpressing miR-208 to not develop cardiomyocyte fibrosis despite being exposed to an increased afterload. Opposite, miR-208a has been identified as a regulator of endoglin expression and increases myocardial fibrosis in volume overloaded hearts[62]. Comparable results were reported in a model of cultured rat myoblasts[59].

miR-133a was reported to be down-regulated in a mouse model of aortic constriction-induced hypertrophy[63]. Expression of miR-133a prevented this down-regulation while the authors found less myocardial fibrosis along with improved diastolic function of the analyzed mouse hearts[63]. Similar results were reported from analyses of CF in canines. Shan *et al*[64] described reduced miR-133 and miR-590 levels in canine hearts after nicotine-induced CF mediated by up-regulation of transforming growth factor β (TGF-β) 1 and TGF-β RII. By transfection of miR-133 or miR-590 into cultured atrial fibroblasts it was possible to reduce fibroblast activity as well as collagen production while this effect was reversible by administration of antisense oligonucleotides against miR-133 or miR-590[64]. Confirming results of reducing CF by miR-133 induction were recently reported in a mouse model of aortic banding-induced hypertrophy[65]. Besides miR-133 also miR-30 was reported to control pro-fibrotic proteins and thus regulate changes in the extracellular matrix of the myocardium[66]. The decrease of miR-133 and miR-30 in a gene-modulated rat model of pathological cardiac hypertrophy was found to be linked with an up-regulation of collagen synthesis and CF[66].

In a transgenic mouse model miR-21 was discovered as a key regulator of signaling pathways in cardiac fibroblasts controlling the extent of cardiac hypertrophy and interstitial fibrosis[55]. A different working group was able to show that elevated miR-21 expression was highly related to CF[67]. These findings are in line with the observation that miR-21 is involved in the regulation pathway of cardiac fibroblasts in infarcted mouse hearts[68]. Cardin *et al*[69] were able to suppress atrial fibrosis in miR-21 knock out mice after induced MI. Also, the authors succeeded in depressing post infarction fibrosis by means of anti-miR-21 reduction of miR-21 availability. More recent studies validate these findings of miR-21 promoting cardiac remodeling and fibrosis[67,70,71]. On the other hand silencing of miR-21 by means of antagomirs resulted in cardiomyocyte necrosis and apoptosis[72,73] indicating the integration of this miRNA in cardiac remodeling.

MI is frequently followed by ventricular remodeling processes and fibrotic structural changes in the infarcted areas. This process eventually leads to HF. A miRNA involved in post-MI CF is miR-24. In a mouse model of induced MI Wang *et al*[74] reported miR-24 down-regulated and found a simultaneous increase of extracellular matrix remodeling. *In-vivo* lentivirus-based intramyocardial elevation of miR-24 levels caused attenuation of fibrosis in the infarct border zone[74]. The authors described TGF-β to mediate the miR-24 modulated effect and concluded miR-24 to be a potential target for the treatment of post-MI remodeling.

miR-29 is involved in fibrotic processes in different types of body tissue[75,76]. It controls a variety of pro-fibrotic genes such as collagens, fibrillins, laminins, integrins and elastin[77]. Furthermore, miR-29 negatively regulates a number of anti-apoptotic genes, including *Tcl-1*, *Mcl-1*, *YY1*, *p85a*, *CDC42* and *DNMT3*[77-81]. In a mouse model of induced MI miR-29 was down-regulated[82]. Further analyses revealed miR-29 being predominantly expressed in cardiac fibroblasts and the *in-vivo* inhibition of miR-29 resulted in an induction of collagen mRNA expression[82]. Raising miR-29 concentrations using Mirmimics lead to a down-regulation of collagen mRNA expression[82]. Affirmative results were recently reported by Yang *et al*[83]. With respect to these results miR-29 can be attributed a key roll in regulation of tissue fibrosis and CF in particular.

Study results like these gave rise to projects analysing the roll of miRNAs in manifest HF.

miRNAS IN HF

In the process of HF development different intracellular signaling pathways are activated including an up-regulation of structural fetal genes, such as β-myosin heavy chain (β-MHC) and down-regulation of adult structural genes, such as α-MHC[84-86]. miRNAs are involved in regulatory processes of activating fetal genes that are known to be up-regulated in failing hearts[87]. The involvement of miR-208 in α- and β-MHC regulation was reported by van Rooij *et al*[57]. *Via* this mechanism miR-208 regulates cardiomyocytes growth under stress conditions as reported by the authors in a transgenic mouse model[57]. The same working group had previously found a set of miRNAs up-regulated not only in a mouse model of induced hypertrophy but also in failing human hearts[36]. They found an increased expression of miR-24, miR-125b, miR-195, miR-199a and miR-214 in both mice and human hearts and postulated that these miRNAs are part of a molecular signature of adverse cardiac remodeling[36].

Besides the development of cardiac hypertrophy and fibrosis as described above a knockout of the key enzyme Dicer, essential for intracellular miRNA

processing, leads to dilatative cardiomyopathy (DCM) and HF[88]. The authors found reduced Dicer expression in human failing hearts and reported a significant increase of Dicer expression in hearts of patients with improved cardiac function after implantation of left ventricular assist device (LVAD) for HF[88]. These results depict the importance of miRNAs in the regulation of pathophysiologic processes involved in the development of HF and have lead to considerations of clinical implications of miRNAs dysregulated in cardiomyopathies and HF in particular. Matkovich *et al*[89] drove the Dicer-related findings by Chen *et al*[88] (mentioned above) a step further and analyzed a miRNA expression profile of cardiac tissue from HF patients with and without LVAD-based recovery compared to healthy controls. The authors found 28 miRNAs up-regulated in failing hearts compared to healthy controls and 20 of these miRNAs returned to near normal levels in the LVAD-treated group with significant improvement of left ventricular performance[89]. While these results link defined miRNAs to clinically apparent HF and suggest their potential in treatment monitoring, a more distinct analysis of miRNAs in different types of cardiomyopathies was performed by Ikeda *et al*[90]. In left ventricular biopsy samples of 67 humans with ischemic cardiomyopathy (ICM), DCM, aortic stenosis and healthy controls they analyzed miRNA expression[90]. Using a genome-wide miRNA expression profiling they detected 87 miRNAs and found their expression profiles significantly altered in the three heart diseases compared to healthy controls. While seven miRNAs were altered in the same direction in all three disease entities, the global pattern of miRNA expression was distinct in different types of HF[90]. miR-19 appeared as the most strongly down-regulated miRNA in DCM and AS but not in ICM, while miR-1 was down-regulated in all three diseases. miR-214 - considered pro-hypertrophic[36] - was most strongly up-regulated. Surprisingly, miR-133 and miR-208 levels, which are associated with myocardial hypertrophy[46,57-59,63] and fibrosis[59,62,64], were unchanged. These reported data suggest miRNAs to be specifically dysregulated in different types of HF pathology. In this regard an interesting study was recently reported by Leptidis *et al*[91] who performed miRNA deep sequencing analyses in myocardial biopsies of end stage DCM, hypertrophic cardiomyopathy (HCM) and healthy controls to analyze the human heart's miRNOME with respect to these two different HF pathologies. They were able to identify a set of ten miRNAs (miR-23b, miR-30d, miR-125a, miR-143, miR-145, miR-193, miR-197, miR-342, miR-365, miR-455) that is differentially expressed in HCM and DCM compared to healthy controls and had not been linked to HF previously[91]. The authors were able to confirm previously described dysregulated levels of miR-133a, miR-1, miR-21, miR-214, miR-212, miR-29, miR-129, miR199a in HCM, while miR-119 and miR-214 expression was reported only to be altered in DCM[91]. miR-145 was identified as a new regulator of pathologic left-ventricular remodeling. Satoh *et al*[92] who analyzed miRNA expression in cardiac tissue from myocardial biopsies of patients with DCM reported higher levels of miR-208, miR-208b and miR-499 than in healthy controls. Follow-up revealed baseline miR-208 levels to be strong predictors of clinical outcome[92] indicating a potential utilization of miRNAs in risk prediction of HF.

Based on these results, the potential applicability of miRNAs as distinct biomarkers for the diagnosis of HF and for different entities of the disease seems possible. miRNAs seem to be promising biomarkers in risk prediction of HF patients. For obvious reasons, though, the availability of heart tissue is limited and therefore different sources of biomaterial for miRNA analysis are needed. In this respect body fluids appear to present an ideal origin to non-invasively win such biomaterial.

CIRCULATING miRNAS

Besides regulating gene expression and phenotypic control in the cell of origin[93] and mediating metabolism on an intracellular level[94] miRNAs are also secreted from the producing cell and capable of transmitting their silencing signals to different cells[95]. miRNAs have been detected in numerous body fluids such as serum and plasma as well as saliva and urine[25,26] and can be found in pericardial fluid of HF patients[96]. Consequently, miRNAs have been tested to function as detectable extracellular messengers in cell-to-cell communication[97]. Their structure prevents miRNAs from early degradation in circulating blood[25,98-102] and their ideal biomarker characteristics including size, abundance and tissue specificity suggest circulating miRNAs as blood-based biomarkers for tissue injury[12,103-105].

CIRCULATING miRNAS IN THE DIAGNOSIS OF HF

The ability to detect and measure miRNAs in a minimal-invasive way has led to their evaluation as potential circulating biomarkers for cardiovascular disease[3,106]. The promising results of disease-specific cellular miRNA dysregulation in HF and their suitable characteristics with regard to circulating biomarker diagnostics have led to their evaluation as blood based biomarkers in HF. In a rat model of induced left ventricular hypertrophy with consecutive development of HF the authors reported significantly elevated plasma levels of miR-16, miR-20b, miR-93, miR-106b, miR-223 and miR-423-5p[107]. These results were in line with earlier findings of Tijsen *et al*[108] who were amongst the first researchers to evaluate circulating miRNAs as diagnostic biomarkers in HF in a clinical approach. The authors reported that besides 6 miRNAs (miR-18b[a], miR-129-5p, miR-1254, miR-675, oncomir HS_202.1 miR-622), that were moderately elevated in plasma of 30 HF patients compared to 20 dyspnea patients and 20 healthy controls, miR-423-5p was found to be a significant predictor of HF diagnosis in a multivariate logistic regression model[108]. Despite the

small sample size and suboptimal matching of baseline characteristics[109] these were promising initial results that were confirmed by Goren *et al*[110] in a similar study setup. The authors were one of the first groups to perform a screening of circulating miRNAs on a larger scale in cardiovascular disease and HF. They screened 186 miRNAs in serum of 30 HF patients compared to 30 healthy controls and were able to detect four miRNAs (miR-423-5p, miR-320a, miR-22, miR-92b) that were up-regulated in the serum of HF patients compared to the control group[110]. Furthermore, the authors succeeded in generating a score out of these miRNAs that discriminates HF patients from healthy controls. The group was able to describe a significant association between the miRNA score and several established prognostic HF parameters such as NT-proBNP, a wide QRS complex and left ventricular (LV) dilatation underlining the significance of these results not only with respect to diagnostic but also to prognostic applicability of circulating miRNAs[110]. At that time the analyzed combination of miRNAs miR-320 and miR-423-5p had previously been associated with HF[86,111,112].

Ellis *et al*[113] analyzed miRNA plasma levels of 44 HF patients compared to 32 Chronic obstructive pulmonary disease (COPD) patients, 59 patients with breathlessness for other diagnoses and 15 healthy controls after an initial miRNA screening phase. Not only were seven miRNAs (miR-103, miR-142-3p, miR-342-3p, miR-199a, miR-23a, miR-27b, miR-324-5p) associated with the diagnosis of HF in regression and receiver operating characteristics (ROC) analysis, plasma levels of four miRNAs (miR-103, miR-142-3p, miR-30b and miR-342-3p) were able to distinguish between HF and exacerbation of COPD, other causes of dyspnea and controls[113]. Although miR-423-5p could not be identified as a predictor of HF diagnosis, the addition of miR-423-5p to NT-proBNP significantly improved the area under the operating receiver curve (AUC) for predicting the diagnosis HF[113]. These findings confirm previous results of the potential applicability of miR-423-5p as circulating biomarker in HF diagnosis.

In a larger clinical trial serum miRNA levels of 81 HF patients were compared to 60 non-HF patients and 15 healthy subjects[114]. The authors reported a set of 24 miRNAs significantly down-regulated in the HF group compared to controls. miR-26b-5p, miR-145-5p, miR-92a-3p, miR-30e-5p and miR-29a-3p inversely correlated with NT-proBNP and directly correlated with EF, while ROC analysis to predict differentiation of HF patients from non-HF cases revealed strong AUC values between 0.84 and 0.91, suggesting these miRNAs to be potentially strong circulating biomarkers in the diagnosis of HF[114].

Recently, Wong *et al*[115] identified miR-1233, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p, miR-211-5p, miR-494 and miR-671-5p to be able to distinguish HF from healthy controls in plasma levels of 60 HF patients and 30 healthy subjects.

Circulating blood cells and endothelial cells contain higher miRNA concentrations than serum and plasma[116]. Thus, further approaches in quantification efforts of circulating miRNAs with respect to HF were aimed at analyzing their concentration in circulating blood cells such as peripheral blood mononuclear cells (PBMCs). Gupta *et al*[117] analyzed miRNA concentrations in PBMCs of 44 DCM HF patients compared to 48 healthy controls. Real time polymerase chain reaction (RT-PCR) revealed miR-548c and miR-548i significantly down-regulated in PBMCs of DCM patients, while miR-138 was up-regulated in PBMCs of those patients. ROC analysis showed an AUC of 0.85 for miR-548c with respect to its discriminatory power to distinguish DCM from controls[117].

Frequently, HF consecutively develops after ischemic events such as MI. Corsten *et al*[10] analyzed whole blood samples, plasma and urine of 32 acute myocardial infarction (AMI) patients compared to 36 non-AMI controls and reported plasma miR-208b and miR-499 to correlate with cardiac injury markers and, hence, to correlate with myocardial damage. miR-499 was significantly up-regulated in a subgroup of patients with acute HF. Another group that analyzed a predefined set of circulating miRNAs in plasma of 12 post-MI patients compared to 12 healthy controls was able to find levels of miR-1, miR-21, miR-29a, miR-133a and miR-208 altered in the time course after MI[118]. These miRNAs had previously been described to affect myocardial growth, hypertrophy, fibrosis and viability[118] implying that the same miRNAs that have been shown to be associated with these pathophysiological processes preceding HF can be found dysregulated in plasma of patients with cardiovascular disease.

Independently from the pathophysiological cause miRNAs have been reported to complement biomarker-based prediction of outcome in HF. In a study including 20 clinically stable and 22 decompensated HF patients as well as 15 healthy controls the authors performed a microarray-based miRNA profiling and reported a large number of miRNAs to be quantitatively dysregulated in HF patients compared to controls[119]. More importantly, Cox regression identified miR-182 to be able to predict cardiovascular mortality. Remarkably, the prognostic value of miR-182 was identified to be superior to NT-proBNP as well as high-sensitive C-reactive protein by ROC analysis[119]. Table 1 gives an overview of cellular and circulating miRNAs dysregulated in heart failure.

The above studies suggest blood-based circulating miRNAs as potential strong tools in the diagnosis and risk evaluation of HF. On the other hand, most trials included rather small sample sizes and most identified miRNAs were not confirmed in repetitive studies.

miRNA SIGNATURES IN HF

Combining two or more biomarkers as a defined set for diagnostic purposes can enhance discriminatory power compared to the use of single biomarkers. In the field of miRNA biomarker research the assessment of sets (so-called signatures) of miRNAs might deliver superior

Table 1 Systematic overview of microRNAs dysregulated in heart failure

miRNA	Study type	Bio-material	Group/size	Detection method	Effect	Value as biomarker	Ref.
Single miRNAs							
Let-7	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Up-regulated in DCM and ICM	Diagnostic	[90]
miR-1	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-15b	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Up-regulated in DCM	Diagnostic	[90]
miR-16	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-17-5p	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-18b	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic	[108]
miR-20b	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-21[1]	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-22	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-23a	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-24	Experimental	Tissue	Mice, human hearts	Micro-array	Up-regulated in HF, CH	Experimental	[36]
miR-26b-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-28	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-29a-3p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-30b	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-30e-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-92b	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-92a-3p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-93	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-103	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-106a	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM	Diagnostic	[90]
miR-106b	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-125b	Experimental	Tissue	Mice, human hearts	Micro-array	Up-regulated in HF, CH	Experimental	[36]
miR-126	Clinical	Plasma	HF n = 10 HC n = 17	qRT-PCR	Down-regulated in HF	Diagnostic	[150]
miR-133	Clinical	Tissue	LVAD-patients	Micro-array	Up-regulated in HF	Experimental	[89]
miR-138	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Up-regulated in DCM	Diagnostic	[117]
miR-142-3p	Clinical	Plasma	HF n = 44 COPD n = 32	RT-PCR	Down-regulated in HF	Diagnostic	[113]

			Dyspnea n = 59 HC n = 15				
	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in stable and decompensated DCM	Diagnostic	[121]
miR-145-5p	Clinical	Plasma	HF n = 81 HC n = 15	qRT-PCR	Down-regulated	Diagnostic	[114]
miR-182	Clinical	Serum	HF n = 42 HC n = 15	Micro-array	Up-regulated in HF	Prognostic	[119]
miR-183-3p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-190a	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-193b-3p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-193b-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-195	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-199a	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-208	Experimental Clinical	Tissue Tissue	Mice DCM n = 82 HC n = 21	Micro-array qRT-PCR	Up-regulated in HF, CF, CH Up-regulated in DCM	Experimental Diagnostic/ prognostic	[57] [92]
miR-208b	Clinical	Tissue	DCM n = 82 HC n = 21	qRT-PCR	Up-regulated in DCM	Diagnostic	[92]
miR-211-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]
miR-214 miR-222	Experimental Clinical	Tissue Tissue	Mice, human hearts ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	Micro-array qRT-PCR	Up-reglated in HF, CH Down-regulated in DCM and ICM	Experimental Diagnostic	[36] [90]
miR-223	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
miR-320a	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
miR-342-3p	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Diagnostic	[113]
miR-422b	Clinical	Tissue	ICM n = 10 DCM n = 25 AS n = 13 HC n = 10	qRT-PCR	Down-regulated in DCM and ICM	Diagnostic	[90]
miR-423-5p	Experimental	Plasma	Rats, hypertension-induced HF	qRT-PCR	Up-regulated in HF	Diagnostic	[107]
	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic/ prognostic	[108]
	Clinical	Serum	HFrEF n = 30 HC n = 30	qRT-PCR	Up-regulated in HFrEF	Diagnostic/ prognostic	[110]
	Clinical	Plasma	HF n = 44 COPD n = 32 Dyspnea n = 59 HC n = 15	RT-PCR	Down-regulated in HF	Prognostic when combined with NT-proBNP	[113]
miR-494[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Down-regulated in HF	Diagnostic	[115]

miR-499	Clinical	Tissue	DCM n = 82 HC n = 21	qRT-PCR	Up-regulated in DCM	Diagnostic	[92]
	Clinical	Plasma	Acute HF n = 33 HC n = 34	qRT-PCR	Up-regulation in acute HF	Diagnostic	[10]
miR-548c	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Down-regulated in DCM	Diagnostic	[117]
miR-548i	Clinical	PBMC	DCM n = 44 HC n = 48	qRT-PCR	Down-regulated in DCM	Diagnostic	[117]
miR-671-5p[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Up-regulated in HF	Diagnostic	[115]
miR-675	Clinical	Plasma	HF n = 30 Dyspnea n = 20 HC n = 20	qRT-PCR	Up-regulated in HF	Diagnostic	[108]
miR-1233[1]	Clinical	Plasma	HF n = 60 (HFpEF n = 30; HFrEF n = 30) HC n = 28	qRT-PCR	Up-regulated in HF	Diagnostic	[115]
miRNA signatures							
miR-520d-5p miR-558 miR-122* miR-200b* miR-622 miR-519e* miR-1231 miR-1228*	Clinical	Whole blood	HFrEF n = 53 HC n = 39	qRT-PCR	Dysregulated in HF - superior to single miRNAs	Diagnostic	[120]

[1]See also Table 2; *Most microRNAs have two mature products, one derives from the 5′ arm of the miRNAs hairpin and the other from the 3′ arm of the hairpin; the latter is marked "*". miRNA: microRNA; HF: Heart failure; CF: Cardiac fibrosis; CH: Cardiac hypertrophy; HC: Healthy control; ICM: Ischemic cardiomyopathy; DCM: Dilated cardiomyopathy; AS: Aortic stenosis; COPD: Chronic obstructive pulmonary disease; MI: Myocardial infarction; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; PBMC: Peripheral blood mononuclear cell; LVAD: Left ventricular assist device; qRT-PCR: Quantitative real time polymerase chain reaction.

results compared with the application of single miRNAs. In order to assess circulating miRNAs as biomarkers for HFrEF, Vogel *et al*[120] performed miRNA quantification measures in whole blood samples of 53 HFrEF patients with non-ischemic HF compared to 39 healthy controls. In a two-step screening-validation study the authors found a signature of eight miRNAs (miR-520d-5p, miR-558, miR-122*, miR-200b*, miR-622, miR-519e*, miR-1231 and miR-1228*) which reliably predicted the diagnosis of HFrEF with an AUC of 0.81[120] (Table 1). Compared to the most powerful single miRNAs miR-558, miR-122[a], and miR-520d-5p (AUC between 0.7 and 0.71) this miRNA signature further improved discrimination of HFrEF patients from controls[120] confirming the idea to improve sensitivity and specificity when utilizing combinations of more than one miRNA.

miRNAS IN THE DIFFERENTIATED DIAGNOSIS OF HF WITH PRESERVED EJECTION FRACTION

Pathophysiologically, HFpEF is the clinical manifestation of LV diastolic dysfunction as a major differentiating factor from HFrEF. Therefore, diastolic dysfunction appears as a useful parameter in the early diagnosis of HFpEF.

Initial results proving the involvement of miR-21 in cardiac remodeling and fibrosis[55,67-73] (see above) suggested its roll in HFrEF. These findings were taken up by Dong *et al*[67] in order to analyze this miRNA with respect to HFpEF. The authors created a rat model of aortic constriction-induced HFpEF. HFpEF was diagnosed *via* echocardiographic parameters and quantitative RT-PCR (qRT-PCR) analyses showed higher cellular miR-21 levels in HFpEF rats compared to healthy controls[67]. These results confirm the former pathophysiologic miR-21 findings and indicate their potential to be transferrable to a functional level in HFpEF. In order to assess whether circulating miRNAs as opposed to cellular miRNAs can be utilized as biomarkers in the detection of HFpEF and in a differentiated diagnosis compared to HFrEF, results were published recently by Nair *et al*[121]. They analyzed miRNA plasma levels of HF patients with diastolic dysfunction. The authors found miR-454, miR-500 (both down-regulated) and miR-1246 (up-regulated) significantly dysregulated in diastolic dysfunction indicating that circulating miRNAs can serve as biomarkers for diastolic dysfunction[121]. This suggests itself to further considerations for miRNA-based diagnostics to differentiate HFrEF from HFpEF. Wong *et al*[115] performed a miRNA quantification of whole blood and plasma samples in 39 HFrEF and 19 HFpEF patients as well as 28 healthy controls and identified 344 miRNAs dysregulated between the three groups. Of these, 90 serum derived miRNAs were identified that showed high correlation with or an AUC > 0.7 for LVEF. Again a selection of 32 miRNAs with considerably high detection levels was made. These analytical steps allowed for a qualitative selection of promising miRNAs and those, that can easily be detected in serum. Further analyses of these 32 miRNAs in plasma of an

independent cohort of 30 HFrEF and 30 HFpEF patients as well as 30 controls identified 12 miRNAs that could segregate HFrEF and HFpEF from non-HF controls as well as HFrEF from HFpEF[115]. In detail, miR-125a-5p, miR-183-3p, miR-193b-3p, miR-211-5p, miR-494, miR-638 and miR-671-5p differed significantly between HFrEF and controls while miR-1233, miR-183-3p, miR-190a, miR-193b-3p, miR-193b-5p and miR-545-5p showed significant differences in expression between HFpEF and controls[115]. miR-125a-5p (up-regulated in HFrEF - normal in HFpEF), miR-190a (down-regulated in HFpEF - normal in HFrEF), miR-550a-5p (directionally opposite expression pattern between HFrEF and HFpEF) and miR-638 (down-regulated in HFrEF - normal in HFpEF) were revealed to distinguish between HFrEF and HFpEF. Clinically relevant, the combinatory use of NT-proBNP with miR-125a-5p improved the AUC value to differentiate HFrEF from HFpEF from 0.83 for NT-proBNP alone to 0.91 for the combinatory use and thus significantly increased NT-proBNP's discriminative diagnostic abilities[115]. Another aspect that was addressed in this study was the application of panels of miRNAs. The authors reported that miRNA panels had comparable performance to NT-proBNP with respect to the discrimination of HFrEF from HFpEF while single miRNAs tended to perform slightly inferior to NT-proBNP[115].

In order to identify a miRNA signature helping to differentiate HFpEF from HFrEF Watson *et al*[122] performed miRNA quantification analyses in sera of 90 HFpEF patients compared to 90 HFrEF patients and 90 healthy controls. The diagnosis of HFrEF and HFpEF was made echocardiographically. In an initial miRNA screening in serum samples of 15 individuals per group five candidate miRNAs (miR-30c, miR-146a, miR-221, miR-328 and miR-375) were identified as differentially expressed between the three groups and validated in an independent study cohort of 225 individuals[122]. The authors performed AUC analyses to differentiate HFpEF from HFrEF and reported an equal predictive value of any of the single miRNAs compared with the use of brain natriuretic peptide (BNP)[122]. Importantly, combinations of two or more of miR-146a, miR-221, miR-328 and miR-375 with BNP significantly improved the predictive power to differentiate HFpEF from HFrEF as compared with BNP alone in the AUC model[122]. The latter two very recent studies were the first to investigate circulating miRNAs as promising new biomarkers to differentiate HFpEF from HFrEF. They similarly provide evidence that combinatory utilization of miRNAs can improve discriminative power compared to single miRNAs. Critically evaluated, though, there was no overlap in the identified miRNAs in these studies that were able to distinguish between HFrEF and HFpEF. Schmitter *et al*[123] discussed potential explanations for these differences. The authors regarded several explanations relevant in this respect. First, methodological variances such as the choice of body liquid, detection methods and the importance to perform microarray screenings prior to qRT-PCR analyses were identified to be contributors to a lack of comparability. Also, pre-analytical variations like sample storage, degree of hemolysis, extraction efficiency and standardization methods are mentioned as important contributors to a reduced comparability[123]. The authors furthermore define the need for more large-scale studies with well-defined control- and validation cohorts limiting the influence of different HF etiologies, concomitant diseases, and treatments. Another important factor to be considered when interpreting miRNA quantification results is the influence of confounding medications and classical cardiovascular risk factors[123].

Taken together the very recent results in miRNA-based diagnostics of HFpEF and HFrEF are highly promising but urgently need verification in large-scale studies with harmonized methods and well-defined study samples.

Table 2 gives an overview of miRNAs in the diagnosis of HFpEF and HFrEF.

miRNAS IN DISEASE TREATMENT

Molecular diagnostics and therapeutics represent an important contributor to improve outcome for HF[123]. In contrast to traditional treatments, gene therapy is capable of modifying the genetic structure of the cell and can modulate the disease phenotype[124]. In this respect miRNAs are promising new players in the development of molecular therapeutics in cardiovascular disease and HF in particular. The regulation of selected miRNAs highly involved in cardiac remodeling could be a key factor in influencing the development of HF by controlling hypertrophy and fibrosis.

The concept of miRNA related disease treatment bases on the idea to specifically influence miRNA levels by raising or suppressing miRNA levels. Several different approaches have successfully been tested[125]. The major method to raise miRNA levels is the miRNA mimic technology (miR-mimic), which operates *via* miRNA substitution by artificially generated double-stranded miRNA-like RNA fragments[126]. They "mimic" endogenous miRNAs and bind - unlike endogenous miRNAs - gene-specifically to their target mRNA[126]. On the other hand, so called antagomirs can be used to suppress miRNA levels[127]. Antagomirs are chemically engineered oligonucleotides that competitively bind to and thus inhibit the mature target miRNA[127,128]. This mechanism leads to an up-regulation of specific mRNAs and gene expression[129]. Furthermore, miRNA sponges (also referred to as "target mimicry") are competitive inhibitors that contain binding sites for a miRNA family and thus inactivate miRNAs of that particular family[130-132]. As opposed to antagomirs, sponges are specific only to the seed region of a miRNA and thus can interfere with a whole family of miRNAs[133]. Masking (also called "target occupiers") describes a mechanism to prevent specific miRNAs from binding to their very binding site[134]. Consequently, fewer miRNAs remain to bind

Table 2 microRNAs in the diagnosis and differentiation of heart failure with preserved ejection fraction and heart failure with reduced ejection fraction

miRNA	Study type	Bio-material	Groups/size	Detection method	Effect	Value as biomarker	Ref.
miR-21[1]	Experimental	Tissue	Mice - HFpEF *vs* HC	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[67]
miR-30c	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-125a-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFrEF, unchanged in HFpEF	Diagnostic in HFrEF + differentiating HFrEF from HFpEF	[115]
miR-146a	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-183-3p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF and HFpEF	Diagnostic	[115]
miR-190a*	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFpEF, unchanged in HFrEF	Diagnostic in HFpEF + differentiating HFrEF from HFpEF	[115]
miR-193b-3p[1]	clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF and HFpEF	Diagnostic	[115]
miR-193b-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[115]
miR-211-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF	Diagnostic	[115]
miR-221	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-328	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-375	Clinical	Serum	HFpEF n = 90 HFrEF n = 90 HC n = 90	qRT-PCR	AUC analyses	Differentiating HFrEF from HFpEF	[122]
miR-454	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]
miR-494[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF	Diagnostic	[115]
miR-500	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10 Decompensated DCM n = 13 HC n = 8	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]
miR-545-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[115]
miR-550a-5p	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30	qRT-PCR	Up-regulated in HFrEF Down-regulation in HFpEF	Differentiating HFrEF from HFpEF	[115]
miR-638	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Down-regulated in HFrEF, unchanged in HFpEF	Diagnostic in HFrEF + differentiating HFrEF from HFpEF	[115]
miR-671-5p[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFrEF	Diagnostic	[115]
miR-1233[1]	Clinical	Plasma	HFpEF n = 30 HFrEF n = 30 HC n = 28	qRT-PCR	Up-regulated in HFpEF	Diagnostic	[115]
miR-1246	Clinical	Plasma	HFpEF n = 8 Stable DCM n = 10	qRT-PCR	Down-regulated in HFpEF	Diagnostic	[121]

	Decompensated DCM n = 13 HC n = 8

[1]See also Table 1. HC: Healthy control; miRNA: microRNAs; DCM: Dilated cardiomyopathy; HFpEF: Heart failure with preserved ejection fraction; HFrEF: Heart failure with reduced ejection fraction; qRT-PCR: Quantitative real time polymerase chain reaction; AUC: Area under the operating receiver curve.

to the target and their effect is lessened. Erasers are oligonucleotides complementary to a specific miRNA. By binding to the miRNA the eraser inhibits its endogenous function[127].

miRNA therapeutics in HF

Up to now there are no clinical trials published dealing with miRNA therapeutics in humans. Therefore, the following results relate to *in-vitro* studies and animal models. Initial studies in the field of miRNA therapeutics in HF were designed to identify differential regulations of miRNAs in HF. Sucharov *et al*[135] extracted miRNAs from 6 nonfailing, 5 idiopathic dilated cardiomyopathy (IDC) and 5 ischemic dilated cardiomyopathy (ISC) patients. The authors were able to find a set of miRNAs dysregulated in both IDC and ISC. In order to further evaluate the function of increased or decreased expression of those miRNAs the group introduced virus-delivered mirmimics as well as antagomirs against miR-92, miR-100 and miR-133b into neonatal rat ventricular myocytes and was able to cause dramatic down-regulation or up-regulation of the particular miRNAs[135]. Pathophysiologically, an up-regulation of miR-100 resulted in repression of adult genes α*MyHC* and *SERCA* while fetal genes *ANF* and β*MyHC* were up-regulated. These observations suggest the involvement of miR-100 in the specific regulation of gene expression involved in the repression of adult isoforms. The study shows that an artificial dysregulation of miR-100 is able to affect HF associated gene expression.

Raising miRNA levels as a therapeutic approach in HF

An initial project studying miRNA-associated therapeutic aspects in HF with respect to specifically raising miRNA levels was performed by Karakikes *et al*[136]. The authors addressed previous findings that proved miR-1 to be a key regulator of cardiac hypertrophy[44-47,90,137] and analyzed whether the restauration of miR-1 expression has protective effects on maladaptive cardiac remodeling. They established a hypertrophy and ventricular dilatation model in rats by ascending aortic banding before they raised miR-1 expression *in-vivo* by systemically administered adeno-associated virus-mediated gene transfer[136]. The authors were able to detect improved systolic as well as diastolic LV function in the miR-1 restauration group as measured by echocardiography and catheter-based pressure-volume loop analyses[136].

A similar approach was pursued by Pan *et al*[138] who induced an adenovirus-mediated overexpression of miR-101a in rats with chronic MI and were able to find a significant improvement of cardiac performance in those subjects treated with miR-101a overexpression. Recently, these results were confirmed in a rat model of induced MI[139]. The authors found decreased miR-101a levels at the site of the infarction and were able to verify this observation in cultured cardiac fibroblasts exposed to hypoxia and linked this effect to a TGF-β-modulated fibrotic effect. An administration of miR-101a mirmimics reduced the expression of TGF-β[139] indicating that miR-101a mimicry might negatively regulate fibrosis in ischemic cardiac tissue. These findings point out a potential applicability of mirmimics in the field of HF therapy and ignited studies further evaluating this aspect. In a recent study the authors succeeded in modulating myocardial fibrosis and apoptosis in a hypertrophic mouse model by regulating miR-455 levels[140]. Tail vein injection of viral delivered miR-455 resulted in aggravated cardiac hypertrophy on the one hand but also reduced myocardial fibrosis and inhibited apoptosis suggesting that this treatment can prevent maladaptive ventricular remodeling[140].

A different approach was addressed by Dakhlallah *et al*[141]. The authors used mirmimics to raise miR-133a levels in mesenchymal stem cells (MSC) and implanted these into ischemic rat hearts. Compared to non-miR-133a treated MSCs these rat hearts were shown to have increased cardiac function, decreased fibrosis and presented with improved cell engraftment due to better survival of miR-133a treated MSCs[141]. These study results indicate a potential roll of miRNAs in HF treatment with respect to an improvement of bioengineering of stem cells and are an example of the broad potential applicability of miRNAs in the field of HF treatment.

Lowering miRNA levels as a therapeutic approach in HF treatment

Reduced miR-29 levels were observed to be associated with a decrease of cardiac remodeling in mice[142]. The authors used an ischemia/reperfusion model in mice to analyze the effect of miR-29 on post-infarction remodeling. They found that an antisense inhibition of miR-29 implemented by an antagomir against miR-29 inhibited post infarction/reperfusion apoptosis and necrosis and led to a reduction of cardiac remodeling[142]. In a rat model of aortic constriction-induced HFpEF revealing higher cellular miR-21 levels in HFpEF rats compared to sham operated animals[67] (see above), Dong *et al*[67] performed further analyses after administering a miR-21 antagonist. The authors were able to find a reduction of fibrosis in those rats' cardiac tissues that were transfected with anti-miR-21 and attributed this effect to a reduction of Bcl-2 expression - an anti-apoptotic

factor involved in the apoptosis of cardiac fibroblasts[67]. The same miRNA was analyzed in a transgenic mouse model of cardiac failure. Thum *et al*[55] were able to show that *in-vivo* silencing of miR-21 by a systemically applied specific antagomir inhibits interstitial fibrosis by a reduction of mitogen-activated protein kinase activity when applied to pressure-overload-induced cardiac dysfunction in mice.

In order to assess the therapeutic potential of miR-652 in another mouse model with established pathological hypertrophy and cardiac dysfunction due to induced pressure overload Bernardo *et al*[143] first proved miR-652 expression to be elevated in pressure overloaded hearts compared to healthy controls. The authors then systemically administered antimiR-652 and found the expression of miR-652 effectively silenced in heart tissue of treated mice. The authors were able to show that antimiR-652 treated mice had better cardiac function and improved cardiac diameters compared to controls[143].

The first discovered miRNA Let-7 recently was found to be a potential therapeutic target in the treatment of deteriorated cardiac function after MI[144]. After induced MI in mice Let-7 was inhibited with a specific systemically applied antagomir. Molecularly, the expression of pluripotency-associated genes *Oct4* and *Sox2* was increased in cardiac fibroblasts *in vitro* and *in vivo*. Let-7 antagomir treated mice showed preserved LVEF and improved cardiac output compared to controls[144].

Addressing the hallmark of phathological hypertrophy and HF - the reactivation of fetal cardiac genes, in which miR-208 is highly involved, study results were reported in a model of antagomir-based silencing of miR-208. Montgomery *et al*[145] were able to silence miR-208 in a rat model of diastolic HF (Dahl salt-sensitive rats) by means of systemically administered locked nucleic acid-modified antimirs. On the one hand the authors found pathological myosin switching and cardiac remodeling lessened in antagomir-208 treated animals. More important from a clinical point of view was the observation that in diastolic HF therapeutic silencing of miR-208 resulted in lessened HF symptoms, a reduction of cardiac remodeling and an improved cardiac function as well as longer survival compared to control animals[145].

Current treatment strategies in HF are predominantly focused on HFrEF and no distinct therapy is established with respect to HFpEF[146-149]. The current non-specific therapy of HFpEF is limited and requires development and improvement of more distinct diagnostic and therapeutic options. Molecular diagnostics and therapeutics might provide the foundation for differential therapeutic approaches with regards to HFpEF and HFrEF.

CONCLUSION

Numerous studies have proven miRNAs to be key regulators and moderators in the development of HF and its pathophysiological precursors hypertrophy and fibrosis. Their molecular construction and integration in cellular and intercellular transport mechanisms define miRNAs as ideal circulating biomarkers for diagnostic and prognostic purposes while they can easily be collected and analyzed. Therefore, the application of miRNAs as circulating biomarkers represents a promising tool to complement established protein-based biomarkers of HF such as NPs on the one hand or novel stand-alone biomarkers in the diagnosis and prognosis of HF. In the differential diagnostics of HFrEF and HFpEF miRNAs can reliably differentiate between these two disease entities, although this has to be confirmed in larger samples. This is especially interesting considering the fact that the diagnosis of HFpEF at an early stage might significantly improve secondary prevention and established biomarkers of HF still lack precision in the differentiated diagnosis of HFpEF. Nevertheless, looking at the large number of studies only few of them confirmed previous findings with identical results and still different miRNAs are identified to be linked to HF presumably reflecting the complex interaction of miRNAs and their target sites. In this respect analysis of combinations of several miRNAs - miRNA signatures - represent a promising way to increase diagnostic and prognostic accuracy. An important aspect that should get attention when performing miRNA analyses is the comparability and standardization of analytical methods and the need for well-defined study samples.

Over the past years several different possibilities have been identified to alter levels of circulating miRNAs by systemically administering agents such as mirmimics or antagomirs. This therapeutic approach has been reported to significantly reduce hypertrophy and CF and improve LV function in animal models. It represents a promising approach to complement existing therapeutic options in the treatment of HF. Nevertheless, results of *in-vitro* and *in-vivo* models have not yet led to an application in clinical studies. A successful implementation of those insights in clinical trials represents the next step towards realizing this idea.

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REVIEW

Myocardial perfusion echocardiography and coronary microvascular dysfunction

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Abstract

Our understanding of coronary syndromes has evolved in the last two decades out of the obstructive atherosclerosis of epicardial coronary arteries paradigm to include anatomo-functional abnormalities of coronary microcirculation. No current diagnostic technique allows direct visualization of coronary microcirculation, but functional assessments of this circulation are possible. This represents a challenge in cardiology. Myocardial contrast echocardiography (MCE) was a breakthrough in echocardiography several years ago that claimed the capability to detect myocardial perfusion abnormalities and quantify coronary blood flow. Research demonstrated that the integration of quantitative MCE and fractional flow reserve improved the definition of ischemic burden and the relative contribution of collaterals in non-critical coronary stenosis. MCE identified no-reflow and low-flow within and around myocardial infarction, respectively, and predicted the potential functional recovery of stunned myocardium using appropriate interventions. MCE exhibited diagnostic performances that were comparable to positron emission tomography in microvascular reserve and microvascular dysfunction in angina patients. Overall, MCE improved echocardiographic evaluations of ischemic heart disease in daily clinical practice, but the approval of regulatory authorities is lacking.

Key words: Contrast echocardiography; Myocardial perfusion; Myocardial ischemia; Microvascular angina; Coronary flow

Core tip: Diagnostic work-up of coronary heart disease is evolving to include evaluations of the coronary microcirculation in addition to the imaging of obstructive atherosclerosis of coronary arteries and its eventual effects. Functional assessments of coronary microvasculature have become the challenge. Myocardial contrast echocardiography (MCE) emerged as a promising tool several years ago to detect myocardial perfusion abnormalities and quantify coronary blood flow. MCE compared favorably with other expensive techniques, and it accurately evaluated coronary microvascular

reserve and dysfunction in research studies. However, its daily use in clinical practice is not established. Therefore, the future of this technique is questionable.

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INTRODUCTION

The past paradigm of ischemic heart disease was the relationship between myocardial ischemia and obstructive atherosclerosis of the epicardial coronary arteries[1]. Coronary angiography was the gold standard to evaluate the severity and extent of coronary artery disease (CAD). Clinical data in the last two decades challenged this paradigm and shifted the attention to the possible role of anatomical and functional abnormalities of the coronary microcirculation throughout the clinical spectrum of myocardial ischemia.

Functional assessments of coronary microvasculature have become the challenge. Direct visualization of the coronary microcirculation is not possible with any currently available technique, but descriptions of its function are possible. The thrombolysis in myocardial infarction (TIMI) frame count is a qualitative method to evaluate coronary blood flow[2], and intracoronary thermodilution and intracoronary Doppler wire, which are based on thermal dilution curves and the Doppler principle, respectively, measure myocardial blood flow (MBF). Transthoracic Doppler echocardiography is a noninvasive technique that is widely used to measure coronary blood flow reserve primarily in the left anterior descending coronary (LAD) artery territory[3].

The well-documented diagnostic accuracy of single-photon emission computer tomography (SPECT) myocardial perfusion imaging for CAD[4] promoted its widespread clinical use[5]. Myocardial perfusion is generally evaluated in a qualitative or semi-quantitative manner[6] that suffers from several limitations: Attenuation and Compton scatter effects; the plateau effect of ^{99m}Tc uptake, which limits the detection of further increase in flow, and the limited spatial resolution[7]. Recent technical and methodological advances, such as dynamic SPECT using a SPECT/computed tomography camera[8-10], allow measurements of absolute MBF and its reserve, but the approximately 12-mSv exposure to the patient limits its clinical use[11].

Positron-emission tomography (PET) allows the calculation of blood flow per unit of mass, which quantifies microvascular function[12]. Contrast-enhanced cardiac magnetic resonance (CMR) imaging is the other technique that accurately quantifies MBF[13,14].

Myocardial contrast echocardiography (MCE) is a bedside and relatively low-cost tool to detect myocardial perfusion abnormalities and quantify regional and global coronary blood flow. The clinical use or MCE is limited despite growing evidence to support its reliability[15].

This review discusses research results and the established settings in which MCE use may support clinical decision making.

MCE PROTOCOLS

Ultrasound contrast agents

The following contrast agents are commercially available: Optison (Amersham Health AS, Oslo, Norway), Definity (Bristol-Myers Squibb Billerica, Massachusetts), and Sonovue (Bracco, Milan, Italy). These agents consist of a shell of albumin, lipids or galactose filled with a gas to form microspheres smaller than 10 μm. The shell allows the low diffusible and low solubility gas the resistance to intravascular pressure and the ability to share erythrocyte rheology in the intravascular compartment, including the transpulmonary passage. Therefore, microspheres reach the left heart cavities and opacify the left ventricle and myocardium.

Physical principles

Microspheres are strong ultrasound scatterers. Microsphere behavior in an ultrasound field depends on the energy of the ultrasound source. Very low-energy ultrasound induces linear oscillations of the microspheres, and its fundamental frequency is reflected. Non-linear oscillations of microspheres emerge with increasing incident ultrasound energy when compression and rarefaction waves of variable magnitude are produced and generate harmonics, *i.e.*, ultrasounds of higher-order frequencies than the fundamental frequency. High-intensity ultrasound is used in medical imaging and disrupts microbubbles (Figure 1). In contrast, enhanced ultrasound imaging uses low-energy ultrasound in which the myocardium primarily exhibits linear responses and generates few harmonic frequencies, as opposed to the contrast agent. Selective reception and amplification of harmonic echoes allows sensitive detection of the contrast with good signal-to-noise ratio.

Imaging modalities

High-intensity ultrasounds (mechanical index-MeI > 0.3) in standard echocardiographic imaging destroy microbubbles. The acquisition of one ECG-triggered systolic frame every several cardiac cycles improves the contrast effect by reducing microbubble destruction, and it allows time for the replenishment of myocardial microvasculature with contrast for each subsequent triggered frame. Low-MeI real-time contrast echocardiography greatly improved left ventricular and myocardial opacification compared to low-frame rate high-MeI intermittent contrast imaging. Contrast enhancement in pulse inversion Doppler technique is obtained by the transmission of two pulses of the same amplitude and inverted phase: The subtraction of reflected ultrasounds of a linear scatterer (the myocardium) generates

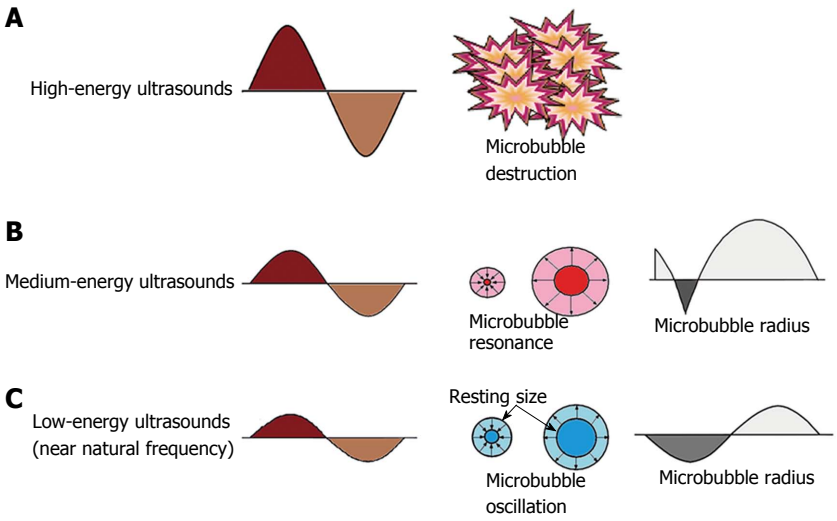

Figure 1 Behavior of microbubbles in an ultrasonic field. An acoustic wave generated by an ultrasound system consists of alternating high and low pressures: The positive pressure compresses the microbubble, and the negative pressure expands it. High-energy ultrasound (within the energy levels used for diagnostic echocardiographic imaging) destroys microbubbles (A); Intermediate energy ultrasound triggers asymmetrical nonlinear oscillations of microbubbles so that the magnitude of compression and rarefaction waves are not the same with each oscillation, and frequencies other than (*e.g.*, multiple of) the intrinsic fundamental frequency are generated (B); Low-energy ultrasound causes microbubbles to oscillate linearly, which reflects ultrasound at their intrinsic fundamental frequency (C).

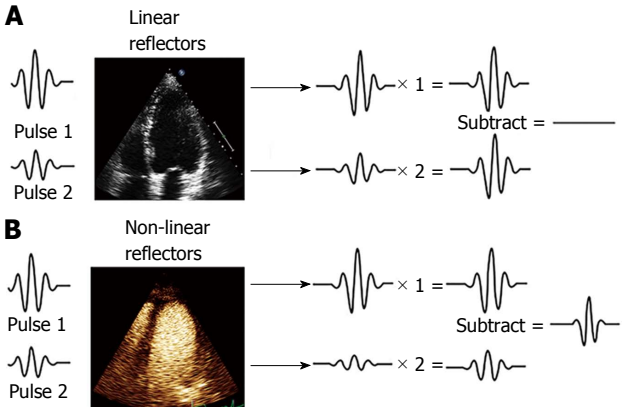

Figure 2 Pulse inversion harmonic imaging signal processing: Effects on linear and non-linear reflectors. This technique consists of the transmission of a first pulse and a second inverted replica of the first pulse. Any linear target, such as blood in conventional echocardiography, responds equally to positive and negative pressures (A) and reflects back to the transducer equal but opposite echoes, which will be canceled (blood displayed in black in 2-dimensional echocardiography); B: Pulse 1 and pulse 2 excite microbubbles generating fundamental and higher order harmonic responses with different phases that constructively add.

no signal, and the summated signals result from nonlinear scatterers (microspheres) (Figure 2). Other multi-pulse techniques were developed to improve the signal-noise ratio. The amplitude of one pulse can be increased (Power modulation, Philips, Andover, Massachusetts) (Figure 3), or amplitude and phase can be modulated (Cadence™ contrast pulse sequencing, Siemens Acuson Sequoia; Mountain View, California) to generate ultraharmonic oscillations. Both techniques exhibit excellent spatial resolution to assess myocardial contrast in real time.

Qualitative assessment

Low flow constant contrast infusion produces homogeneous opacification of the myocardial wall in patients without significant stenosis of epicardial coronary arteries because new microbubbles uniformly replaced those that are destroyed by a flash of high-intensity ultrasound. In contrast, the rate of contrast replenishment is reduced at rest in myocardial regions where microcirculation is damaged by previous infarction or during stress in regions supplied by a significantly stenosed epicardial vessel. Myocardial flow increase during pharmacological or physical stress enhances regional differences of myocardial opacification and allows the quantification of regional perfusion as normal, reduced, or severely reduced.

Quantitative assessment

The basis for myocardial flow quantification in MCE studies is the complete intravascular compartmentalization of microbubbles, which allows reaching a steady state concentration of microbubbles during continuous infusion. Replenishment of myocardial microvasculature after complete destruction of microbubbles using a flash of high-intensity ultrasounds may be assessed as a time-intensity curve. Intensity values "y" fitted to a monoexponential function: $y = A \times (1-e^{-\beta t})$ gives a measure of mean myocardial microbubble velocity (β), whereas the microvascular cross-sectional area is obtained from the plateau value of the replenishment curve (A). The product of A and β represents MBF.

Vogel *et al*[16] proposed the use of ratio of myocardial video intensity to the adjacent left ventricular cavity as an adjustment for the inhomogeneous contrast enhancement of the myocardium due to attenuation or other technical factors. An excellent correlation was found between absolute MBF (mL/min per gram of myocardium) measured with MCE and the values obtained using PET.

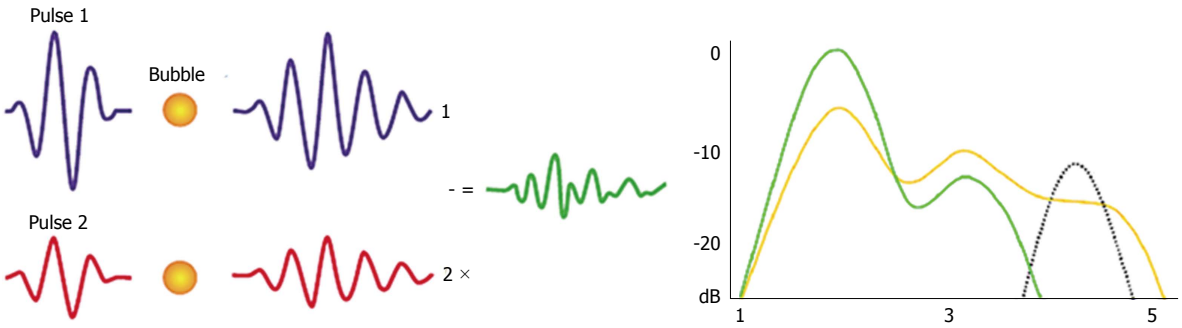

Figure 3 Power modulation imaging. This technique changes the amplitude of each successive pulse in a group of transmitted pulses and detects the differential nonlinear responses generated from two different excitations. Microbubbles' response to multipulse cancellation technique produces ultra-harmonic oscillations that are detected in the field of higher frequencies.

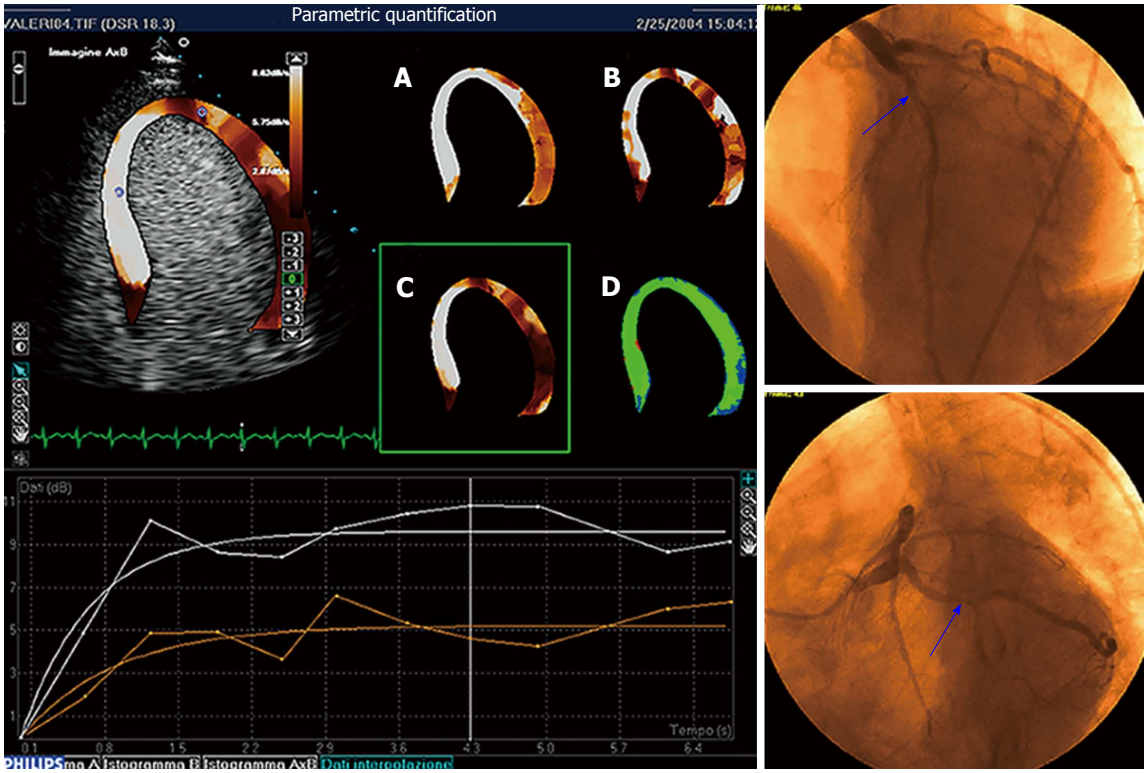

Figure 4 Parametric quantification of myocardial perfusion. Critical stenosis of the circumflex artery is present at coronary angiography (right panels). Parametric images of peak dipyridamole stress echo reported in panels A through D represent, respectively, the plateau value A of the contrast replenishment curve, the slope β of the replenishment curve, the A × β value, and the goodness of fitting. The A × β value parametric imaging is also displayed superimposed onto the apical four-chamber view in the top left panel. Replenishment time-course curves (sampling and interpolation) relative to the septum (white curves) and the apico-lateral wall (red curves) are reported in the graph at the bottom. Flow is reduced in the territory perfused by circumflex artery.

A fast and easy method to quantify MCE is represented by parametric images (Figure 4), which furnished visual information on the maximal intensity of contrast (A), rate of replenishment (β) and quality of acquisition[17]. Four, two and three chamber apical acquisitions of MCE create feasible computations of MBF.

CORONARY ANATOMY AND REGULATION

A detailed description of coronary anatomy and regulation is beyond the scope of this review. However, some features must be described to appreciate the potential of MCE. Figure 5 shows the complexity of coronary anatomy. Coronary circulation comprises the large epicardial conduit vessels and resistance vessels. Resistance to flow is very low in conduit vessels and progressively increases as resistance vessel diameter decreases to the arteriolar bed (from 300 to 100 μm). The so-called "coronary driving pressure", *i.e.*, the pressure gradient between the aortic root and the right atrium, is the main determinant of blood flow across the myocardium. The coronary driving pressure under normal conditions reduces little, if any, along the epicardial conduit vessels, but it declines progressively along the microvasculature, particularly in 300-100 μm

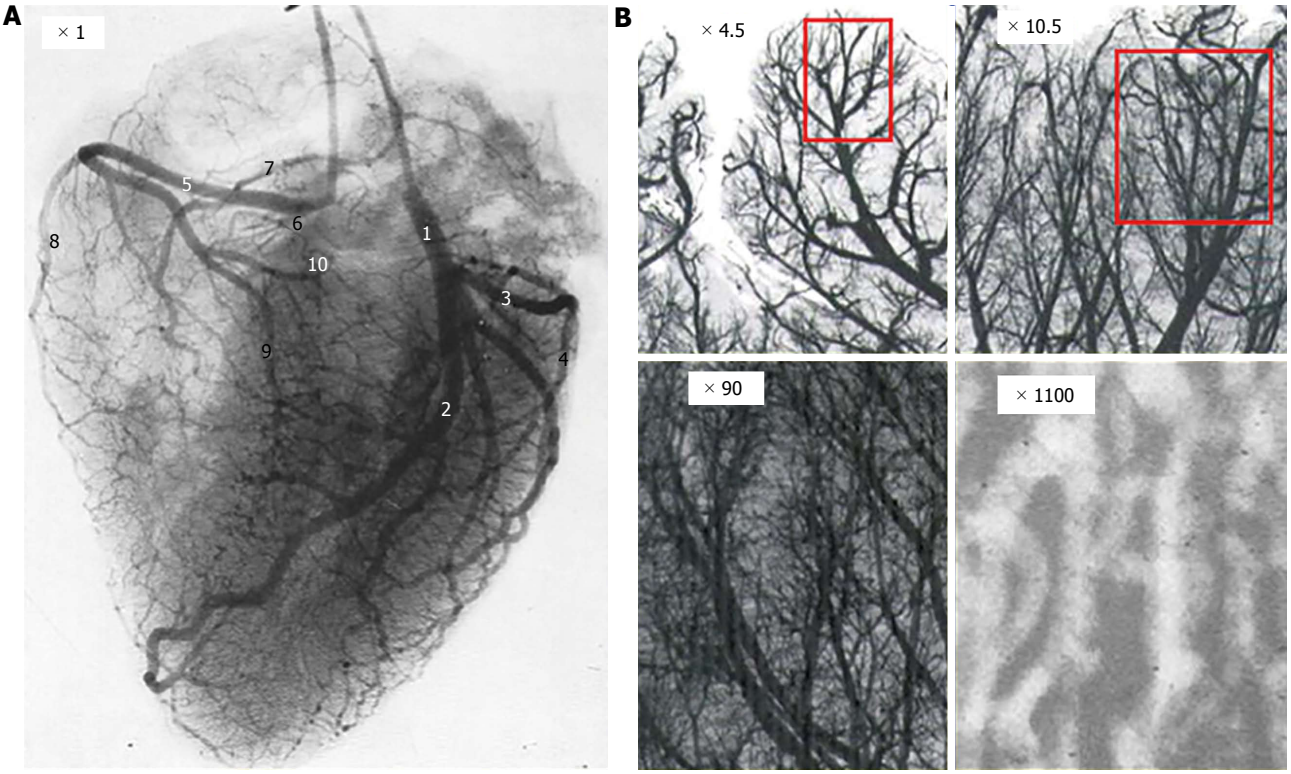

Figure 5 Coronary artery tree arborization. A: Angiographic still frame of left and right coronary arteries of an isolated human heart after injection of radiopaque dye; B: Progressive magnification of a region of interest showing the coronary tree fractal anatomy.

diameter arterioles, until reaching the 20-30 mmHg level. Extravascular resistive forces, which are related to the development of left ventricular systolic pressure, inotropism of the myocardium, and heart rate are additional determinants of the resistance to flow across the myocardium.

Coronary circulation has two main characteristics. First, oxygen extraction at rest is approximately 75%, but coronary venous oxygen saturation during strenuous exercise may decrease to approximately 10%. The increase in flow needed to meet the 4- to 5-fold increase in oxygen demand is accomplished by vasodilation of resistance coronary arteries, which is mediated by mechanisms that are intrinsic to the vascular wall and metabolic and neuro-humoral factors[18]. Second, coronary flow is pulsatile, *i.e.*, it is high in diastole and low in systole.

Coronary arterial pressure and myocardial oxygen consumption are the major determinants of coronary flow, but coronary autoregulation allows the flow to be relatively independent of the driving pressure at fixed oxygen consumption level, whereas at any given coronary arterial pressure a metabolic adaptation of coronary flow to oxygen requests may occur[19].

The hemodynamic impact of a focal diameter reduction of epicardial vessels is modulated by the vasodilation of resistance coronary arteries. Maximal flow reduces at maximal vasodilation because coronary perfusion pressure decreases with the square of flow through the stenosis. This law of coronary hemodynamics is the basis for dynamic assessment of coronary stenosis by pharmacological or physical stress that can elicit a reversible perfusion defect and eventually a transient regional asynergy of contraction.

Experimental canine models, in which external constriction was applied to normal coronary arteries, led to the concept of "critical" coronary stenosis. In these studies, an 85% diameter narrowing caused a fall in resting coronary flow, but a 50% diameter narrowing reduced maximum coronary flow. In surgical decision-making, the angiographic criterion of > 70% coronary stenosis was appropriate to define eligibility for revascularization.

However, this paradigm failed to describe the correlation between coronary flow reserve (CFR) and percent coronary stenosis in human studies[20], and it does not consistently identify patient groups whose prognosis can be improved by primary coronary interventions. A further confirmation of the dissociation between anatomic and functional severity of stenosis came from the poor correlation between quantitative percent stenosis on invasive or computed tomography coronary angiograms and fractional flow reserve (FFR) using pressure flow wire[21]. FFR is a validated, reproducible measurement of relative CFR. The reference model for FFR is the hyperemic pressure-flow relation with the assumptions that a discrete stenosis induces proportional changes of perfusion pressure and flow, and downstream of a stenosis minimal coronary resistance equals that of a normally perfused region[22]. In practice, FFR derives from pressure measurements upstream and downstream of a given coronary stenosis at maximal

pharmacological arteriolar vasodilation. FFR was proposed as the best available technique to guide clinical decision making (whether mechanical revascularization or medical therapy), and FFR-guided PCI is the way to improved prognoses with respect to PCI based on angiographic severity[23-26].

Absolute CFR equals the ratio of maximal to baseline flow for any given arterial distribution with or without a stenosis or diffuse narrowing[27]. Relative CFR equals the ratio of maximal stress flow in the diseased artery to maximal flow in non-diseased arterial segments either in the same or adjacent arterial distribution. CFR in healthy volunteers with no risk factors is up to 4.5 ± 0.7. Values for mild disease in patients with coronary risk factors average 2.7 ± 0.6[28]. CFR < 2.0 is the proposed threshold for inducible ischemia[29]. FFR pressure ratio for a single discrete stenosis in the absence of diffuse disease also equals relative CFR using flow or flow velocity measurements. However, flow-based CFR and pressure-based FFR may not give evidence of comparable stenosis severity in approximately 40% of lesions[30]. Diffuse narrowing may reduce CFR significantly, with only a minimal fall in segmental pressure gradients or FFR. In situations of mixed diffuse and segmental disease, which are common in the clinical setting, noninvasive absolute maximal perfusion and CFR together likely define the severity of each coronary stenosis. Gould[31] provides the following example: "A 38% diffuse diameter narrowing in the absence of arterial remodeling would reduce CFR to 1.4 whereas the same diffuse disease plus a discrete angiographic 60% stenosis without remodeling would reduce CFR to 1.0, both without significant fall in FFR". Therefore, FFR is not a direct measure of low-flow ischemia, and it does not reflect absolute flow or absolute CFR, which are the determinants of ischemia. Further, FFR and CFR results may be inconsistent because microvascular resistance during hyperemia causes FFR and CFR to change in opposite directions. Higher hyperemic resistance means reduced maximal flow and higher distal pressure, *i.e.*, reduced CFR and increased FFR. Vice versa, low hyperemic microvascular resistance for equal stenosis means higher maximal flow and increased pressure gradient through the stenosis, which may result in low FFR.

Therefore, the microvascular compartment must be considered in an integrated view of coronary perfusion because its functional anatomy is complex. The branching of vessels in the myocardial wall, the different structure of subendocardium and subepicardium vessel wall, and the incremental vascular capacity across the myocardial wall depth describe this complexity, which is the substrate for more important functional differences that are physiologically intended to compensate the effects of myocardial contraction on subendocardial perfusion[32]. More intensely in early systole, the development of intraventricular pressure squeezes intramural vessels and reduces intramural blood volume, which causes coronary venous flow to increase and arterial flow to decrease[33].

The diagnosis of coronary microvascular disease likely occurs when CFR is significantly reduced in the absence of segmental perfusion defects. Coronary microvascular endothelial dysfunction may be assessed noninvasively by evaluating CFR with perfusion imaging in combination with cold pressor stress or demonstrating improvements in resting perfusion heterogeneity using a vasodilator stress. These methods may help correctly evaluate the physiological meaning of anatomical coronary stenosis when FFR and CFR results are inconclusive.

PHARMACOLOGICAL STRESS TESTING

Two possible pharmacological methods of challenging coronary circulation evolved over the years: (1) pharmacological interaction with adenosine receptors of vascular smooth muscle cells (dipyridamole, adenosine, and regadenoson[34,35]); and (2) pharmacological inotropic stimulation (dobutamine[36]).

Adenosine vasodilator stress induces maximal increases in blood flow (three- to five-fold in healthy subjects) by reducing vascular resistance. The hyperemic flow is dependent on systemic pressure and the residual resistance at the microcirculation level *via* relaxation of smooth muscle cells, which uncouples coronary flow (supply) and myocardial work (demand). The mismatch between coronary flow and myocardial demand may induce the stealing phenomenon[34]. However, hyperemia induced by adenosine is mediated by endothelium receptors and neuronal-mediated mechanisms, which was elegantly demonstrated in two studies using L-nitroarginine methyl ester (L-NAME). Intracoronary L-NAME attenuated the hyperemic dilation in healthy volunteers *via* inhibition of endothelial nitric oxide synthase[37], but systemic L-NAME counterbalanced CFR increase *via* the neuronal response[38]. This effect may explain the limited accuracy of the ratio of hyperemic to rest MBF that exists in pathological conditions, such as hypertension, which is characterized by elevated flow at rest.

Dobutamine stressor is an alternative to physical stress. This synthetic sympathomimetic amine stimulates β- and α-adrenoreceptors, increases myocardial oxygen consumption (inotropic effect) and MBF[39-41]. Thickening of subendocardial myocardial layers primarily contributes to resting wall thickening, but catecholamines also stimulate a thickening of subepicardial layers. This effect is useful to detect myocardial viability, and it explains why patients with resting wall motion (WM) abnormalities consequent to subendocardial infarction still exhibit improved contractility during dobutamine infusion. This pharmacological effect is beneficial for the diagnosis of viability, but it may mask the subendocardial ischemia in patients with normal resting WM and significant coronary stenosis[42].

The cold pressor test deserves some attention for its capacity to interact with the sympathetic system[43-45].

Sympathetic stimulation using cold exposure induces a sharp rise in heart rate and systolic arterial pressure

and a norepinephrine release in the coronary circulation from adrenergic nerve terminals. This increase in rate-pressure product induces a similar increase in MBF, and norepinephrine vasoconstriction is balanced by endothelium-related vasodilation of epicardial coronary vessels and myocardial microcirculation. This mechanism is related to flow augmentation, and it exhibits a similar extent as pharmacologically induced hyperemia.

Therefore, the cold pressor test produces a similar increase in rate-pressure product and MBF. The effects on sympathetic endothelium modulation and endothelium-related flow augmentation suggest the utility of this stressor in evaluating microvascular function.

CORONARY ARTERY STENOSIS DETECTION

The ischemic cascade concept, whereby abnormal perfusion precedes abnormal mechanical function during increased demand-induced ischemia, was demonstrated in an experimental setting. The spatial extent of perfusion abnormality is greater than the contraction abnormality, and the mismatch between perfusion and function is more evident in single-vessel *vs* multi-vessel disease[46].

Pharmacological and physical stress echocardiography is a mainstay of the noninvasive assessment of CAD. Technological advances and the use of contrast agents provided echocardiography the ability to increase and match the perfusion information to the classic evaluation of WM abnormalities that are induced by adrenergic or adenosinic mechanisms[47].

The use of echocardiographic contrast agents improved the accuracy of stress echocardiography[48-50]. Shah *et al*[48] demonstrated the usefulness of myocardial contrast stress echocardiography in 193 patients (88%) in a prospective clinical study, and it provided an incremental benefit over WM analysis in 25% of patients and greater confidence with WM evaluations in 62% of patients.

Thomas *et al*[51] randomized 1776 patients to real-time myocardial contrast (RTMCE) dobutamine, physical stress echocardiography or standard stress echocardiography in which contrast was used for opacification of the left ventricle only (non-RTMCE). Myocardial perfusion tests exhibited a higher positivity (22% for RTMCE *vs* 15% with non-RTMCE, $P = 0.0002$) with similar positive values to predict > 50% diameter stenosis using quantitative coronary angiography (67% for non-RTMCE, 73% for RTMCE). The higher positivity of RTMCE was related to the detection of subendocardial wall thickening abnormalities that were missed in non-RTMCE studies that only examined transmural wall thickening[51] (see the representative case in Figure 6).

At variance with a previous study[51], a large multicenter study[7] demonstrated a higher sensitivity of myocardial contrast stress echocardiography *vs* SPECT (75.2% *vs* 49.1%, $P < 0.0001$) for the detection of $\geqslant$ 70% or $\geqslant$ 50% stenosis, but the specificity was lower (52.4% *vs* 80.6%, $P < 0.0001$). Sensitivity for the detection of $\geqslant$ 70% single-vessel stenosis was higher for MCE (72.5% *vs* 42.7%, $P < 0.0001$) and the detection of proximal vessel disease (80% *vs* 58%, $P = 0.005$), but a cautionary note must be placed relative to fair inter-reader agreement ($k = 0.37$ for MCE, 0.34 for SPECT in the mentioned study)[15]. The sensitivity of MCE was greater for the detection of LAD and multivessel disease[15].

Dobutamine-atropine stress RTMCE and CMR exhibited comparable diagnostic accuracies for significant CAD detection due to the incremental values of myocardial perfusion imaging over WM analysis for RTMCE and CMR[52].

Myocardial contrast stress echocardiography also facilitates the measurement of the CFR-LAD using transthoracic Doppler, and CFR-LAD exhibited incremental value for WM analysis[53-55].

The prognostication potential of myocardial contrast stress echocardiography requires further research. MCE detects stress-induced perfusion defects that are uncoupled to WM abnormalities and identifies a sub-group of patients who are at a higher risk of coronary events among patients without inducible WM abnormalities at stress echocardiography, who are by definition at low risk[49,56-60].

Porter *et al*[60] studied 2014 patients with intermediate to high pre-test probability of CAD who were randomized to dobutamine or exercise stress RTMCE or conventional stress echocardiography (CSE) and followed prospectively for a median of 2.6 years. They demonstrated that patients with abnormal RTMCE studies had higher death rates, nonfatal myocardial infarction (MI), or subsequent revascularization rates than patients with abnormal CSE studies. No difference emerged in primary end-point rates following normal CSE or RTMCE studies. Notably, patients with perfusion defects and WM abnormalities and patients with perfusion defects only exhibited similar rates death/nonfatal MI (7.2% and 6.5%, respectively)[60].

Gaibazzi *et al*[61] confirmed these data in 718 patients in a multicenter cohort study who were followed for 16 mo after high-dose dipyridamole MCE with measurements of LAD flow reserve, and patients who underwent revascularization after the diagnostic test were censored[61].

NO REFLOW

The "no reflow" phenomenon, first described by Ito *et al*[62] and subsequently confirmed by several investigators in MCE studies[63-67], refers to the situation when myocardial tissue perfusion is not restored despite a grade 3 TIMI flow on coronary angiography after primary coronary intervention for acute MI. The no reflow phenomenon influences the eventual infarct size and affects up to one third of patients who undergo reperfusion coronary interventions. This phenomenon

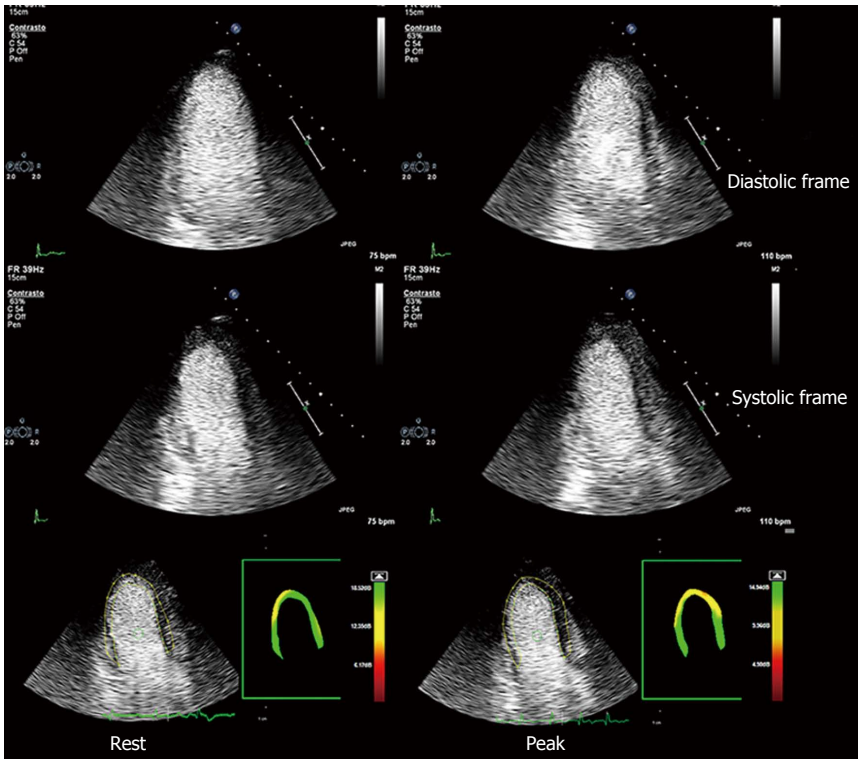

Figure 6 Myocardial contrast stress echocardiography. Apical three-chamber view of a patient with previous by-pass graft (left internal mammary artery graft onto left anterior descending coronary artery). Baseline diastolic and systolic frames on the left, top and intermediate rows, respectively; peak stress diastolic and systolic frames on the right, top and intermediate rows, respectively. Baseline and peak stress myocardial perfusion parametric quantification are displayed, respectively, in the bottom left and right panels. At baseline, akinesis of the infero-apical region is evident, which is concordant with a transmural defect of perfusion of the same region. At peak stress, no new wall motion abnormalities are detected, whereas parametric quantification of myocardial perfusion shows a large transmural defect of perfusion of all the apical regions and a subendocardial defect of perfusion of middle and basal anterior septum. A critical stenosis of distal mammary graft anastomosis was found on coronary angiography.

results from microvascular obstruction caused by the embolization of thrombus and plaque debris during balloon angioplasty and stent deployment in the setting of short duration myocardial ischemia (< 45 min). Other mechanisms are major determinants in prolonged ischemia, in which the no reflow correlates to microvascular damage. There may be areas of low reflow all around the no reflow area, whose salvage may be the potential target of treatment options. The presence of collaterals and the dynamics of the target vessel occlusion, whether it was an abrupt event or a chronic intermittent reduction of flow, influence the low reflow area.

Addressing the no reflow phenomenon may affect therapeutic intervention outcome in acute coronary syndromes. Several studies demonstrated that thrombus aspirations and occlusive protection devices may increase microvascular perfusion, and platelet inhibitors may reduce the no reflow area and infarct size. Pharmacological interventions (nicorandil, verapamil, adenosine) may also increase the chance to reduce myocardial infarct size[68-71].

ISOLATED MICROVASCULAR DYSFUNCTION

Abnormalities of coronary microcirculation beyond myocardial ischemia caused by atherosclerosis of the epicardial coronary arteries are an alternative cause of, or may contribute to, myocardial ischemia in several conditions. Coronary microvascular disease (MVD) is a unique cause of symptoms in several patients with angina. This condition is known as microvascular angina (MVA)[72], and it is better defined as primary MVA to distinguish it from secondary MVA, which occurs in the setting of specific diseases (Table 1)[73].

The clinical presentation of MVA covers the entire spectrum of coronary syndromes, from chronic to unstable angina and acute syndromes.

Stable primary MVA is characterized by angina episodes that are exclusively or predominantly related to effort, and it can be identified with the clinical entity that is generally known as cardiac syndrome X[74]. No cardiac or systemic diseases should be detectable by definition. However, patients with uncomplicated hypertension or diabetes mellitus are often classified as syndrome X patients because these pathological conditions confer a risk for MVD similar to atherosclerosis obstructive CAD[74].

Functional abnormalities of resistive coronary vessels were documented in numerous studies on MVD. Blunted endothelium-dependent vasodilation due to impaired nitric oxide release is the most commonly proposed mechanism for MVD in stable MVA patients,

Table 1 Pathogenetic classification of cardiac microvascular dysfunction

MVD in the absence of myocardial and obstructive coronary artery diseases
MVD in the presence of myocardial disease
MVD in the presence of obstructive coronary artery disease
MVD caused by coronary recanalization interventions

MVD: Microvascular dysfunction.

and it is based on a reduced coronary blow flow (CBF) response to acetylcholine[75]. A reduced CBF response to endothelium-independent vasodilators, such as adenosine, dipyridamole, and papaverine, was repeatedly reported[76-78], which suggests an important role of primary impaired relaxation of small vessels. Other studies demonstrated enhanced vasoconstrictor activity in coronary microcirculation in several patients with stable MVA. Ergonovine injection, mental stress, and hyperventilation resulted in impairments of CBF[79]. Tests to diagnose MVD in the clinical setting should explore the vasodilation and vasoconstriction responses of coronary microcirculation. Vasodilator tests are the first choice in patients with stable MVA, but the response to vasoconstrictor stimuli should be assessed when the former tests are normal or inconclusive. Transthoracic Doppler echocardiographic evaluation of CBF may be used as a first-line method to identify MVD in the LAD territory of patients with normal coronary arteries with suspected MVA. Contrast stress echocardiography may represent the frontier method to detect MVD in the entire myocardial circulation[80].

Unstable primary MVA should be suspected in patients with non-ST-segment elevation acute coronary syndrome and normal coronary arteries on angiography. *De novo* abnormalities on standard ECG in these patients (*e.g.*, ST-segment depression, negative T waves), their gradual normalization, and mild elevation of serum markers of myocardial damage (troponins) indicate a cardiac ischemic origin of symptoms. Diagnosis requires the exclusion of epicardial coronary spasm and transient coronary thrombosis as the cause of angina together with evidence of MVD.

Multiple studies evaluated the prognosis in MVD and demonstrated more cardiac events in patients with reduced CFR. However, there is no consensus in the literature on the best prognostic CFR cutoff (range 1.5-2.5). Murthy *et al*[81] demonstrated a 5.6-fold increased risk of cardiac death in patients with suspected CAD and CFR < 1.5.

Two forms of unstable MVA are described, microvascular variant angina and stress-related cardiomyopathy. Mohri *et al*[82] described the first form in Japanese patients with angina attacks at rest (less frequently with associated effort angina) in the presence of normal coronary arteries. Intracoronary acetylcholine reproduced angina and ST-segment changes in these patients. The absence of vasospasm of epicardial coronary vessels suggested diffuse coronary microvascular spasm.

Stress-related cardiomyopathy (also known as apical ballooning syndrome or takotsubo disease) is generally triggered by sudden emotional or even physically intense stress[83]. Acute chest pain may be associated with abrupt heart failure or cardiogenic shock. The clinical picture includes normal epicardial coronary arteries, depressed left ventricular function, left ventricular ballooning at angiography or echocardiography due to apical and mid-ventricular akinesia with preserved contraction of basal segments, relatively minor elevations of troponins and creatine kinase-MB and a favorable clinical course with recovery of all abnormalities in 1 to 3 mo. The disease is considered adrenergic-mediated because of the cause-effect relationship with stress. Findings that support this hypothesis include increased catecholamine levels, histological signs of catecholamine-mediated cardiotoxicity in endomyocardial biopsy specimens[84], and the unique distribution of cardiac WM abnormalities, which may reflect the variable distribution of adrenergic innervation in the myocardium[84]. Sustained intense coronary microvascular constriction or spasm induced by excessive adrenergic stimulation that results in myocardial ischemia and stunning may be an alternative pathological mechanism, at least in some patients[85]. Some reports demonstrated abnormal myocardial perfusion in the affected myocardial segments[86], and MVD was documented by evidence of reduced CBF responses to vasodilator stimuli in the acute phase[87]. MVD subsided in several weeks, which paralleled clinical improvement[87], but subclinical microvascular dysfunction persisted long after the acute phase. This persistence was demonstrated by the abnormal response of coronary flow to the cold pressor test and left ventricular regional contraction on contrast echocardiography[88]. Our follow-up study of patients with takotsubo syndrome demonstrated transient WM abnormalities and reduced CFR without regional myocardial perfusion abnormalities in response to the cold pressor test, which suggests the persistence of microvascular dysfunction (Figure 7).

CONCLUSION

Second-generation echocardiographic contrast agents received regulatory authorities' approval for clinical use in left ventricular opacification studies with the only limitation of patients with intracardiac shunts or pulmonary hypertension. In contrast, MCE did not receive approval for clinical use, and the technique remains an option for research. MCE is a demanding technique in the technical skills that are required of sonographers and physicians and the investment of software of analysis systems. The reimbursement issue may represent an adjunctive drawback. Reimbursement for MCE studies is approximately $60 in the United States, but reimbursement covers only the cost of the drug in most European countries. Analysis of cost/effectiveness and cost saving based on the procedural

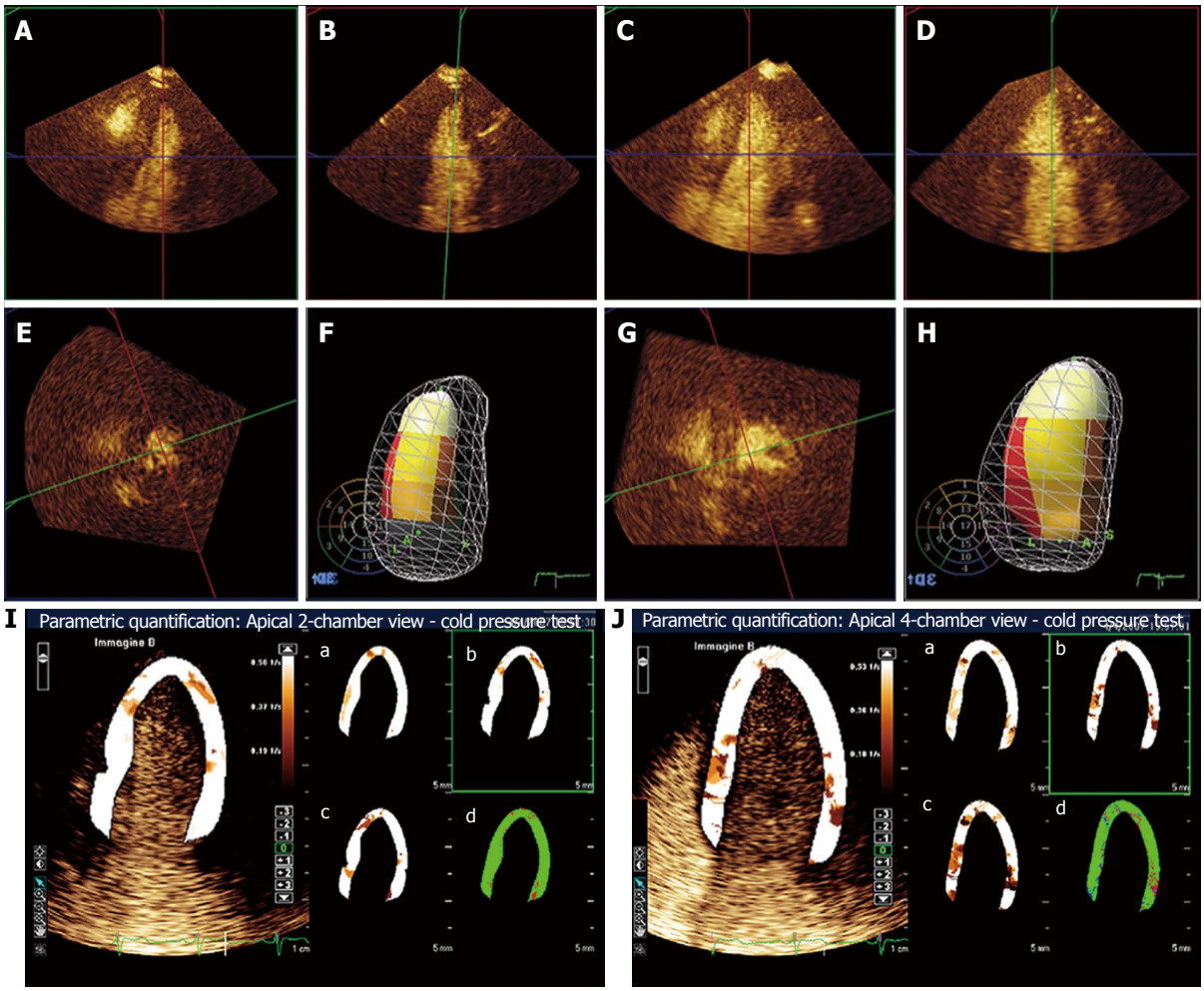

Figure 7 Real-time 3-dimensional myocardial contrast echocardiography during follow-up using cold pressor test in a patient who recovered from apical ballooning syndrome. A, B and E: Reconstructed 4-chamber (A), 2-chamber (B) and short-axis (E) end-systolic frames at baseline; F: 3-dimensional systolic volume rendering as left ventricular cast inside the diastolic mesh volume rendering. The American Society of Echocardiography 17-segment model of the left ventricle is reproduced as a bulls-eye in the background, and superimposed color-coded onto the left ventricular cast; C, D and G: Reconstructed 4-chamber (C), 2-chamber (D) and short-axis (G) end-systolic frames using the cold pressor test; H: The diastolic and systolic 3-dimensional casts as in panel 1d. Note that wall motion is normal at baseline, whereas apical akinesia develops during the cold pressor test; I and J: Apical 2-chamber and 4-chamber, respectively, parametric myocardial contrast echocardiography quantification using the cold pressor test; the slope β of the replenishment curve is superimposed onto the left ventricular wall in 2-chamber and 4-chamber views, respectively. Perfusion parameters A, β and A × β are superimposed onto the same left ventricular wall as in panels I and J, respectively, in panels a, b and c; panels d represents the goodness of fit. Parametric images demonstrate homogeneous perfusion during the cold pressor test. The coronary flow reserve in this patient was 1.10 (normal range 2.77 ± 0.70).

and downstream investigation costs, as the one conducted by the Medical Services Advisory Committee of Australia on the use of second-generation contrast agents in patients with suboptimal echocardiograms[89], is lacking for MCE. However, the diagnostic potentials of MCE, which covers the entire spectrum of cardiac circulation physiopathology and clinical presentation of coronary syndromes, may reduce overall costs.

Research has established that the application fields for MCE represent the clinical perspective to pursue this technique in the future daily workflow of echo laboratories. The integration of FFR with quantitative MCE offers the opportunity to demonstrate the effects of anatomical diffuse non-critical coronary stenosis and furnishes an integrated vision of the extent of ischemic burden that is comprehensive of collateral flow contribution. MCE in the setting of acute coronary syndromes identifies complex anatomo-functional features, such as no reflow and low flow, within and around the infarct area, respectively, which foresees the potential for functional recovery of stunned myocardium to guide therapeutic interventions. In an era when typical angina is less frequently associated with significant coronary stenosis[90] and acute coronary syndromes are dissociated from intracoronary thrombosis or significant coronary stenosis in up to 10% of cases, MCE is a relatively low-expensive, bedside technique to examine microvascular reserve and identify patients with MVD.

In conclusion, current research provides good evidence that MCE improves comprehensive echocardiographic evaluations of ischemic heart disease. The approval of regulatory authorities and the availability of quantitative operator-independent analysis software will hopefully prompt physicians and sonographers to

implement MCE into the daily work flow of echo laboratories.

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MINIREVIEWS

Mechanical valve obstruction: Review of diagnostic and treatment strategies

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Abstract

Prosthetic valve obstruction (PVO) is a rare but feared complication of mechanical valve replacement. Diagnostic evaluation should focus on differentiating prosthetic valve thrombosis (PVT) from pannus formation, as their treatment options differ. History of sub-optimal anticoagulation and post-op time course to development of PVO are useful clinical characteristics in differentiating thrombus from pannus formation. Treatment of PVT is influenced by the patient's symptoms, valve location, degree of obstruction and thrombus size and may include thrombolysis or surgical intervention. Alternatively, pannus formation requires surgical intervention. The purpose of this article is to review the pathophysiology, epidemiology, diagnostic approach and treatment options for aortic and mitral valve PVO.

Key words: Prosthetic valve thrombosis; Pannus overgrowth; Thrombolysis; Prosthetic valve obstruction; Echocardiography

Core tip: Prosthetic valve obstruction (PVO), while rare, is a dreaded complication of mechanical valve replacement. Careful clinical and multiple non-invasive imaging modalities are necessary to assess suspected PVO and evaluate for pannus overgrowth or valve thrombosis. Unlike pannus overgrowth, prosthetic valve thrombosis is more common, occurs earlier in the post-op period, is frequently related to inadequate anticoagulation, and can often be treated through non-invasive thrombolysis. While the current understanding of pannus overgrowth remains elusive, future clarification of its pathophysiology may allow for the development of non-invasive therapeutic options.

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INTRODUCTION

A 60-year-old male underwent 1-vessel coronary artery bypass graft and a 31 mm bileaflet St. Jude's mechanical mitral valve (MV) replacement for newly diagnosed ischemic cardiomyopathy and functional mitral regurgitation. His post-op course was uneventful and he reported self-compliance with all his medications. Three months after his surgery he was admitted for shortness of breath and was found to be hypotensive with jugular venous distention, warm extremities with pitting edema bilaterally, and a new 3/6 holosystolic murmur with a 2/4 diastolic rumble- both radiating to the axilla. His international normalized ratio (INR) was 1.3.

Transthoracic echocardiography (TTE) revealed an unchanged ejection fraction and a fixed closed mitral leaflet disc with a transmitral Doppler mean gradient of 13 mmHg. His calculated MV area was 0.41 cm^2 (*via* continuity equation), maximum MV E wave velocity of 1.7 m/s and new severe right ventricle dilatation, dysfunction, and tricuspid regurgitation were also present. Transesophageal echocardiography (TEE) confirmed a fixed mitral leaflet (Figure 1), and a soft thrombus in left atrial appendage. A small soft non-mobile mass (5-6 mm) adjacent to the sewing ring on the fixed leaflet was identified. Follow up TTE and cine fluoroscopy (CF) confirmed residual immobility of the posterior occluded prosthetic leaflet.

He was treated with intravenous furosemide with symptomatic improvement. Tissue plasminogen activator was administered (10 mg bolus centrally through Swan Ganz catheter followed by a 90 mg infusion peripherally over 5 h). Follow up TTE transmitral gradient *via* Doppler interrogation demonstrated a significant decrease to 4 mmHg. A decision was made to pursue redo-mitral valve replacement with a 31 mm St. Jude's porcine bioprosthesis since the valve remained in the closed position. Gross sample revealed residual organized thrombus on the mitral valve disc (Figure 2). Three-month follow-up TEE showed no change in transmitral gradient.

Pathophysiology

Prosthetic valve replacement whether mechanical or bioprosthetic carries an inherent risk for serious, sometimes devastating complications. Obstruction of prosthetic valves can result from thrombus, pannus overgrowth, vegitations or combination of thrombus and pannus formation.

Prosthetic valve thrombosis: Prosthetic valve obstruction (PVO) is a rare but dreaded post-surgical complication, with the most common cause being prosthetic valve thrombosis (PVT). PVT occurs more commonly in mechanical, as compared to biologic prostheses, likely related to the underlying pathophysiology of thrombus development[1,2].

Post-surgical endothelization after prosthetic valve surgery occurs over weeks to months. During this time, the exposed and healing endothelium may serve as a nidus for clot formation. Typically, an initial small thrombus may develop and act as a further substrate for additional layering of new thrombus[3]. In addition, the post op course of a newly placed mechanical valve results in the development of turbulent flow and stasis which is an additional contributor to thrombus development. This relative stasis and aberrant flow helps explain why tricuspid valve thrombosis is 20 times as common as left sided thrombosis, and MV thrombosis is more common than aortic valve (AV) thrombosis[3]. Similarly, increased prosthetic surface area has been correlated to a greater formation of both thrombi and pannus[4].

The intrinsic prothrombotic milieu post valve replacement requires strict anticoagulation to avoid complications. Thus, multiple investigators have observed a significantly higher incidence of thrombotic complications among patients with subtherapeutic anticoagulation, which has been validated as the best clinical tool to differentiate pannus from thrombus, as discussed below[1,5,6].

Pannus overgrowth: Although less common than thrombus formation, pannus may develop over prosthetic valves. A biologic reaction to the prosthesis material with unknown mechanism is thought to cause fibroelastic and collagen overgrowth, with subsequent infiltration of endothelial cells, myofibroblasts, and chronic inflammatory cells resulting in fibrinous ingrowth around the prosthetic valve annulus[1,4,7].

The precise trigger for pannus formation remains unclear at this time, further limiting the ability to prevent and treat this phenomenon. Peripheral blood samples of patients with pannus formation have elevated levels of the proliferation and cell differentiation signaling protein transforming growth factor-beta (TGFβ) when compared to a control cohort (87.7 ng/mL *vs* 73.7 ng/mL, $P < 0.05$)[7]. A careful immunohistochemical analysis of these patients' surgical valve specimens revealed endothelial cells, myofibroblasts and macrophages-each with a cell specific expression profile at the left ventricular pre-annular septum. While the profile differed based on cell type, two of the three cells had increased expression of TGFβ, with all three having increased expression of TGFβ receptor 1. Thus, it would appear that aortic valve pannus originates from the healing process occurring at the junction of the neointima, which is mediated by TGFβ.

It should be noted that many investigators have identified mechanical valve obstructions with both elements of pannus and thrombus. It is likely that pannus serves as a nidus for thrombus, with pannus formation being the underlying cause[1]. The prevalence of these concomitant factors has been reported to be between 12%-75% of all PVO[1,3,4].

Epidemiology

The overall incidence of PVO ranges from 0.4%-6.0%

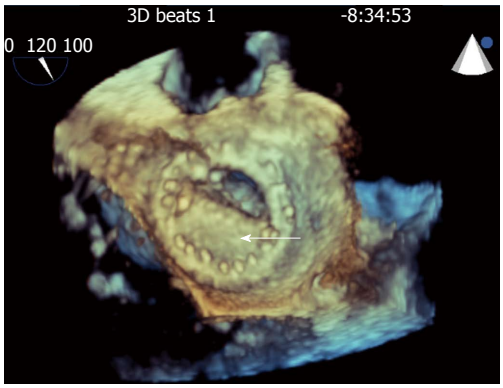

Figure 1 Still frames of 3-dimensional transesophogeal echocardiographic rendering of the mechanical bi-leaflet mitral valve as visualized from the left atrial perspective during diastole showing fixed mitral leaflet (arrow).

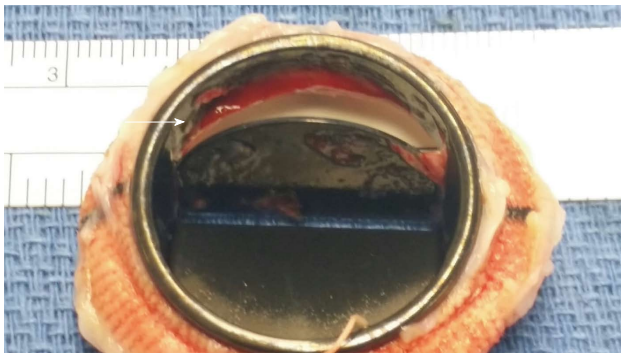

Figure 2 Gross sample of explanted mechanical mitral valve revealing the transesophogeal echocardiography finding residual organized thrombus, apparent on the mitral valve disc (arrow).

annually with the difference in rates depending on the type and location of prosthetic valve replacement[1,8-10]. This may be underestimated as routine post-op screening for PVO is not typically performed, unless patients become symptomatic. For instance, in observing 680 consecutive patients who underwent prosthetic valve surgery, Laplace *et al*[11] observed 64 patients (9.4%) with evidence of significant valve thrombosis starting as early as 9 d post-op, a significantly higher rate as compared to those who present with symptoms.

An observational study by Deviri *et al*[1] found thrombus associated obstruction in 78% of cases (both MV and AV), pannus formation in 10.7% cases, and combination of thrombus and pannus for the remaining 11.6% cases. Overall, the time from valve replacement to obstruction ranged from 6 wk to 13 years (median 4 years)[1]. Alternatively, a report by Vitale *et al*[4] found pannus in 31%, thrombus in 24%, and both pannus and thrombus in 45% of MV PVO. When comparing mitral to aortic valve complications, aortic valves appear to have a higher incidence of pannus, while mitral valves more commonly have PVO from thrombus[1,5,8,12].

The annual incidence of PVT ranges between 0.03%-5.7%[1,3,10,13,14]. PVT can occur in mechanical or bioprosthetic valves and can result in non-obstruction to complete obstruction[9-12]. PVT is more common in mechanical compared to bioprosthetic valves, with the immediate post-op period being the time of highest risk. Although PVT can occur any time after valve replacement, 24% occur within one year postoperatively with subsequent decreases in incidence with each year that follows[1,3]. Compared to pannus formation, thrombotic valvular dysfunction appears to occur at an earlier time with larger masses on imaging[4,5,8,15]. As mentioned above, larger valves, valves exposed to decreased flow (*i.e.*, mitral *vs* aortic, tricuspid *vs* left sided valves) and subtherapeutic anticoagulation status have been shown to be significant risk factors for PVT development[3,6,16].

Diagnosis

While a patient's clinical presentation may suggest a possible prosthetic valve complication, diagnosis of PVO, and differentiating its etiology, requires direct visualization of the valve by various imaging modalities.

Valvular obstruction should be considered when an unexpected rise in trans-valvular gradient is observed on Doppler echocardiography. Non-invasive visualization utilizing modalities such as TTE, TEE, and CF are necessary to accurately diagnose and guide treatment strategies. Since the etiology of obstruction may guide choice of therapy, the differentiation of thrombus from pannus is an essential but often challenging task. Initial diagnostic evaluation should commence with TTE in order to assess valve motion, degree of obstruction, and clot burden but also exclude non-acquired obstruction like patient prosthetic mismatch (PPM).

Echocardiography: TTE with color Doppler is regarded as the initial step for diagnosis of PVO and is required to determine hemodynamic severity and impact on valve function[5,12,17]. Sudden increases in transvalvular gradients from baseline are indicative of valvular obstruction. However, it is important to consider other causes of increased prosthetic valve gradients such as high cardiac output states, pressure recovery [in AV replacement (AVR)], regurgitation, and PPM. Furthermore, TTE may be limited by prosthetic reverberation artifacts. In this scenario, the use of spectral Doppler may detect a stuck valve due to aberration of opening and closing spikes. More importantly, image optimization despite these limitations, can be attempted through the use of 3-dimensional (3D) TEE allowing a more precise and realistic visualization[18]. Girard *et al*[12] found that TTE correctly identify the pathological mechanism of mechanical AVR obstruction in only 10% of cases but 63% of bioprosthetic AVR. While TTE is usually inadequate for valvular leaflet investigation and often not sensitive enough to identify thrombi as compared to pannus, it is an essential screening modality and may accurately identify obstructive masses in > 80% of cases[5].

MV Doppler echocardiographic evaluation should focus on measuring the mean transmitral gradient and pressure half time (PHT), in addition to the use of continuity equation to calculate valve area[19,20]. PHT

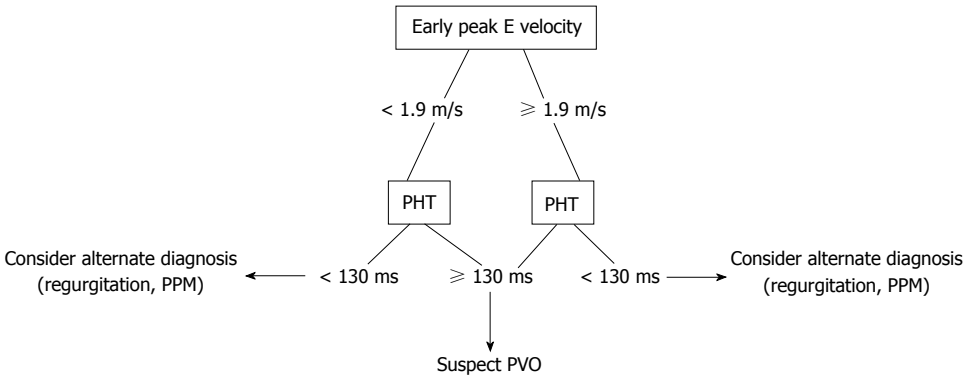

Figure 3 Proposed echocardiographic evaluation for suspected prosthetic mitral valve obstruction. PHT: Pressure half-time; PVO: Prosthetic valve obstruction; PPM: Prosthetic patient mismatch.

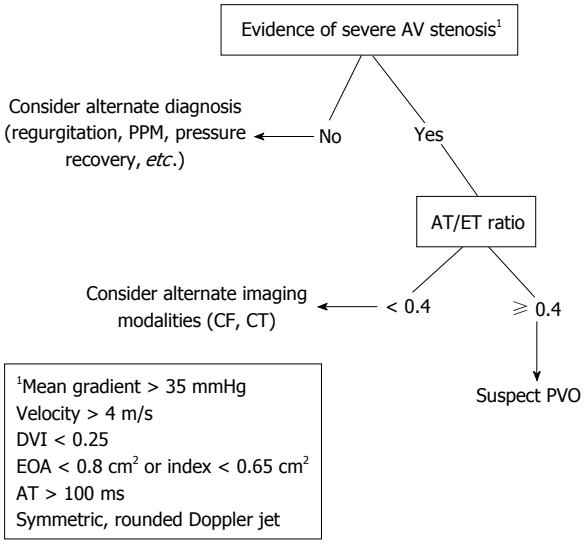

Figure 4 Proposed non-invasive evaluation for suspected prosthetic aortic valve obstruction. AT: Acceleration time; ET: Ejection time; PPM: Prosthetic-patient mismatch; CF: Cine fluoroscopy; CT: Computed tomography; PVO: Prosthetic valve obstruction; EOA: Effective orifice area; DVI: Doppler velocity index; AV: Aortic valve.

⩾ 130 ms has been shown to identify MV PVO in 99% of patients, however, its sensitivity is limited due to its relationship with atrial and ventricular compliance in addition to heart rate[2,21]. While peak early E velocity (PEV) ⩾ 1.9 m/s has also been shown to be a useful screening tool for PVO (OR = 3.51; 95%CI: 1.62-7.57; for every 10-unit increments of peak E velocity in cm/s), even with a peak E < 1.9 m/s, the presence of HT ⩾ 130 ms still correlates well with PVO. In addition, PHT ⩾ 130 ms is especially helpful in differentiating PVO from prosthetic valve dysfunction and regurgitation. Thus, while PEV may suggest PVO, PHT ⩾ 130 ms is necessary to indicate PVO, irrespective of PEV (Figure 3)[21].

Aortic valve PVO investigation by echocardiography should begin with a Doppler peak and mean transvalvular gradients. These acquired values should then be compared to known brand and size specific published values[2]. Severe prosthetic AV stenosis is suggested (assuming a normal stroke volume) with the presence of a rounded symmetric Doppler jet, a peak velocity ⩾ 4 m/s, mean gradient ⩾ 35 mm/Hg, Doppler velocity index < 0.25, effective orifice area < 0.8 cm^2 (or an indexed area to surface body area of < 0.65 cm^2), and an acceleration time ⩾ 100 ms. A ratio of acceleration time to ejection time of ⩾ 0.4 has been demonstrated as a reliable angle-independent variable that is consistent with PVO (Figure 4)[22]. In addition, careful assessment should be made for any abnormal echo densities or valve motions. Other non-valvular parameters should also be closely measured and compared to prior studies, including left ventricular size, function, and hypertrophy[2].

If there is a clinical suspicion for PVO but TTE Doppler is equivocal then stress TTE can be considered for further evaluation. While there is limited data regarding strict ranges and diagnostic cutoffs for PVO on stress TTE, a mean transmitral gradient rise of ⩾ 15 mmHg (or ⩾ 18 mmHg with AV prostheses) with stress has been suggested as a reliable marker to suggest PVO, even if the resting mean gradient is normal[2].

Use of TEE remains the gold standard for diagnosis of PVO and is required to determine the etiology of PVO as well as identifying candidates for thrombolytic therapy *vs* surgical intervention[12,23,24]. There is an additional benefit in using 3D echocardiography to more precisely visualize and evaluate the anatomy of both aortic and mitral prosthetic valves. As compared to standard 2-dimensional echo, 3D echocardiography allows a more detailed and accurate assessment of valve leaflets, prosthetic rings and struts. However, AV visualization still remains relatively difficult to image, as compared to the MV, given its distance from the transducer and its oblique angle of incidence as related to the ultrasound beam. Additionally, 3D TEE has been shown to have a high correlation with surgical findings, especially in regards to MV pathology[25-27].

Characteristics on TTE and TEE that differentiate pannus from thrombus include a larger size (2.8 cm *vs* 1.7 cm) and a soft mass-like appearance, as compared to pannus. A quantitative evaluation of mass characteristics can be done by comparison to myocardium

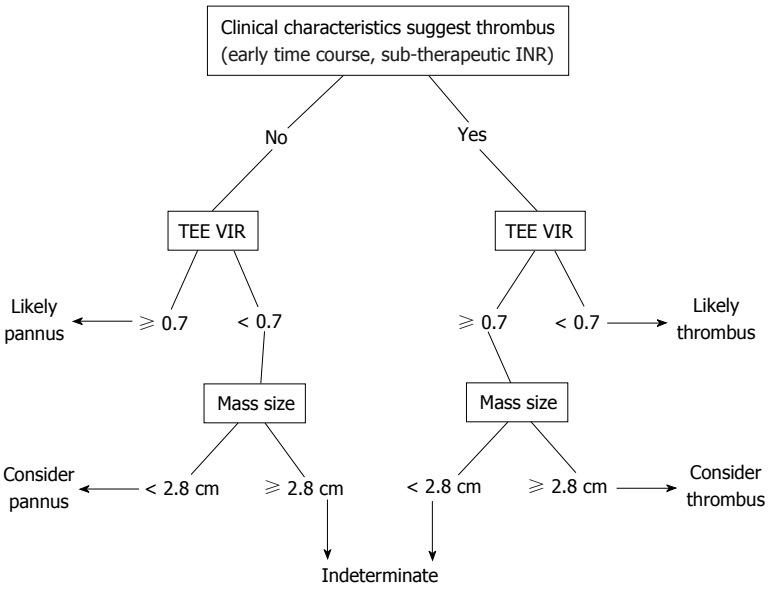

Figure 5 Proposed non-invasive evaluation for differentiating thrombus from pannus as underlying cause of prosthetic valve obstruction. TEE: Transesophogeal echocardiography; VIR: Video intensity ratio; INR: International normalized ratio.

using a video intensity ratio (VIR), with a VIR < 0.7 as being similar to myocardium (VIR; video intensity of the mass in relation to the prosthetic material). A VIR < 0.7 has a positive predictive value (PPV) 87% and negative predictive value (NPV) 89% and specificity of 80% with a sensitivity of 93%, slightly better than identification of soft mass alone (NPV 80%, PPV 86%, sensitivity 86%, specificity 80%). Interestingly, a clinical history of inadequate anticoagulation alone had a specificity of 92% and a sensitivity of 79% for thrombus. When it was combined with imaging findings thought to be thrombus-specific on TEE (either soft mass-like or VIR < 0.7), sensitivity and specificity remained the same at 93% and 80% respectively. Furthermore, in the mitral position, unlike pannus formation, thrombi on TEE characteristically extend into the left atrium and appendage (Figure 5)[5].

In addition to its diagnostic role, TEE, unlike TTE, has been shown to contribute to risk stratification for embolic phenomenon which in turn may assist to guide therapeutic decision making. A thrombus area of < 0.85 cm^2 on TEE has the lowest risk of systemic embolization[17]. As discussed below, fibrinolytic therapy has emerged as a therapeutic option in PVO.

Cine-fluoroscopy: While CF was historically the original imaging technique to evaluate for PVO, it has remained a useful tool to this day. CF allows for direct visualization of the radiopaque valve disks and allows comparison of the opening and closing angles of leaflets to normal or baseline angles, something that has limited usage with tissue valves[2,28]. Abnormal tilting of the ring base may suggest valve dehiscence, which can be confirmed with the injection of contrast dye. Valve obstruction is suggested with incomplete seating of the valves moving parts or impaired excursion[2].

TEE may appear to provide more robust information as compared to CF due to its ability to evaluate valve motion, structure, and hemodynamic parameters. However, CF is an essential complimentary examination to TEE, especially when TTE is insufficient at determining the difference between PVO and PPM[24]. In a comparison study between TTE and CF in the evaluation for PVT, sensitivity and specificity were 75% and 64% for TTE, 87% and 78% for CF[24]. Positive and negative predictive values for TTE and CF were 57%/78% and 80%/91% respectively. When used together, CF and TTE correctly diagnosed PVT in 85% of cases with TEE only required in 15% of cases.

Computed tomography scan: While computed tomography (CT) may appear to have a limited diagnostic role in the evaluation for PVO due to its incomplete evaluation of valve motion and hemodynamics, it may afford superiority over echocardiography when imaging pannus, especially in the atrial position[29]. At this time, there are no comprehensive comparative studies of echocardiography and CT in evaluating PVO. While CT may not be primarily indicated in the evaluation of PVO, it should be considered as an adjunct to TEE and CF, especially if the results are inconclusive[2].

Treatment

Treatment options for PVO include either a medical or surgical approach. In general, medical treatments are favored as an initial therapy, as the mortality of repeat valve surgery can be extremely high, depending on patient specific factors. However, pannus, due to its highly fibrotic makeup does not respond to medical therapy. When indicated, thrombolysis affords a non-invasive approach to clot dissolution and valve restoration. Thrombolysis should be considered based on the level of obstruction, ejection fraction (in aortic obstruction) and symptomatic burden (*i.e.*, NYHA class

Ⅲ-Ⅳ)[1,6]. Thrombolysis, when used appropriately, has shown complete resolution of valvular obstruction in 71%-82% of patients with 17% showing a partial hemodynamic resolution. If unsuccessful, a second dose of thrombolysis shows an additive effect and further hemodynamic benefit[30-32]. Thrombolysis may be more effective in aortic valves as compared to mitral valves, however, at this time the data is limited[32]. A multicenter registry has demonstrated complications from PVT thrombolysis treatment in 18% of patients, with death occurring at a rate 6%. Specifically, prior history of stroke and increased thrombus area (for every 1 cm^2 $\geqslant$ 0.8 cm^2, defined by TEE) are independent predictors of complications to thrombolysis for PVT[17].

It remains to be seen if medical therapies can be applied to treatment of pannus obstruction. While a monoclonal antibody targeting TGFβ may seem sensible, it would likely require intervention at the very early stages of pannus formation in order to be fully effective. However, early identification remains difficult and, to date, such an approach has yet to be attempted.

The 2006 ACC/AHA guidelines for management of valvular disease[23] suggest prioritizing surgery over medical therapy in select situations. With left sided valvular obstruction, surgery is considered a first line treatment for valve dysfunction if a patient has significant symptoms (NYHA Ⅲ-Ⅳ), or a large clot burden. The ACCP has suggested a cutoff thrombus area of $\geqslant$ 0.8 cm^2[10]. Patients at high surgical risk should first be considered for thrombolysis administration. Right sided valvular dysfunction should first be considered for thrombolysis, even if NYHA class is Ⅲ-Ⅳ. Importantly, after treatment, whether medical or surgical, ACC/AHA recommend a new higher chronic INR goal of 3.5 for aortic valve and 4 for mitral valve. Patients that undergo treatment for PVO (especially fibrinolysis, as discussed below) should undergo serial Doppler echocardiography to ensure there is no change in transvalvular gradients that may suggest rethrombosis[2]. The use of novel oral anti-coagulants has yet to be studied in this population and cannot be recommended at this time.

CONCLUSION

PVO, while rare, is a dreaded complication of mechanical valve replacement. Careful clinical and multiple non-invasive imaging modalities are necessary to definitively evaluate a patient with suspected PVO. As compared to pannus formation, PVT is more common, occurs earlier in the post-op period, is commonly related to inadequate anti-coagulation, and in many patients can be treated by thrombolysis. While the pathophysiology of pannus formation remains elusive, a better understanding of pannus may allow for the development of non-invasive therapeutic options.

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MINIREVIEWS

Space radiation and cardiovascular disease risk

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Abstract

Future long-distance space missions will be associated with significant exposures to ionizing radiation, and the health risks of these radiation exposures during manned missions need to be assessed. Recent Earth-based epidemiological studies in survivors of atomic bombs and after occupational and medical low dose radiation exposures have indicated that the cardiovascular system may be more sensitive to ionizing radiation than was previously thought. This has raised the concern of a cardiovascular disease risk from exposure to space radiation during long-distance space travel. Ground-based studies with animal and cell culture models play an important role in estimating health risks from space radiation exposure. Charged particle space radiation has dense ionization characteristics and may induce unique biological responses, appropriate simulation of the space radiation environment and careful consideration of the choice of the experimental model are critical. Recent studies have addressed cardiovascular effects of space radiation using such models and provided first results that aid in estimating cardiovascular disease risk, and several other studies are ongoing. Moreover, astronauts could potentially be administered pharmacological countermeasures against adverse effects of space radiation, and research is focused on the development of such compounds. Because the cardiovascular response to space radiation has not yet been clearly defined, the identification of potential pharmacological countermeasures against cardiovascular effects is still in its infancy.

Key words: Space radiation; Cardiovascular disease risk; Experimental models; Countermeasures; Ionizing radiation

Core tip: This review article provides an overview of studies in experimental models that have begun to shed light on the potential risks of damage in heart and blood vessels after exposure to space radiation.

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INTRODUCTION

Participants of future long-distance space missions will be exposed to significant doses of ionizing radiation in space, and the health risks of these exposures need to be assessed. Because the cardiovascular system has recently been shown to be more sensitive to ionizing radiation than was previously thought, there is current concern that exposure to radiation during long-distance space travel may be associated with a cardiovascular disease risk. This review article provides an overview of studies in experimental models of ionizing radiation exposure relevant to that found in space that have started to shed light on the potential risks for heart and blood vessels.

Characteristics of ionizing radiation

Exposure of living cells and tissues to ionizing radiation, forms of radiation that can remove electrons from the atoms in these cells or tissues, may result in molecular damage, which can eventually lead to early and late injury. Exposure of cells or tissues to ionizing radiation causes DNA damage, which has long been considered as the primary cause of cellular injury and cell death. However, additional mechanisms are now recognized as important in normal tissue radiation injury[1]. Doses of ionizing radiation are indicated in Gray (1 Gy equals 1 Joule of absorbed energy per kilogram of mass, *e.g.*, tissue). Because equal doses of different types of ionizing radiation may not have equal biological effects, one can express radiation exposure as equivalent dose in Sieverts (Sv), which is the absorbed dose multiplied by a unit-less radiation weighting factor and accounts for difference in the biological response[2].

Ionizing radiation can take many forms, including electromagnetic waves and high energy charged particles; the latter deposit their energy along densely ionizing cylindrical tracks. These forms of radiation can be distinguished, among other characteristics, by the amount of energy the radiation transfers to the target material per unit of track length, or linear energy transfer (LET)[2]. Ionizing radiation in the form of electromagnetic waves, such as X-rays or γ-radiation, are considered forms of low-LET radiation and deposit their energy uniformly in target volumes, while high energy charged particles release their energy along dense tracks of ionization and are considered high-LET radiation. Space travel is associated with low-dose-rate exposure to high-LET radiation if the form of galactic cosmic rays (GCR) and occasional high dose rate solar particle events (SPEs)[3].

IONIZING RADIATION AND THE CARDIOVASCULAR SYSTEM

Ionizing radiation has long been known to cause injury in heart and blood vessels. These effects first became apparent from follow-up of patients after radiation therapy, which delivers high doses of low-LET radiation locally to the tumor but in some cases also exposes normal (non-cancer) tissues such as the heart and blood vessels[4-8]. Several previously published review articles[9-11] have provided a comprehensive overview of the effects of low-LET radiation on the cardiovascular system. In short, manifestations of radiation-induced heart disease as a result of exposure to high doses of ionizing radiation include accelerated atherosclerosis, myocardial fibrosis, and cardiac conduction and valve abnormalities. Most deleterious effects in heart and blood vessels are observed years to decades after exposure to ionizing radiation. Therefore, long post-radiation follow-up is required for a full assessment of cardiovascular risk. Mechanisms by which ionizing radiation has its effects in the cardiovascular system are not yet fully known.

Recent reports of health assessments in atomic bomb survivors[12-15] have shown an increased incidence of cardiovascular disease, including ischemic heart disease and stroke, in people several decades after exposure to doses of γ-radiation as low as 2 Gy. Moreover, other epidemiological studies in occupational exposure and low-dose exposure due to medical treatments indicate that cardiovascular disease may occur after lower doses of ionizing radiation than was previously thought[16-20]. The main cardiovascular effects seen in atomic bomb survivors include hypertension and ischemic heart disease, suggesting that after low-dose radiation exposure a vascular component may play a central role in the cardiovascular disease risk.

These recent reports on health effects from exposure to low doses of low-LET radiation have raised the concern about potential risk of cardiovascular disease from exposure to ionizing radiation during space travel[21]. However, care should be taken when the results of terrestrial radiation exposures such as those from atomic bombs are used to support the potential for a cardiovascular disease risk from space radiation, since certain conditions such as dose rate are different

between atomic bomb events and radiation exposure in space. The remainder of this review is focused on studies in experimental models that have aimed to shed light on the cardiovascular risk of exposure to space radiation.

SPACE RADIATION

Characteristics of space radiation

While astronauts in the International Space Station are somewhat protected from exposure to space radiation due to the earth's magnetosphere, future long-distance space travel (beyond low-Earth orbit) will be accompanied by exposure to higher cumulative doses of space radiation, and short-term and long-term health risks need to be assessed[22,23].

GCR and solar emissions are dominated by protons and iron, silicon, oxygen, and carbon that are highly energetic. The greatest particle abundance is found for particles with energies ranging from hundreds of MeV per nucleon (MeV/n) up to about 1 GeV/n[24]. Practical levels of current shielding materials cannot easily protect against these particles[25]. Chronic exposure occurs at a dose rate of 1.3 mGy/d, or the dose equivalent of 4.8 mSv/d, when assuming the radiation weighting factors of the International Commission on Radiological Protection Publication 60 outside the earth's magnetosphere[26,27]. The exposure is characterized by the traversal of most cells in the body by one or more protons and electrons per day, with infrequent traversals (days to weeks) by ions of higher atomic number (Z).

SPEs consist predominantly of protons, and exposure to the largest SPEs occurs at dose rates up to 0.5 Sv/h over hours to a few days[28]. Energies of SPE protons are less than those for GCR and therefore have shorter ranges in material, which may enable effective shielding inside a spacecraft but not inside a thin spacesuit. These higher dose rate exposures may put an astronaut at risk for acute radiation effects, sometimes collectively called acute radiation sickness[29]. Both SPEs and GCR may also cause long-term degenerative disease in various tissues, including the heart and blood vessels.

Experimental data obtained from animal and cell culture models play an important role in estimating health risks from exposure to space radiation. Appropriate simulation of the space radiation environment, including the long-term low-dose rate exposures to various charged particles and the appropriate energy of these particles, and the choice of the most relevant animal or cell culture model are challenging but key to providing relevant estimates of health risks[30-32]. The concern of adverse cardiovascular effects of exposure to space radiation is relatively new, and studies on the cardiovascular effects in animal models of space radiation exposure are not yet abundant. An overview of existing studies on heart and blood vessels is given below. Since much of this work is ongoing, we have had to occasionally refer to proceeding abstracts, but hope to find the results in peer-reviewed publications in the near future.

Cardiac response in animal models of charged particle exposure

Studies in animal models of charged particle exposure have shown cardiovascular effects at doses lower than those required to cause cardiovascular changes if low-LET radiation is used. This may not be surprising, since high-LET radiation typically causes more damage per unit of absorbed dose. Among studies with charged particles, some previous research has focused on the cardiac response to fission spectrum neutrons in animal models[33-36]. More recently, studies were designed to provide answers about the cardiovascular risk from exposure to high-LET radiation in space. Exposure of male C75Bl/6NT mice at 8-10 mo of age to protons (1 GeV, 0.5 Gy) or iron ions (1 GeV/n, 0.15 Gy) induced cardiac infiltration of CD68-positive cells (monocytes and macrophages), increased DNA oxidation, myocardial fibrosis, and modified cardiac function, both at baseline and in response to myocardial infarction, in a radiation-type specific manner[37-39]. Exposure of male CBA/CaJ mice at 10-12 wk of age to silicone ions (300 MeV/n) at doses between 0.1 and 0.5 Gy caused prolonged apoptosis and increased expression of the common pro-inflammatory cytokines interleukin (IL)-1β, IL-6, or tumor necrosis factor-α in the heart[40]. Low doses of high-LET radiation have been shown to cause long-term alterations in DNA methylation in various organ systems *in vivo* and cells in culture[41-43]. Similarly, we recently found changes in cardiac DNA methylation in male C57BL/6J mice exposed at 10 wk of age to protons (150 MeV, 0.1 Gy) or iron ions (600 MeV/n, 0.5 Gy) (Figure 1), suggesting that epigenetic alterations may contribute to the cardiac radiation response[44]. Analysis of the response in individual cardiac cell types is also ongoing[45].

Vascular response in animal models of charged particle exposure

Whole-body exposure of rats to iron ions at doses of 0.5 and 1 Gy induced long-term indications of endothelial dysfunction and increased aortic stiffness[46]. It is difficult to assess the effects of ionizing radiation on atherosclerosis when using regular rodent models, due to the low prevalence of atherosclerosis in these animals. Targeted exposure of the atherosclerotic-prone apolipoprotein E-deficient ($Apo^{-/-}$) mouse model to iron ions (600 MeV/n) at doses of 2 and 5 Gy caused accelerated atherosclerosis in the exposed parts of the aorta[47]. Additional studies with lower doses of particle irradiation may provide a more comprehensive estimate of cardiovascular risk in this mouse model. Studies on adhesiveness of endothelium in charged particle-exposed animal models are also underway[48].

The microvasculature also plays an important role in normal organ function, degenerative tissue effects, and tissue injury from ionizing radiation[49,50]. Exposure of 10-wk old male C57BL/6 mice to iron ions (600 MeV/n) at doses between 0.5 and 2 Gy caused a long-term loss of endothelial cells in the hippocampus[51].

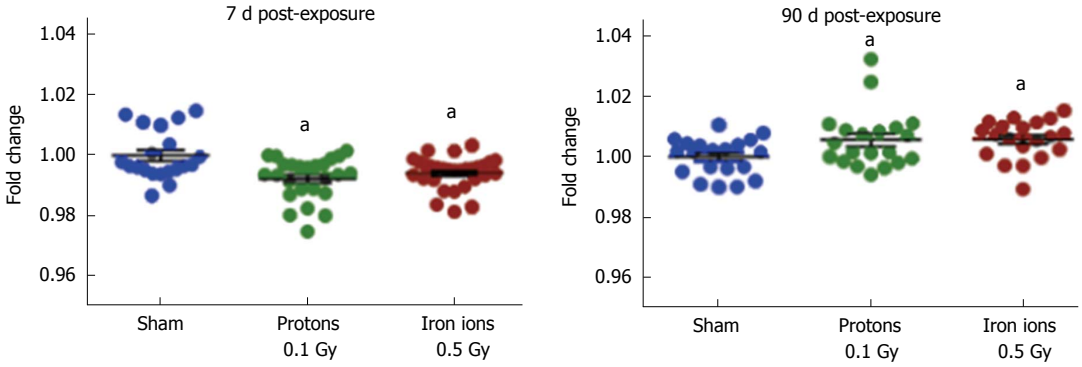

Figure 1 Methylation of genomic DNA isolated from hearts of male C57Bl/6 mice at 7 d and 90 d after exposure to protons (150 MeV, 0.1 Gy) or iron ions (600 MeV/n, 0.5 Gy). DNA methylation of the open reading frame 1 of long interspersed nuclear element-1, a transposable element that comprises about 20% of the mouse genome, as assessed by pyrosequencing and indicated as fold change compared to sham-irradiated controls. Each group contained 4-5 animals. Horizontal lines indicate average ± standard error of the mean. [a]$P < 0.05$ *vs* the sham-irradiated control group.

More research is required to assess the effects of space radiation on the microvasculature.

Studies on charged particle exposure in cells culture models

Endothelial cells are considered to play a central role in the cardiovascular response to ionizing radiation. Endothelial dysfunction, which is characterized by a pro-inflammatory and profibrogenic phenotype of endothelial cells, is a critical contributor to the patho-physiological manifestations of radiation injury[52-54]. Experimental models of exposure to low-LET radiation have shown that ionizing radiation can cause prolonged endothelial dysfunction, thereby sustaining a detrimental tissue environment that leads to chronic inflammation and adverse remodeling[55,56].

Because of the central role of endothelial cells in the radiation response, studies are addressing the effects of space radiation on endothelial cells in cultures[57]. Various tissue-relevant cell culture models are being used[58]. For instance, in three-dimensional culture models of human endothelial cells, protons (1 GeV) and iron ions (1 GeV/n) at doses up to 3 Gy caused alterations in vasculogenesis and endothelial cell death in a radiation-type specific manner[59,60]. These results raise the concern of damage of the human vasculature from exposure to charged particles *in vivo*.

Potential countermeasures against the cardiovascular response to space radiation

Astronauts could potentially be administered pharmacological countermeasures against adverse effects of space radiation, when the countermeasure is safe, stable during long-term space flight, and has a relatively light weight. Therefore, research is focused on the development of countermeasures against various biological effects of space radiation[29]. Interestingly, pharmacological countermeasures are being developed for low-LET radiation in exposure scenarios on earth and may point to potential countermeasures against adverse effects of space radiation. Neupogen [filgrastim, recombinant human granulocyte colony stimulating factor (G-CSF)], for instance, was recently approved by the American Food and Drug Administration as a countermeasure against acute injury from accidental radiation exposure. G-CSF has also been shown to protect animal models against acute injury from exposure to SPE-like protons[61].

Because the cardiovascular response to space radiation has not yet been clearly defined, the identification of potential pharmacological countermeasures against cardiovascular effects is still in its infancy. Nonetheless, similar to the acute response scenario, potential countermeasures against cardiovascular effects of terrestrial radiation exposure, albeit not yet approved for clinical use, may be pursued in space radiation models. For example, the angiotensin converting enzyme (ACE) inhibitor captopril has been shown to reduce cardiac injury in animal models of localized irradiation of the heart[62,63]. In addition, the vitamin E analog γ-tocotrienol is one of the most potent dietary countermeasures to radiation injury currently known. It is safe and nontoxic and has no known drug interactions. It is commercially available, requires no specific storage conditions, and is currently in advanced stages of development for terrestrial applications in radiation protection[64,65]. In addition, γ-tocotrienol has several beneficial effects in the cardiovascular system. It is a potent inhibitor of the cholesterol biosynthesis pathway, thereby reducing the isoprenylation of Rho proteins that modify a wide range of cellular functions, including stress fiber formation, hypertrophy, regulation of NOS, and production of cytokines and growth factors[66]. Indeed, γ-tocotrienol reduces vascular oxidative stress and protects against vascular radiation injury at least in part *via* HMG-CoA reductase inhibition[67,68]. The protective properties of agents such as ACE inhibitors or γ-tocotrienol against cardiovascular effects of space radiation need to be assessed.

CONCLUSION

The cardiovascular system may be more sensitive

to ionizing radiation than was previously thought, which raises the concern of a cardiovascular risk from exposure to ionizing radiation during long-distance space missions. Animal and cell culture models have started to shed light on risk of cardiovascular complications from exposure to charged particle irradiation. Additional studies, including those that employ low radiation doses/dose rates and mixed particle fields to mimic GCR are required to aid in assessing the cardiovascular risk of space radiation.

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MINIREVIEWS

Thrombus aspiration during primary percutaneous coronary intervention for acute myocardial infarction: A review of clinical evidence and guidelines

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Abstract

Acute ST segment elevation myocardial infarction (STEMI) is characterized by complete thrombotic occlusion of a major coronary artery. Early recanalization of the infarct-related artery is most efficiently delivered by primary percutaneous coronary intervention (PPCI), however this does not always restore normal myocardial perfusion, mainly due to distal embolization of the thrombus and microvascular obstruction. Early evidence for manual thrombus aspiration during PPCI was promising and this was once considered an important aspect of the procedure, especially in patients with a high thrombus burden. However, a large body of evidence from recent major randomized controlled trials (notably TASTE and TOTAL) does not support the routine use of manual thrombus aspiration in patients with STEMI undergoing PPCI.

Key words: Primary percutaneous coronary intervention; Clinical evidence; Stroke; Acute myocardial infarction; Thrombus aspiration

Core tip: The role of manual thrombus aspiration during primary percutaneous coronary intervention (PPCI) for acute ST segment elevation myocardial infarction (STEMI) has been a matter of significant research and intense debate recently. The rationale for manual thrombus aspiration during PPCI is the removal of intracoronary thrombus, thus avoiding the complication of downstream embolization leading to impaired myocardial perfusion. In this review article, we present the data from early clinical trials and meta-analyses of thrombus aspiration during PPCI, and the more recent evidence from larger multi-center randomized controlled trials that have had a major influence on clinical practice. We highlight the relevant major society guidelines for thrombus aspiration during PPCI and provide the reader with an overview of this technology and its role in contemporary management of STEMI.

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INTRODUCTION

The common pathophysiological mechanism of acute coronary syndrome is sudden disruption of a coronary arterial plaque due to rupture, fissuring or superficial erosion leading to obstructive intracoronary thrombosis. Other less frequent mechanisms include acute plaque expansion, embolism, spontaneous dissection or coronary inflammation[1]. Acute ST segment elevation myocardial infarction (STEMI) is characterized by complete thrombotic occlusion of a coronary artery, with a great potential to cause a large myocardial infarction if not treated promptly. The primary therapeutic goal in STEMI, therefore, is early restoration of normal coronary blood flow, most efficiently delivered by primary percutaneous coronary intervention (PPCI) in combination with adjunctive pharmacological treatment. PPCI aims to achieve myocardial salvage, electrical stability and preserve left ventricular function, improving both early and late outcomes after STEMI.

However, restoration of epicardial coronary artery patency does not always equate with normal myocardial reperfusion. The hallmarks of reperfusion failure despite achieving arterial patency are microvascular obstruction and the no-reflow phenomenon. A high burden of intracoronary thrombus and subsequent distal embolization during PCI are possible major contributors to these events. Myocardial reperfusion failure clinically manifests as persistent ST segment elevation, poor myocardial blush grade (MBG) and low thrombolysis in myocardial infarction (TIMI) flow grade[2].

Earlier investigations revealed that angiographically distal embolization occurred in around 15% of patients undergoing PPCI[3]. Distal embolization was associated with impaired myocardial reperfusion, larger infarct size and an unfavorable prognosis. Further evidence of distal embolization and its impact on myocardial reperfusion is provided by intravascular ultrasound analysis (IVUS). In a study of 35 patients undergoing PCI for myocardial infarction, Kotani *et al*[4] applied volumetric IVUS analysis before and after PPCI to assess the plaque reduction as evidence of distal embolization. Plaque reduction following PPCI was associated with impaired myocardial reperfusion. "The enhanced myocardial efficacy and recovery by aspiration of liberated debris (EMERALD)" trial investigators, while investigating a distal balloon occlusion and aspiration system, demonstrated that visible debris was retrieved in 73% of the patients undergoing PPCI[5]. Avoidance of distal embolization is hence a considerable therapeutic challenge during STEMI.

PHARMACOLOGICAL AND MECHANICAL MEANS OF REDUCING THROMBUS

Pharmacological agents (especially glycoprotein Ⅱb/Ⅲa inhibitors), mechanical thrombectomy devices, embolic protection devices and manual aspiration thrombectomy catheters have been investigated over the past couple of decades as adjunctive therapies during PPCI with the aim of reducing thrombus burden and subsequent distal embolization. Glycoprotein Ⅱb/Ⅲa inhibitors inhibit the final common pathway of platelet activation and are a useful adjunct to PPCI, albeit with an increased risk of bleeding. While theoretically attractive, the clinical value of mechanical thrombectomy and embolic protection devices during PPCI is unproven, after several negative trials. Manual thrombus aspiration (thrombectomy) during PPCI is the focus of this review article.

A major technical advantage of a manual thrombus aspiration device is its simplicity, consisting of a monorail catheter containing a central lumen that connects one or more large holes at the distal end to an aspiration syringe at the proximal end. The commonly used aspiration devices in clinical practice are Export® (Medtronic, MN, United States), Eliminate™ (Terumo), Pronto™ (Vascular solutions, MN, United States) Diver™ CE (Invatec, Italy), QuickCat (Spectranetics Inc, United States) and Hunter® (IHT Cordynamic, Barcelona, Spain). All these devices are formed on the same principle and convincing clinical advantage of one particular device over the other is lacking.

CLINICAL EVIDENCE

Randomized controlled trials

A number of studies, including randomized clinical trials and subsequent meta-analyses have evaluated the clinical efficacy of routine manual thrombus aspiration during PPCI. In the initial "randomized evaluation of the effect of mechanical reduction of distal embolization by thrombus-aspiration in primary and rescue angioplasty (REMEDIA)" trial, 100 patients with STEMI were randomized to PPCI with or without manual thrombus aspiration (Diver™ CE). More patients in the manual thrombus aspiration group achieved MBG 2 or more and ST segment resolution (STR) of 70% or more (46% *vs* 25%)[6]. In "Thrombectomy with Export Catheter in Infarct-Related Artery During Primary Percutaneous Coronary Intervention" (EXPIRA) trial, 175 patients with STEMI were randomized to PCI with or without manual thrombus aspiration. The primary end points of MBG 2 or more (88% *vs* 60%) and STR of 70% or more (64% *vs* 39%) occurred more often in PCI with thrombus aspiration group compared with standard PCI. Patients in the aspiration group had less microvascular obstruction and smaller infarcts[7]. After 24 mo, major adverse cardiac events were 4.5% *vs* 13.7% and cardiac death was 0% *vs* 6.8%, respectively, in patients with PCI with manual thrombus aspiration compared with standard

PCI[8].

INFUSE-AMI was a multicenter, single-blind trial of 452 patients presenting within 4 h of anterior STEMI undergoing PPCI with bivalirudin who were randomized in a 2 × 2 factorial design to bolus intracoronary abciximab *vs* no abciximab and to manual thrombus aspiration (using the Export® catheter) *vs* no thrombectomy. To maximize the likelihood of demonstrating a reduction in infarct size, enrollment was limited to the patients with proximal or mid LAD occlusion and baseline TIMI 0-2 flow. There was no significant difference in the infarct size at 30 d, the primary end point, in the thrombus aspiration *vs* no aspiration arm (17% *vs* 17.3% respectively, $P = 0.51$) as assessed by cardiac magnetic resonance imaging[9].

Thrombus aspiration during primary percutaneous coronary intervention trial

The first large randomized controlled trial (RCT) evaluating use of manual thrombus aspiration (Export® catheter) during PPCI was "thrombus aspiration during primary percutaneous coronary intervention (TAPAS)". In this single-center all-comers RCT, 1071 patients with STEMI were randomized, to either thrombus aspiration during PCI or standard PCI alone, prior to coronary angiography. The primary end-point was the post-procedural frequency of a MBG of 0 or 1. All patients received standard pharmacological therapy including the glycoprotein Ⅱb/Ⅲa inhibitor abciximab, unless contraindicated. Ninety-two percent patients underwent stent implantation in both groups. A MBG of 0 or 1 occurred less frequently in the thrombus aspiration group compared with the conventional PCI group (17% *vs* 26%, $P < 0.001$). Complete ST-segment resolution was more frequent in the manual thrombus aspiration group (56% *vs* 44%, $P < 0.001$). Atherothrombotic material was retrieved in 73% of the patients in thrombus aspiration group. Clinical outcomes at 30 d, including the rate of death and major adverse cardiac events, were significantly related to the MBG and ST-segment resolution. Rates of target vessel revascularization were similar between the two groups[10]. A 1-year follow-up study showed reduced rates of cardiac death (3.6% *vs* 6.7%) and cardiac death or non-fatal reinfarction (5.6% *vs* 9.9%) in the thrombus aspiration group[11]. The benefit of manual thrombus aspiration was irrespective of vessel size, infarct-related coronary artery or visible thrombus on the angiogram. A total ischemic time of less than 180 min was associated with a trend towards increased benefit ($P = 0.09$). Angiographically proven acute stent thrombosis (< 24 h) occurred with a similar frequency between both groups (0.2%) but subacute (1-30 d) and late stent thrombosis (> 30-365 d) was observed less frequently in the thrombus aspiration cohort (RR = 0.5, 95%CI: 0.19-1.32). The findings of TAPAS form the basis for major society guidelines recommending manual thrombus aspiration as an adjunct for PPCI. The trial, however, was criticized for being underpowered for clinical events and susceptibility to selection bias (single center study).

Thrombus aspiration in STEMI in Scandinavia and the Trial of Routine Aspiration Thrombectomy with PCI vs PCI alone in patients with STEMI trials

TASTE trial: The above inconsistent results were followed by the two major randomized controlled trials in the field, thrombus aspiration in STEMI in Scandinavia (TASTE) and the Trial of Routine Aspiration Thrombectomy with PCI *vs* PCI alone in patients with STEMI (TOTAL). TASTE was a multi-center (29 PCI centers in Sweden, 1 each in Iceland and Denmark), randomized study that utilized the platform of population-based "Swedish coronary angiography and angioplasty registry". A total of 7244 STEMI patients were randomized to PCI with manual thrombus aspiration or standard PCI alone[12]. The primary end point of all-cause mortality at 30 d was not different between the two groups (2.8% for thrombus aspiration with PCI *vs* 3% for PCI alone, $P = 0.63$). The majority of patients in TASTE had a low thrombus burden (thrombus grade 0-3). Bailout thrombus aspiration was performed in 4.9% patients assigned to PCI alone. The 30-d rates of secondary end-points (hospitalization for recurrent myocardial infarction, target-vessel revascularization, target-lesion revascularization, stent thrombosis and the composite of all-cause mortality or recurrent myocardial infarction) were not statistically different. The rate of stroke or neurological complication was identical (0.5%) in each group. The incidence of stent thrombosis, although statistically not significant, was lower (0.2% *vs* 0.5%, $P = 0.06$, HR = 0.47, 95%CI: 0.20-1.02) in the thrombus aspiration group. Similarly, hospital length of stay, incidence of heart failure or left ventricular dysfunction were all unaffected by manual thrombus aspiration. The failure to influence the primary end-point was consistent across all subgroups, including patients with diabetes, previous myocardial infarction, smokers and various measures of ischemic time. Outcomes in TASTE were similar irrespective of the infarct-related coronary artery, intra-arterial culprit segment (proximal *vs* non-proximal), TIMI flow grade before PPCI, use of glycoprotein Ⅱb/Ⅲa drugs and importantly thrombus burden. All-cause mortality at 1 year was a pre-specified secondary end-point of the study, which later reported no benefit of thrombus aspiration across all the major subgroups[13]. There were concerns that TASTE was underpowered to detect a difference in its primary end-point and also for its registry-based design (it was the first major trial ever to use this concept) with no separate, dedicated data monitoring and adjudicating set-up.

TOTAL trial: The most recent and so far the largest trial evaluating the benefit of manual thrombus aspiration in PPCI is TOTAL. This multi-center, prospective, randomized controlled trial assigned 10732 patients with acute STEMI to routine upfront manual aspiration thrombectomy *vs* PCI alone[14]. Almost 80% patients

had a high thrombus burden as assessed by TIMI thrombus grade 4 or 5. The primary outcome (composite of death from cardiovascular causes, recurrent myocardial infarction, cardiogenic shock, or New York Heart Association class Ⅳ heart failure within 180 d) was similar between the two groups (6.9% in the thrombus aspiration group *vs* 7% in the PCI alone group). The key safety outcome of stroke within 30 d occurred more frequently in the thrombectomy group compared to PCI alone group (0.7% *vs* 0.3%, *P* = 0.02). Within 180 d, stroke had occurred in 1% of patients with thrombectomy *vs* 0.5% in those without. The incidence of definite stent thrombosis within 180 d was similar between both groups (1.3% for thrombectomy *vs* 1.4%, *P* = 0.72). Bailout manual thrombus aspiration was performed in 7.1% patients originally assigned to PCI alone. As noted in TASTE, the negative primary trial outcome was consistent across all pre-specified subgroups, including those with high thrombus burden, initial TIMI flow, time of symptom onset and anterior *vs* non-anterior myocardial infarction. The strength of the trial was the study design and the large study population. Concerns were raised towards potential selection bias of a lower-risk population (in view of lower than expected event rates for the primary outcome) and bailout thrombus aspiration in PCI alone group[15] The finding of increased incidence of stroke in the thrombectomy group is potentially significant, however, the absolute number of stroke events was small. The trial was also underpowered to detect a difference in stroke. It is possible that the higher risk of stroke in the thrombus aspiration group was not directly related to the thrombectomy procedure, supported by the observation that the increased stroke risk was not confined to the periprocedural period.

Observational studies: In a single-center retrospective analysis of 2567 consecutive STEMI patients treated with PPCI, aspiration thrombectomy (*n* = 1095, using Export catheter in 93%) was associated with improved post-procedure TIMI 3 flow as well as reduced in-hospital (adjusted OR = 0.51, 95%CI: 0.29-0.93, *P* = 0.027) and long-term (adjusted HR = 0.69, 95%CI: 0.48-0.96, *P* = 0.028) mortality rates (4.5% *vs* 9.0%), over a mean follow-up of 9.9 mo. The study identified that the mortality benefit of thrombus aspiration was driven by results in patients with a total ischemic time of less than 180 min[16]. However, critics of the study called the extent of mortality reduction excessive and implausible[17].

In a retrospective observational cohort study of 10929 STEMI patients treated with PPCI at 8 centers across London, United Kingdom, manual aspiration thrombectomy (32.7%, *n* = 3572) was associated with a higher procedural success rate (90.9% *vs* 89.2%; *P* = 0.005) and lower in-hospital major adverse cardiac event rates (4.4% *vs* 5.5%; *P* = 0.012). However, no significant differences in the primary outcome of all-cause mortality were evident between patients with or without manual thrombus aspiration (14.8% *vs* 15.3% respectively; *P* = 0.737) during the median follow-up of 3 years[18].

Meta-analyses: A pooled analysis of 2686 patients enrolled in 11 thrombectomy trials (7 trials using manual aspiration devices such as TAPAS and EXPIRA and 4 non-manual devices trials) similarly concluded that thrombectomy (especially manual aspiration thrombectomy) significantly improves clinical outcomes, including lower all-cause mortality, in STEMI patients undergoing PPCI[19]. However, the suggestion of improved clinical outcome with thrombectomy was questioned by a meta-analysis of 21 trials (including 16 with manual thrombus aspiration devices) involving 4299 PPCI treated STEMI patients which concluded that adjunctive thrombectomy, despite improving the early markers of myocardial reperfusion, does not significantly affect 30-d mortality, reinfarction or stroke[20]. A meta-analysis of 21 trials involving 4514 patients (50% randomized to thrombectomy, either manual or mechanical) concluded that while both types of thrombectomy did improve myocardial perfusion, a trend towards short-term mortality benefit was evident only with manual aspiration. The meta-analysis also observed a trend towards higher risk of stroke with thrombectomy (*P* = 0.06)[21]. Another meta-analysis of PPCI-treated STEMI patients included data from 25 trials, including 18 trials with manual aspiration thrombectomy; this study suggested that use of manual thrombus aspiration, but not mechanical thrombectomy, was associated with reduced major adverse cardiovascular events, including mortality, at 6 to 12 mo. A trend towards a higher risk of stroke was noted with mechanical thrombectomy[22].

Unlike the previous meta-analyses, two recent meta-analyses have included data from the large TASTE trial however both were performed before the publication of the largest and most reliable trial investigating the use of manual thrombus aspiration in PPCI (TOTAL). A recent meta-analysis of 26 PPCI randomized trials in 11943 patients (thrombus aspiration *n* = 5969, PCI alone *n* = 5974) and a weighted maximum follow-up duration of 10.4 mo concluded that the routine unselected use of adjunctive thrombus aspiration during PPCI does not significantly reduce all-cause mortality (polled RR = 0.88; 95%CI: 0.74-1.04; *P* = 0.124), reinfarction, target-vessel revascularization or definite stent thrombosis. Although thrombus aspiration was noted to be associated with reductions in failure to achieve TIMI 3 flow, MBG 3, incomplete ST-segment resolution and distal emobolization, these effects were less obvious among the larger, higher quality recent trials. The risk of stroke was noted to be similar between both groups[23]. In another recent meta-analysis of 16 randomized trials in PPCI including 10518 patients (thrombus aspiration *n* = 5256, PCI alone *n* = 5262), routine use of manual thrombus aspiration compared to PCI alone did not reduce the rate of all-cause mortality (6.6% *vs* 7.4% respectively, *P* = 0.149), reinfarction, target vessel

revascularization/target lesion revascularization and stent thrombosis. The rate of stroke was similar between the two groups (0.5% *vs* 0.5%, P = 0.819). Thrombus aspiration was associated with improved rates of post-procedural TIMI 3 flow, MBG 2-3 and ST-segment resolution[24].

GUIDELINES

The 2014 ESC/EACTS guidelines on myocardial revascularization suggest that while routine use of manual thrombus aspiration is not essential in patients undergoing PPCI for STEMI, selected use may be useful to improve TIMI 3 flow or prevent stent thrombosis. Thrombus aspiration in selected patients during PPCI has a class Ⅱb indication (level of evidence A). These guidelines take into account the evidence including the TASTE trial but predate the publication of TOTAL trial, so far the largest trial addressing this question[25].

The 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction consider manual thrombus aspiration reasonable in patients undergoing PPCI. Thrombus aspiration in a PPCI setting is a class Ⅱa indication in these guidelines (level of evidence B). These guidelines predate the publication of TASTE and TOTAL trials[26].

CONCLUSION

The success of PPCI for STEMI is marred by suboptimal myocardial reperfusion, despite achieving epicardial coronary patency, mainly secondary to distal embolization of the thrombus and microvascular obstruction. Early evidence for manual thrombus aspiration during STEMI was promising and this was once considered an important aspect of PPCI, especially in patients with a high thrombus burden. However, recent clinical evidence from major randomized controlled trials (notably TASTE and TOTAL) does not support the routine use of manual thrombus aspiration in patients with STEMI undergoing PPCI.

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MINIREVIEWS

Inequalities in care in patients with acute myocardial infarction

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Abstract

Coronary heart disease is the single largest cause of death in developed countries. Guidelines exist for the management of acute myocardial infarction (AMI), yet despite these, significant inequalities exist in the care of these patients. The elderly, deprived socioeconomic groups, females and non-caucasians are the patient populations where practice tends to deviate more frequently from the evidence base. Elderly patients often had higher mortality rates after having an AMI compared to younger patients. They also tended to present with symptoms that were not entirely consistent with an AMI, thus partially contributing to the inequalities in care that is seen between younger and older patients. Furthermore the lack of guidelines in the elderly age group presenting with AMI can often make decision making challenging and may account for the discrepancies in care that are prevalent between younger and older patients. Other patients such as those from a lower socioeconomic group, *i.e.*, low income and less than high school education often had poorer health and reduced life expectancy compared to patients from a higher socioeconomic group after an AMI. Lower socioeconomic status was also seen to be contributing to racial and geographical variation is the care in AMI patients. Females with an AMI were treated less aggressively and had poorer outcomes when compared to males. However even when females were treated in the same way they continued to have higher in hospital mortality which suggests that gender may well account for differences in outcomes. The purpose of this review is to identify the inequalities in care for patients who present with an AMI and explore potential reasons for why these occur. Greater attention to the management and a better understanding of the root causes of these inequalities in care may help to reduce morbidity and mortality rates associated with AMI.

Key words: Coronary artery disease; Dual antiplatelet

therapy; Inequalities; Guidelines; Myocardial infarction

Core tip: Coronary heart disease is the leading cause of death in developed countries. Guidelines exist for the management of acute myocardial infarction (AMI), yet despite these, significant inequalities exist in patient care. The elderly, deprived socioeconomic groups, females and non-Caucasians are the patient populations where practice tends to deviate from the evidence base. The purpose of this review article is to identify the inequalities in those who present with an AMI and explore potential reasons for this. Greater attention to the management and a better understanding of the root causes of these inequalities may help to reduce morbidity and mortality rates.

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INTRODUCTION

Coronary heart disease is the leading cause of death in the world and accounted for 7.4 million deaths in 2012[1]. In Europe the mortality per annum from coronary heart disease is 20%[2] and 25% in the United States[3]. Guidelines exist to aid with the management of patients who present with acute myocardial infarction (AMI)[4-7] yet inequalities in the management and outcomes of these patients are clearly apparent.

The World Health Organisation defines inequality in health care as avoidable inequalities in health between groups of people within countries and between countries[8]. Factors that drive care inequality can be defined as biological for instance age and gender, socioeconomic, race/ethnicity or geographical. In this review article, we describe the inequalities in care for patients presenting with AMI and explore the potential reasons for inequity in AMI care.

Age differences

Life expectancy is increasing in the United Kingdom and much of the developed world. From 2003 to 2010 life expectancy increased from 76.5 to 78.1 years for males and 80.9 to 82.1 years for females. AMI accounted for the majority of deaths in the United Kingdom in 2012 especially in those individuals over the age of 85 years[9]. Age is a risk factor for AMI and poorer prognosis thereafter, and with the rising life expectancy this will lead to a greater number of patients presenting with AMI with potentially greater morbidity and mortality[9-11].

Data from the Myocardial Ischemia National Audit Project (MINAP), a multicentre clinical registry for patients who have been hospitalised with a myocardial infarction in England and Wales demonstrated that in-hospital mortality in 2010 following a myocardial infarction was 20.4% in those $\geqslant$ 85 years compared to 0.9% in those < 55 years old[10,11]. In part, greater frailty and co-morbidity in older patients explain this variation in outcome. However, the provision of evidenced based care to older patients was significantly lower than that provided to younger patients following an AMI. For instance, MINAP data showed that up to 75% of patients $\geqslant$ 85 years presenting with a ST segment myocardial infarction (STEMI) were less likely to receive thrombolysis or primary percutaneous coronary intervention (PPCI) compared to those < 55 years[11,12]. Furthermore, although evidence suggests benefit in the use of anti-platelets and statins in the elderly following an AMI[13], the use of such therapies are not equivocal across age groups. For example, in-hospital use of aspirin for those < 65 years was 95% *vs* 87% in those $\geqslant$ 85 years of age[14,15]. Thus, strategies targeting improved adherence to evidenced based treatments in the elderly may narrow the inequality in outcomes.

Nevertheless, outcomes after an AMI have improved over time across all age groups. This is partly due to better therapeutic options and treatments, such as, PPCI for STEMI that has a better safety profile in elderly patients compared to thrombolytic therapy[16]. In 2004 PPCI rates for all elderly STEMI patients was 2.0% *vs* 36.1% in 2009. During the same time period, 11.5% and 25.5% of elderly patients had coronary angiography following AMI. The rate of prescription of secondary medications increased in all age groups but a greater rate of change was observed in those $\geqslant$ 85 years compared to those < 55 years old from 2003 to 2010: 28% to 89% *vs* 56% to 97%, respectively[12].

In addition to higher mortality following an AMI, elderly patients have more frequent complications and greater risk of physical de-conditioning compared to younger patients[17]. Indeed, the latter can be improved by interventions such as cardiac rehabilitation (CR) programmes, yet the overall rates of participation in CR are relatively low[18,19] especially in the elderly[20,21]. Greater awareness by the caring Physician of the benefits of CR might help improve care and outcomes post AMI as recommendation by a Physician was a strong predictor of CR uptake[21].

The better evidenced based care provided to younger patients following an AMI might explain the continued variation in outcome compared to older counterparts. Understanding the reasons for this inequality is paramount if care and outcomes are to be improved. It is likely that the reasons for this variation are multifactorial. For instance, elderly patients have a greater degree of co-morbidity such as anaemia, cerebrovascular disease and dementia which potentially provides a further barrier to the use of AMI therapies[14,22]. For instance, older patients with dementia had lower rates of evidenced based treatment than elderly patients without dementia, 44% *vs* 62%, respectively. Such variation may occur as

patients with dementia often need prompting to ensure compliance with medications. Non-compliance with dual antiplatelet therapy after percutaneous coronary intervention (PCI) may lead to significant implications such as a further AMI. Advanced dementia may signify to the caring Physicians that evidence based therapies have less long term benefit and subsequently have a higher threshold to prescribe such treatments. Other factors such as frailty may preclude invasive treatment[23] as there is the perception that the patient is likely to gain more harm than benefit from advanced treatments. Clinicians may be reluctant to prescribe dual antiplatelet therapy or list patients for cardiac catheterisation in the presence of anaemia[24], more commonly seen in the elderly. Such clinical concerns highlight the potential risk of therapies and may encourage decisions that are risk adverse, aiming to do no harm in the first instance.

There is little evidence base to help guide care decisions in elderly patients with AMI. For instance, advanced age is often an exclusion criteria in coronary heart disease trials[25]. Only 9% of all trials included those $\geqslant$ 75 years and 2% included those $\geqslant$ 85 years of age. The elderly patients included in these trials had less risk factors for coronary heart disease, fewer co-morbidities, better kidney function and haemodynamics on presentation to hospital compared to similar aged patients that are seen in real life practice[14]. This highlights a lack of clarity in the evidence base for managing older patients and underpins further the uncertainty in treating patients in the more conventional way seen in younger patients with an AMI.

Additionally, elderly patients are more likely to present with atypical symptoms of an AMI. For example, only 40% of those $\geqslant$ 85 years with an AMI had chest pain compared to 77% of those < 65 years. Indeed, they were more likely to present with dyspnoea (49%) but less commonly with nausea and vomiting (24%) or syncope (19%)[14]. Due to the atypical presentation, these patients are often misdiagnosed and therefore not receive timely recommended therapies which maybe contributing to the greater morbidity and mortality[26]. Furthermore, 40% of patients with an AMI $\geqslant$ 85 years of age did not have diagnostic electrocardiogram changes compatible with AMI compared to 25% of patients < 65 years[14]. Even when the diagnosis of AMI has been established, those > 80 years were less likely to be admitted under cardiology care compared to patients < 65 years of age (39.1% *vs* 64%, respectively)[27], despite evidence to suggest care by a cardiologist improved outcomes[25].

Guidelines recommend using risk scoring systems to identify those individuals at high risk after an AMI[5-7,28,29] and who may potentially benefit from invasive and aggressive therapies. Clinicians often subjectively assess individuals risk by taking into account other co morbidities that are not incorporated into clinical risk scores[30]. There is poor correlation between the perceived risk judged by physicians and actual validated risk scores[31]. Equally age is a major driver of heightened risk in risk scores, such as, the Global Registry of Acute Coronary Events (GRACE) and thus most elderly patients would be in a high risk group post AMI[32]. This suggest that physician decisions are potentially influenced by other factors not represented in the risk scores, such as perceived frailty, and highlights the difficult in driving optimum AMI care in the elderly.

With the aging population, it is likely that the number of elderly patients presenting with AMI will increase. This will have a significant impact on morbidity and mortality as well as health care resources. Several societies including the American College of Cardiology, American Heart Association and the European Society of Cardiology guidelines on non STEMI (NSTEMI) advocate the use of intensive and early interventional therapies in high risk groups[5-7], such as the elderly, who are likely to achieve better outcomes if therapies for AMI are advocated, although this is confounded by the lack of a strong evidence base. Thus, uncertainty of risk *vs* benefit of AMI therapies in elderly patients makes decisions around the use of effective therapies difficult. In part, unmodifiable risk factors associated with age drive the disparity in outcomes post AMI between young and old. However, whilst inequalities in care exist across age groups there is still potential to narrow the gap in adverse outcomes by improved provision of evidence based care to older patients post AMI.

Socioeconomic factors

The socioeconomic status of patients can be defined according to the patient's occupation, income wealth, education or where they live[33]. Lower socioeconomic status, *i.e.*, low income, less than high school education, is a key determinant of inequality in care and results in these individuals experiencing poor health and reduced life expectancy. Studies from Sweden, Finland, Canada and the United States have found that prognosis is worse after an AMI in patients from a lower socioeconomic status group[34-36]. In a study conducted in Finland, a higher number of patients with lower socioeconomic status had AMI's and lower prescription of secondary prevention medication compared to the higher socioeconomic group. These patients often presented later to hospital with chest pain[21,36] and higher numbers were treated in urban hospitals compared to their counterparts who were treated in specialist hospitals[36]. This may have been because specialist centres were situated in more affluent areas. In addition poorer patients were likely to refuse invasive procedures[37]. Mortality rates were also significantly higher in patients with a low income and basic education. Males with a low income had a 28 d mortality rate of 49.5% compared with 14.5% of those with higher income. In males who had a basic level of education 28 d mortality rate was 80.3% and 19.7% in those with higher education. A similar trend was also seen in females[36]. This suggests that low income and potentially a lack of understanding of physical health contributes to these findings.

In the United States, patients of a lower socioe-

conomic status were less likely to proceed to a coronary angiogram within 24 h of a STEMI compared to those with a higher socioeconomic status (69.5% *vs* 73.7%) or within 48 h of a NSTEMI (47.6% *vs* 51.8%). Probable reasons included, those from a lower socioeconomic group were less educated about their co-morbidities, did not have consistent medical records and were unable to obtain anti-platelets therapies reliably. Therefore physicians often spent longer trying to establish if there were any contraindications to anti-platelet therapy. In contrast, individuals from a higher socioeconomic status had higher expectations for early treatment to be instituted when presenting with chest pain. They also tended to receive more frequently drug eluting stents, possibly because their level of insurance covered the cost of the procedure. However, the perception of the operating physician, that a patient was of a higher socioeconomic status, was independently related to higher drug eluting stent use than level of insurance as this was not checked prior to procedure in all patients[38].

Poor health was further contributed by factors such as occupational stress, social isolation and depression which are seen more frequently in the lower socioeconomic groups[35]. On the other hand, some therapies were equally provided across socioeconomic groups. For example, there were no significant differences in patients referred for coronary artery bypass grafting (CABG) post AMI in the United States. It was speculated that patients of a lower socioeconomic status were likely to have severe and more complex coronary artery disease making them appropriate for CABG rather than PCI. This would also resolve the problem of compliance with dual antiplatelet therapy which is required post PCI[38]. On the other hand, if the lower socioeconomic group had coronary disease more suitable for CABG, one would expect the referral rate for CABG to exceed the higher socioeconomic group. However, rates of CABG referral between the two groups were comparable. This might suggest that either those of lower socioeconomic class were under-referred for CABG or those in higher socioeconomic were over-referred for CABG.

Intriguingly, MINAP data from 2003 to 2007 in England and Wales, suggested that there was no socioeconomic differences in the management of patients with AMI[39]. This is likely explained by differences in the healthcare systems with the United States being predominantly paid for through medical insurance whilst in the United Kingdom, a nationally funded service offers universal access to care at the point of need.

Racial and ethnic factors

Within racial and ethnic groups there is variation in the way AMI patients are treated. In the United States, the national registry of myocardial infarction from 1994-2002 showed that black (not well defined as to whether these were Afro-Caribbean patients or black United States patients) patients received less coronary reperfusion therapy and coronary angiography compared to Caucasian patients[37,40,41]. Compared to Caucasians, Afro-Caribbean females were less aggressively treated and had higher in hospital mortality[40]. Reasons for such differences were unclear, but may be related to socioeconomic factors rather than race alone.

Data from hospital discharges in the state of Pennsylvania between 2003 and 2004 showed that 46% of Caucasian patients underwent PPCI compared to 40% of African Americans because more than often African American patients presented later to hospitals at which point the benefit of PPCI had elapsed[37].

In Singapore and Malaysia, ethnic variations in the treatment of patients with an AMI were prevalent. Ethnicity is defined as groups of people who identify with each other based on social and cultural experience. Malays had the least invasive treatment and had the highest mortality rate after an AMI compared to the Indians and the Chinese[42,43]. The level of education and household income may have contributed to these differences. In 2000, less than 5% of Malays in Singapore progressed to higher education compared to nearly 20% of Chinese and Indians[43]. Education and income both act together to enhance health and reduce the need for health care. Provision of education may serve as a key strategy to reduce disparities in AMI care.

In the United Kingdom there is a paucity of data regarding AMI care between different racial groups.

Gender differences

Studies in the United States, Switzerland, United Kingdom and France demonstrate gender differences in AMI treatment. PCI rates were lower in females compared to males (14.2% *vs* 24.4% respectively)[44]. Females that presented with an AMI were generally older than men with greater co-morbidities and presented later to hospital[45,46]. The latter point may drive some of the variation seen with PCI rates as late presenters would derive less benefit. Furthermore, females with an AMI more frequently had non obstructive coronary atheroma therefore precluding the need for any interventional therapy[44]. There is also evidence to suggest that females are less inclined to consent to coronary angiography compared to males[47].

Similar findings were seen in England, with females compared to males, less likely to be given thrombolytic therapy (37% *vs* 46%), aspirin (83% *vs* 90%), have angiography, exercise testing or revascularisation. However when adjusted for age these inequalities were less apparent but poor outcome was statistically higher in females than males yet, despite females being higher risk partly due to age and co-morbidity they were treated less aggressively than males[46,48,49]. However, it is difficult to know if this now represents contemporary practice as this data precedes 2000.

Even when females were treated in the same way as males there was still higher in hospital mortality despite correction by age, co-morbidities, haemodynamic status and time to treatment. Mortality rates in females were 2.3 times higher in comparison to males (7.9% *vs* 2.3% respectively). Furthermore hypotension and shock was

more prevalent in females despite the degree of left ventricular systolic impairment being the same in the male group. This suggests that gender in itself may account for the differences in outcomes[50]. Inequalities in care between the genders are not fully understood and like elderly patients, females have historically been under represented in clinical trials.

Geographic variation

GRACE looked at the management of patients with AMI from 95 hospitals from 14 countries including Europe, North and South America, Australia and New Zealand. Aspirin and ACE inhibitor use was similar across all regions with over 91% receiving Aspirin on admission. There was geographical variation in the discharge use of statin, ranging from 26% to 57%. This was due to the uncertainty about the benefit of statins acutely. Furthermore the United States appeared to use more Glycoprotein Ⅱb/Ⅲa inhibitors compared to other countries, explained by United States GRACE centres having more direct access to coronary angiography facilities[51]. Post AMI 30 d and 1 year mortality varied in 458 hospitals across 24 countries, 5.0% to 13.9% and 4.9% to 14.8%, respectively. However, patient level factors, such as socioeconomic status accounted for most of this variation (96% to 99%) whilst hospital level factors at most accounted for 4% of variation in post AMI outcome[48]. Similar findings were reported in other studies[52,53].

Old practice data from 1998 comparing AMI treatments in the United States and the United Kingdom revealed that coronary angiography was performed in 61% *vs* 22% of cases respectively. United States patients were more likely to receive coronary revascularisation, 69% *vs* 41%, respectively, although the extent of coronary disease was similar between the two patient groups. The greater availability in coronary angiography and revascularisation in the United States may have accounted for these findings at the time. There were no significant differences in the primary end points of recurrent angina, myocardial infarction and death in the United States (29%) compared to the United Kingdom (25%)[54]. It is not clear how differences in the two healthcare systems and how they are funded affected the variation in care provided to AMI patients.

Geographical variation in AMI care is likely driven by several factors including the economical strength of countries or the way healthcare systems are funded, which makes comparisons difficult.

CONCLUSION

Inequalities in the treatment of an AMI are described with regards to age, socioeconomic factors, race, gender and geographical location. Age is known to be a risk factor for an AMI and with the aging population more patients are predicted to have an AMI, resulting in a significant impact on morbidity, mortality and healthcare resources. The inequality in care between younger and older patients suggests that older patients may still gain a survival benefit by equalising the disparity in care by simple measures such as ensuring guideline recommended care being provided to more elderly patients. There is an increasing need for further research to guide optimum care of elderly patients post AMI. Clinicians taking a more proactive role in the treatment of these patients may further narrow the gap between the young and the old. A similar model in the care of elderly patients following orthopaedic surgery has been successful with the evolution of the Ortho-geriatrician. Females had the highest mortality and given that they make up 50% of the global population it is imperative that treatment is equalised. Further research is required to help understand the inequality of care that exists amongst females and ultimately guide further AMI management.

Further discrepancies were seen between the higher and lower socioeconomic groups with the latter experiencing poor healthcare. Furthermore, Lower socioeconomic status probably accounted for geographical and racial variation. Socioeconomic status is strongly linked to education which also potentially allows the understanding and prevention of illness, control of risk factors and compliance to medications as well as a determinant of higher income. This would therefore suggest that education is a fundamental component but outside the influence of the medical sphere.

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MINIREVIEWS

Predicting mortality in patients with acute heart failure: Role of risk scores

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Abstract

Acute heart failure is a leading cause of hospitalization and death, and it is an increasing burden on health care systems. The correct risk stratification of patients could improve clinical outcome and resources allocation, avoiding the overtreatment of low-risk subjects or the early, inappropriate discharge of high-risk patients. Many clinical scores have been derived and validated for in-hospital and post-discharge survival; predictive models include demographic, clinical, hemodynamic and laboratory variables. Data sets are derived from public registries, clinical trials, and retrospective data. Most models show a good capacity to discriminate patients who reach major clinical end-points, with C-indices generally higher than 0.70, but their applicability in real-world populations has been seldom evaluated. No study has evaluated if the use of risk score-based stratification might improve patient outcome. Some variables (age, blood pressure, sodium concentration, renal function) recur in most scores and should always be considered when evaluating the risk of an individual patient hospitalized for acute heart failure. Future studies will evaluate the emerging role of plasma biomarkers.

Key words: Acute heart failure; Prognosis; Scoring; Risk stratification; Outcome

Core tip: We present a review of the most relevant scores developed for the risk stratification of patients hospitalized for acute heart failure. For each score, the strengths, weaknesses, statistical pertinence and applicability in a real-world situation are evaluated. Furthermore, we revisit the general criteria and statistical metrics that should be considered in the design and analysis of prognostic studies.

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INTRODUCTION

Acute heart failure (AHF) is a complex and heterogeneous clinical syndrome defined as the rapid onset or change in symptoms and signs of heart failure requiring immediate medical attention and urgent therapy[1]. It is a leading reason for hospitalization and is burdened by high short-(intra-hospital) and long-term (6 to 12 mo) mortality.

Most often, the first triage of patients with AHF is performed in the emergency department (ED), where these patients present to receive initial care. Then, on the basis of the clinical profile and risk stratification, patients are discharged, admitted to a medical ward or cardiac division, or transferred to an intensive care unit. At the end of hospitalization, a structured follow-up is planned to reduce the risk of early rehospitalization (a major issue in health care system) and improve long-term survival.

Therefore, the risk stratification of patients with AHF is a pivotal medical task aimed to improve the outcome of patients with AHF and the efficiency of the health care delivery system. Physicians involved in the care of acute heart failure patients should be able to evaluate the risk profile especially in two critical turning points: (1) at the time of hospital admission, for choosing the best hospital setting according to the risk profile and for identifying low-risk patients who can be safely discharged to home, thus pursuing both the best outcome of patients and the correct allocation of resources; and (2) at the time of discharge, for planning disease management of patients for a given risk profile, and for the selection of patients suitable for more advanced therapies.

Physicians always determine an initial prognosis by integrating the patient's characteristics, clinical signs and laboratory tests. The prediction is inherently multivariable; however, the relative weight that a doctor assigns to each variable, which relies on his clinical judgment, previous experiences, personal beliefs and, eventually, on his current mood, could be inaccurate and misleading.

Even the most skilled physician might incorrectly estimate the risk of death in heart failure patients or be uncertain about prognosis[2]. Furthermore, the precision of risk estimate based on clinical judgment might be reduced by the urgency of making a critical decision in the case of more severe clinical scenarios[3-5]. An incorrect prognosis might generate a mismatch between intensity of care and the risk profile of the patient.

Risk score are multivariable predictive models in which relative weights are assigned to each variable in order to calculate the probability that a specific event (death, rehospitalization) will occur in the future. They are tools that help doctors estimate prognosis in a more unbiased way, translating the result of prognostic studies in clinical practice.

Beyond the benefit to an individual patient, the research of valid prognostic models is fundamental for public health policy, for comparative effectiveness and health service research, for quality of care outcome assessment, for health technology assessment of therapies and laboratory tests, and for studying new approaches, mechanisms and targets for clinical trials[6].

Methodological issue and critical points of risk stratification of AHF patients

A risk model is the final output of prognostic research, which is a three-step course that calls for (1) development studies aimed to identify relevant predictors entering the model and their relative weights. In this phase the performance of the models estimated by evaluating the calibration and the discrimination, and the model is adjusted for overfitting; an internal validation should be performed by bootstrapping techniques in the same population from which the model is derived; (2) external validation studies, in which the model is validated in new populations; and (3) impact studies designed to evaluate if the decision making for a single patient, driven by the risk status assigned according to the predictive model, could improve clinical outcome[7].

Correct statistical metrics should be used for reporting prognostic studies. To measure the ability of a model to discriminate patients for a binary outcome, the C-statistic (equivalent to the area under the receiver operating characteristics curve) is calculated[8]; it ranges from 0.50 (no discrimination) to 1 (perfect discrimination).

Calibration measures the correlation between observed and predicted events, and it is generally assessed with the Hosmer-Lemeshow statistic[9]. Recently, the standardization of reporting of a multivariable prediction model has been proposed[10]. Many reasons make the development of a prognostic score in the setting of acute heart failure a challenging task.

The validity of a risk score depends on the population from which it is derived and on the choice of the variables; AHF syndromes include different clinical scenarios: (1) decompensated HF and worsening chronic heart failure; (2) pulmonary oedema; (3) cardiogenic shock; (4) hypertensive HF; and (5) right HF[11]. Moreover, each class could undergo a further classification; for example, worsening chronic heart failure patients could have preserved or reduced ejection fraction as well. It is unlikely that the same prognostic model could fit miscellaneous clinical patterns, as each of one is endowed with peculiar physiopathological aspects.

Another relevant issue is the source of the data set from which the model is derived. Community-based settings and clinical trial populations are often very divergent; the latter generally includes younger people with a lower rate of co-morbidities that might have a relevant role in driving prognosis, especially in older

Table 1 Main risk scores in acute heart failure

Risk score	Data source	Publication year	Sample size (derivation)	Sample size (validation)	Validation	Model development	Endpoint
In-hospital mortality							
ADHERE[15]	Registry	2005	33046	32229	External	Classification trees	In-hospital mortality
AHFI[16]	Statewide databases	2005	33533	8384	External	Classification trees	In-hospital mortality and complications
OPTIMIZE-HF[19]	Registry	2008	37548	181830	Internal/external	Logistic regression model	In-hospital mortality
GWTG-HF[20]	Registry	2010	27850	11933	Internal/external	Logistic regression model	In-hospital mortality
EHMRG[21]	Population-based cohort	2012	7433	5158	Internal/external	Logistic regression model	7 d mortality
PROTECT[23]	Clinical trial	2012	2015	1435	Internal/external (clinical trial population)	Cox proportional hazards model	Composite endpoint of death, worsening heart failure or heart failure rehospitalization
Post-discharge mortality							
EFFECT[24]	Community	2003	2624	1407	Internal/external	Logistic regression model	30 d mortality/1 yr mortality
OPTIME-CHF[25]	Clinical trial	2004	949	-	Internal	Cox proportional hazards model	60 d mortality
OPTIMIZE-HF[26]	Registry	2008	4402	949/433	Internal/external (clinical trial population)	Cox proportional hazards model	60-90 d post-discharge mortality
APACHE-HF[27]	Community (single centre)	2014	824	-	-	Cox proportional hazards model	90 d mortality
ELAN[28]	Pooled data of seven cohorts	2014	1301	325	External (clinical trial population)	Cox proportional hazards model	180 d mortality
ADHF/NT-proBNP[29]	Community	2013	453	371	External	Logistic regression model	1 yr mortality
ESCAPE[30]	Clinical trial	2010	433	471	Internal/external (clinical trial population)	Cox proportional hazards model	6 mo mortality

ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; GWTG-HF: Get with the guidelines-heart failure; EHMRG: Emergency heart failure mortality risk grade; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; EFFECT: Enhanced feedback for effective cardiac treatment; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; APACHE-HF: Acute physiology and chronic health evaluation in heart failure; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; ELAN: European collaboration on acute decompensated heart failure.

populations. The external validity of a model derived from clinical trial is, at minimum, controversial.

Another critical point is the choice of variables used to calculate the score. A huge number of determinants of survival for AHF have been studied; many variables have been associated with prognosis in univariate and multivariate analysis, including clinical characteristics, hemodynamic markers, serum biomarkers, and medication use[12]. If a stepwise selection is used, then the availability of so many variables could lead to the inclusion of too many parameters in the model, causing overfitting, with the model generating random error or noise and resulting in a spurious prognostic association.

A model that has been overfit will have a poor predictive performance in other populations. Parsimony in the number of parameters and developing the simplest model with the highest accuracy are proper ways to improve the applicability of the model to other populations[13]. In AHF syndrome, clinical, laboratory and hemodynamic variables might suddenly change during the clinical course. Some variables could be associated with short-term improvement but worse long-term survival (for example, use of inotropic drugs); therefore, the timing of data collection and the timeline for the endpoint survey are pivotal.

RISK SCORES

Several prognostic models combining different variables have been developed to predict in-hospital mortality and to estimate outcomes between 30 d up to 6 mo post-discharge. Table 1 summarizes methodological characteristics of the risk scores, Table 2 the variables entering different models, and Table 3 the models' performances.

In-hospital risk models

Acute decompensated heart failure national

Table 2 Variables used in the risk score models

Risk score	Variables
ADHERE[15]	BUN, creatinine, SBP
AHFI[16]	Gender, CAD, diabetes, lung disease, SBP, HR, respiratory rate, temperature, blood urea nitrogen, sodium, potassium, white blood cell count, acute myocardial infarction o myocardial ischemia at ECG, pulmonary congestion or pleural effusion on radiographic examination
OPTIMIZE-HF[19]	Creatinine, sodium, age, HR, liver disease, previous CVA/TIA, peripheral vascular disease, race, left ventricular systolic dysfunction, COPD, SBP, previous HF hospitalization
GWTG-HF[20]	Older age, low SBP, elevated heart rate, presence of COPD, and non-black race
EHMRG[21]	HR, creatinine, systolic blood pressure initial oxygen saturation, serum troponin
PROTECT[23]	BUN, respiratory rate, HR, albumin, cholesterol, diabetes, previous HF hospitalization
EFFECT[24]	Age, SBP, BUN, sodium concentration, cerebrovascular disease, dementia, COPD, hepatic cirrhosis, cancer, hemoglobin
OPTIME-CHF[25]	Age, BUN, SBP, sodium, NYHA class
OPTIMIZE–HF[26]	Age, weight, SBP, serum creatinine, history of liver disease, history of depression history of reactive airway disease
APACHE-HF[27]	Mean blood pressure, HR, serum sodium, serum potassium, creatinine, haematocrit, Glasgow coma scale, age
ELAN[28]	NT-proBNP at discharge, NT-proBNP reduction, age, peripheral oedema, SBP, sodium, serum urea, NYHA class
ADHF/NT-proBNP risk score[29]	COPD, SBP, eGFR, serum sodium, hemoglobin, NT-proBNP; left ventricular ejection fraction, tricuspid regurgitation
ESCAPE[30]	Age, BUN, six-minute walk test, sodium, CPR/mechanical ventilation, diuretic dose at discharge, no-blocker at discharge, BNP

BUN: Blood urea nitrogen; SBP: Systolic blood pressure; CAD: Coronary heart disease; HR: Heart rate; CVA: Cerebral vascular accident; COPD: Chronic obstructive pulmonary disease; CPR: Cardiopulmonary resuscitation; eGFR: Estimated glomerular filtration rate; NT-proBNP: N-terminal brain natriuretic peptide; ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; EHMRG: Emergency heart failure mortality risk grade; EFFECT: Enhanced feedback for effective cardiac treatment; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; GWTG-HF: Get with the guidelines-heart failure; APACHE-HF: Acute physiology and chronic health evaluation in heart failure; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; ECG: Electrocardiogram; TIA: Transient ischemic attack; ELAN: European collaboration on acute decompensated heart failure.

Table 3 Performances of risk scores

Risk score	Calibration	C-statistic (derivation cohort)	C-statistic (validation cohort)	Low-risk group mortality (%)	High-risk group mortality (%)
In-hospital mortality					
ADHERE[15]	NV	0.75	0.75	2.1	21.9
AHFI[16]	NV	NA	0.59	0.3	NA
OPTIMIZE-HF[19]	NV[1]	0.75	0.746	NA	NA
GWTG-HF[20]	Calibrated	0.75	0.75	0.4	9.7
EHMRG[21]	Calibrated	0.80	0.803	0.3	8.2
PROTECT[23]	Calibrated	0.67	0.67	4.8[1]	28.7[2]
Post-discharge mortality					
EFFECT[24]	Calibrated	0.80 (30 d) 0.77 (1 yr)	0.79 (30 d) 0.76 (1 yr)	0.4 (30 d) 7.8 (1 yr)	59 (30 d) 78.8 (1 yr)
OPTIME-CHF[25]	NV[1]	0.77	0.76	2	30
OPTIMIZE-HF[26]	NV	0.72	NA	NA	NA
APACHE-HF[27]	NV	0.78			20[2]
ELAN[28]	NV	0.77	NA	3.6	51.1
ADHF/NT-proBNP risk score[29]	Calibrated	0.84	0.77	3.7	89.5
ESCAPE[30]	NV[1]	0.76	0.65[3]	7	100

[1]A graphic plot of the predicted *vs* observed probability of outcome was reported; [2]Relative risk of death in the high-risk group *vs* the low-risk group; [3]In the validation cohort, the model did not include brain natriuretic peptide and diuretic dose. NA: Not available; NV: Calibration was not verified by statistical tests; ADHERE: Acute decompensated heart failure national registry; AHFI: Acute heart failure index; OPTIMIZE-HF: Organized program to initiate lifesaving treatment in hospitalized patients with heart failure; GWTG-HF: Get with the guidelines-heart failure; OPTIME-CHF: Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure; EHMRG: Emergency heart failure mortality risk grade; EFFECT: Enhanced feedback for effective cardiac treatment; BNP: Brain natriuretic peptide; ADHF/NT-proBNP: Acutely decompensated heart failure n-terminal pro-brain natriuretic peptide; ESCAPE: Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness; PROTECT: Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function; ELAN: European collaboration on acute decompensated heart failure.

registry: The "acute decompensated heart failure national registry" (ADHERE) provides a risk stratification model to predict in-hospital mortality in patients admitted with acutely decompensated heart failure[14]. The authors analysed the clinical, demographic and biochemical data of 33046 patients from the Acute Decompensated Heart Failure National Registry in order to develop a risk stratification model. The model

was prospectively tested using data from 32229 hospitalizations, which comprised the validation cohort. Statistical analysis revealed that blood urea nitrogen (BUN) level of 43 mg/dL or higher was the best single predictor for mortality. The second best predictor was admission systolic blood pressure (SBP) < 115 mmHg. Serum creatinine levels of 2.75 mg/dL or higher provided additional prognostic value in patients with BUN levels ⩾ 43 mg/dL and SBP ⩽ 115 mmHg. The authors employed the CART method to derive a risk tree identifying acutely decompensated heart failure (ADHF) patients at low, intermediate and high risk for in-hospital mortality in the validation cohort. Heart rate and age did not improve the risk stratification of patients in the final algorithm.

Finally, ROC curves were used to assess the accuracy of the models. The study provided a useful and validated tool for mortality risk stratification by employing signs and laboratory data evaluated on hospital admission. The combination of two different markers of renal function confirms the established link between the heart and kidney and thus the association between clinical outcomes and markers of renal function[15]. Mortality in the low- and high-risk group was 2.1% and 22%, respectively.

The ADHERE algorithm was derived from a real-world population, the model was adequately validated in an additional cohort of patients, and it meets parsimony criteria requiring only three variables, which are easily measured at the time of hospital admission. A major criticism of the ADHERE algorithm is that the registry entries reflect individual hospitalizations, and repeated hospitalizations of the same patient are entered as separated records. This is a clear violation of the fundamental research principle of independence of experimental units, which limits the internal validity of the study. Another limit is the overly high mortality of the low-risk group in comparison with other models. However, the ADHERE algorithm might allow for immediate and simple triage at admission in the emergency department, not requiring complex calculations.

AHF index: Auble *et al*[16] analysed 33533 patients admitted from the ED with a diagnosis of heart failure. The authors derived a prediction rule to identify patients at low-risk of in-hospital death and serious medical complications. The proposed prediction rule resulted from a combination of demographic, biochemical and non-invasive diagnostic tools.

The performance of this algorithm, named the AHF index, was further examined, and the index was validated in an independent group of 8383 patients admitted to the ED with heart failure, with respect to inpatient mortality, serious medical complications before hospital discharge, and 30 d mortality. The mortality rates in the low-risk group were significantly higher in the validation cohort compared to the two derivation cohorts (0.7%-1.7% *vs* 0.3%)[17,18].

Organized program to initiate lifesaving treatment in hospitalized patients with HF: Beginning with an analysis of a national hospital-based registry and quality improvement program [organized program to initiate lifesaving treatment in hospitalized patients with HF (OPTIMIZE-HF) registry], predictors of in-hospital mortality were identified, and a practical risk-prediction tool of in-hospital mortality that is applicable in routine clinical practice for patients hospitalized for heart failure was derived. The identification of the most important predictors from the multivariate logistic regression analysis allowed the development of a point scoring system to predict in-hospital mortality. The ability of the logistic regression model to discriminate mortality was tested by a classification and regression tree (CART) analysis. The model combined multiple variables, and the final risk-prediction normogram included age, heart rate, SBP, serum creatinine, serum sodium, primary cause of admission (heart failure or other), and left ventricular systolic dysfunction. For each value of each variable, a score associated with the probability of in-hospital mortality is calculated. The model had a good performance, with a C-statistic of 0.75; however, no validation of the score has been reported[19].

Get with the guidelines-HF: Another useful risk model has been provided by the American Heart Association's "get with the guidelines-heart failure" programme. The score combines clinical variables to predict in-hospital mortality. The programme involved 39783 patients, with a derivation sample of 27850 and a validation sample of 11933 patients, and can be applied to heart failure patients, with both preserved and reduced left ventricular ejection fraction. The proposed score combined 7 clinical factors routinely collected at the time of admission. The 7 predictor variables (older age, low SBP, elevated heart rate, presence of chronic obstructive pulmonary disease, and non-black race) were identified in the multivariate model. The estimation of in-hospital mortality can be carried out by summing points assigned to each predictor, with a total score ranging from 0 to 100. The inclusion of race among the predictors might limit the application of the model in different countries. The risk score had good discrimination: C-index was 0.75 in both derivation and validation data set.

In-hospital mortality in the lower and higher risk group was 0.4% and 9.7%, respectively. The model was thought to be helpful in patient triage and in the use of evidence-based therapy in the highest-risk patients, reducing resource allocation in those at low risk[20].

Emergency heart failure mortality risk grade: Lee DS *et al*[21] proposed a multivariate risk index for 7-d mortality using initial vital signs, clinical and presenting features and readily available laboratory tests, with the aim of predicting acute mortality and guiding acute clinical decision making for patients with HF who present to the ED. The derivation cohort was comprised of 7433

patients, and the validation cohort was comprised of 5158 patients. The authors developed the "emergency heart failure mortality risk grade" (EHMRG), which comprises multiplicative and additive variables with an available online calculator. The EHMRG encompassed all patients presenting to the ED, regardless of whether they were hospitalized or discharged, providing a useful tool to guide hospitalization-*vs*-discharge decisions based on prognosis. A higher heart rate and creatinine concentration, a lower SBP and oxygen saturation, and non-normal serum troponin levels were associated with an increased mortality risk and were entered into the score. The area under the receiver-operating characteristic curves of the model was 0.805 for the derivation data set and 0.826 for the validation data set. Despite the fact that left ventricular ejection fraction and natriuretic peptide analysis have been validated as predictive variables in both acute and chronic heart failure, they were not included in the model because they are not frequently assessed in the ED.

Placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function: The international "placebo-controlled randomized study of the selective A1 adenosine receptor antagonist rolofylline for patients hospitalized with acute decompensated heart failure and volume overload to assess treatment effect on congestion and renal function" (PROTECT) trial enrolled 2033 patients hospitalized with AHF and mild or moderate impairment of renal function[22]. Of the 2033 patients, 2015 had complete data for the analysis, and a risk score was developed for predicting the composite endpoint (death, worsening heart failure, rehospitalization for HF) at 7 d; points assigned to each predictor were summed, for a total point score ranging from 0 to 100 points. All variables employed were collected within 24 h of admission. The strongest predictor of the outcome was higher BUN concentration. Other predictors of an adverse outcome were lower values of serum albumin, serum cholesterol, and SBP, as well as higher heart and respiratory rates. The variables employed in the model demonstrate the role of metabolic status, neurohormonal activation and reduced cardiac performance in influencing patient outcomes. The model underwent an external validation in a study population of another clinical trial; the C-index in the derivation and validation population was 0.67.

The study population of the derivation data set was enrolled in the trial with strict inclusion and exclusion criteria: Patients taking inotropic agents and those with severe pulmonary disease, recent ischemia or preserved ejection fraction were not included; therefore, the applicability of the PROTECT risk score to a wide range of community-based populations is limited[23].

Post-discharge risk models

In addition to prediction of in-hospital mortality, attempts to assess short-, medium- and long-term prognosis, as well as the risk of events, in patients hospitalized for AHF, has led to the proposal of different risk models.

Enhanced feedback for effective cardiac treatment: The "enhanced feedback for effective cardiac treatment" study analysed multiple variables available at the time of hospital presentation of more than 4000 patients hospitalized for heart failure. The authors identified predictors of mortality, and they developed and validated a model that could predict all-cause 30 d and 1 year mortality. Age, lower SBP, higher respiratory rate, higher BUN level, hyponatremia, and co-morbidities were independent predictors of mortality at both 30 d and 1 year. Very low risk scores (< 60) identified patients with a mortality rate of 0.4% at 30 d and 7.8% at 1 year. Patients with very high-risk scores (> 150) had a mortality rate of 59% at 30 d and 78.8% at 1 year. The authors suggested the importance of assessing selected variables during the first hours of hospital presentation in order to help the physician to identify patients with a high risk of events and optimize patient management[24].

Outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure: The data from the "outcomes of a prospective trial of intravenous milrinone for exacerbations of chronic heart failure" (OPTIME-CHF) study were analysed to develop a model predicting the post-discharge outcome of inpatients hospitalized for acute decompensated heart failure[25].

A multivariate model allowed the assessment of variables predictive of mortality or the composite endpoint of death and re-hospitalization at 60 d. Age, lower SBP, New York Heart Association class Ⅳ symptoms, elevated BUN, and decreased sodium were predictors of death at 60 d. The C-index for mortality at 60 d was 0.77. As for other models derived from clinical trials, the populations used to derive the models represent only a subgroup of AHF patients; the study populations have consisted entirely of patients with reduced ejection fraction, whereas patients with renal dysfunction or who required inotropes were excluded from the studies.

OPTIMIZE-HF: O'Connor et *al*[26] developed a clinical model predictive of short-term clinical outcome in patients discharged after hospitalization for HF. The authors employed logistic regression analysis that initially included 45 potential variables and finally identified 8 significant risk factors to predict the risk of mortality within 60 d after discharge, with a C-index of 0.72. Co-morbidities (liver disease, depression, reactive airway disease) have a major role in the score.

In addition to the risk score, the study confirmed the

importance of evidence-based therapies prescribed at discharge; β-blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blocker and lipid-lowering therapies were associated with decreased mortality and rehospitalization.

Acute physiology and chronic health evaluation in HF: This score, constructed by Okazaki *et al*[27] includes all factors significantly predictive of survival after discharge and assigns one point for each factor. The parameters considered in the scoring system are the mean blood pressure, pulse, sodium, potassium, creatinine, haematocrit, age and glasgow coma scale; these parameters exhibited a high sensitivity and specificity and an adequate area under the curve. The score was able to predict all-cause death or readmission due to heart failure at 90 d. The study did not include NYHA class, left ventricular ejection fraction, BUN, hemoglobin and brain natriuretic peptide (BNP), which has been found to be predictive of prognosis in previous studies. Acute physiology and chronic health evaluation in HF has other major limitations: It was derived from a single centre population, all patients were admitted to an intensive care unit for respiratory or circulatory support, and the score has not been validated.

European collaboration on acute decompensated-HF: The data from seven cohorts of prospective studies of patients admitted due to acutely decompensated heart failure were pooled by Salah *et al*[28] to develop a predictive discharge score based on different predictors of mortality, including the absolute value at discharge and percentage reduction of NT-proBNP. The European collaboration on acute decompensated-HF score assigned one point for each factor but 3 points for n-terminal (NT)-proBNP values at discharge ranging from 5001 to 15000 pg/mL and 4 points for values > 15000 pg/mL. The score showed that the absolute values of NT-proBNP at discharge and the percentage reduction during hospitalization, combined with other established risk markers, might improve the risk stratification for adverse events within 180 d after discharge.

ADHF/NT-proBNP risk score: Confirming the relevance of natriuretic peptide measurements in patients with acutely decompensated heart failure, Scrutinio *et al*[29] studied the improvement in the risk reclassification of patients with AHF by adding NT-proBNP to other common clinical variables. The authors proposed the ADHF/NT-proBNP risk score, with a possible total score ranging from 0 to 22. The score proved to be effective in predicting one-year mortality in patients hospitalized for acutely decompensated heart failure, providing clinicians with a validated and easy-to-use predictive tool in daily clinical practice. Adding NT-proBNP to the reference model did not improve discrimination, but resulted in significant risk reclassification.

Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness: The evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness (ESCAPE) trial enrolled 433 patients hospitalized with ADHF, and it analysed the relationship between clinical factors at discharge and 6 mo mortality. The aim of the analysis was to create a score, potentially useful to identify patients at high and low risk of recurrent events. Among the variables analysed, a high discharge BNP level showed the strongest association with death. The proposed score included 8 variables, with 1 point possible for each variable, except for BUN and BNP, for which additional points were assigned for the highest value, with a maximum 13 possible points. The C-index for 6 mo mortality was 0.78 in the derivation data set, but it was reduced to 0.65 in the validation population[30].

CLINICAL APPLICATIONS AND FUTURE DIRECTIONS

The great number of validated prognostic models, each combining different variables, suggests how difficult it is to estimate risk in patients with AHF. Nevertheless, efforts to develop risk models are justified by the evidence that the risk of in-hospital mortality, early post-discharge mortality, and re-hospitalization remains high[31]. Approximately 12%-15% of patients hospitalized for AHF die within 12 wk, and 30% of these patients die within 12 mo of admission[32].

The accurate estimation of risk is essential for proper in-hospital and post-discharge treatment plans and outpatient follow-up. Nevertheless, despite all the proposed prognostic models, the clinical application remains challenging, and clinical scores are not considered part of the standard of care[33].

A major limit of the risk scores approach is that these tools evaluate a "class risk", that is to say, the risk of a cohort of patients sharing common characteristics. In addition, the scores' applicability in evaluating the risk of an individual patient remains elusive. Lemeshow demonstrated that valid predictive models might produce markedly different prognosis for an individual[34], suggesting that they should not be used for individual patient decision making. Due to the great number of prognostic variables, the discordance between prognosis for an individual by different scores might be substantial.

Risk stratification by scoring methods should support rather than replace medical judgment in the clinical decision making process concerning the single patient. Physicians involved in the care of patients with AHF should be familiar with a number of risk scores and should choose the most suitable on the basis of the patient's profile according to the characteristics of the derivation population of the score.

Beyond the evaluation of an individual patient, risk scores are useful tools for managing the process of care, defining diagnostic and therapeutic pathway,

and identifying possible subjects to include in a clinical trial. In patients with chronic advanced heart failure, the Heart Failure Survival Score was able to identify medium- and high-risk patients who benefit from heart transplantation in comparison with a low-risk group in which heart transplantation was not associated with a survival benefit[35]. Currently, no study has evaluated if allocation of patients, driven by risk status according to a predictive model, could improve the clinical outcome in acute heart failure, and currently, no pharmacological intervention has been able to reduce mortality in AHF. Appropriate risk stratification could allow targeting of patients who could benefit from established or new therapies.

Even if the phenotypic heterogeneity of AHF patients makes difficult to find a risk model suitable for all patients, some parameters recur in most of the models. Age, low blood pressure, reduced cardiac performance, low sodium renal concentration due to neurohormonal activation, and decreased renal function are included in most risk models.

Notably, baseline renal dysfunction is a relevant predictor of short and long-term outcome in AHF patients. Worsening renal function, which occurs in 20%-30% of patients hospitalized for AHF, is associated with a poor outcome[36], and the possible role of new therapies for AHF in patients with worsening renal function has recently been investigated[37].

Regarding biomarkers, the role of natriuretic peptides is well-known, and it has a significant prognostic value at both baseline and discharge. Nevertheless, new plasma biomarkers are continuously being identified and validated but have yet to enter in clinical practice[38-41]. In the MOCA trial, biomarkers such as sST2, MR-proADM, natriuretic peptides and CRP provided incremental value for risk stratification of ADHF patients when added to a clinical variables-based model. Further studies are needed to determine if a multi-marker strategy could improve the prognosis and outcome of acute heart failure patients[42].

How to choose a risk score? To choose a risk score, statistical and methodological pertinence should be evaluated. Models have a high grade of evidence when they are derived from large community or registry populations, when they have been validated in an external population, and when they show good discrimination (c-statistic > 0.70) in both derivation and validation cohorts; eventually, adequate calibration is crucial.

Clinicians should be suspicious of risk models derived from clinical trials and that were not validated in an external population and that were not calibrated. Risk models in which in-hospital mortality is the outcome must be used at the time of hospital admission. Obviously, when patients with AHF are admitted to the emergency department, risk stratification based on models with few easily measurable variables is preferred. Risk models that evaluate long-term mortality are useful during hospitalization and at discharge to plan the follow-up or to select patients for advanced therapies.

CONCLUSION

Scores for the risk stratification of AHF patients are useful tools that might support, not replace, clinical judgment and supply a rational approach for prognostication of the individual patient. Further studies are necessary to evaluate if the outcome of patients with acute heart failure can be improved with the use of these tools.

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MINIREVIEWS

Current status of high on-treatment platelet reactivity in patients with coronary or peripheral arterial disease: Mechanisms, evaluation and clinical implications

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Abstract

Antiplatelet therapy with aspirin or clopidogrel or both is the standard care for patients with proven coronary or peripheral arterial disease, especially those undergoing endovascular revascularization procedures. However, despite the administration of the antiplatelet regiments, some patients still experience recurrent cardiovascular ischemic events. So far, it is well documented by several studies that *in vitro* response of platelets may be extremely variable. Poor antiplatelet effect of clopidogrel or high on-treatment platelet reactivity (HTPR) is under investigation by numerous recent studies. This review article focuses on methods used for the *ex vivo* evaluation of HTPR, as well as on the possible underlying mechanisms and the clinical consequences of this entity. Alternative therapeutic options and future directions are also addressed.

Key words: Coronary disease; Clopidogrel; Aspirin; High on treatment platelet reactivity; Peripheral arterial disease; Antiplatelet therapy; Ticagrelor; Prasugrel

Core tip: Recent data related high on-treatment platelet reactivity (HTPR) with adverse clinical outcomes, such as stent thrombosis and repeat procedures, following coronary or peripheral endovascular revascularization procedures. Notably, the incidence of patients suffering from peripheral arterial disease demonstrating inadequate response to clopidogrel is around 50%, which is much higher than the approximately 30% reported for patients suffering from coronary artery disease. Novel more potent antiplatelet P2Y12 agents seem to overcome the phenomenon of HTPR decreasing ischemic events with the cost of increased bleeding risk. Until today no major trial demonstrated clinical improvement for patients undergoing platelet function test-guided individualized antiplatelet therapy. Prescription of new antithrombotic agents aims in avoiding major cardiovascular adverse events, as well as sustaining vessel patency following revascularization. Therefore, improving antiplatelet therapy, considering the risk/benefit ratio, is imperative

especially in HTPR patients. Further large-scale studies are awaited to elucidate the role of individualized therapy.

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INTRODUCTION

Thrombus generation resulting from platelet activation and aggregation is the established main process involved in atherosclerotic vascular disease, including coronary artery disease (CAD) and peripheral arterial disease (PAD)[1,2].

Therefore, antiplatelet therapy has been the cornerstone therapy in patients with documented arterial disease, especially in those undergoing coronary or peripheral percutaneous endovascular procedures[3-5]. However, a variable amount of these patients continue to experience recurrent ischemic events[6,7]. This clinical phenomenon has been correlated with various parameters among which poor antiplatelet effect of clopidogrel or aspirin, described by consensus as high on-treatment platelet reactivity (HTPR) initially identified in patients with CAD[8,9]. HTPR in patients with PAD, especially those undergoing percutaneous peripheral angioplasty (PTA) has recently been documented by several studies[10,11].

This review focuses on the clinical significance of HTPR, the possible mechanisms and the common tests used to measure the phenomenon, as well as future perspectives of novel antiplatelet agents and platelet function-guided antiplatelet therapy.

HTPR DEFINITION

Despite the fact that the optimal method to define HTPR has not been clarified in the literature the clinical challenge of inter-individual variability of the inhibitory effect of antiplatelet agents on platelet function, initially named non-responsiveness or resistance, should be definitely considered as failure of the antiplatelet drug to inhibit its target of action[12]. HTPR has been strongly associated with an increased incidence of major adverse cardiovascular events (MACEs) especially for patients on clopidogrel. Clopidogrel is an adenosine diphosphate (ADP)-receptor antagonist that obstructs platelet activation and aggregation by irreversibly binding to both ADP receptors (P2Y1 and P2Y12)[13]. Therefore, the basic principles of assessing HTPR are to quantify the activity of the target receptor after administration of the antiplatelet agent by using a laboratory method and to determine consensus HTPR cut-off values for various assessment methods[14].

MEASURING HTPR

Numerous tests are available for measuring HTPR. Light transmission aggregometry is the most well established laboratory method for the determination of HTPR. It evaluates the response of the platelet to ADP agonist as an increase in light transmittance measuring as maximal platelet aggregation. However, because it is time and labor intensive, today it is seldom used for monitoring response to clopidogrel[15]. Many other platelet function assays are now available but the most common platelet function tests (PFTs) used in everyday clinical practice are the flow cytometric vasodilator-stimulated phosphoprotein phosphorylation (VASP) analysis and the VerifyNow P2Y12 assay[16].

The VASP assay uses flow cytometry to measure inhibition of VASP phosphorylation by ADP *via* the P2Y12 receptor. The ratio of VASP phosphorylation is indicative for the receptors' activity and reported as platelet reactivity index (PRI). Several studies reported high correlation between high PRI values and recurrent stent thrombosis after percutaneous coronary intervention (PCI)[17]. However, the specific method has been gently criticized for its lack of standardization and therefore the inability of establishing a universal PRI cut-off value[18,19].

The most widely used method of routinely monitoring platelet function is the VerifyNow bedside assay. It is a very practical, rapid and well-standardized point-of-care test that measures platelet-induced aggregation to fibrinogen-coated beads in whole blood in response to an ADP induced stimulus[20,21]. Results are expressed as P2Y12 reaction units (PRU) reflecting P2Y12 mediated platelet reactivity. Published studies using this instrument have demonstrated the relationship between HTPR values and long-term cardiovascular events after PCI[14,22].

Several additional PFTs are also available but rarely used in clinical research: PFA-100, Impedance Aggregometry (Multiplate Analyzer) and whole blood thromboelastography[23-25]. Wisman *et al*[26] in a recent meta-analysis of 59 studies using 15 different tests stated that HTPR was associated with a significant 2.8 times higher risk of MACE. Based on all the available evidence and according to the most recent expert consensus paper issued by the Working Group on Thrombosis of the European Society of Cardiology, the recommended assays for monitoring P2Y12 platelet inhibition are the VerifyNow P2Y12 assay, the Multiplate device with the ADP kit and the VASP assay[27].

HTPR CUT-OFF VALUES

In order to overcome the lack of universally defined cut-off values for the various PFTs for HTPR, Bonello *et al*[14] based on numerous studies using receiver operating characteristic (ROC) established consensus values for HTPR for every major platelet function test: (1) > 46% maximal for a 5-μmol/L ADP-induced aggregation; (2) > 50% PRI using the Platelet VASP test; and (3)

Table 1 Common platelet function assays

Test	LTA	VASP	VerifyNow
Function	Increase in light transmittance	Flow cytometric measurement of VASP phosphorylation	Measurement of platelet-induced aggregation to fibrinogen-coated beads
Receptor	P2Y1 and P2Y12	P2Y12	P2Y12
Results	MPA	PRI	PRU
Cut-off value	> 46%	> 50%	230-240

LTA: Light transmission aggregometry; VASP: Vasodilator-stimulated phosphoprotein phosphorylation; MPA: Maximal platelet aggregation; PRI: Platelet reactivity index; PRU: P2Y12 reaction units.

230-240 P2Y12 reaction units PRU by the VerifyNow P2Y12 assay (Table 1).

However, the majority of the data for this consensus were extrapolated from the coronary studies, given the lack of data from PAD patients. The PRECLOP study, a prospective single-center trial was the first study suggesting the optimal HTPR cut-off value exclusively in patients with PAD using the VerifyNow test[28]. ROC analysis performed in this trial revealed an identical to CAD patients' cut-off value (PRU $\geqslant$ 234; area under the curve 0.883; 95%CI: 0.811-0.954; P = 0.0001).

HTPR MECHANISMS

The antiplatelet effect of clopidogrel is based on the inhibition of platelet aggregation by irreversibly binding to the P2Y12-ADP receptor. It is basically an inactive prodrug that undergoes two consecutive oxidations by the hepatic cytochromes P450 (CYP) to create an active metabolite. This accounts for 15% of the drug metabolism[29]. Multiple potential factors for HTPR have been proposed mainly correlated with distorted activity of cytochrome P450 isoenzymes[30,31].

Genetic factors

Several studies initially documented that poor response to clopidogrel may be greatly heritable[32]. Specifically, genetic polymorphisms to the hepatic CYP450 enzymes, especially to CYP2C19 that is involved in both steps of clopidogrel's biotransformation might disturb the metabolism and therefore the effect of the drug[33]. It has been described that carriers of at least one low function CYP2C19 allele experience a reduction of the active metabolite in plasma up to 32.4% in comparison to healthy gene carriers[34]. The most notorious *2 allele follows an autosomal co-dominant inheritance[35]. Therefore, the highest risk profile group links with those who are homozygous for *2 allele[36]. Latest clinical trials have also suggested that alternative alleles (CYP2C19*3 and *4), as well as polymorphisms in alternative CYP450 enzymes (CYP2C9 and CYP2B6), may also induce HTPR[37]. Another genetic factor responsible for low response to clopidogrel is the ABCB1 gene polymorphisms responsible for reduced enteric absorption of the drug[33]. Notably, the Food and Drug Administration issued a boxed warning on clopidogrel stating that the clinical antiplatelet effectiveness is reduced for poor metabolizers, indicating that genetic tests are available to identify poor metabolizers and highlighting their emerging role in clopidogrel treatment decisions. Nonetheless, genotype accounts for approximately 2% to 12% of inter-individual variability of response to clopidogrel and various demographic and clinical factors largely contribute to the phenomenon[27].

Clinical factors

Beside the genetic background, a major issue in the field of HTPR has been the interaction with other concomitant drugs that are also metabolized by the CYPP450 system. Proton-pump inhibitors, especially omeprazole, were the first class of drugs to be investigated for possible interference with clopidogrel metabolism in early studies. Initial data outlined high incidence of HTPR in patients with CAD after PCI[38]. However, a large randomized control trial investigating clopidogrel with or without concomitant use of omeprazole following PCI revealed no significant difference in terms of MACEs between the two groups[39]. Drug-drug interactions between antiplatelet agents and calcium-channel blockers or statins were also originally reported[40,41] but additional studies demonstrated conflicting findings[42,43]. As a result according to updated guidelines there is no contraindication for the concomitant use of the above mentioned drugs with clopidogrel[27].

On the other hand, clinical entities such as chronic kidney disease (CKD) and diabetes mellitus (DM) seems to be associated with HTPR according to recent studies[44,45]. CKD, an established cardiovascular risk factor, has been recognized as an independent factor of HTPR in patients with CAD[46], while several studies also revealed poor response to clopidogrel and high incidence of stent thrombosis in diabetic patients after PCI, especially those requiring insulin therapy[47]. The possible causes include various pharmacokinetic processes such as the increased platelet turnover and the up-regulation of P2Y12 pathway in these patients[48,49]. Finally, body mass index (BMI) may be another contributing factor to attenuated platelet inhibition. Limited studies reported that overweight patients (BMI > 25 kg/m^2) while on clopidogrel demonstrated reduced antiplatelet effect[50]. However, available data are scarce and further data from larger trials are awaited.

HTPR IN CAD

Numerous studies have demonstrated that the insuffi-

cient response to clopidogrel may lead to adverse clinical outcomes, such as stent thrombosis (acute or subacute) and myocardial infarction. Moreover, recent meta-analysis including thousands of patients treated with PCI either for ST-elevation myocardial infarction (STEMI) or non-STEMI using several platelet function tests reported the correlation between high on-clopidogrel platelet reactivity and MACE, while the incidence of CAD patients detected with HTPR is approximately 35%[26,51].

Müller *et al*[52] published one of the first studies associating poor response to clopidogrel among patients experiencing MACEs after stent implantation in 2003. Successively, Gurbel *et al*[53], in a thorough analysis of the CREST study identified HTPR as a risk factor for stent thrombosis. Subsequently, the possible correlation of the phenomenon with stent thrombosis was investigated by numerous studies[54-56]. The ARMYDA-PRO study (Antiplatelet therapy for Reduction of Myocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) was the first study investigating HTPR by using the user-friendly point-of-care assay VerifyNow and overcoming technical limitations of previous traditional platelet function methods[57]. The authors supported the concept of bedside monitoring platelet inhibition in clinical practice by proving a strong correlation between HTPR and MACEs at 30-d follow-up after PCI. These findings were amplified by latter similar studies with longer follow-up periods. Price *et al*[58] documented HTPR as a risk factor for cardiac death and stent thrombosis after drug eluting stent (DES) implantation at 6 mo follow-up. The authors also noted the perspective of modifying the antiplatelet regimen.

Of note, all the previously mentioned studies reported the correlation between high on-clopidogrel platelet reactivity and MACE, while until today there are no data indicating an analogous correlation between response to aspirin and stent thrombosis or adverse clinical events[27]. Notably, in 2014 the French VERIFRENCHY, multi-center, prospective trial published data regarding the prognostic value of testing antiplatelet response to clopidogrel and aspirin with the VerifyNow assay, in an intermediate-risk population (1.001 patients) undergoing elective stent implantation due to stable coronary disease or non-ST-segment elevation acute coronary syndrome. Overall 36.0% and 8.6% of the patients demonstrated HTPR to clopidogrel or aspirin, respectively. According to one year results, although ischemic events were numerically more in patients with high on-clopidogrel platelet reactivity (composite endpoint 3.9% of *vs* 2.3% and definite or probable stent thrombosis: 1.1% *vs* 0.3%) results did not reach statistical significance, while there was no difference in rates of major bleeding. In patients receiving aspirin there was also no significant difference in ischemic endpoints[59]. These results indicate that either HTPR may not affect clinical outcomes or that it is difficult to statistically prove the role of HTPR in populations at low to intermediate risk of stent thrombosis, due to the low number of ischemic events.

HTPR in PAD

Contrary to CAD, there is a lack of high quality evidence demonstrating the possible correlation between HTPR and adverse clinical events in patients with PAD undergoing peripheral endovascular procedures. The MIRROR single-blinded, single-center, randomized controlled trial was the first to report the existence of low response to clopidogrel in patients undergoing PTA[11]. The authors alongside with the clinical superiority of dual antiplatelet therapy following femoropopliteal angioplasty or stenting, also reported a 30% HTPR rate, similar to that identified in coronary studies. Subsequently, Pastromas *et al*[60] in a retrospective audit of 113 patients treated with clopidogrel after angioplasty or stenting, noticed an even higher HTPR incidence rate (approximately 54%). The authors speculated that this difference was mainly driven by high comorbidity rates and advanced arterial disease characteristic in critical limb ischemia (CLI) cohorts. The specific study also originally associated HTPR with significantly higher re-intervention rates. In American College of Cardiology (ACC) 2012, Kliger *et al*[61] presented the results from a study investigating responsiveness in patients undergoing PCI or PTA, which also detected a higher HTPR incidence in PAD patients.

Following these initial results, Spiliopoulos *et al*[28] further investigated the phenomenon in the PRECLOP study (NCT01744613) and established the optimal cut-off value for HTPR in PAD patients using the VerifyNow assay (PRU $\geqslant$ 234). In total 100 patients were screened with the VerifyNow assay and were stratified according to PRU values in four quartiles. The study's primary endpoint was the 1-year composite of cardiovascular death, major amputation and re-intervention events. Results revealed patients with HTPR demonstrated a less than 40% event-free survival at 1-year, while an approximately 90% event-free survival at 1 year was noted in patients with an adequate response to clopidogrel. Moreover, high on-clopidogrel platelet reactivity was identified as an independent predictor of increased events (mainly repeat revascularization procedures; HR = 16.9; 95%CI: 5-55; $P = 0.0001$). The incidence of HTPR was 51%, considerably higher than that reported in CAD trials, and was again correlated to CLI, DM and chronic kidney disease[28].

High on-aspirin platelet reactivity has been also investigated by several authors and its incidence has be reported to range between 4%-40%, a variability attributed to the multiplicity of methods used and the small sample studied. Moreover, Karnabatidis *et al*[62] and Spiliopoulos *et al*[63] reported that nearly 12% of PAD patients on dual antiplatelet therapy, demonstrated HTPR for both clopidogrel and aspirin. However, the clinical implication of low response to aspirin remains controversial and more data are needed.

NOVEL ANTIPLATELET AGENTS

Recently, novel and stronger antiplatelet agents, such as prasugrel and ticagrelor, have been introduced in

everyday clinical practice in patients suffering from acute coronary syndrome (ACS) undergoing PCI[64,65].

Prasugrel, a third generation thienopyridine agent is also a prodrug that requires metabolism before its active metabolite will bind to ADP receptor and inhibits platelet aggregation. The PRINCIPLE-TIMI 44 trial proved that prasugrel promotes platelet inhibition more rapidly and effectively in comparison with clopidogrel, showing that the degree of inhibition of platelet aggregation achieved with prasugrel within 30 min after treatment is comparable to the peak effect of clopidogrel 6 h after administration[66].

The first trial dedicated to the clinical outcomes of prasugrel was the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel-Thrombolysis in Myocardial Infarction (TRITON-TIMI) 38[67]. Over 13000 patients with ACS receiving prasugrel or clopidogrel scheduled for endovascular treatment were enrolled in this large multi center trial. The results demonstrated a significant reduction in the rate of periprocedural myocardial infarction and stent thrombosis. However, high incidence of major bleeding in some patients of the subgroup receiving prasugrel was noted. The authors concluded that prasugrel reduces the rate of recurrent ischemic events compared with clopidogrel, but with a significantly higher bleeding risk. Based on these results, the 2011 updated ACC/American Heart Association guidelines do not recommend the use of prasugrel in patients > 75 years old, or weight < 60 kg (with a recommended decreased dose of 5 mg), history of stroke or pathologic active bleed[68]. Moreover, Bonello *et al*[69] investigating the clinical effect of prasugrel in CAD patients after PCI identified a persistent high rate of HTPR (approximately 25%) correlated with high incidence of MACEs at 30 d follow-up.

Ticagrelor, in contrast to clopidogrel, binds reversibly to the P2Y12 receptor and therefore prevents binding of ADP. Another major advantage of this novel P2Y12 antagonist is that it does not require metabolic activation, in order to exert its effect.

The DISPERSE-2 trial examined the effect of ticagrelor *vs* clopidogrel in non-STEMI patients with ACS and documented higher rate of platelet inhibition in the subgroup of the patients' cohort receiving ticagrelor[70]. Following these results, the PLATO (Platelet Inhibition and Patient Outcomes) multi-center, randomized, controlled trial compared the clinical outcomes of loading doses of ticagrelor *vs* clopidogrel in patients with ACS admitted to the hospital for prevention of cardiovascular death[71]. The results demonstrated significantly less incidence of the primary endpoint (time of occurrence of CV death, MI or stroke) in ticagrelor group than in clopidogrel group. Furthermore, the rates of major bleeding were not significantly different between the two groups. Nevertheless, after carefully analyzing bleeding events ticagrelor was associated with an increase in combined major and minor PLATO bleeding rates by 11% ($P = 0.008$)[72].

PFT-GUIDED INDIVIDUALIZED ANTIPLATELET THERAPY

Given the possibility to measure the response to clopidogrel and to use alternative antiplatelet agents in selected patients, investigators began to investigate PFT-guided antiplatelet protocols. The GRAVITAS study, a multicenter randomized double blind control trial, investigated the effect of high-dose *vs* standard-dose clopidogrel using the VerifyNow assay to identify HTPR in 2.214 patients undergoing PCI. Patients with HTPR were given high-dose platelet (600 mg loading dose and 150 mg daily doses) *vs* the standard-dose (300 mg loading dose and 75 mg daily doses). The study showed that although double-dose clopidogrel significantly reduced - but not completely abolished-HTPR, it failed to reduce MACEs at 6 mo follow-up. Specifically, HTPR was reduced by only 22% at one month[73]. This observation was further demonstrated by Alexopoulos *et al*[74] reporting that although double clopidogrel dose further inhibits platelet reactivity compared to standard dose, 35.8% of the patients under double dose remained non-responders, while for HTPR patients switching to prasugrel the percentage of non-responders was reduced to 7.0% ($P < 0.0001$).

However, two recent multi-center randomized controlled trials failed to demonstrate a clinical benefit PFT-guided antiplatelet therapy in CAD patients. The testing platelet reactivity in patients undergoing elective stent placement on clopidogrel to guide alternative therapy with prasugrel (TRIGGER-PCI) compared HTPR patients (PRU > 208) with stable CAD receiving prasugrel or clopidogrel following PCI using DES the study was prematurely terminated as the primary endpoint of death or MI at 6 mo occurred only in one patient of the clopidogrel group. The authors concluded that although prasugrel significantly reduced HTPR (mean PRU values from 245 to 80 at 3 mo) the small incidence of adverse events in elective DES procedures would not allow to prove the effectiveness of PFT-guided antiplatelet therapy[75].

The ARTIC trial compared conventional (1227 patients) *vs* PTF-guided (1213 patients) antiplatelet therapy after PCI for the composite endpoint of cardiovascular death, MI, stent thrombosis stroke and revascularization at one year follow-up. In total 37% of the patients suffered a non-ST-segment elevation acute coronary syndrome (NSTE-ACS), while the remaining had severe stable CAD. In the conventional therapy group 6% of the patients received prasugrel and only 12% in the PFT-guided group, while in the rest of non-responders double clopidogrel dose was used to treat HTPR. There was no significant difference in the primary outcome or bleeding events between the two study groups at one year follow-up[76] (Table 2). Nevertheless, the fact that the vast majority of the patients were offered double clopidogrel dose to overcome HTPR, a strategy previously reported as less effective compared

Table 2 Highlighted multicenter randomized control trials investigating platelet function tests-guided antiplatelet therapy

Study	Gravitas	Arctic	Trigger-PCI
Study population (*n*)	2214	2440	423
PFT assay	VerifyNow	VerifyNow	VerifyNow
High-dose clopidogrel	100%	80%	-
High-dose Aspirin	-	45%	-
Prasugrel	-	12%	100%
Results (primary endpoint)	2.3% *vs* 2.3%	31.1% *vs* 34.6%	0.0% *vs* 0.5%

PFT: Platelet function tests; PCI: Percutaneous coronary intervention.

to switching to novel antiplatelet agents (prasugrel or ticagrelor) in overcoming HTPR, as well as the small percentage of patients with ACS enrolled, probably negatively influenced outcomes in the PFT-guided group.

Finally, Aradi *et al*[77], conducted a meta-analysis to investigate the safety and efficacy of tailored antiplatelet therapy based on platelet reactivity testing in patients after PCI. The authors included 10 randomized controlled trials (5 multi-center, 2 double-center and 3 single-center) with a total of 4213 patients and concluded that PFT-guided intensified antiplatelet therapy was associated with decreased cardiovascular mortality and stent thrombosis. Nevertheless, the authors emphasized that the net benefit of personalized antiplatelet therapy depends on the risk of stent thrombosis and should be applied in patients at high risk. This extremely significant observation may also explain the early termination of the TRIGGER-PCI trial, where no stent thrombosis occurred in more than 400 HTPR patients, the results of the French VERIFRENCHY trial investigating patients of intermediate risk presenting with stable CAD and NSTE-ACS, as well as the negative results of the ARTIC trial where less than half of the patients suffered from ACS[78]. Of note, the ARTIC study was not included in this meta-analysis.

In the PAD arena, data about the clinical efficacy of novel antiplatelet agents and PFT-guided antiplatelet therapy modification are scarce. Torngren *et al*[78] in a study including PAD patients receiving ticagrelor because of previous ACS reported enhanced peripheral endothelial function compared to clopidogrel or prasugrel, while in a recently published post hoc analysis of the PLATO trial involving 1.144 patients with peripheral arterial disease, ticagrelor reduced the rate of cardiovascular death and MI to 16.7% compared to 21.5% in the clopidogrel group (P = 0.045)[79]. Spiliopoulos *et al*[80], recently published a study observing the clinical effect ticagrelor in 37 consecutive HTPR patients suffering from CLI undergoing angioplasty or stenting of complex lesions (long occlusions, advanced infrapopliteal disease). According to this initial experience switching therapy from clopidogrel to ticagrelor managed to overcome HTPR in all patients with documented increased platelet aggregation (PRU $\geqslant$ 234). Specifically, mean PRU during clopidogrel therapy (308.4 ± 41.8) was significantly reduced when switched to ticagrelor (67.0 ± 52.8; P < 0.0001). This was accompanied with very satisfactory clinical outcomes for the specific CLI cohort where major amputation can usually reach 25% at one year. Kaplan-Meier analysis estimated that the one-year primary composite endpoint of event-free survival was 92.0%, while revascularization-free survival rate was 67.3% at one year follow-up.

Currently, a global, multi-center, double blind, randomized, controlled, trial involving 900 sites in 25 countries (EUCLID trial; sponsored by AstraZeneca), enrolled approximately 13500 symptomatic PAD patients in order to investigate the safety and efficacy of ticagrelor *vs* clopidogrel. Primary outcome measures will be cardiovascular death, MI and ischemic stroke and results are expected within 2016. The authors speculate that individualized therapy using PTF as to identify CAD or PAD patients on increased ischemic or bleeding risk, will gradually earn its way in everyday clinical practice as long as future well-designed large-scale trials demonstrate its utility. Novel antiplatelet agents should be prescribed with consciousness as they have been related with increased bleeding events.

CONCLUSION

Following the CAPRIE trial in which clopidogrel achieved a further 24% relative risk reduction and 0.51% per year absolute risk reduction (P = 0.043) in major cardiovascular events compared to aspirin in symptomatic PAD patients, its use in every day clinical practice has been remarkably increased over the years[81]. It is generally a safe and effective drug commonly combined with aspirin, in selected patients undergoing coronary or peripheral revascularization procedures, to prevent cardiovascular ischemic events. However, a notable percentage of vascular patients present poor response to traditional antiplatelet therapy. High on-clopidogrel platelet reactivity, a clinical entity has recently emerged in the ambit of coronary and peripheral arterial disease seems to negatively affect clinical outcomes and certainly merits further investigation. The same phenomenon of low response to aspirin has been also described, however until today its clinical significance remains unproven. As modern clinical practice can support the routine use of platelet monitoring, given the fact that today platelet function tests are user-friendly, accurate and affordable in the immediate future personalized antiplatelet therapy could become a safe and efficient option in patients with low response to clopidogrel. Nonetheless, the potential risk of bleeding should always be under concern, especially in patients at high hemorrhagic risk. Consideration of the individual's genetic profile could also be an appropriate tool regarding tailored antiplatelet therapy. However, it is a fact that until today the benefit of PFT-guided personalized therapy in clinical outcomes remains to be determined and more data from meticulously designed trials are necessary.

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ORIGINAL ARTICLE

Prospective Study

Comparison of partners-heart failure algorithm *vs* care alert in remote heart failure management

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Abstract

AIM: To compare the utility of the partners-heart failure (HF) algorithm with the care alert strategy for remote monitoring, in guiding clinical actions oriented to treat impending HF.

METHODS: Consecutive cardiac resynchronization-defibrillator recipients were followed with biweekly automatic transmissions. After every transmission, patients received a phone contact in order to check their health status, eventually followed by clinical actions, classified as "no-action", "non-active" and "active". Active clinical actions were oriented to treat impending HF. The sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of the partners-HF algorithm *vs* care alert in determining active clinical actions oriented to treat pre-HF status

and to prevent an acute decompensation, were also calculated.

RESULTS: The study population included 70 patients with moderate to advanced systolic HF and QRS duration longer than 120 ms. During a mean follow-up of 8 ± 2 mo, 665 transmissions were collected. No deaths or HF hospitalizations occurred. The sensitivity and specificity of the partners-HF algorithm for active clinical actions oriented to treat impending HF were 96.9% (95%CI: 0.96-0.98) and 92.5% (95%CI: 0.90-0.94) respectively. The positive and negative predictive values were 84.6% (95%CI: 0.82-0.87) and 98.6% (95%CI: 0.98-0.99) respectively. The partners-HF algorithm had an accuracy of 93.8% (95%CI: 0.92-0.96) in determining active clinical actions. With regard to active clinical actions, care alert had a sensitivity and specificity of 11.05% (95%CI: 0.09-0.13) and 93.6% respectively (95%CI: 0.92-0.95). The positive predictive value was 42.3% (95%CI: 0.38-0.46); the negative predictive value was 71.1% (95%CI: 0.68-0.74). Care alert had an accuracy of 68.9% (95%CI: 0.65-0.72) in determining active clinical actions.

CONCLUSION: The partners-HF algorithm proved higher accuracy and sensitivity than care alert in determining active clinical actions oriented to treat impending HF. Future studies in larger populations should evaluate partners-HF ability to improve HF-related clinical outcomes.

Key words: Heart failure; Cardiac resynchronization therapy; Defibrillators; Remote monitoring

Core tip: This is a multicenter observational registry that compared the utility of the partners-heart failure (HF) algorithm with the care alert strategy for remote monitoring, in guiding clinical actions oriented to treat impending HF in a population of 70 cardiac resynchronization therapy recipients followed over a mean follow-up period of 8 ± 2 mo. The partners-HF algorithm displayed high sensitivity (96.9%), specificity (92.5%), positive (84.6%) and negative (98.6%) predictive values for active clinical actions oriented to treat impending HF. The care alert exhibited lower sensitivity (11.1%), positive (42.3%) and negative (71.1%) predictive values.

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INTRODUCTION

Heart failure (HF) is a primary public health problem, with mortality and hospitalization rates of approximately 7.2% and 31.9% at one-year respectively[1]. Outpatient management, symptoms and daily weight often do not identify patients in time to prevent imminent HF. Modern implantable cardiac resynchronization therapy-defibrillators (CRT-D) with remote monitoring (RM) capabilities, continuously assess parameters, including heart rate, patient's activity (PA), intra-thoracic impedance, atrial fibrillation (AF), ventricular arrhythmias (VA), shock therapy delivered and system integrity[2-5].

Previous studies have demonstrated the ability of individual device diagnostic data, to predict HF events, to reduce the time from clinical event to treatment, length of hospitalization and quantity of in-office visits[6-10].

Earlier studies have shown that implantable device-measured parameters, such as intra-thoracic impedance, AF burden, mean heart rate, heart rate variability (HRV), patient activity (PA), frequency of premature ventricular contractions (PVCs), VA episodes, implantable cardioverter defibrillators (ICD) shocks and percentage of pacing of cardiac resynchronization therapy (%CRT), individuate subjects at risk of HF and facilitate early interventions[6-12]. Variations of intrathoracic impedance[3] as well as HRV and PA[2] occurs nearly two weeks before HF exacerbation. Low HRV indicate a sympathetic dominance in cardiac autonomic control and may be associated with exacerbation of atrial and VAs[13]. A prolonged AF duration, a rapid ventricular rate (VR) during AF and an increase in the burden of PVCs reduce %CRT[14] and are warning signs of HF, together with ICD shocks[15].

Each HF device diagnostic parameter, although validated in various studies, has several limitations. A previous study[16] showed that sensitivity values of individual parameters, ranged from 23.6% to 50.0%, whereas their combination displayed 65.4% sensitivity and 99.5% specificity for cardiovascular hospitalizations and deaths.

The partners-HF[11] is the largest cohort study to have evaluated the ability of combined HF device diagnostics, including Optivol™ Fluid index, AF duration, rapid VR during AF, low PA, high nocturnal heart rate (NHR), low HRV, low CRT pacing percentage, and ICD shocks, to identify patients at risk of acute HF in the subsequent 30 d. The retrospective analysis of the prospectively collected data of the partners-HF study, demonstrated that subjects with a positive partners-HF algorithm were at a greater risk (HR = 5.5; $P < 0.001$) of HF hospitalization during the next month.

The purpose of this multicenter, observational registry was to prospectively assess the utility of the partners-HF criteria, implemented within Discovery Link™, in guiding clinical actions oriented to treat pre-HF status and to prevent an acute decompensation in a population of HF individuals implanted with a Medtronic CRT-D device.

MATERIALS AND METHODS

Registry population and design

This study has been approved by our Institutional Review Board and is conform to the guiding principles of the Declaration of Helsinki.

Consecutive CRT-D candidates were enrolled by three Italian cardiology centers. The clinical status of the patients, including NYHA class, was initially assessed by the cardiologists involved in the project. All patients underwent implantation of a Medtronic CRT-D system (Model: Consulta™, Concerto™ Ⅱ, VIVA XT™, PROTECTA XT™; Medtronic Inc., Minnesota) equipped with the CareLink Medtronic®-RM system for RM.

Inclusion criteria were: Left ventricular ejection fraction ⩽ 35% + NYHA class Ⅱ, Ⅲ and ambulatory Ⅳ and broad QRS (> 120 ms if left bundle branch block was present, or otherwise > 150 ms + optimal pharmacological treatment for HF). Exclusion criteria were: acute coronary syndrome within 40 d, coronary artery revascularization within 3 mo, end-stage HF requiring inotropic support, ventricular assist devices or dialysis.

Each patient received a wireless CareLink Monitor which provided automatic transmission of clinical and technical parameters stored in the implanted device's memory to a Service Center where information was decrypted, uploaded to a secure website and periodically accessed by the nurses. Patients were followed up for at least 6 mo. Data were prospectively collected between January 2012 and October 2012 and classified on the basis of both the care alert and the partners-HF algorithms at the same time. Automatic "scheduled" transmissions were programmed every 15 d. "Care alert"-triggered transmissions and transmissions activated manually by the patients were also collected. Patients were instructed to manually activate transmissions in case of occurrence or exacerbation of HF-related symptoms (including shortness of breath, dyspnea, orthopnea, asthenia, pre-syncope or syncope) or signs (including weight increase, peripheral edema enlargement).

The project, including data collection, was approved by the Hospital Ethics Committees of each cardiology center involved in the registry using the Medtronic Clinical Service Project®, and every individual enrolled gave written informed consent to enrolment in the registry.

Partners-HF algorithm

The partners-HF application, based on the algorithm described by Whellan *et al*[11], was implemented within the Discovery Link™. The latter is a web environment enabling elaborated and aggregated information from the Medtronic CareLink Network® to be shown in interactive JavaScript charts. The partners-HF algorithm was adopted to process information in order to select the last transmission (including both manually and automatically triggered ones) from each device and to perform analysis. Statistics were calculated over the last 28 consecutive days[11]. Every two weeks, the first partners-HF profile of those patients who satisfied the partners-HF criteria was directly logged into the Discovery Link.

The partners-HF algorithm was considered positive in the following cases: Optivol™ Fluid index ⩾ 100 or any 2 of the following criteria met during a one-month period of evaluation: Long AF duration, rapid VR during AF, Optivol™ fluid index ⩾ 60, low PA, high nocturnal NHR, low HRV, low %CRT, and ICD shocks (Appendix).

Care alert

The Carelink system automatically triggered alerts in case of shocks delivered or if the following clinical and technical parameters exceed a programmable threshold: OptiVol™ Fluid Monitoring Index (> 60), AF duration (> 24 h), VR rate during AF (> 100 bpm), lead impedance, integrity and battery voltage alert (out of predefined range).

Adjudication of impending heart failure and classification of clinical actions

After every transmission, all patients received a phone contact and their health status was checked by nurses experienced in HF. At time of enrollment, patients were instructed to measure frequently their body weight and to check their pulse in order to identify HF-related signs (increase of heart rate, weight and/or peripheral edema) and symptoms (increase in shortness of breath, cough and/or asthenia, reduction of exercise tolerance, needing use of extra pillows during the night). Data on vital status, symptoms, quality of life, adherence to pharmacological treatment, hospitalizations and mortality were collected by nurses at every phone contact. Pre-specified boundaries for weight, blood pressure, pulse and symptoms were previously established for every patient. Adjudication of impending HF was based on the development of early HF-related signs and symptoms (see above) and on the exceeding from the prespecified boundaries, but still not requiring hospitalization[12]. RM transmissions suggestive of worsening HF or device malfunctioning were submitted to physicians.

Clinical actions performed as a result of transmissions, according to each center's clinical practice, were registered on a Medtronic Clinical Service®-form and were classified as follows: "no-action", "non-active" and "active". No action: (1) consisted on telephonic contact; (2) non-active clinical action; (3) consisted on clinical examination without pharmacological treatment modification (PTM). Active clinical actions included PTM during telephonic contact; or (4) during clinical examination. In the event of manual or care alert transmissions, physicians could decide either to undertake clinical action immediately or to wait until the first partners-HF data from those specific transmissions

Table 1 Study population

Clinical characteristics of the study population	
Age (yr)	70.3 ± 8.3
Male (%)	78.3
EF (%)	27.5 ± 6.5
Etiology post-ischemic DC (%)	63.2
Idiopathic DC (%)	33.7
Valvular DC (%)	2
Congenital DC (%)	1.1
NYHA Ⅱ (%)	17.3
Ⅲ (%)	78.6
Ⅳ (%)	4.1
Optimized pharmacological treatment (%)	63.2[1]
Prevention: Primary (%)	73.7
Secondary (%)	18.3
SVT (%)	3.7
Syncope (%)	3.2
Cardiac arrest (%)	5.1
AF permanent (%)	16.3
Persistent (%)	7.4
Paroxysmal (%)	4.1
Devices: Consulta™ CRT-D	37.4
Concerto™ Ⅱ CRT-D (%)	26.3
Viva XT™ CRT-D (%)	22.1
Protecta XT™ CRT-D (%)	14.2

[1]The low percentage of optimized pharmacological treatment is related to reduce aldosterone antagonists administration in patients affected by chronic kidney disease. EF: Ejection fraction; DC: Dilated cardiomyopathy; NYHA: New York Heart Association; SVT: Sustained ventricular tachycardia; AF: Atrial fibrillation; CRT-D: Cardiac resynchronization therapy-defibrillator.

became available in the Discovery Link environment.

Study endpoints

The aim of this study was to determine the sensitivity, specificity, positive and negative predictive values and diagnostic accuracy of the partners-HF algorithm and of care alert in determining active clinical actions oriented to treat pre-HF status and to prevent an acute decompensation. Analyses of sensitivity, specificity, predictivity and accuracy were performed with respect to overall active clinical actions (3 + 2) *vs* the sum of clinical actions and no actions (1 + 0).

Statistical analysis

Continuous variables are summarized as mean ± SD and categorical variables as counts and percentages. Positive transmissions by the partners-HF algorithm and/or care alert were considered true positive when they were associated with acute HF and/or with pharmacological treatment modification due to impending HF. Positive transmissions by the partners-HF algorithm and/or care alert were considered as false positive in the remaining cases. Negative transmissions by the partners-HF algorithm and/or care alert were considered true negative when they were not associated to acute HF or PTM due to pre-HF (see above), and as false negative when they were not. The sensitivity of the partners-HF algorithm and of care alert was calculated as the ratio between the number of true positive transmissions and the sum of true positive and false negative transmissions. Specificity was calculated as the ratio between true negatives and the sum of true negatives and false positives. Positive predictive value was calculated as the ratio between true positives and the sum of true positives and false positives. Negative predictive value was calculated as the ratio between true negatives and the sum of true negatives and false negatives. Accuracy was calculated as the ratio between the sum of true positive and true negative transmissions and total transmissions. All the tests were performed by means of R 2.11.1 for Windows.

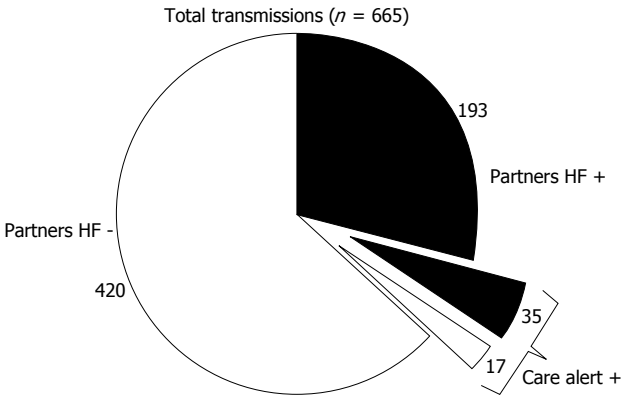

Figure 1 Distribution of total transmissions. Legend total transmissions are depicted in a pie-chart; positive partners-HF transmissions are shown in black; negative partners-HF transmissions are shown in white. Care alert transmissions are represented in separate slices. HF: Heart failure.

RESULTS

Study population

The characteristics of the study population are presented in Table 1. Patients were predominantly males and had mostly a moderate to advanced HF. All patients had QRS duration longer than 120 ms. The relatively low (63.2) percentage of optimized pharmacological treatment is due to reduced aldosterone antagonists administration in patients affected by chronic kidney disease.

Transmissions

During a mean follow-up of 8 ± 2 mo, 665 transmissions were received from 70 patients. Transmissions were classified as follows: 52 (7.8%) care alert, 149 (22.4%) manual and 464 (69.8%) scheduled.

Of all transmissions, 228 (34.3%) fulfilled the partners-HF criteria. Positive partners-HF transmissions were classified as: scheduled (136; 59.6%), manual (57; 25%), and care alert (35; 15.4%). Of the 437 negative partners-HF transmissions, 328 (75.1%) were scheduled, 92 (21%) were manual and 17 (3.9%) were triggered by a care alert. Figure 1 shows the distribution of partners-HF positive and negative transmissions, contemporarily triggered or not by care alert. Overall, the "care alert" transmissions met the partners-HF criteria in 67.3% of cases (Figure 1).

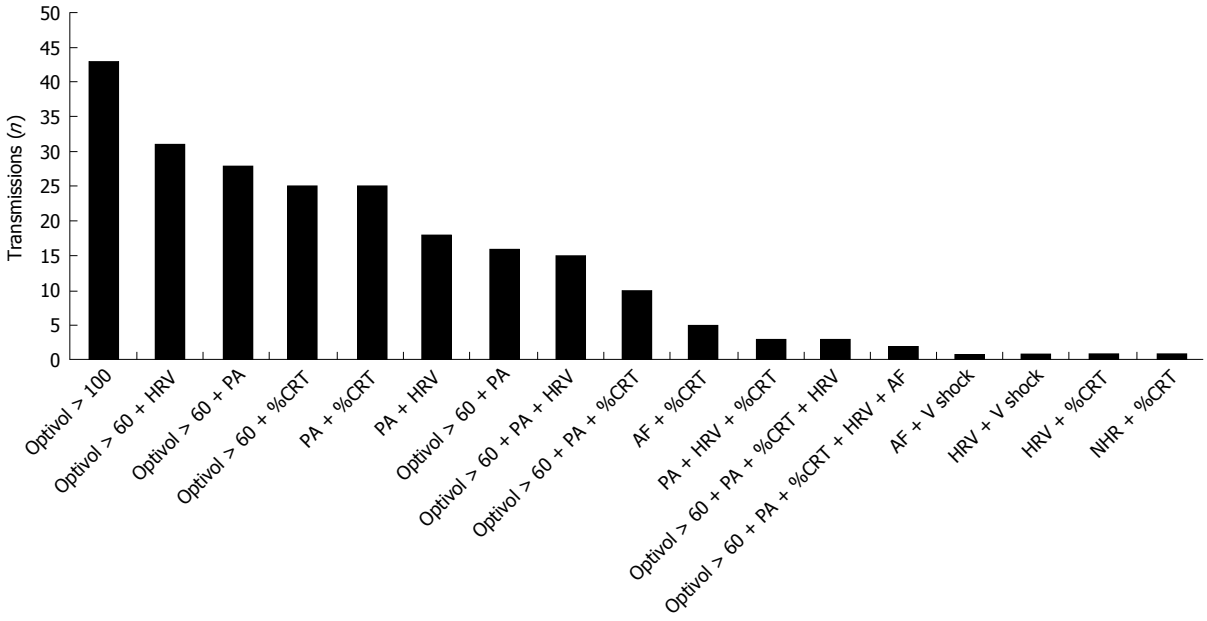

Figure 2 Positive partners-heart failure transmissions. HRV: Heart rate variability; PA: Patient activity; %CRT: Percentage of cardiac resynchronization therapy pacing; AF: Atrial fibrillation; V shock: Ventricular shock; NHR: Night heart rate.

Table 2 Pharmacological treatment modifications following positive or negative partners-heart failure transmissions and care alert

Active clinical actions	Partners-HF +	Care alert +
Pharmacological treatment modification during telephonic contact		
Diuretic dosage increase	120	25
BB dosage increase	57	22
AAD administration	2	2
ACE-I and ARA dosage increase	4	0
OAC administration	5	5
Clinical examination and pharmacological treatment modification		
Diuretic dosage increase	9	4
BB dosage increase	8	3
AAD administration	1	1
ACE-I and ARA dosage increase	1	1
Anti-platelet administration	0	0

+: Positive; BB: Beta-blockers; AAD: Anti-arrhythmic drugs; ACE-I: Angiotensin-converting-enzyme inhibitors; ARA: Angiotensin Ⅱ-receptor antagonists; OAC: Oral anti-coagulant; HF: Heart failure.

The most common reasons triggering a positive partners-HF transmission: Optivol fluid index ≥ 100 (18.8%) or optivol fluid index > 60 plus one of the following parameters: reduced HRV (13.6%), low PA (12.3%), or reduced %CRT (11%) (Figure 2). The 52 "care alert"-triggered transmissions (35 partners-HF positive and 17 partners-HF negative) were generated by OptiVol fluid index in 43 (82.7%) cases, AF duration and/or AF VR in 7 (13.5%) cases and shock for VAs in 2 (3.8%) cases.

Clinical actions following transmissions

During follow-up, no deaths or HF hospitalizations occurred. Of overall transmissions, 16 (2.4%) were followed by clinical examination and PTM, 183 (27.5%) by PTM during telephonic contact and 7 (1%) by clinical examination without PTM.

Of the 228 positive partners-HF transmissions, 11 (4.8%) were followed by clinical examination and PTM, 182 (79.8%) by PTM during telephonic contact and 6 (2.7%) by clinical examination without PTM (Figure 3A). PTM consisted of 19 drug dosage up-titrations and/or new treatment administrations during clinical examination and 188 during telephonic contact (Table 2). No pharmacological down titration was done.

Of the 437 negative partners-HF transmissions, 5 (1.1%) were followed by clinical examination and PTM, 1 (0.2%) by PTM during telephonic contact and 1 (0.2%) by clinical examination without PTM (Figure 3B). PTM consisted of 7 drug dosage up-titrations and/or new treatment administrations during clinical examination and 2 telephonic PTM, made as a consequence of a single care alert transmission. In these cases, diuretic and beta-blocker dosages were increased; no pharmacological down titration was reported.

Of the 52 care alert transmissions, 4 (7.7%) were followed by clinical examination and PTM and 18 (34.6%) by PTM during telephonic contact (Figure 3C). PTM consisted of 9 drug dosage up-titrations and/or new treatment administrations during clinical examination and 54 without in-office clinical examinations (Table 2).

Clinical actions following negative care alert transmissions consisted of 12 (1.9%) clinical examinations and PTM, 165 (27%) PTM during telephonic contact, 7 (1.1%) clinical examinations without PTM and 429 (70%) telephone contacts alone. PTM consisted of 17 drug dosage up-titrations and/or new treatment administrations during clinical examination and 136 without in-office clinical examinations.

Diagnostic accuracy of partners-HF and care alert

True positive, true negative, false positive and false

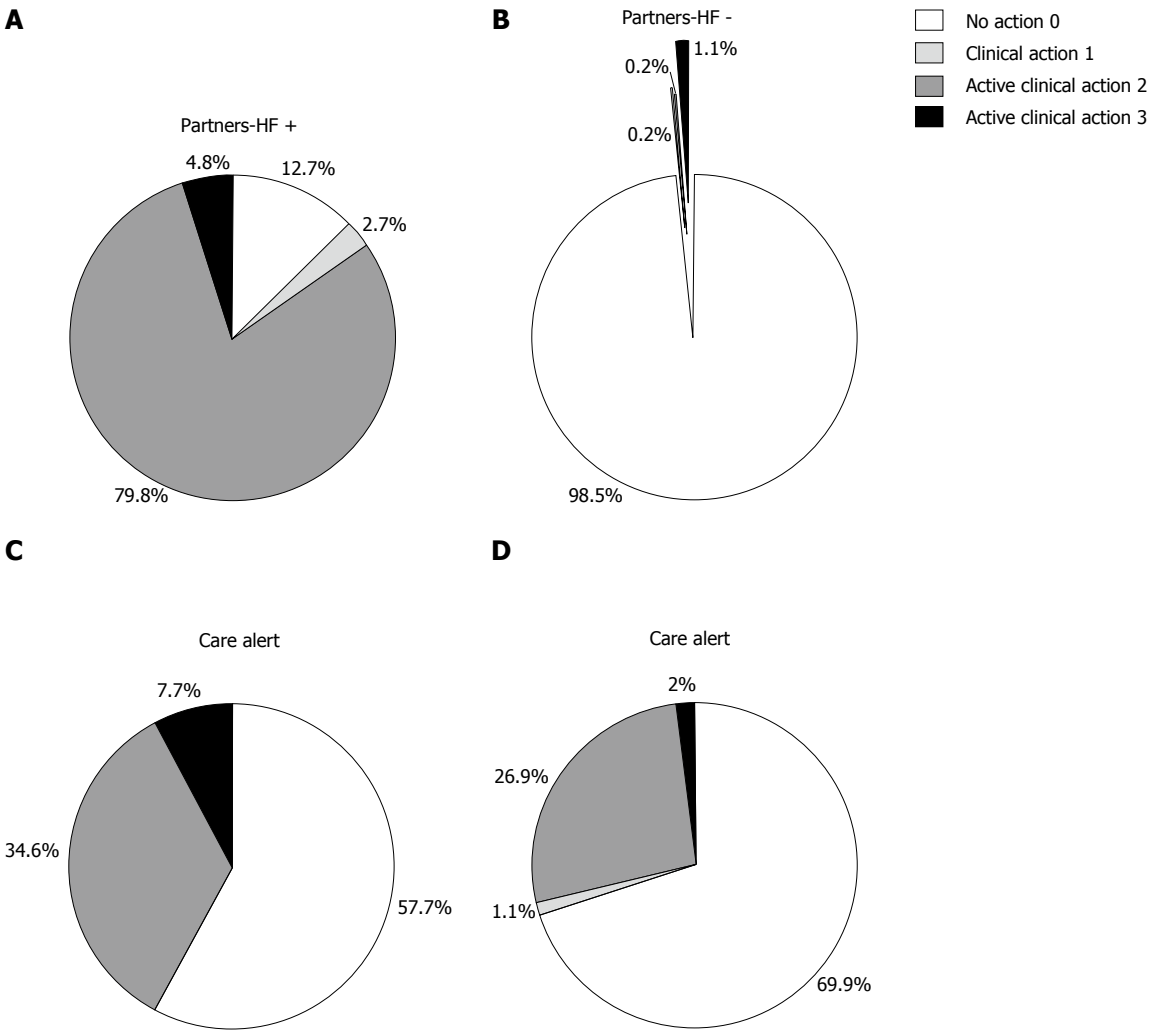

Figure 3 Clinical actions following positive partners-heart failure, negative partners-heart failure, positive care alert and negative care alert transmissions. No actions are depicted in white; clinical actions are depicted in light gray; active clinical actions are depicted in dark gray (pharmacological treatment modifications during telephonic contacts) and black (clinical examination and pharmacological treatment modifications). HF: Heart failure.

negative partners-HF transmissions with respect to active clinical actions are depicted in Figure 4A. The sensitivity and specificity of the partners-HF algorithm for active clinical actions (classes 2-3) were 96.9% (95%CI: 0.96-0.98) and 92.5% (95%CI: 0.90-0.94) respectively (Table 3). The positive and negative predictive values were 84.6% (95%CI: 0.82-0.87) and 98.6% (95%CI: 0.98-0.99) respectively. The partners-HF algorithm had an accuracy of 93.8% (95%CI: 0.92-0.96) in determining active clinical actions (Table 3).

Care alert true positive, true negative, false positive and false negative transmissions with respect to active clinical actions are depicted in Figure 4B. With regard to active clinical actions (classes 2-3), care alert had a sensitivity and specificity of 11.05% (95%CI: 0.09-0.13) and 93.6% respectively (95%CI: 0.92-0.95). The positive predictive value was 42.3% (95%CI: 0.38-0.46); the negative predictive value was 71.1% (95%CI: 0.68-0.74). Care alert had an accuracy of 68.9% (95%CI: 0.65-0.72) in determining active clinical actions (Table 3).

DISCUSSION

Main findings

In this registry we observed that: (1) The partners-HF algorithm has high sensitivity (96.9%), specificity (92.5%) and diagnostic accuracy (93.8%) in identifying patients with early HF-related symptoms and signs (pre-HF), at risk of acute HF, who benefit from active clinical actions; (2) The care alert displays good specificity (93.5%) but very low sensitivity (11.1%) in identifying patients with pre-HF who benefit from active clinical actions; (3) Of all the CRT-D remote transmissions, 34.3% fulfilled the partners-HF criteria and 7.8% were triggered by a care alert. Positive partners-HF transmissions also determined a care alert in 15.4% of cases, and care alert transmissions met partners criteria in 67.3% of cases; (4) The most common reasons triggering a Positive partners-HF transmission were: Optivol fluid index $\geqslant$ 100 (18.8%) or optivol fluid index

Table 3 Diagnostic accuracy of partners-heart failure and care alert in determining active clinical actions

	Active clinical actions (2-3)	Non-active/no clinical actions (0-1)	Total transmissions
Positive partners-HF transmissions	193	35	228
Negative partners-HF transmissions	6	431	437
Positive care alert transmissions	22	30	52
Negative care alert transmissions	177	436	613
Overall transmissions followed by an action	199	466	665
Appendix partners-HF algorithm			
Parameters	Criterion		
Fluid index	⩾ 60 d		
AT/AF duration	⩾ 6 h and not persistent AT/AF		
VR during AT/AF	AT/AF ⩾ 24 h and VR ⩾ 90 bpm		
Patient activity	< 1 h over 1 wk		
NHR	⩾ 85 bpm for 7 consecutive days		
HRV	< 60 ms for 7 consecutive days		
%CRT pacing	< 90% for 5 of 7 d		
Shock (s)	⩾ 1 shock		

AT: Atrial tachycardia; AF: Atrial fibrillation; VR: Ventricular rate; NHR: Night heart rate; HRV: Heart rate variability; CRT: Cardiac resynchronization therapy; HF: Heart failure.

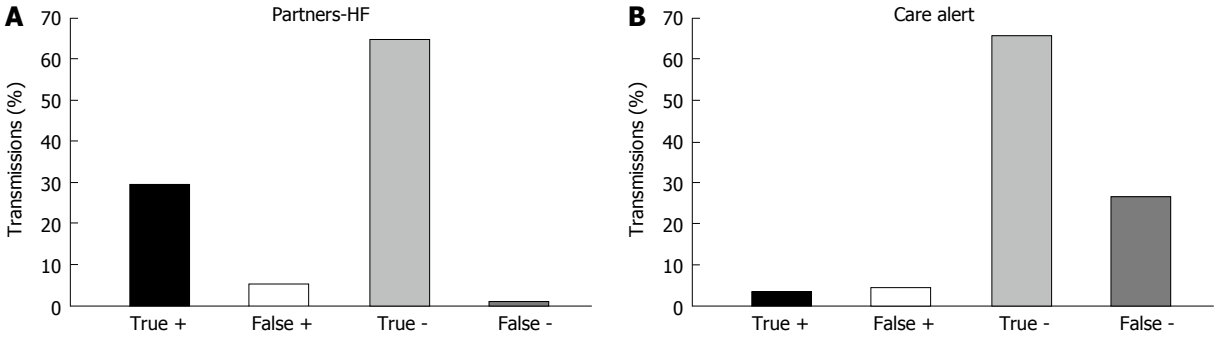

Figure 4 True and false positive and negative transmissions according to active clinical actions. A: True +: Positive partners-HF transmissions followed by active clinical actions (class 2 or 3); False +: Positive partners-HF transmissions followed by no actions or non-active clinical actions (class 0 or 1); True -: Negative partner-HF transmissions followed by no actions or non-active clinical actions (class 0 or 1); False -: Negative partners-HF transmissions followed by active clinical actions (class 2 or 3); B: True +: Positive care alert transmissions followed by active clinical actions (class 2 or 3); False +: Negative care alert transmissions followed by no actions or non-active clinical actions (class 0 or 1); True -: Positive care alert transmissions followed by no actions or non-active clinical actions (class 0 or 1); False -: Negative care alert transmissions followed by active clinical actions (class 2 or 3). All values are expressed in terms of percentage of total transmissions. HF: Heart failure.

> 60 plus one of the following parameters: reduced HRV (13.6%), low PA (12.3%), or reduced %CRT (11%); and (5) The most common active clinical action was HF-therapy titration, particularly of diuretics and beta-blockers, and the introduction of oral anticoagulation in patients with asymptomatic AF.

Despite advances in treatment of HF, it is still a major cause of cardiovascular mortality and hospitalization, especially in the early period after hospital discharge[1]. Prevention of HF relapses is important not only to reduce HF mortality and morbidity, but also health care costs[1]. Cardiac implantable electronic devices have nowadays remote monitoring capabilities that allow clinicians to have remote access to the complete device diagnostic information.

Previous studies

Earlier studies have shown that implantable device-measured variables, including intra-thoracic impedance, AF burden, mean heart rate, HRV, PA, frequency of PVCs, VA episodes, ICD shocks and %CRT, individuate subjects at risk of HF and facilitate early interventions[6-10]. Intrathoracic impedance[3], HRV and PA[2] reduction, occurs nearly two weeks before HF exacerbation. Low HRV indicate a sympathetic dominance in cardiac autonomic control and may be associated with exacerbation of atrial and VAs[13]. A prolonged AF duration, a rapid VR during AF and an increase in the burden of PVCs reduce %CRT[14] and are warning signs of HF, together with ICD shocks[15].

Although validated in various studies, the use of each device parameter in HF patients, is restricted by some limitations. In particular, variations of intrathoracic impedance may be related to lung inflammation; increased AF burden and prolonged AF duration are not useful in subjects with permanent AF; reduced mean heart rate, HRV or patient activity may reflect difficulty walking secondary to orthopedic diseases. Consequently, there is great interest in combining HF device diagnostic parameters for the management of CRT-D recipients.

The partners-HF[11] was a large cohort study explor-

ing the ability of the partners-HF criteria algorithm to dynamically stratify patients' risks of HF. A cohort of 694 CRT-D recipients with advanced HF (NYHA Ⅲ-Ⅳ) was prospectively evaluated in 100 centers. The retrospective evaluation of diagnostic CRT-D data demonstrated that subjects with a positive partners-HF algorithm had greater risk of hospitalization due to HF in the next month (adjusted HR = 5.5; $P < 0.001$). Moreover, the study demonstrated that increasing the frequency of reviewing the HF device diagnostics from quarterly (90 d) to monthly (30 d) but not to semimonthly (15 d), improved the ability to identify individuals at higher HF risk.

The prospective, multicenter observational Home Monitoring in CRT (Home-CARE) study[16] followed up for 1 year 377 CRT-D recipients who had been hospitalized for HF at least once within the 12 mo before enrollment. The following data were automatically retrieved every 24 h by the Home Monitoring (Biotronik, Berlin, Germany) algorithm: Mean heart rate, heart rate at rest, PA, frequency of PVCs, HRV, right ventricular pacing impedance, and painless shock impedance. The retrospective sensitivity values of individual parameters ranged from 23.6% to 50.0%, whereas their combination displayed 65.4% sensitivity and 99.5% specificity for cardiovascular hospitalizations and deaths.

Some studies have demonstrated favorable effects of RM in improving HF treatment, with potential benefits on clinical outcomes[6-10]. However, few and inconclusive data are available on the RM use in routine clinical practice and its impact on HF clinical outcomes. The Home Guide[17] registry proved RM highly effective in detecting clinical events, excluding deaths, with a sensitivity and a positive predictive values of 89% and 97%, respectively. RM sensitivity for atrial and VAs and device-related issues was > 90%, while it was < 35% for stroke, syncope and acute coronary syndromes and displayed an intermediate sensitivity (59%) for HF detection. Interestingly, 3 out of 4 events needing clinical intervention were asymptomatic and were effectively detected by RM, allowing a prompt reaction.

In our study the most common clinical reaction to partners-HF transmissions was drug therapy adjustment, while HF therapy titration and oral anticoagulation introduction in patients with asymptomatic AF were the most prevalent therapy interventions.

Clinical implications

This is the first multicenter observational registry prospectively assessing the clinical utility of partners-HF algorithm for risk stratification of HF patients in clinical practice. Remote monitoring of CRT-D recipients trough partners-HF algorithm, was not compared with usual care and this registry was not powered to explore the impact of the partners-HF algorithm on HF-hospitalizations and mortality. Our results prove that the partners-HF has significant diagnostic accuracy in determining active clinical actions oriented to treat pre-HF status and to prevent an acute decompensation. Given the high positive and very high negative predictive values, clinicians could contact only patients with positive partners-HF transmissions, thus avoiding a significant number of unnecessary telephone contacts.

Care alert displays very low sensitivity and a poor ability to identify patients needing active clinical action oriented to treat pre-HF status. Moreover, given its low positive predictive value, clinicians should be aware that an active clinical action oriented to preventing acute HF may be not necessary in case of care alert triggered transmissions.

Another important aspect is that this prospective analysis was conducted in patients with advanced HF (82.7%: NYHA classes Ⅲ/Ⅳ; mean EF: 27.5% ± 6.5%). This may explain the high percentage of manual and care alert transmissions collected and the high prevalence of positive partners-HF transmissions (35%). Considering that no HF hospitalization occurred in a population of advanced HF during a 6 mo follow-up, the partners-HF algorithm appears to be a powerful tool to identify and consequently treat pre-HF status in order to prevent acute decompensation.

According to the partners-HF study[11], positive partners transmissions were mostly triggered by the optivol fluid index, alone or in combination with low HRV, low PA or a low %CRT. The weight of each partners-HF criterion in the risk stratification of HF patients was not considered in this registry. A combined algorithm of HF diagnostic parameters could be utilized to stratify patients into high, medium and low risk of HF by using a specific risk stratification score, calculated by attributing a specific weight to each partners-HF criterion on the basis of its ability to detect pre-HF status. Finally, whether therapeutic interventions based on the partners-HF algorithm are effective in improving outcomes in HF, was not investigated.

The partners-HF algorithm proved to be a powerful predictor of a pre-HF status and was able to guide clinical actions oriented to avoiding acute HF. Future larger randomized prospective trials should be performed to confirm our results, to develop and validate a dynamic HF risk score based on the partners-HF algorithm and to ascertain whether the use of this algorithm for RM can improve the main clinical outcomes of HF patients.

COMMENTS

Background

Heart failure (HF) is a principal cause of death hospitalization and health care costs. The partners-HF algorithm retrospectively identified cardiac resynchronization-defibrillator (CRT-D) recipients at risk of HF relapses in the subsequent 30 d. However no studies have validated this algorithm prospectively and have compared it with the care alert strategy, that is commonly adopted for CRT-remote monitoring.

Research frontiers

Remote monitoring has emerged as a useful tool to prevent HF relapses, and to reduce cardiac hospitalization and mortality.

Innovations and breakthroughs

This is the first multicenter observational registry prospectively assessing the

clinical utility of partners-HF algorithm for risk stratification of HF patients in clinical practice.

Applications

The authors' prospective study showed that the partners-HF algorithm has significant diagnostic accuracy in determining active clinical actions oriented to prevent HF relapses. Moreover, it has a high positive and a high negative predictive value, allowing clinicians to contact only patients with positive partners-HF transmissions, thus avoiding a significant number of unnecessary telephone contacts.

Terminology

Remote monitoring: Wireless remote monitoring of cardiac electronic devices, including cardiac defibrillators and CRT.

Peer-review

This is a valuable research, because status of clinical actions is very important for patient's therapy and outcomes. Herein the traits of the partners-HF algorithm *vs* care alert in determining active clinical actions were explored and observed the effect of different methods on treatment or prevent heart failure.

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Salmonella Berta myocarditis: Case report and systematic review of non-typhoid *Salmonella* myocarditis

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Abstract

AIM: To study trends in the epidemiology, clinical presentation, microbiology and prognosis of non-typhoid *Salmonella* (NTS) myocarditis.

METHODS: We performed a systematic literature search for all reported NTS cases. The search yielded 838 publications. A total of 21 papers were deemed eligible. No language restrictions were enforced. Articles that were not written in English were translated. Pre-specified data such as clinical presentation, electrocardiogram (ECG) changes, transthoracic echocardiographic findings, cardiac magnetic resonance findings, microbiology cultures, *Salmonella* species, inflammatory markers (erythrocyte sedimentation rate and C-reactive protein), cardiac biomarkers and severity of illness were collected using data extraction sheets. Cases were classified by age into 2 groups; pediatric cases (defined as < 18 years old) and adult cases (defined ⩾ 18 years old). The mean age of patients and standard deviations were calculated. The data was analyzed with IBM SPSS Statistics (Windows, Version 20.0. Armonk, NY: IBM Corp.) for demographic characteristics, presenting symptoms, microbiology, diagnostic methods, treatment modalities and outcome.

RESULTS: From the selected articles, we identified a total of 24 individual cases with verifiable data. There were 20 males with a male to female ratio of 5:1. The mean age at presentation was 30.8 years (range 1 mo-67 years), 16% of cases were children aged < 18 years. Most patients presented with chest pain, fever,

and abdominal pain. The most common ECG finding was ST elevation. Cardiac biomarkers were elevated in around 70% of cases. *Salmonella* Enteritidis was the most common NTS isolated. Definitive diagnosis was established by blood and stool cultures in most of the cases. The pediatric and adults cases had similar incidence of bacteremia (40% *vs* 36.8%) while the pediatric group had more stool cultures positive compared to the adult group (100% *vs* 63.1%). Eighty-three percent of patients received antibiotics and 58% were successfully treated through conservative management. The overall mortality was 24% and 42% of patients required intensive care.

CONCLUSION: This systematic review of published cases shows that NTS myocarditis occurs predominantly in young adults and carries a poor prognosis.

Key words: Diarrhea; Myocarditis; *Salmonella*; Non-typhoid

Core tip: Myocarditis is a rare extra-intestinal manifestation of non-typhoid *Salmonella* infection. In our review, the most common presenting symptoms were fever, abdominal pain, and chest pain and the most frequent electrocardiogram finding was ST segment elevation. Around 70% of patients had positive cardiac biomarkers (creatine kinase and/or troponin). *Salmonella Enteritidis* was the most common pathogen identified. Mortality appears to be high as is seen with all bacterial myocarditis, and intensive care unit admission is warranted in a large number of cases.

Villablanca P, Mohananey D, Meier G, Yap JE, Chouksey S, Abegunde AT. Salmonella Berta myocarditis: Case report and systematic review of non-typhoid *Salmonella* myocarditis. *World J Cardiol* 2015; 7(12): 931-937 Available from: URL: http://www.wjgnet.com/1949-8462/full/v7/i12/931.htm DOI: http://dx.doi.org/10.4330/wjc.v7.i12.931

INTRODUCTION

Salmonella species are gram-negative bacilli that are responsible for significant morbidity and mortality in both developing and developed nations. They are responsible for a wide spectrum of disease including enteric fever or typhoid fever [*Salmonella Typhi* (*S. Typhi*) and (*S. Paratyphi*)], as well as a range of clinical syndromes including diarrheal illness caused by a group of bacteria known as non-typhoid *Salmonella* (NTS)[1], *Salmonella* associated myocarditis is a rare entity described in case reports. Most case reports have described myocarditis associated with *S. Typhi* and Para-typhi infections. However, myocarditis associated with NTS, the species most commonly found in the western hemisphere, has been infrequently reported. Also, there is no existing structured analysis on this subject. We report an illustrative case and carefully analyze the available literature on NTS myocarditis in order to describe the epidemiological distribution, diagnostic trends and prognosis.

A 19-year-old male with no significant past medical history presented to the emergency department (ED) with a 2-d history of watery non-bloody diarrhea associated with diffuse abdominal cramping, fever, nighttime chills and sweats. He recalled eating jerk chicken from a local restaurant and a sausage-egg biscuit prior to onset of diarrhea. He ate alone and denied any sick contacts or recent travel. On admission his vitals were as follows: Temperature 38.8 ℃, heart rate (HR) 103/min and blood pressure (BP) 122/94 mmHg. Physical exam was unremarkable except for mild abdominal tenderness and dehydration. He received intravenous fluids and was discharged with a diagnosis of possible viral gastroenteritis. Forty-eight hours after discharge, he developed acute-onset chest pain (CP) and shortness of breath (SOB). The patient described the CP, as a retrosternal "squeezing" pain, 8/10 in severity, not radiating and associated with SOB at rest along with intermittent palpitations. He did not recognize any aggravating or relieving factors. When the symptoms persisted for 24 h, the patient came to the ED again and this time his vitals were as follows: BP 112/65 mmHg, HR 81/min, temperature 37.3 ℃ and respiratory rate 18 breaths/min with oxygen saturation of 97% on room air. Physical examination was unrevealing. Initial electrocardiogram (ECG) was significant for ST segment depression in leads V1 and V2 with ST segment elevations in leads V5 and V6 (Figure 1). Chest X-ray (CXR) did not show any cardiopulmonary process. Initial troponin I was 6.23 ng/mL (0.000-0.034 ng/mL) and a repeat assay 6 h later was 13.2 ng/mL. CBC showed hemoglobin of 12.5 g/dL, white blood cell count (WBC) of 6.9 × 10^9/L (4.4 × 10^9-10.6 × 10^9/L) with 26% bands. Kidney function and electrolytes were within normal limits. Liver enzymes showed alkaline phosphatase of 33 IU/L (50-120 U/L), aspartate aminotransferase of 42 IU/L (0-40 IU/L) and lactate dehydrogenase of 325 IU/L (85-210 IU/L). Table 1 summarizes the laboratory and imaging investigations for this patient. Within a few hours of admission, the patient became hypotensive with BP of 90/49 mmHg and the troponin went up to 18.9 ng/mL. He was subsequently transferred to the cardiac intensive care unit (ICU). Transthoracic echocardiogram (TTE) showed an ejection fraction (EF) of 40% with no regional wall motion abnormalities or pericardial fluid. Due to suspicion for myocarditis, a cardiac magnetic resonance imaging (CMRI) was done which showed multiple areas of abnormal sub-epicardial and mid-myocardial contrast hyper enhancement involving the posterior, inferior and anterior walls of the left ventricle, the anterior wall of right ventricle and the inter-ventricular septum reflecting multifocal biventricular myocarditis (Figure 2). Stool culture came back positive for *Salmonella*

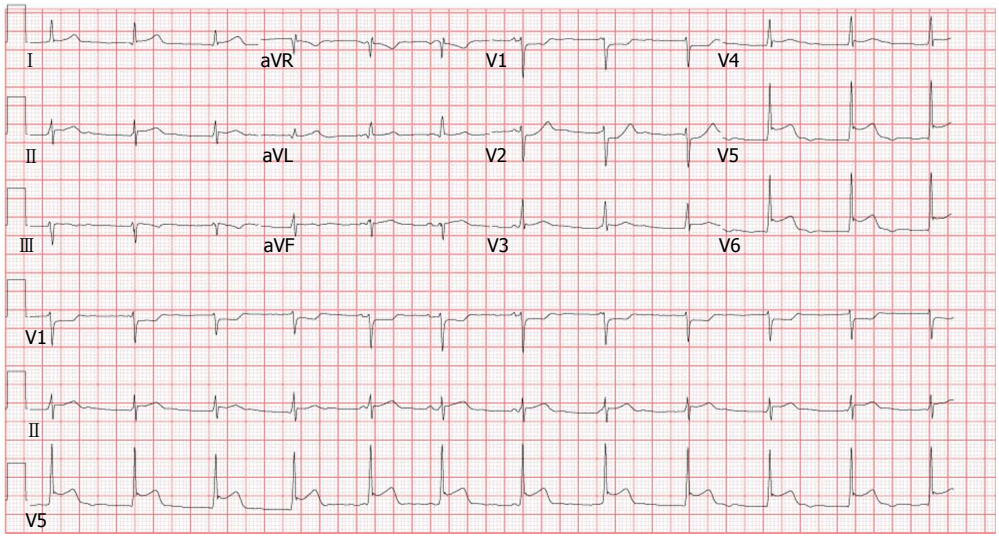

Figure 1 Electrocardiogram on admission with ST segment changes; ST segment depression in V1 and V2 with ST segment elevations in V5 and V6.

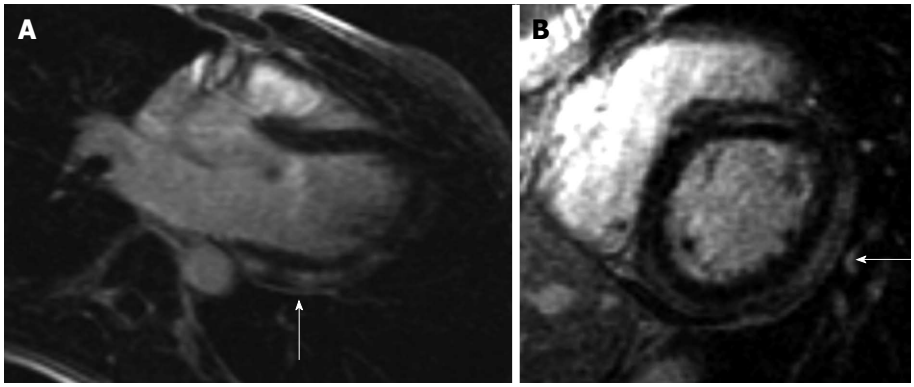

Figure 2 Cardiac magnetic resonance imaging findings. Cardiac magnetic resonance demonstrates pathological delayed gadolinium enhancement (as indicated by arrows). A: Long axis view: Delayed enhancement of the myocardium demonstrates subepicardial and mid myocardial enhancement indicative of myocarditis; B: Short axis view: Delayed enhancement of the myocardium demonstrates subepicardial and mid myocardial enhancement indicative of myocarditis.

Berta and therapy was initiated with sulfamethoxazole/trimethoprim. The patient gradually improved over the next 4 d and his CP resolved. He received 14 d of therapy during which symptoms resolved completely and EF normalized.

MATERIALS AND METHODS

This systematic review was conducted according to the PRISMA guidelines[2]. A computer-assisted literature search of PubMed, EMBASE CENTRAL and Google search engine was conducted. We also performed manual searches of the reference lists of studies, reviews, editorials, and letters, as well as related conference proceedings. Search terms keywords included "*Salmonella* myocarditis", "bacterial myocarditis", "non-typhoidal *Salmonella*" as well as combinations of these terms. No language restrictions were enforced. Articles that were not written in English were translated.

Inclusion criteria for publications in this systematic review: (1) Articles reporting original data; (2) Articles including patients with at least 1 blood and/or stool culture or tissue finding confirming diagnosis of *Salmonella*; (3) Articles including patients with clinical, electrocardiographic, or imaging evidence suggesting myocardial involvement; and (4) Articles providing data on at least one of the following: Clinical presentation, ECG description or original ECG, serum cardiac markers, any radiologic images. Exclusion criteria are listed as follows: (1) Articles including patients with myocarditis and infection with *Salmonella* typhi or para-typhi; (2) Articles reporting conditions that might present with clinical and imaging abnormalities similar to *Salmonella* myocarditis; (3) Articles on *Salmonella* infection affecting other organs; and (4) Review articles.

The following data was extracted: age, gender, presenting complaints (CP SOB, diarrhea) fever > 37.5 ℃, white blood cell count, serum cardiac markers [creatine kinase (CK) and Troponin], ECG characteristics, microbiology cultures, inflammatory markers [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], TTE findings, CMRI findings, CXR

Table 1 Diagnostic testing for reported case

Diagnostic test	Result
Hemoglobin	12.5 g/dL
WBC	6.9×10^9/L
Kidney function and electrolytes	Within normal limits
Alkaline phosphatase	33 IU/L
Aspartate aminotransferase	42 IU/L
Lactate dehydrogenase	325 IU/L
Troponin I (at presentation)	6.23 ng/mL
Troponin I (6 h later)	13.2 ng/mL
ECG	ST depression in V1 and V2 with ST elevations in V5 and V6
TTE	EF of 40% with no regional wall motion abnormalities or pericardial fluid
CMR	Multiple areas of abnormal sub-epicardial and mid-myocardial contrast hyper enhancement involving the posterior, inferior and anterior walls of the left ventricle, the anterior wall of right ventricle and the inter-ventricular septum reflecting multifocal biventricular myocarditis

WBC: White blood cell count; ECG: Electrocardiogram; TTE: Transthoracic echocardiogram; CMR: Cardiac magnetic resonance imaging; EF: Ejection fraction.

findings, hemodynamics (BP and HR), *Salmonella* species and possible source of infection, need for ICU admission and outcomes (dead or alive). There was no age restriction for inclusion of cases in the study. Cases were classified by age into 2 groups; pediatric cases (defined as < 18 years old) and adult cases (defined ⩾ 18 years old). The mean age of patients and standard deviations were calculated. All available ECG descriptions for each case were obtained and original data was analyzed. The ECG data was grouped into the following categories: ST segment elevation, ST segment depression, affected walls, T wave inversion, and others findings that included specific abnormalities such as prolonged QT, atrioventricular blocks (AVB), premature ventricular complex (PVC) were also recorded. Serum cardiac markers (CK and/or troponin) inflammatory markers (CRP and/or ESR) were classified as normal or elevated. Fever was defined as temperature > 37.5 ℃, leukocytosis as WBC count > 10000/mm^3, reduced EF as < 50%, hypotension as systolic BP < 90 mmHg and diastolic BP < 60 mmHg, tachycardia as HR > 100/min. If data was reported without quantification but the description matched the criteria it was considered a positive finding. CXR and CMRI findings were obtained from the report or direct data analysis if there was an available image. The prevalence of the different measured variables was calculated from the extracted data. The "not available" data cases were not considered in the calculation. Data was analyzed with IBM SPSS Statistics (Windows, Version 20.0. Armonk, NY: IBM Corp) for demographic characteristics, presenting symptoms, microbiology, diagnostic methods, treatment modalities and outcome. The analysis was reviewed by

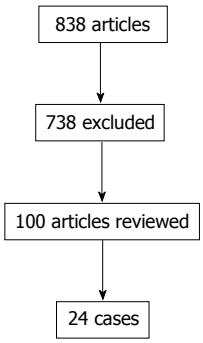

Figure 3 Flow chart of literature review.

Villablanca P (MD MS-Clinical Research).

RESULTS

The literature search yielded a total of 838 publications. Following the exclusion criteria, 816 citations were excluded after examining titles and abstracts, leaving twenty-one articles with 24 patients for detailed evaluation (Figure 3)[3-23].

Demographic characteristics

There were 19 adult and 5 pediatric cases, 20 males with a male-to-female ratio of 5:1. Female prevalence was higher in pediatric population (60%) as compared to adults (5.2%). The age of the patients ranged from 1 mo to 67 years. The mean age at presentation was 30.8 years. The mean age for adults and pediatrics cases was 36.6 years (range: 18-67 years) 9.6 years (range: 0.1-16 years) respectively.

Diagnostic evaluation

NTS species were identified either by blood cultures or stool cultures; only one patient had both cultures positive. One pediatric case was confirmed with myocardial biopsy after the patient died (Table 2). The pediatric and adults cases had similar incidence of bacteremia (40% *vs* 36.8%) while the pediatric group had more stool cultures positive compared to the adult group (100% *vs* 63.1%). *S. Enteritidis* was the most common pathogen found among all the reported cases, with a total of 10 cases (41.6%). *Salmonella typhymurium* was the most frequently reported pathogen in the adult group (36.8% of cases) and *S. Enteritidis* was the most frequently reported pathogen in the pediatric group (80% of cases) (Figure 4).

Presenting signs and symptoms

Fever, abdominal pain, and CP were the most common reported symptoms. Fever was present in 66.6% of the cases. SOB, chills and sweating were less prevalent. Of note, less than 25% of the cases had associated diarrhea. More than half of the cases of NTS presented with tachycardia and around 20% with hypotension. There was history of recent travel in 8 cases with a wide distribution around the world including Pakistan,

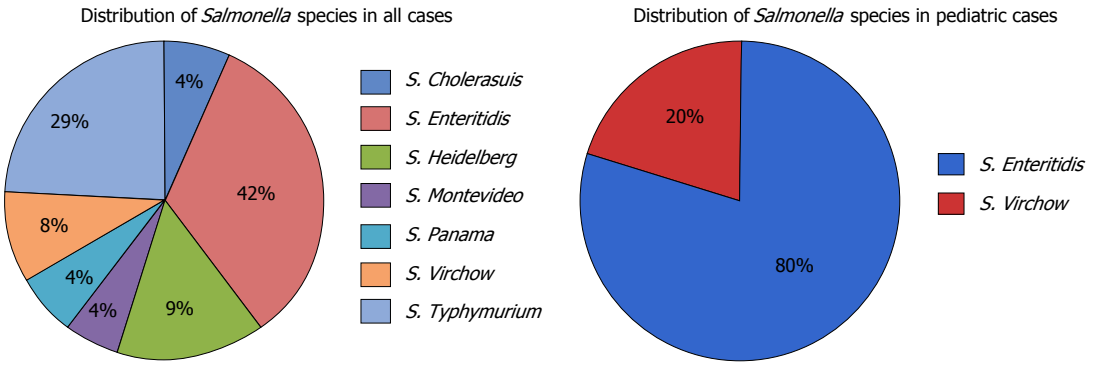

Figure 4 Distribution of *Salmonella* species in all cases, including pediatric cases.

Table 2 Clinical, electrocardiographic, laboratory, and imaging findings of non-typhoid *Salmonella* myocarditis

	n (%)	*n*[1]
Culture		
Blood	7 (29.1)	24
Stool	16 (66.6)	24
Myocardial biopsy	1 (4.1)	24
Presenting sign or symptom		
Dyspnea	6 (25)	24
Chest pain	15 (62.5)	24
Fever	16 (66.6)	24
Diarrhea	5 (22.7)	22
Abdominal pain	15 (71.4)	21
Hypotension	5 (21.7)	23
Tachycardia	13 (56.5)	23
ECG abnormalities		
ST elevation	12 (52.1)	23
ST depression	4 (17.3)	23
T-wave inversion	6 (26)	23
Infero-lateral	12 (52.1)	23
Antero-lateral	6 (26)	23
Inflammatory markers		
WBC > 10000/mm^3	8 (40)	20
Elevated troponin	9 (69.2)	13
Elevated CK	10 (66.6)	15
Elevated ESR or CRP	9 (100)	9
TTE		
Reduced ejection fraction	4 (36.4)	11
Regional wall motion abnormality	5 (45.5)	11
Chest X-ray		
Cardiomegaly	3 (27.7)	11
Pulmonary edema	3 (27.7)	11
CMR	2 (100)	2

[1]Based on reported data. ECG: Electrocardiogram; WBC: White blood cell count; CK: Creatine kinase; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; TTE: Transthoracic echocardiogram; CMR: Cardiac magnetic resonance imaging.

Bali, Dominican Republic, Spain and Eastern Europe. Average prodrome was 4.6 d. Duration of prodromal symptoms was slightly longer in the pediatric cases (8.1 d). Four cases recalled a possible source of infection, which included eating chicken, rice-eggs, sausages with spoiled meat and dumplings. Table 2 illustrates the frequency of presenting signs and symptoms.

Electrocardiographic findings

The most common finding on ECG was the presence of ST segment elevation (52.1%). ST segment depression was seen in a small number of the cases with only one pediatric case. Adults had a higher prevalence of more serious ECG findings including 3rd degree AVB (10.5%, *n* = 2) and ventricular fibrillation (5.2%, *n* = 1). More benign findings like 1st degree AVB, prolonged QT, PVC, right bundle branch block and low voltage were present in both groups. Table 2 shows the frequency of different ECG abnormalities along with area of involvement.

Markers of inflammation and cardiac injury

Variability in the choice of biomarkers was seen in the cases reviewed. Older cases used CK since troponin was not available. The prevalence of elevated CK and/or troponin in patients presenting with *Salmonella* myocarditis was near 70%. Prevalence of reported elevated troponin was high, in both, adults (71.4%, *n* = 10) and pediatric cases (40%, *n* = 2). However, troponin assay was not available at that time when most of the pediatric cases were reported. Most of the cases measured troponin I (55%, *n* = 5), followed by troponin T (33%, *n* = 3), and one case did not specify the kind of troponin used. Among the cases reviewed, the prevalence of elevated white count > 10000/mm^3 was 40% of which 25% had a left shift. Where it was measured, elevation in ESR or CRP was noted in 100% of cases of *Salmonella* myocarditis. Frequency of abnormalities in inflammatory and cardiac biomarkers is illustrated in Table 2.

Imaging

Almost 30% patients had evidence of pulmonary edema on CXR and less than 1/3 had cardiomegaly. On TTE, 36.4% reported reduced EF (less than 50%). More than 50% of the adults had regional wall motion abnormalities. Pericardial effusion was seen exclusively in children (3 cases). Out of the 24 cases, only 2 received CMRI scans, both of which revealed delayed gadolinium enhancements. Frequency of abnormal imaging studies is shown in Table 2.

DISCUSSION

Myocarditis is an inflammatory condition of the myocardium with both infectious and non-infectious etiologies.

It most commonly presents as non-ischemic cardiomyopathy but manifestations can range from sudden death, new onset atrial or ventricular arrhythmias, complete heart block or an acute myocardial infarction[24]. Viral infections are the most common cause of myocarditis with an epidemiological change from Coxsackie B virus and adenovirus being identified in the past to parvovirus B19 being the most common etiological agent currently[24]. Bacterial myocarditis is uncommon with a prevalence ranging from 0.2% to 1.5%[25]. However, it should always be considered in patients with sepsis and ventricular dysfunction[26].

Salmonella species are gram-negative bacilli that are responsible for significant morbidity and mortality in both developing and developed nations. Both typhoidal and NTS can have extra-intestinal manifestations however myocarditis is an uncommon extra-intestinal manifestation[27]. NTS are food-borne pathogens that are responsible for diarrheal illness. There is about a 5% incidence of invasion beyond the gastrointestinal tract present most commonly in immunocompromised hosts[28]. In our review, the major presenting symptoms were fever and CP with only around 20% having diarrhea. A large study of over 7000 human *Salmonella* infections noted cardiac involvement in the form of endocarditis in 20 patients all of whom had a rapidly fatal outcome[29]. It can be postulated that myocardial damage occurs secondary to involvement of endocardium or due to direct bacterial invasion from bacteremia. In addition to this, sepsis induced myocardial depression and subsequent remodeling may also play a part as it does in other bacterial myocarditis[26].

ECG analysis in myocarditis usually shows sinus tachycardia with non-specific ST segment changes and T wave abnormalities[24]. In our review, the most common ECG abnormality was ST elevation. Troponins have been shown to have high specificity (89%) and low sensitivity (34%) for diagnosis of myocarditis[30]. In our review troponins were elevated in majority of the patients with NTS myocarditis. Even though the gold standard for diagnosis remains endomyocardial biopsy, CMRI is slowly replacing the need for more invasive procedures[31]. In a recent study of 82 patients with troponin elevation without significant coronary artery disease, late gadolinium enhancement CMRI established a diagnosis of myocarditis in 80% of the patients in comparison to 88% diagnosed with endomyocardial biopsy[31]. In our review, only 2 patients received CMRI with both cases showing evidence of myocarditis. TTE showed a reduced EF in around 36% of the cases with around 50% of adult cases showing regional wall motion abnormalities. Interestingly, all of the pediatric cases showed pericardial fluid suggestive of increased incidence of pericardial disease in pediatric population affected by *Salmonella*.

The most common etiological agent overall was *S. Enteritidis* (40%). This is in concordance with a Malaysian retrospective analysis of 55 patients with NTS bacteremia, which showed that *S. Enteritidis* had the maximum blood invasiveness[28]. The overall mortality in our patients was around 20% with 40% of patients requiring ICU stay. Antibiotic therapy is not recommended for NTS gastrointestinal infections. However, it should be considered if patients are at risk for invasive disease (age > 50 or neonates, immunosuppressed, sickle cell patients and those with vascular abnormalities)[27]. Treatment of myocarditis caused by NTS has not been detailed in any study, but in essence it can be treated as NTS bacteremia or as a life threatening infection. In those cases affected by life threatening infections, treatment should be started with both, a third-generation cephalosporin and a fluoroquinolone until the susceptibility is known[32]. Ninety percent of the patients included in this review received antibiotic treatment.

Results of this systematic review of published cases shows that NTS myocarditis occurs predominantly in young adults and carries a poor prognosis. The initial diagnostic approach is similar to myocarditis due to other etiologies and includes ECG, TTE and CXR. Upon diagnosis, patients should receive supportive therapy for myocarditis in addition to antibiotics. Mortality appears to be high as with all bacterial myocarditis and ICU admission is warranted in a large number of cases. *Salmonella* infections are a rare cause of myocarditis but should always be considered in cases presenting with features of myocarditis and evidence of *Salmonella* infection in the absence of viral etiology.

COMMENTS

Background

Salmonella species are bacteria that are responsible for significant morbidity and mortality in both developing and developed nations. They are responsible for a wide spectrum of disease including enteric fever or typhoid fever (*S. Typhi* and *S. Paratyphi*) and a range of clinical syndromes including diarrheal illness caused by a group of bacteria known as non-typhoid *Salmonella* (NTS). In rare circumstances *Salmonella* can cause inflammation of the myocardium (myocarditis).

Research frontiers

To the best of our knowledge, no systematic review of NTS myocarditis has previously been published. The authors carefully analyze the available literature on NTS myocarditis in order to describe the epidemiological distribution, diagnostic trends and prognosis of this condition.

Innovations and breakthroughs

Salmonella Enteritidis was the most common pathogen identified in these cases. Around 30% of patients had bacteremia and 100% of pediatric patients had either stool or blood culture positive for *Salmonella*. Fever, abdominal pain and chest pain were the most common presenting symptoms and ST segment elevation was the most frequent electrocardiogram finding. Around 70% of patients had positive cardiac biomarkers (creatine kinase and/or Troponin). Mortality appears to be high as with all bacterial myocarditis and intensive care unit admission is warranted in a large number of cases.

Applications

Salmonella infections are a rare cause of myocarditis but should always be considered in cases presenting with features of myocarditis and evidence of *Salmonella* infection in the absence of viral etiology.

Terminology

Salmonella species are gram-negative bacteria that cause a wide range of

diseases. *S. Berta*, *S. Typhi*, *S. Paratyphi*, *S. Enteritidis, etc*., are all serotypes of the Genus *Salmonella*. However, based on clinical syndromes, *i.e.*, causation of enteric fever, salmonella can be divided into typhoid salmonella and NTS. Myocarditis is an inflammatory condition of the muscular wall of the heart.

Peer-review

NTS infection involving myocarditis is rare, and the current case presentation and systemic review of the disease is therefore unique.

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SYSTEMATIC REVIEWS

Adherence to cardiovascular medications in the South Asian population: A systematic review of current evidence and future directions

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Abstract

AIM: To review methods of assessing adherence and strategies to improve adherence to cardiovascular disease (CVD) medications, among South Asian CVD patients.

METHODS: We conducted a systematic review of English language studies that examined CVD medication adherence in South Asian populations from 1966 to April 1, 2015 in SCOPUS and PubMed. Working in duplicate, we identified 61 studies. After exclusions, 26 studies were selected for full text review. Of these, 17 studies were included in the final review. We abstracted data on

several factors including study design, study population, method of assessing adherence and adherence rate.

RESULTS: These studies were conducted in India (*n* = 11), Pakistan (*n* = 3), Bangladesh (*n* = 1), Nepal (*n* = 1) and Sri Lanka (*n* = 1). Adherence rates ranged from 32%-95% across studies. Of the 17 total publications included, 10 focused on assessing adherence to CVD medications and 7 focused on assessing the impact of interventions on medication adherence. The validated Morisky Medication Adherence Scale (MMAS) was used as the primary method of assessing adherence in five studies. Three studies used validated questionnaires similar to the MMAS, and one study utilized Medication Event Monitoring System caps, with the remainder of the studies utilizing pill count and self-report measures. As expected, studies using non-validated self-report measures described higher rates of adherence than studies using validated scale measurements and pill count. The included intervention studies examined the use of polypill therapy, provider education and patient counseling to improve medication adherence.

CONCLUSION: The overall medication adherence rates were low in the region, which suggest a growing need for future interventions to improve adherence.

Key words: Assessing medication adherence; South Asia; Cardiovascular disease medication

Core tip: The overall adherence rate in South Asia is quite low. Only 7 of 17 publications conducted interventions geared toward improving adherence. Even fewer (*n* = 3) utilized community health care workers, which provide a unique resource in these resource constrained environments. Just over half of the studies found in our review utilized validated or gold standard methods (*n* = 9) with the rest using non-validated self-reported measures. Additionally, there was a lack of usage of technology despite the majority of these countries benefitting from a high cell phone density.

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, with an estimated 17.5 million people dying from CVD in 2012[1]. Approximately one-fifth of the global population resides in South Asia (India, Pakistan, Bangladesh, Nepal, and Sri Lanka), where patients suffer from a disproportionately high rate of CVD-related morbidity and mortality[2-6]. In a large international, case-control study of first myocardial infarction (MI), results indicated that the mean age for first MI was significantly lower in South Asian participants (53.0 years) than in participants from other countries (58.8 years)[5]. In approximately 10% of these cases, first MI in South Asian participants occurred in those aged 40 or below. These data indicate a growing epidemic of premature CVD in South Asian populations.

Use of beta-blockers, angiotensin-converting-enzyme (ACE) inhibitors, statins and antiplatelet drugs are successful means of secondary prevention of CVD. However, the use of these drugs varies widely by population. Results of a large-scale epidemiological study examining use of secondary prevention drugs for CVD in high-income, middle-income, and low-income countries showed that use was highest in high-income countries [antiplatelet drugs 62.0%, β blockers 40.0%, ACE inhibitors or angiotensin Ⅱ receptor blockers (ARBs) 49.8%, and statins 66.5%] and lowest in low-income countries (including India, Bangladesh and Pakistan) (8.8%, 9.7%, 5.2%, and 3.3% for antiplatelets, β blockers, ACE inhibitors or ARBs, and statins, respectively)[7].

An important factor in the use of appropriate medications to manage CVD risk is medication adherence. Adherence is critical to the effectiveness of all drug therapies, but is particularly important for medications prescribed for chronic conditions[8]. In a study of 37154 patients with established atherothrombotic disease, non-adherence to medications at baseline and one year were both significantly associated with increased risk of cardiovascular death, myocardial infarction, or stroke at 4 years[9]. This is of particular importance to South Asian countries for various reasons. First, low availability of electronic medical records in most health care settings precludes accurate assessment of medication adherence by health care providers in these countries and therefore, poses specific challenges in the assessment of medication adherence. Second, the overall health literacy and the opportunities to improve provider and patient awareness of the importance of medication adherence may be limited. Lastly, low availability of pharmacy records and medication refill data also limit the use of traditional measures used to assess medication adherence (*i.e.,* medication possession ratio or proportion of days covered).

Therefore, the overall aim of this review is to examine the current methods of assessing adherence to CVD medications as well as explore current interventional strategies to improve medication adherence, among CVD patients in South Asia.

MATERIALS AND METHODS

Search strategy and study selection

To identify eligible studies, we conducted a systematic search of the literature using the electronic databases PubMed (1966 to April 1, 2015) and SCOPUS (1966 to

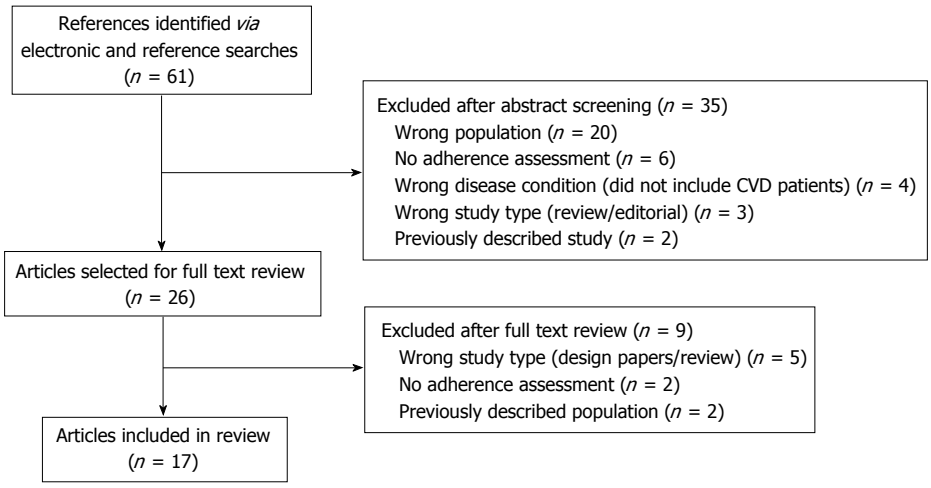

Figure 1 Results of systematic search. CVD: Cardiovascular disease.

April 1, 2015) and reviewed reference lists for relevant articles. Major search terms included "medication adherence" OR "adherence medication" OR "enhancing medication adherence" OR "measuring medication adherence" OR "medication adherence scale" OR "Morisky medication adherence scale" OR "interventions for enhancing medication" AND "South Asia" OR "South Asian" OR "India" OR "Pakistan" OR "Sri Lanka" OR "Nepal" OR "Bangladesh" AND "cardiovascular disease" OR "cardiovascular disease medication" OR "cardiovascular disease medicine".

Working in duplicate, reviewers screened all abstracts and full-text publications for eligibility. The following details were abstracted from included publications: Patient population, sample size, country, study design, adherence measure, definition of adherence and adherence rate.

Eligibility criteria

Eligible studies were defined as fully published (English language) studies that examined CVD medication adherence as the primary or secondary outcome in South Asian populations. English is widely spoken in post-colonial South Asia, and there are very few scientific studies published in native languages in this region. We excluded studies conducted outside of the target population (India, Pakistan, Sri Lanka, Nepal or Bangladesh), studies not reporting the method of adherence assessment, studies not focused on CVD medications, studies with previously described populations, published reviews and editorials, and studies reporting no results (rationale/design papers) (Figure 1).

RESULTS

Results of our systematic search of the literature are shown in the Figure 1. A total of 61 studies were identified through electronic and reference searches, and 35 were excluded after abstract review. The majority of abstracts were excluded because the studies were conducted outside of South Asia (*n* = 20), there was no assessment of adherence reported (*n* = 6), the therapeutic area was not CVD (*n* = 4), the paper was a review or editorial (*n* = 3), or the study population was already included in our review through a different publication (*n* = 2). Of the 26 articles included in the full-text review, we further excluded 9 articles. The reasons for exclusion of full text articles were that the papers were design/rationale or review papers (*n* = 5), no adherence assessment was reported (*n* = 2) and the study population was previously described in an included paper (*n* = 2). Therefore, the final review included 17 articles. A summary of the included studies is shown in the Table 1.

The majority of the studies included in this review were conducted in India (*n* = 11). The remainder of the studies were conducted in Pakistan (*n* = 3), Bangladesh (*n* = 1), Nepal (*n* = 1) and Sri Lanka (*n* = 1). Below, we provide a synthesis of these results in terms of strategies used to measure adherence followed by interventions to improve adherence to CVD medications in South Asian populations.

Adherence assessments

Adherence is defined as "the extent to which a person's behaviour - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider"[10]. Of the 17 publications included in this review, 10 focused on assessing adherence to CVD medications. Adherence was defined and measured using a variety of methods across studies. These studies largely used self-report to determine adherence rate, including the use of the Morisky Medication Adherence Scale (MMAS)[11] and other validated questionnaires, interview questions and pill counts.

Five studies used the validated MMAS[11] as their primary method of adherence assessment. These studies utilized the 4-item MMAS, which scores 1 point for each "no" response and 0 points for each "yes" response, with total scores ranging from 0 (non-adherent) to 4 (fully adherent). Hashmi *et al*[12] used the 4-item MMAS

Table 1 Summary of studies included in the systematic review

Ref.	Patient population	Sample size	Country	Design	Adherence measure	Definition of adherence	Adherence rate
Joshi *et al*[25]	Hypertensive patients seeking treatment	139	India	Cross-sectional	Pill count	⩾ 80% pills taken	79% in controlled hypertensives 39% in uncontrolled hypertensives
Ponnusankar *et al*[32]	Patients with chronic conditions (hypertension, diabetes mellitus, cardiovascular conditions, bronchial asthma)	90	India	Cross-sectional	Pill count Self-assessment	Prescribed doses taken Rating system	92.29% ± 4.5% for counseled group 84.71% ± 11.8% for usual care
Hashmi *et al*[12]	Patients prescribed anti-hypertensive medication for at least 1-mo prior	438	Pakistan	Cross-sectional	Pill count 4-item MMAS	⩾ 80% pills taken ⩾ 0 scores	Pill count : 77% Morisky: Mean overall score = 2.5 ± 1.3
Qureshi *et al*[29]	Patients on anti-hypertensive medications	178	Pakistan	Randomized controlled trial	MEMS bottles	Prescribed doses taken	Intervention arm: 48.1%, 95%CI: 35.8%-60.4% Control arm: 32.4%, 95%CI: 22.6%-42.3%
Kar *et al*[30]	Adults with SBP ⩾ 140	1010	India	Cross-sectional	Self-report	All prescribed doses taken daily in the past 15 d	Baseline: 37.9% Follow-up: 58.3%
Bahl *et al*[26]	Adults with hypertension	1175	India	Prospective	Self-report	If all doses were taken since last visit	100% at each follow-up point
Palanisamy *et al*[31]	Post discharge patients prescribed at least 1 anti-hypertensive medication	43	India	Cross-sectional	4-item MMAS	At least 1 yes response was classified as non-adherent	95.4%
Dennis *et al*[19]	Hypertensive adults with at least 6 mo treatment history	608	India	Cross-sectional	BMQ	⩾ 1 indicates non-adherence	50.33%
Saleem *et al*[21,22]	Hypertension adults using anti-hypertensive drugs for last 6 mo	385	Pakistan	Cross-sectional	DAI-10	⩾ 0 survey scores	Overall mean score: -1.74 ± 2.154
Soliman *et al*[27]	Adults with an estimated 10-yr total CVD risk score greater than 20%	203	Sri Lanka	Randomized clinical trial	Pill count	Not reported	Intervention arm: Over 80% with > 80% pill compliance Usual care arm: Results not provided
Simkhada[16]	Hypertensive patients taking anti-hypertensive medication for at least 6 mo	147	Nepal	Cross-sectional, prospective study	6-item questionnaire	⩽ 1 scores	17.34%
Fathima *et al*[13]	Patients at a CVD clinic	162	India	Cross-sectional	4-item MMAS	⩾ 0 scores	Mean score = 3.2
Thom *et al*[28]	Adults with established CVD, or estimated 5-yr CVD risk ⩾ 15%	2004	Europe and India	Randomized clinical trial	Self-report	Taking medication at least 4 d during the week preceding visit	Intervention arm: 86.3% Usual care arm: 64.7%
Khanam *et al*[23]	Randomly selected hypertensive adults from 3 rural surveillance sites	29960	Bangladesh	Cross-sectional	Self-report	Continued medication use at time of interview	73.8%
Kumar *et al*[15]	Adults on anti-hypertensive medication for more than 6 mo	120	India	Cross-sectional	8-item MMAS Self-reporting	⩽ 2 scores Not-defined in paper	54.2% 96.7%
Rao *et al*[24]	Adults with hypertension and/or diabetes	426	India	Cross-sectional	Self-report	⩾ 80% pills taken	Hypertensive 82.2% Diabetic 83.6%
Venkatachalam *et al*[14]	Adults with hypertension for ⩾ 1 yr	473	India	Cross-sectional	4-item MMAS	⩾ 0 scores	24.1%

MMAS: Morisky medication adherence scale; MEMS: Medication event monitoring system; SBP: Systolic blood pressure; CVD: Cardiovascular disease; BMQ: Brief medication questionnaire; DAI: Drug attitude inventory.

to evaluate adherence to anti-hypertensive therapy in 460 patients from two tertiary care hospitals in Pakistan. Additionally, patients were asked to report the number of pills they were prescribed each week and the number of pills they took and missed over the previous 3, 5, and 7 d. Adherence rate was calculated as pills taken divided by pills prescribed for each time point, with patients taking 80% or more of their prescribed medication classified as adherent, and those taking less than 80% classified as non-adherent. According to the 80% cutoff level, 77% of patients were adherent (mean = 98% ± 5%) and 23% were non-adherent (mean = 39% ± 29%). The mean overall MMAS score was 2.5. Results indicated that adherence by pill count was significantly associated with MMAS score (β = 0.016 for the linear relationship between pill count and

MMAS; $P < 0.001$). Fathima *et al*[13] used the 4-item MMAS to evaluate adherence to prescribed medications among 162 patients with hypertension, diabetes or ischemic heart disease in Bangalore, India. Total scores ranged from 0 (non-adherent) to 4 (fully adherent), with scores of 1, 2, and 3 classified as moderately adherent. The mean MMAS score was 3.2, with 40.1% classified as fully adherent, 58.6% classified as partially adherent, and 1.3% classified as non-adherent. Results showed a significant association between age and adherence, with a significantly higher proportion of patients 60 years of age and older fully adherent (48.1%) compared to those under 60 (32.5%), $P < 0.05$. Similarly, a significantly higher proportion of patients who perceived that their medication was not expensive were fully adherent (51.2%) compared to those who perceived their medication as expensive (27.6%), $P < 0.05$. Venkatachalam *et al*[14] examined determinants of adherence to hypertension medication in 473 individuals residing in South India. Adherence was assessed using the 4-item MMAS, where patients were classified as non-adherent if they failed to meet any one of the four MMAS criteria. Overall adherence was 24.1%, with 51.6% of patients forgetting to take medication regularly, 59.8% having difficulty remembering to take their medication, 53.6% stopping medication upon feeling better, and 55.2% stopping medication upon feeling worse. Kumar *et al*[15] looked at factors associated with medication adherence in 120 hypertensive patients at a tertiary care hospital in India. Adherence was assessed using the 8-item MMAS and through self-report (not well defined in the publication). The 8-item MMAS was scored 1 point for every "yes" response and 0 points for every "no" response, with a score of 2 or greater classified as low adherence, 1-2 as medium adherence, and 0 as high adherence. Despite a self-reported adherence rate of 96.7%, MMAS scores indicated that 45.8% of the study sample had low adherence, 54.2% had medium adherence, and 0% had high adherence.

Simkhada[16] conducted a cross-sectional prospective study of blood pressure control and predictors among 147 hypertensive patients in Nepal. Adherence was assessed using a 6-item questionnaire adapted from Choo *et al*[17] and Morisky *et al*[11] as used by Rose *et al*[18] in a previous publication. The 6 questions assessed difficulty taking medications, forgetting to take medications, number of days medications were missed in the past week, number of days an extra pill was taken in the past week, medications taken less because the patient felt better, and medications taken more because the patient felt worse. Patients who responded "yes" to two or more questions were classified as non-adherent. Results showed that only 29.9% of patients were adherent to blood pressure medications.

Two studies included in this review used validated questionnaires other than the MMAS to assess adherence. Dennis *et al*[19] examined barriers to medication adherence in 608 hypertensive patients in India. Adherence was measured using the Brief Medication Questionnaire (BMQ), and through detailed patient interviews. The BMQ is a self-reported survey that measures barriers to adherence through four screens. The regimen screen (previously validated against Medication Event Monitoring System caps)[20] consists of 5 questions assessing patient behavior towards taking medication. Each question is worth 1 point, where scores greater than 1 indicate non-adherence. Overall, 50.3% of patients were adherent to anti-hypertensive therapy. Many patients believed that either their medications were not working (37.8%), or that medications would bother them (5.9%). Access to medication was also an issue, with 78.6% of patients reporting difficulty paying for medication and 54.9% reporting difficulty getting refills on time.

Saleem *et al*[21] examined the association between knowledge and medication adherence among 385 hypertensive patients in Quetta City, Pakistan. Adherence was measured using the 10-item drug attitude inventory (DAI-10). Scores ranged from -10 to 10, with negative scores indicating non-adherence, 0-5 indicating moderate adherence, and 6-10 indicating high adherence. The DAI-10 was pilot-tested with 40 hypertensive patients for reliability and validity (Cronbach's $\alpha = 0.7$) and translated from English to Urdu. The hypertension fact questionnaire was used to assess patient's knowledge of hypertension, its causes, treatment and management. The overall mean score of the DAI-10 was -1.74 ± 2.15. In this study, 64.7% of patients were classified as non-adherent, 35.3% as moderately adherent, and none as highly adherent. There was a significant, inverse relationship between knowledge and adherence, which investigators noted was a conflicting outcome. Saleem *et al*[22] published another study on the same population, using the same assessment of adherence, looking at the association between adherence and quality of life. These results showed no relationship between quality of life and medication adherence.

Two of the included studies used interview questions to assess adherence. Khanam *et al*[23] measured adherence to anti-hypertensive medication in 29960 patients residing in rural Bangladesh. Adherence was measured through self-reported interview questions using a two-part questionnaire on chronic disease lifestyle risk factors and management. The questionnaire was translated from Bangla to English and back in order to check for consistency of the meaning. Additionally, the questionnaire was piloted and pre-tested. Non-adherence was defined as discontinuation of medication at the time of the interview and was classified as a dichotomous various variable (yes or no) based on this definition. The overall rate of non-adherence in this population was 26.2%. Non-adherence was higher among men (29.2%) than women (24.3%), ($P < 0.001$), decreased with age ($P < 0.001$) and was less common among wealthy people. Non-adherence was greater when hypertension was diagnosed by unqualified providers (community health workers or informal health providers) (OR = 1.67, 95%CI: 1.42-1.97). Those who reported cardiovascular

comorbidities (angina, heart attack or stroke) were more likely to be adherent to medication (OR = 0.78, 95%CI: 0.64-0.97 for non-adherence). Rao *et al*[24] examined medication adherence among 426 patients with either hypertension or type-2 diabetes mellitus in southern India. Adherence to either hypertension or diabetes medication was assessed through self-reported interview questions. Patients were asked to report how they had been taking their medication in the week preceding the interview. Patients who reported taking less than 80% of their prescribed medications in the week preceding the interview were classified as non-adherent. The interview questions were pilot tested and the validity was appraised by experts. Among hypertensive patients, 82.2% reported adherence to treatment, while 83.6% of diabetics reported adherence to treatment. Adherence was higher among females (87.4%) than males (72.2%) ($P < 0.05$). High cost of treatment and asymptomatic disease were the most commonly cited reasons for non-adherence among those with hypertension (39% and 35% respectively) and diabetes (30% and 48%, respectively).

Joshi *et al*[25] examined the relationship between medication adherence and blood pressure control in 156 hypertensive patients in urban India. Adherence was assessed at the end of three month follow-up by pill count. Subjects who took less than 80% of their medication were classified as non-adherent. Adherence was 79% among patients with controlled hypertension, and 39% among patients with uncontrolled hypertension. Additionally, non-adherence was a significant predictor of uncontrolled hypertension in this population (OR = 6.23, 95%CI: 2.36-16.48, $P > 0.0001$).

Strategies for improving adherence

Of the 17 publications included in this review, 7 publications examined interventions aimed at improving adherence to CVD medications in South Asian populations as a primary or secondary outcome. Three of these interventions examined the use of combination or polypill therapy to improve adherence. Bahl *et al*[26] conducted an observational, open-label study to examine the use of a fixed combination therapy of perindopril and amlodipine on the management of hypertension among 1250 patients in India. Adherence was measured *via* self-report through interview questions asked on days 15, 30 and 60 of follow-up. Patients were asked if they had missed any doses and what time they took their medication the day prior to follow-up. All patients who completed the study (94%) adhered to their treatment as specified in the study protocol. It is important to note that there was no usual care group in this study. It is also important to point out that this is a clinical trial, in which participants are generally more adherent than free-living populations. Soliman *et al*[27] examined the effects of a polypill (75 mg aspirin, 20 mg simvastatin, 10 mg Lisinopril and 12.5 mg hydrochlorothiazide) *vs* usual care (not specified) on the prevention of CVD in 216 patients without established CVD. Adherence was assessed *via* monthly self-reports using pill counts (no further details provided). Results showed that the polypill intervention arm had 80% of patients with greater than 80% adherence, 6% with 60%-79% adherence, 3% with 40%-59% adherence, and 10% with < 40% adherence. Results for the usual care group were not reported. Thom *et al*[28] studied the effects of a fixed-dose combination (FDC) therapy (either aspirin, 75 mg; simvastatin, 40 mg; lisinopril, 10 mg; and atenolol, 50 mg or aspirin, 75 mg; simvastatin, 40 mg; lisinopril, 10 mg; and hydrochlorothiazide, 12.5 mg) *vs* usual care (treated at the discretion of their usual physician) on medication adherence and risk factors in patients at high risk of CVD. They utilized data from the Use of a Multidrug Pill in Reducing Cardiovascular Events randomized clinical trial of 1004 patients from Europe, and 1000 from India. Adherence was measured through self-report during a follow-up visit and defined as taking medication at least 4 d during the week before the visit. For patients in India, adherence in the FDC group *vs* the usual care group was 89.1% and 63.6%, respectively (RR = 1.40; 95%CI: 1.30-1.51). For patients in Europe, adherence in the FDC group *vs* the usual care group was 83.6% and 65.8%, respectively (RR = 1.27; 95%CI: 1.18-1.37). The effects of the FDC strategy on adherence did not differ significantly between patients from India *vs* patients from Europe ($P = 0.07$ for interaction).

Qureshi *et al*[29] used MEMS bottles to determine the impact of an education package aimed at general practitioners to increase adherence to anti-hypertensive drugs in 200 patients in Karachi, Pakistan. Components of the education package included non-pharmacological (diet, exercise, weight loss, smoking cessation) and pharmacological interventions (prescribing low cost and appropriate generic drugs; preferential use of single dose drug regimens; scheduled follow-up visits; stepped care approach for titration of drugs to achieve target blood pressure levels; and satisfactory consultation sessions for patients, with explanations of treatment and use of appropriate communication strategies). Adherence, defined as percentage of prescribed doses taken, was measured using MEMS bottles, which electronically monitored and recorded the time each cap was opened. Adherence was recorded by community health workers at the end of weeks 1, 3 and 6 of the study. Adherence was significantly better in the intervention group (unadjusted mean percentage days with correct dose) *vs* the standard of care group (48.1% *vs* 32.4%, $P = 0.048$) over the 6 wk period. Greater adherence was significantly associated with higher levels of education ($P < 0.001$), patients being encouraged by family members to take medications ($P < 0.001$), patient's belief in drug effects ($P < 0.001$) and having the purpose of the drug explained to the patient ($P < 0.001$).

Three small scale interventions used counseling to improve adherence. Kar *et al*[30] conducted a community intervention to implement the World Health Organization (WHO) CVD risk management package in India. The

intervention was implemented among 1010 adults from a randomly chosen cluster of households, and 79 hypertensive patients were followed at 1, 3, and 5 mo to reinforce risk prevention and adherence to medications. Adherence was assessed by self-report and defined as daily intake of all prescribed anti-hypertensive medication in the past 15 d. In the intervention households, regular intake of medication increased from 37.9% to 58.3% ($P < 0.05$), while in the control households, it decreased from 43.5% to 34.8%. Palanisamy *et al*[31] conducted a non-randomized intervention to improve adherence among 43 post discharge hypertensive patients in India, and analyzed differences in adherence pre and post intervention. After discharge, patients were given counseling on drugs and lifestyle modification, were provided with a medication schedule reminder and received frequent telephone reminders from study pharmacists. Adherence was assessed using a version of the Morisky scale, which included a 5-point response option (never/rarely/sometimes/often/always) and a set of open-ended questions regarding reasons for non-adherence. Scores ranged from 0-4 for the response option questions and 0-16 for the open-ended questions, with higher scores indicating poorer adherence. All patients who answered "yes" to at least one question were considered non-adherent. At baseline, 100% of the patients were considered non-adherent, whereas at the second interview, 51.2% were non-adherent and at the third interview, only 4.6% were non-adherent. Ponnusankar *et al*[32] conducted a randomized study of 90 patients with chronic conditions to assess the impact of patient medication counseling on adherence. Patients in the intervention group received a 15-20 min medication counseling session from a pharmacist. In order to determine adherence, patients were asked to bring back all remaining medications and empty foils along with medication receipts, as well as to complete an adherence self-assessment form. As measured by pill count at follow-up, adherence was 92.2% ± 4.5% for counseled group and 84.7% ± 11.8% for the usual care group. As measured by the self-assessment form, 75% of patients in the intervention group and 67% of patients in the usual care group rated themselves as adherent.

Two additional interventions were identified, however, only the design/rationale papers have been published at this point. Fathima *et al*[33] reported the design of the Primary pREvention strategies at community level to Promote Adherence of treatment to pREvent cardiovascular diseases (PrePare) study, a multi-center, household-level, cluster-randomized trial to improve systolic blood pressure (BP) and medication adherence in at-risk households in India. The intervention consists of household visits by community health workers (CHWs) every two months. The CHWs will assess adherence by administering a questionnaire and inspecting the empty blister packets or purchase receipts, measure BP and ascertain if BP values meet the preset targets, ensure that individuals adhere to the prescribed treatment by using educational messages to target tobacco use, adherence to medication, and promote lifestyle changes and set goals for BP and weight and reduction in tobacco use and weight reduction goals for the next visit. Kamath *et al*[34] reported the design of the Secondary Prevention of Acute Coronary Syndromes study, a hospital-based, open-label, randomized trial of community health worker interventions *vs* usual care in India. The intervention includes a patient diary with information on ischemic heart disease risk factors, treatments and importance of treatment compliance, as well as calendar checklist, on which patients mark every time they take a dose of their medications. Adherence will be calculated based on the percentage of doses taken of those prescribed. The 4-item MMAS will also be used to assess adherence at follow-up visits. If patients prematurely stopped taking their medications, the reasons for doing so will be elicited.

DISCUSSION

In this systematic review, we provide a summary of the methodologies used to assess and interventions to improve adherence to CVD medications in South Asians. The most common method for assessing adherence was patient self-report ($n = 16$). While self-reporting does open the door to recall bias, it provides one of the most economically feasible methods for data collection. All of these studies were conducted in developing countries or in resource limited settings, which could be a contributing factor to the use of self-report rather than "gold standard" methods such as MEMS caps or pharmacy refill data. Usage of MEMS caps in resource-limited settings is most likely constrained due to their high costs (approximately $100 USD per cap)[35-37]. Only 1 study in our review included the use of MEMS caps, in a region where the gross national income per capita is $1483 USD, which is the lowest in the world[38]. As expected, pharmacy refill data, which relies on complete pharmacy records, was not used by any of the included studies. Without reliable and interoperable electronic pharmacy records, tracking where patients get their medication from can prove to be largely difficult and resource intensive.

In addition to primarily relying on self-report, several of the studies did not use validated instruments. Of the 16 studies using self-reported measures, 7 used validated questionnaires, while 9 studies used non-validated interview or survey questions. Furthermore, there was a general lack of information on these interview or survey questions. Details on how adherence rates were calculated or how data was collected were missing in several of these publications. There was also a general lack of detail on what class and type of medications was being measured for adherence. From the studies that did report medication type, the majority were anti-hypertensive medications. There was little data on other CVD medications such as anti-platelets and cholesterol lowering medications.

It is important to note that the adherence rates from non-validated, self-reported interview or survey

questions were typically higher than those of the validated scale measurements. The most glaring of disparities appears in Kumar *et al*[16] where 96.7% of patients were classified as adherent according to a self-report assessment form and only 54.2% of patients were classified as medium adherent and 0% classified as highly adherent according to the 8-item MMAS. Therefore, researchers in this region should consider using validated measures such as the MMAS as opposed to non-validated measures when assessing adherence. Additionally, several of these papers did not assess whether a higher medication adherence to hypertensive medications was associated with an improvement in an intermediate outcome measure such as blood pressure control. Generally the adherence rates were low which shows a growing need for future interventions geared towards improving adherence.

Overall, very few studies (n = 7) evaluated the impact of interventions to improve adherence. One intervention targeted provider education. While important, provider based interventions are typically considered quality improvement and are generally considered weak interventions[39]. Three interventions focused on the use of allied health care providers, such as community health workers or pharmacists, to educate or counsel patients on the importance of adhering to medication. Two of these interventions provided drug counseling from pharmacists to patients in an attempt to improve medication adherence, and both showed promising results. The third followed the WHO CVD risk management package utilizing community health care workers to assess risk and provide counseling on lifestyle changes to manage that risk. Community health care workers are a unique resource available to most if not all of these countries and could form the backbone for interventions directed at improving medication adherence in these low resource environments. The final 3 interventions were geared towards reducing pill burden through the use of polypills. No interventions targeted patient-provider communication, which may be an important determinant of a patient's medication adherence[40].

An important distinction to note is that non-adherence can be classified as either (1) intentional; or (2) unintentional. Intentional non-adherence is related to patient's beliefs and knowledge and health seeking behaviors, while unintentional non-adherence is related to demographics and comorbid illness[41]. Unintentional and intentional non-adherence to medications are related to different patient characteristics. A review of medication adherence in native and immigrant South Asians noted that the primary factors related to non-adherence in the included studies were forgetfulness, side-effects and choosing not to take the medication[42]. This suggests that both unintentional and intentional nonadherence contribute to overall non-adherence in South Asian populations and successful interventions aimed at improving adherence in this population should address both mechanisms. The interventions included in this review did not make such a distinction, and thus the generalizability of the results is limited.

Several studies cited behavioral barriers to medication adherence. Interventions geared towards patient education could improve patient self-efficacy and help break down these barriers. Additionally, there was a general lack of system-wide interventions such as audit and feedback and decision support systems. Although the lack of electronic medical record data in this region would lead to a low overall yield for decision support systems. However, audit and feedback interventions could be utilized more in resource constrained settings. Furthermore, we found a lack of interventions utilizing technology to assist patient adherence. Several of these regions benefit from a high cell phone density[43]. However, none of the interventions included in this review used cell phone reminders through text messages or other means to improve medication adherence. Although most interventions used in these studies showed promising results, further research is needed to determine which interventions or combination of interventions is the most effective strategy for improving medication adherence in this resource constrained environment. This research along with cost-effectiveness of each of the above mentioned strategies would be informative as policy makers decide which of these interventions could be scaled up to the population level.

In conclusion, we found that self-report measures were the most commonly used method to assess adherence. Although several interventions were directed towards providers, allied health care professionals and community health care workers, there is a need to employ strategies directed towards provider-patient communication. Additionally, there is a need to better incorporate technology such as cell phones, which are readily available for most people living in these countries. Examples of this type of intervention include the use of tailored and specific Short Text Message (SMS) reminders to improve medication adherence[44] and the use of text and a voice SMS in local language to improve health literacy and medication adherence[45].

Future research should focus on using more validated self-reported measurements. These scales should be validated in South Asian populations specifically, since health literacy may vary from previously studied populations. Additionally, there is a need to tie these adherence measurements to intermediate outcome measures such as blood pressure or cholesterol control as well as determine their effects on CVD outcomes.

COMMENTS

Background

Cardiovascular disease (CVD) is the leading cause of death worldwide, with an estimated 17.5 million people dying from CVD in 2012. Approximately one-fifth of the global population resides in South Asia, where patients suffer from a disproportionately high rate of CVD-related morbidity and mortality. Adherence to medication is critical to the effectiveness of CVD risk management. Therefore, the aim of this review is to examine current methods of assessing adherence and strategies to improve adherence to CVD medications, among

South Asian CVD patients.

Research frontiers

Current research indicates a growing epidemic of premature CVD in South Asian populations. Establishing suitable strategies to assess adherence to CVD medications is of particular importance to South Asian countries for various reasons. First, low availability of electronic medical records in most health care settings precludes accurate assessment of medication adherence using electronic medication refill data by health care providers. Second, the overall health literacy and the opportunities to improve provider and patient awareness of the importance of medication adherence may be limited. Lastly, low availability of pharmacy records and medication refill data also limit the use of traditional measures used to assess medication adherence.

Innovations and breakthroughs

Understanding adherence to CVD medications in South Asian populations is an important research question. This study focuses on the specific challenges and complexities related to CVD medication adherence in native South Asians.

Applications

This review demonstrates the need to identify a gold standard for assessment of adherence related to CVD medications in South Asians. Additionally, there is a need to employ intervention strategies directed towards provider-patient communication and to tie these interventions to intermediate outcome measures in order to determine their effects on CVD outcomes.

Terminology

Adherence in this review is defined as "the extent to which a person's behaviour - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider". The Morisky Medication Adherence Scale is a 4 or 8-item self-report questionnaire that results in a score ranging from 0 (non-adherent) to 4 or 8 (fully adherent).

Peer-review

In this manuscript, Virani *et al* reviewed mainly adherence to antihypertensives in South Asian populations and the methodology of studies conducted in this field. It is an interesting topic and the presentation of data is impressive.

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SYSTEMATIC REVIEWS

Global myocardial strain assessment by different imaging modalities to predict outcomes after ST-elevation myocardial infarction: A systematic review

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Abstract

AIM: To conduct a systematic review relating myocardial strain assessed by different imaging modalities for prognostication following ST-elevation myocardial infarction (STEMI).

METHODS: An online literature search was performed in PubMed and OVID® electronic databases to identify any studies that assessed global myocardial strain parameters using speckle-tracking echocardiography (STE) and/or cardiac magnetic resonance imaging (CMR) techniques [either myocardial tagging or feature tracking (FT) software] in an acute STEMI cohort (days 0-14 post-event) to predict prognosis [either development of major adverse cardiac events (MACE)] or adverse left ventricular (LV) remodelling at follow-up ($\geqslant$ 6 mo for MACE, $\geqslant$ 3 mo for remodelling). Search was restricted to studies within the last 20 years. All studies that matched the pre-defined search criteria were reviewed and their results interpreted. Due to considerable heterogeneity between studies, meta-analysis was not performed.

RESULTS: A total of seven studies (n = 7) were identified that matched the search criteria. All studies used STE to evaluate strain parameters - five (n = 5) assessed global longitudinal strain (GLS) (n = 5), one assessed GLS rate (GLS-R) (n = 1) and one assessed both (n = 1). Three studies showed that GLS independently predicted the development of adverse LV remodelling by multivariate analysis - odds ratio between 1.19 (CI: 1.04-1.37, P < 0.05) and 10 (CI: 6.7-14, P < 0.001) depending on the study. Four studies showed that GLS predicted the development of MACE - hazard ratio (HR) between 1.1 (CI: 1-1.1, P = 0.006) and 2.34 (1.10-4.97, P < 0.05). One paper found that GLS-R could significantly predict MACE -

HR 18 (10-35, $P < 0.001$) - whilst another showed it did not. GLS < -10.85% had sensitivity/specificity of 89.7%/91% respectively for predicting the development of remodelling whilst GLS < -13% could predict the development of MACE with sensitivity/specificity of 100%/89% respectively. No suitable studies were identified that assessed global strain by CMR tagging or FT techniques.

CONCLUSION: GLS measured acutely post-STEMI by STE is a predictor of poor prognosis. Further research is needed to show that this is true for CMR-based techniques.

Key words: Strain; Speckle tracking; Tagging; Feature tracking; Myocardial infarction; Major adverse cardiac events; Remodelling

Core tip: Global myocardial strain is an objective measure of cardiac function. It can be assessed using post-processing analysis on different imaging modalities such as speckle-tracking echocardiography (STE) and cardiac magnetic resonance imaging (CMR) - tagging and feature tracking. We performed a systematic review that showed global longitudinal strain (GLS) measured acutely by STE following ST-elevation myocardial infarction (STEMI) predicted clinical outcomes and adverse left ventricular remodelling, a surrogate marker of poor prognosis. No relevant studies were found for CMR techniques. GLS may refine risk stratification in the STEMI population but further work is needed to support this.

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INTRODUCTION

Ischaemic heart disease (IHD) presents a significant burden to healthcare services and is one of the leading causes of death worldwide[1]. Acute myocardial infarction (MI) results from spontaneous coronary artery occlusion due to thrombus formation as a result of plaque rupture and subsequent platelet aggregation - most commonly seen with the background of IHD[2]. ST-elevation myocardial infarction (STEMI) is an acute emergency that requires prompt reperfusion by either primary percutaneous coronary intervention (PPCI) or thrombolysis, ideally within two hours of symptom onset[3].

Timely reperfusion has led to a reduction in mortality from acute MI[4]. However, despite receiving current best therapy, a significant number of patients still develop complications post-MI that includes new-onset heart failure (HF)[5] - 20.4% of patients develop HF on admission and 8.6% subsequently[6]. The incidence of HF has increased over the past few decades[7] and it is especially prevalent amongst the elderly[8]. Long-term mortality from HF still remains high, even with the best contemporary pharmacological and non-pharmacological interventions[9]. The increase in HF incidence may partly be a result of improved survival post-MI, albeit with greater morbidity in some survivors.

Outcomes after STEMI

Major adverse cardiac events: Major adverse cardiac events (MACE) are often used in cardiovascular studies as a measure of clinical outcomes after STEMI. It is an umbrella term that includes a variety of measures - including all-cause mortality, hospital readmission due to HF, recurrence of MI, need for revascularisation, and occurrence of stroke. Demographic features associated with poor outcomes post-STEMI include age[10], diabetes[11], hypertension[12], infarct location (*i.e.*, anterior MI)[13], large infarct size (IS)[14] and presence of microvascular obstruction[15].

"Hard events" such as mortality are the best markers of outcome. However, these are relatively rare occurrences and so require a considerable sample size to demonstrate statistically significant association with a biomarker, or effects of intervention[16] and some authors believe that studies reporting these need to have a sample size of $n > 1000$ to be statistically robust[17]. Such large, multi-centre trials are challenging to conduct and need to be carried out over a considerable period of time in order to accrue the required sample sizes and numbers of events. Consequently, surrogate markers of poor outcome such as adverse left ventricular (LV) remodelling can be used in lieu of hard outcomes with much smaller sample sizes to achieve statistically significant results.

Adverse LV remodelling: Adverse LV remodelling post-MI is thought to be the main process underpinning the development of HF and is defined as: "A change in size, shape and function of the heart resulting from cardiac load or injury"[18]. It is a complex process that progresses over a period of weeks to months post-infarct (Figure 1). Adverse LV remodelling post STEMI can be defined as either an increase in end-diastolic volume (EDV) of > 20% or end systolic volume (ESV) of > 15%, at follow-up compared to baseline. However, there is no consensus on which definition is better. Several cellular, extracellular, inflammatory, and neuro-hormonal pathways have been implicated to play a role in development of LV remodelling; these include neutrophils[19], macrophages[19], collagen fibres[20], various metallo-proteinases[20] and activation of the sympathetic nervous system along with the renin-angiotensin-aldosterone system (RAAS)[7,18] amongst others. The exact role of

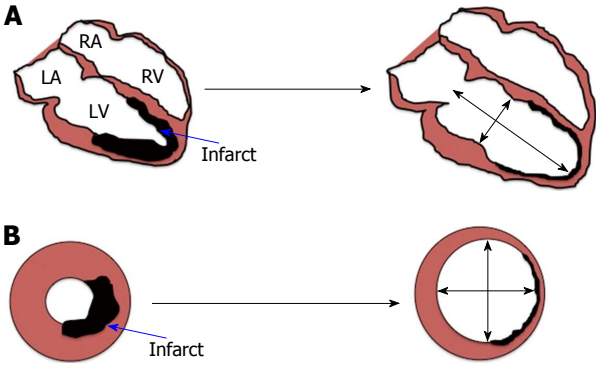

Figure 1 Development of adverse left ventricular remodelling post-myocardial infarction in (A) long axis view and (B) short axis view. LA: Left atrium; LV: Left ventricle; RA: Right atrium; RV: Right ventricle.

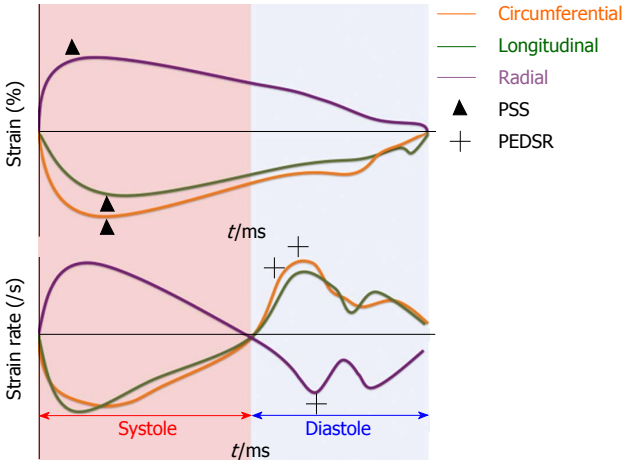

Figure 3 Strain and strain rate values as a function of time - peak systolic strain and peak early diastolic strain rate are annotated. PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate.

these components has not yet been elucidated and there is still some controversy over the initial trigger of remodelling[21]. There is good evidence to suggest that angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and aldosterone antagonists attenuate the process of adverse remodelling by inhibiting RAAS[18].

Early identification of high-risk patients who are likely to undergo adverse LV remodelling may allow targeted therapeutic intervention in these patients to counteract remodelling processes. Parameters that reflect myocardial dysfunction can potentially be utilised to help identify such patients as cardiac function is often affected post-MI, which usually precedes development of overt HF.

LV dysfunction post-infarction

Traditionally, the systolic phase of the cardiac cycle is often used as a measure of LV function in a clinical setting. A region of myocardium affected by an infarct may have impaired contractility due to death of myocytes in that zone. Ejection fraction (EF) is the most commonly used method to assess systolic function and a

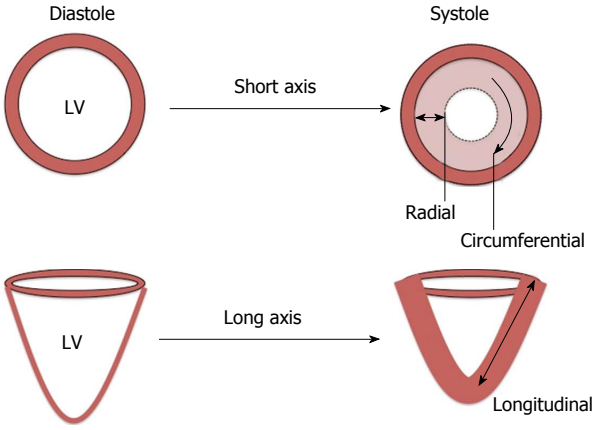

Figure 2 Myocardial contraction in three vectors - circumferential, longitudinal and radial. LV: Left ventricle.

reduced EF, commonly measured by echocardiography, is known to be associated with a poor outcome[22]. However, EF is relatively insensitive to regional differences in myocardial function and has been shown to be a poor predictor of late myocardial dysfunction when measured acutely after reperfusion therapy[23]. Wall Motion Score Index (WMSI) has also been used in addition to EF but it has the inherent shortcoming of being a subjective measure based on the experience of the assessor. WMSI is based on either the 16-segment[24] or the 17-segment model[25] of the LV.

An infarct is also thought to affect LV compliance by increasing wall stiffness and hence reducing active relaxation of the myocardium - this can cause diastolic dysfunction[26]. Recent evidence suggests that diastolic dysfunction post-MI measured by echocardiography confers a poor outcome[27,28].

The optimal marker of LV dysfunction would: (1) Be objective and "angle independent"; (2) Be sensitive to myocardial dysfunction early after an MI; (3) Offer an evaluation of both regional and global LV contractility; (4) Provides an assessment of both systolic and diastolic heart function; and (5) Be reproducible and easy to measure.

Myocardial strain

Strain is defined as the change in length of an object relative to its original length[29]. In the heart, myocardial strain is a sensitive measure of contractility. Strain can be calculated at both the segmental and global level and in the three axes of myocardial contraction - circumferential, longitudinal and radial (Figure 2). Strain rate (SR) measures the change in strain for a given vector as a function of time and can also be assessed. Systolic and diastolic strain rates vary throughout the cardiac cycle (Figure 3).

Anatomically, myocardial fibres are orientated longitudinally in the sub-endocardium and circumferentially in the mid-myocardium[30]. This suggests that longitudinal strain (LS) can provide a reflection of sub-endocardial function whilst circumferential strain can inform mid-myocardial function. Radial strain, whilst

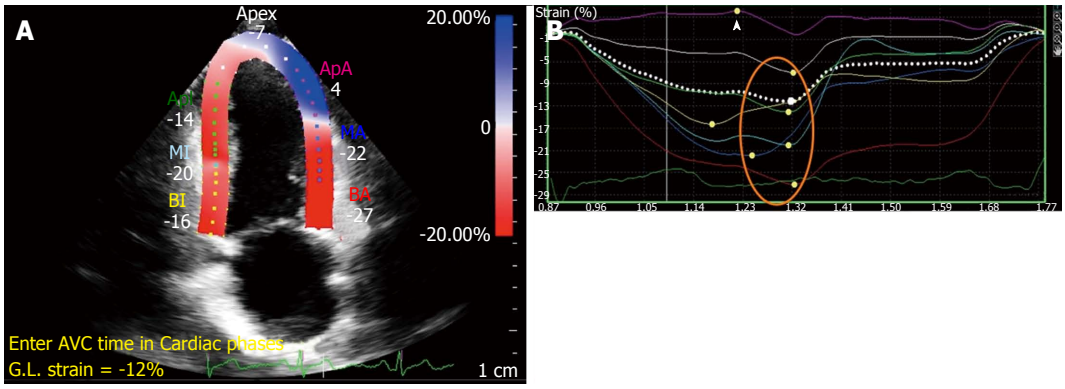

Figure 4 Peak systolic strain calculated by speckle-tracking echocardiography. A: Segmental strain after definition of endocardial and epicardial contours; B: Graphical illustration of segmental peak systolic strain - normal values annotated by orange circle, impaired strain by arrowhead.

being potentially informative of myocardial contraction in the short axis, has been shown to have high intra- and inter-observer variability[31] making it unsuitable for routine clinical practice. Peak systolic strain (PSS) is commonly used to assess myocardial contraction whilst peak early diastolic strain rate (PEDSR) is a marker of diastolic function[32]. Consequently, strain/diastolic strain rate assessment provides a comprehensive evaluation of myocardial contractility and compliance.

Myocardial Strain/strain rates can be assessed by a number of different imaging modalities - most frequently by echocardiography, but also by cardiac magnetic resonance imaging (CMR).

Echocardiography

Tissue Doppler imaging can assess myocardial strain but this technique is extremely angle dependent and has been superseded by speckle tracking echocardiography (STE)[33,34]. The ultrasonic images obtained by echocardiography consist of a large number of "speckles" which have individual properties[35]. These "acoustic markers"[34] can be identified and tracked as they move from one frame to the other throughout the cardiac cycle. Endocardial and epicardial borders are pre-defined by the operator and each speckle within this region of interest (ROI) is tracked. The tracking of such movement can be used to derive measures of strain[36] and strain rate[33]. STE is entirely a post-processing analysis. The only minor requirements are a short duration of breath holding by the patient so that respiratory motion does not affect the tracking of cardiac motion and a high frame rate to optimize temporal resolution.

Common echocardiographic imaging protocols include the acquisition of two-, four-chambered and three chamber views from which global LS (GLS) is derived (Figure 4). Short axis views allow circumferential and radial strain to be derived but it is difficult to accurately obtain global measures due to uncertainty of the imaging plane location.

STE-derived global strain parameters in the setting of an acute STEMI have shown good reproducibility - intra- and inter-observer variability of 0.92 and 0.85 by Intra-class Correlation Coefficient (ICC) respectively[37]. Repeatability is a measure of the "variation in repeat measurements made on the same subject under identical conditions made within a short period of time over which the underlying value can be considered to be constant"[38]. It is another method of establishing reliability. However, no studies to date have reported the repeatability of global strain measured by STE in acute STEMI.

CMR

CMR is another non-invasive imaging modality and is an alternative method of imaging to echocardiography. CMR can be used in the diagnosis, risk-stratification, and prognosis of a number of cardiac disorders[39,40], including acute MI[41-43]. Typically, strain is assessed on CMR using specialised myocardial tissue tagging sequences that involves the superimposition of horizontal and vertical lines on a cine image that appear in the form of a "grid"[44]. These grids or "tags" are formed onto the tissue by changing the local magnetisation through the use of selective radiofrequency saturation pulses perpendicular to the plane of image acquisition[45]. Tags deform along with the myocardium through the cardiac cycle and this deformation can be used to assess strain. Tagged images are commonly acquired using spatial modulation of magnetisation (SPAMM)[46] and complementary SPAMM sequences[47]. Post-processing analysis of tagged data can be performed using Harmonic phase analysis[48] and local sine wave modelling[49] and they have been shown to have good agreement[50]. Tagging has been validated against other invasive methods of strain assessment such as sonomicrometry[51] and has been used in a variety of animal models[52-54]. Tagging-derived strain parameters have a good intra- and inter-observer variability - ICC of 0.8 for both - along with acceptable test-retest repeatability - ICC of 0.74[55].

Tagging sequences however involve relatively long breath holds that may be difficult in the context of a recent STEMI. In addition, analysis is also labour-intensive and time-consuming[56]. Tagging, particularly with SPAMM sequences, cannot reliably calculate diastolic strain as the tags fade after systole especially at the 1.5 T field strength[45,57]. This can be overcome

Table 1 Advantages and disadvantages of speckle-tracking echocardiography *vs* cardiac magnetic resonance imaging

Advantages	Disadvantages
Cheaper than CMR scan	Cannot acquire SAX views easily - needed to calculate circumferential strain
Can be performed at the bedside	Cannot routinely obtain stress imaging as part of acquisition protocol
Short duration: 10-20 min for STE *vs* 45-60 min for CMR	Not possible to ascertain infarct size, oedema, microvascular obstruction
Significant contraindications for CMR - for example, pacemaker/ICD, brain aneurysmal clip, claustrophobia, eGFR < 30 mL/min per 1.73 m^2 - *vs* almost none for STE	CMR has much higher spatial resolution than STE. Consequently, a greater percentage of images are analysable by CMR than STE

CMR: Cardiac magnetic resonance imaging; eGFR: Estimated glomerular filtration rate; ICD: Insertable cardioverter defibrillator; SAX: Short axis; STE: Speckle-tracking echocardiography.

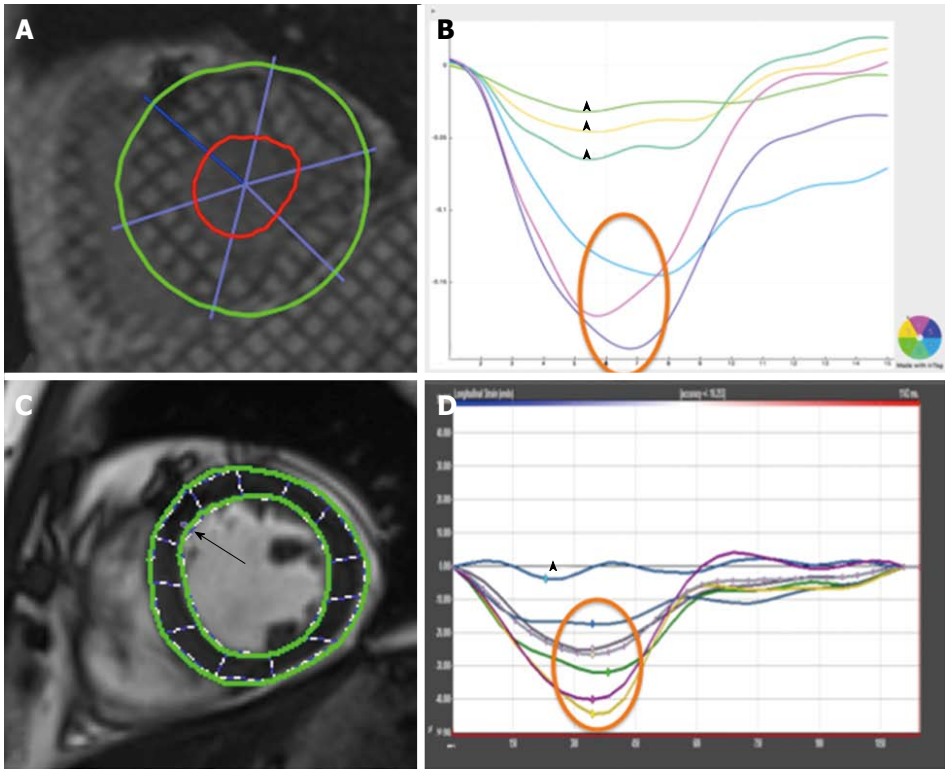

Figure 5 Comparison of tagging (A and B) and feature tracking (C and D) for evaluation of global circumferential strain - normal peak systolic strain annotated by orange circle, impaired peak systolic strain by arrowhead.

by using a stronger magnetic field strength (3.0 T) and Steady State Free Precession (SSFP) sequences[45]. However, true reproducibility is poor at 3.0 T CMR[58]. This may in part be due to the fact that by 3.0 T CMR images are also more susceptible to artefacts due to increase in inhomogeneity within the magnetic field[59].

To overcome the issues of tagging, myocardial motion tracking through the cardiac cycle on routinely acquired cine SSFP sequences can be performed by means of the novel feature tracking (FT) software[60]. FT is analogous to STE - endocardial and epicardial borders are defined and then subsequently propagated through the cardiac cycle. The software tracks the motion of the defined ROI from one frame to the next - PSS and PEDSR can be derived from this motion[60]. FT has shown excellent reproducibility - intra- and inter-observer variability of 0.988 and 0.971 in terms of ICC[61] - and acceptable test-retest repeatability - ICC of 0.77[56] - for PSS. Additionally, PSS by FT can predict global recovery of LV function in terms of EF[62].

Figure 5 illustrates a comparison of global circumferential strain (GCS) evaluation by tagging and FT.

STE vs CMR to assess strain

STE has several advantages over CMR in the assessment of strain (Table 1). There is good agreement between STE-derived and CMR derived global values of strain - this is true both for tagging[63,64] and FT[65]. This suggests that these methods could be used interchangeably in the assessment of global strain. A detailed comparison of different imaging modalities to be used in the setting of an acute MI can be found elsewhere[36].

Aims of systematic review

Global myocardial strain can objectively evaluate LV dysfunction post-STEMI and can be measured by

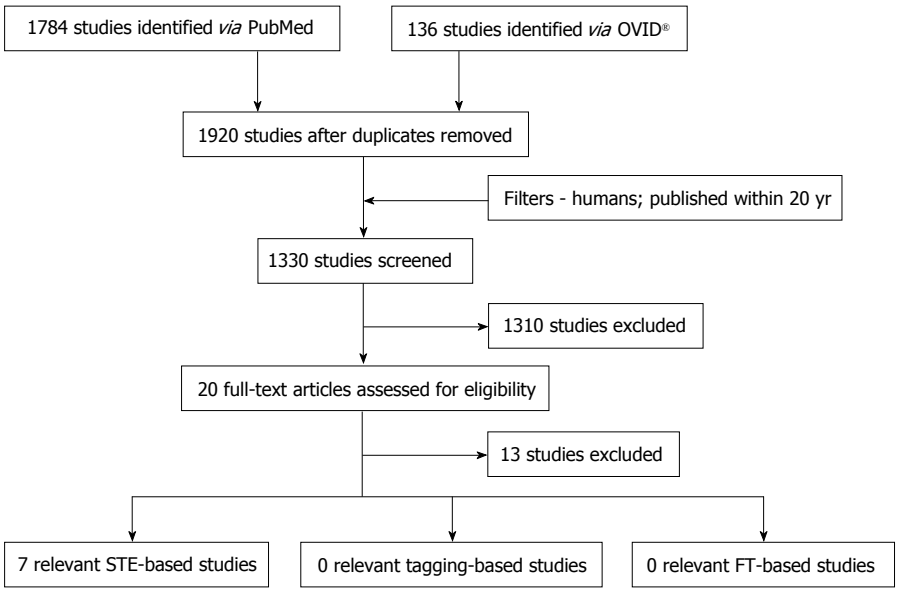

Figure 6 Flowchart illustrating the search for relevant studies. FT: Feature tracking; STE: Speckle-tracking echocardiography.

Table 2 Eligibility criteria for systematic review

Type of characteristic	
Population type	Acute STEMI
Measured parameters	Global longitudinal and/or circumferential strain and/or strain rate - PSS or strain rate (PSS-R) or PEDSR
Imaging modalities	STE or cardiac MRI tagging or cardiac MRI FT
Timeframe for baseline scan	Days 0-14 post-STEMI
Outcomes reported	MACE or adverse LV remodelling
Timeframe for follow-up	MACE - ⩾ 6 mo Adverse LV remodelling - s ⩾ 3 mo
Year published	Within the last 20 yr

STEMI: ST-elevation myocardial infarction; PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate; STE: Speckle-tracking echocardiography; FT: Feature tracking; MACE: Major adverse cardiac events; LV: Left ventricular; MRI: Magnetic resonance imaging.

STE and CMR techniques with good reproducibility and repeatability. We looked to review the literature for studies that evaluated the ability of global strain measured acutely post-STEMI by either STE or CMR to predict either MACE or development of adverse LV remodelling.

MATERIALS AND METHODS

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol[66].

Eligibility criteria

Table 2 highlights the eligibility criteria for the review. Studies were limited to acute STEMI patients to represent the setting of an acute MI - NSTEMI patients were excluded since the diagnosis is more complex, heterogeneous presentations and that their subsequent management is based on risk-stratification[67]. There was no limitation placed on the management of the STEMI - both in terms of method of revascularisation (PPCI or thrombolysis) and success/failure. Strain parameters were restricted to peak systolic GCS and GLS and PEDSR in the same two vectors. Both segmental strain values and radial strain parameters were excluded since they both have been shown to have poor intra- and inter-observer variability[31,58]. We limited the timeframe for the baseline scan to be 0-14 d post- to limit the effects of subsequent remodelling. The timeframe for outcome measures were ⩾ 3 mo for adverse LV remodelling (since it is a dynamic process that takes months to fully develop[68]). Minimum follow-up time for development of MACE was six months. We included studies that quoted either changes in EDV or ESV.

Search protocol

The literature search was performed in PubMed and OVID® electronic databases. The final date on which the online search was performed was January 27th, 2015 (Table 3) for list of keywords used.

Study selection

Figure 6 highlights the process of study selection. Initial electronic search yielded 1920 studies; 1330 remained after addition of relevant filters. The titles and abstracts of these studies were then screened to assess for eligibility for inclusion in the systematic review (Table 2). A majority of the studies were deemed inappropriate for inclusion based on the aforementioned criteria (n = 1310). The remaining 20 papers were further scrutinised by searching for and evaluating the full-text article. A further 13 studies were excluded - some did not actually assess strain at all (n = 4), some assessed torsion (n = 3), three had included NSTEMI patients and the rest did not have full-text articles available as they were presented as posters (n = 3). Consequently,

Table 3 Keywords used for search of electronic databases

"Cardiac MRI" OR "CMR" OR "magnetic resonance imaging [MeSH Term]" OR "cardiac magnetic resonance" OR "feature tracking" OR "tissue tracking" OR "tagging" OR "tag" OR "tagged" OR "SPAMM" OR "CPSAMM" OR "HARP" OR "SinMOD" OR "Echocardiography [MeSH Term]" OR "Speckle tracking", "2D speckle" OR "3D speckle" OR "two dimensional speckle" OR "three dimensional speckle". MIs were searched using "myocardial infarction [MeSH Term]" OR "acute MI" OR "STEMI" OR "ST elevation". Strain was searched using "strain" OR "myocardial strain" OR "strain rate" OR "deformation" OR "myocardial deformation" OR "systolic" OR "diastolic" OR "PSS" OR "PEDSR" OR "longitudinal" OR "circumferential". Outcomes were searched using "Predict" OR "Outcome" OR "Risk" OR "Prognosis" OR "Logistic Models [MeSH Term]" OR "risk" OR "multivariable" OR "multivariate" OR "odds" OR "MACE" OR "mortality [MeSH Term]" OR "remodelling" OR "remodelling" OR "adverse" OR "cardiac" OR "left ventricular"
Note: MeSH terms were only available on PubMed

MRI: Magnetic resonance imaging; CMR: Cardiac magnetic resonance imaging; STEMI: ST-elevation myocardial infarction; PSS: Peak systolic strain; PEDSR: Peak early diastolic strain rate.

there were seven studies that matched our inclusion criteria for the review.

RESULTS

Strain measured by STE

Seven STE-based studies that matched our inclusion criteria were found (Table 4) highlights studies that assessed global strain to predict adverse LV remodelling and Table 5 highlights studies that used global strain to predict MACE. Six studies reported peak systolic longitudinal strain parameters to predict outcomes - only one study used diastolic strain. All the patients were treated with PPCI.

Multivariate analyses in all the studies have shown that peak systolic GLS can independently predict both adverse LV remodelling and MACE. Such analyses have shown that this is independent of factors such as age, diabetes, location of infarct, EF and WMSI. One study showed that global longitudinal SR (GLS-R) also had significant impact on prognosis[69] - patients with impaired GLS-R, and GLS, were 18-times more likely to suffer from composite endpoint of mortality, readmission due to HF, revascularisation, or re-infarction. One study showed that a cut-off GLS > -12.5% (*i.e.*, LV unable to contract more than 12.5% of its original length in the longitudinal vector) could predict development of remodelling - OR 1.19 (1.04-1.37), $P < 0.05$, sensitivity/specificity of 69%/79%[70]. Another showed a cut-off of GLS = 10.85% - OR 0.39 (0.26-0.57), $P < 0.01$, sensitivity/specificity of 89.7%/91.7%[71]. A cut-off for prediction of MACE ranged from GLS > -13% [HR = 2.34 (1.10-4.97), $P < 0.05$, sensitivity/specificity of 100%/89%][72] to GLS > -9.55% [OR = 0.56 (0.34-0.91), $P = 0.02$, sensitivity/specificity of 83.3/83.5%][71].

PEDSR was only measured in one study[73]. There was no significant difference in PEDSR in between patients that reached clinical endpoints and those that did not.

Strain measured by CMR

There were no studies that used CMR-based strain measurement techniques - either tagging or FT - to predict outcomes post-STEMI that matched our eligibility criteria.

DISCUSSION

This systematic review has shown that certain strain parameters measured by STE - namely, GLS[70-72,74,75] and GLS-R[69] - are independent predictors of adverse outcomes post-STEMI. Impaired GLS can predict both clinical endpoints and adverse LV remodelling, a surrogate marker of poor prognosis. When combined with routine clinical functional parameters such as EF and WMSI, strain provides incremental value in the prognostication of STEMI patients.

However, studies that monitored "hard" events such as mortality could not match the large sample size of $n > 1000$ that some authors believe is important for the evidence to be considered statistically robust[17]. Only one of the studies we assessed had such a large sample size - but the authors monitored remodelling and not "hard" events[74].

Some of the studies that monitored MACE had only a small number of patients that had reached their defined endpoints. Despite this, they constructed models for multivariate analysis that included a large number of independent variables (in addition to GLS). It is believed that one variable should be added for every 10 events to ensure that the regression estimates have reasonable precision[76]. Therefore, all of these studies may have included an inappropriately high number of variables to assess independent predictors of clinical endpoints and the models are likely to suffer from over-fitting.

PEDSR does not seem to provide any benefit at predicting these outcomes although has only been assessed in one study. Consequently, further studies are surely needed to determine if diastolic dysfunction has any role to play in prognostication after a STEMI[27].

Data in this review is limited to GLS measured by STE. We cannot comment on whether GCS is of any added value or has similar predictive properties as GLS since no studies assessed these two parameters together.

Evidence suggests that GLS measured by STE is related to IS[37,77]. The question remains as to whether GLS provides additional information to IS in post-

Table 4 All studies that have used speckle-tracking echocardiography-based strain to predict adverse left ventricular remodelling

Ref.	Age (yr)	Sample size (male)	Baseline ejection fraction (%)	Timeframe baseline scan	Timeframe follow-up scan(s)	Definition of adverse remodelling	Other parameters in multivariate model	Results	Limitations
Bochenek *et al*[70]	59.6 ± 10.3	66 (53)	49.7 ± 9.2	4-6 d post-infarct	3 mo	EDV > 20%	Diabetes Anterior MI Leuk. Count Time to reperfusion WMSI Max. Trop ST-elevation max pre-PCI	22 patients remodelled; GLS can predict LV remodelling - OR = 1.19 (1.04-1.37), $P < 0.05$ - shown by multivariate analysis GLS > -12.5% can predict remodelling - AUC = 0.77 for ROC, sensitivity/specificity of 69%/79% respectively	Only longitudinal strain measured. Too many variables in multivariate analysis
Joyce *et al*[74]	60 ± 12	1041 (792)	47.0 ± 9.0	2 d post-PPCI	3 and 6 mo	EDV ⩾ 20%	Male sex LAD infarct Max. Trop Discharge heart rate LA volume index WMSI	GLS > -15% can predict remodelling at 3 and 6 mo *vs* GLS < -15% (both $P < 0.001$): OR = 6.7 (2.8-11) for 3 mo; OR = 10 (6.7-14) for 6 mo	Only longitudinal strain measured; Prognostic data divided categorically - *i.e.*, GLS > -15% or < -15%; Excluded patients with re-infarction before follow-up and cardiogenic shock - could potentially have been used as another endpoint
Cong *et al*[71]	59.9 ± 11.6	127 (103)	51.8 ± 5.1	1 d post-PPCI	6-9 mo	ESV ⩾ 15%	Anterior MI Time to reperfusion ΣST before PPCI ΣST post-PPCI Raised CK-MB/Trops Baseline ESV/EF WMSI	41 patients developed remodelling; GLS predicted remodelling - OR = 0.39 (0.26-0.57), $P < 0.01$; GLS = -10.85% had sensitivity/specificity of 89.7%/91.7% respectively by ROC to predict remodelling	Only longitudinal strain measured; Too many variables in the multivariate analysis

AUC: Area under curve; CK: Creatine kinase; EDV: End diastolic volume; ESV: End systolic volume; GLS: Global longitudinal strain; LA: Left atrium; LAD: Left anterior descending; LV: Left ventricle; OR: Odds ratio; PPCI: Primary percutaneous coronary intervention; ROC: Receiver operator characteristic; WMSI: Wall motion score index; ΣST: Sum of ST-elevation.

STEMI prognostication and it can only be adequately answered using CMR. However, no studies were found that showed global strain measured by CMR could predict development of remodelling or MACE.

Limitations

We could rule out publication bias - unpublished data were not included as part of our review and could possibly affect our results, especially if it contradicted the seven studies that were assessed. We did not include three search results that were presented as posters since we could not access either the poster itself or the full-text articles associated with it. Regardless, we do not feel this exclusion would significant affect the results of the review since the titles of all three posters stated that GLS could predict post-STEMI outcomes. There is outcome data available in strain measured by TDI but we decided to exclude it from our review since its major limitation of "angle dependence" has been superseded by STE.

Conclusion

Global longitudinal strain when measured by STE is an independent predictor of both adverse LV remodelling and MACE after STEMI and provides incremental prognostic value when combined with traditional LV functional parameters such as EF and WMSI. No such data exist for CMR, but this modality could inform us as to whether strain provides prognostic data in addition to IS.

Table 5 All studies that have used speckle-tracking echocardiography-based strain to predict major adverse cardiac events

Ref.	Age (yr)	Sample size (male)	Baseline ejection fraction (%)	Timeframe baseline scan	Follow-up period	Outcome measures	Other parameters in multivariate model	Results	Limitations
Antoni *et al*[69]	60 ± 12	759 (517)	46.0 ± 8.0	2 d post-PPCI	21 ± 13 mo	GLS and/or GL-strain rate to predict: A: Mortality; B: Composite of revascularisation/ readmission for HF/re-infarction	Age (A) HTN (A) Multi-vessel disease (A/B) Peak Trop (A) QRS duration (A/B) EF (A/B) Severe MR (A) Smoking (B) Diabetes (B)	179 patients reached one or more endpoints; GLS independent predictor of all-cause mortality - HR = 1.2 (1.1-1.3), $P = 0.002$; GLS-R independent predictor of B endpoints - HR = 22 (11-48), $P < 0.001$; Both GLS and GLS-R independent predictors of combined A and B endpoints - HR = 1.1 (1 -1.1, $P = 0.006$) and 18 (10-35, $P < 0.001$) respectively	Sample size $n < 1000$ - potentially not large enough to predict "hard" events like mortality; Only longitudinal strain measured; SR analysis feasible in only 89% of segments
Shanks *et al*[73]	59.7 ± 11.6	371 (288)	45.2 ± 8.0	2 d post-PPCI	17.3 ± 12.2 mo	GL-PEDSR to predict: Mortality; Readmission for HF; Re-infarction; Revascularisation	EF TIMI 0-1 ESV-index Iso-volumetric relaxation SR	Combined clinical endpoints occurred in 84 patients; GL-PEDSR does not predict clinical outcomes	Sample size potentially too small to assess "hard" endpoint such as mortality; No measure of GLS; Only longitudinal parameters obtained
Woo *et al*[72]	64.4	98 (65)	52.6 ± 12.0	Pre-PPCI and 3 d post-PPCI	13.1 ± 3.8 mo	GLS to predict: Mortality; Readmission for HF	Initial Trop Initial NT-pro BNP EF (baseline) WMSI (follow-up) E/e'sr EF (follow-up) WSMI (follow-up)	7 patients developed endpoints; Pre-PPCI GLS predictor of outcomes - HR = 1.41 (1.01-1.98), $P < 0.05$; Post-PPCI GLS more likely to predict outcomes - HR = 2.34 (1.10-4.97), $P < 0.05$; Pre-PPCI GLS < 14% had sensitivity/specificity of 85%/75% respectively - post-PPCI GLS < 13% of 100%/89%	Very small sample size; Only longitudinal strain measured; Too many variables in multivariate analysis
Munk *et al*[78]	63.1	576 (446)	50.0 ± 10.0 (without composite endpoint), 47.0 ± 12.0 (with composite endpoint)	1 d post-PPCI	24 (IQ range 13-61) mo	GLS to predict: Mortality/re-infarction/ stroke/hospitalisation for HF; Crude mortality	EF WMSI ESV-index (Separately and in combination with each other)	162 patients experienced composite endpoints; GLS alone predicted outcomes within 1 yr post-MI - HR = 1.2 (1.12-1.29), $P < 0.01$; GLS alone could not predict outcomes later than 1yr post-MI	GLS could only be obtained in 74% of 576 patients - 26% excluded due to poor image quality (no difference in event rates, however); Only longitudinal strain measured
Cong *et al*[71]	59.9 ± 11.6	127 (103)	51.8 ± 5.1	1 d post-PPCI	16.9 ± 1.6 mo	GLS to predict: Mortality; Development of HF	Anterior MI Time to reperfusion ΣST before PPCI ΣST post-PPCI Raised CK-MB/Trops Baseline ESV/EF WMSI	GLS predicted outcomes - OR = 0.56 (0.34-0.91), $P = 0.02$; GLS > -9.55% had sensitivity/specificity of 83.3%/83.5% respectively	Sample size could potentially be too small to significantly predict "hard" events such as mortality

CK: Creatine kinase; ESV: End systolic volume; GLS: Global longitudinal strain; HF: Heart failure; OR: Odds ratio; HR: Hazard ratio; HTN: Hypertension; IQ: Inter-quartile range; MR: Mitral regurgitation; PEDSR: Peak early diastolic strain rate; PPCI: Primary percutaneous coronary intervention; TIMI: Thrombolysis in myocardial infarction; WMSI: Wall motion score index; ΣST: Sum of ST-elevation; MI: Myocardial infarction.

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COMMENTS

Background

Left ventricular (LV) dysfunction is an important determinant of prognosis following ST-elevation myocardial infarction (STEMI). Routinely used measures of LV dysfunction such as ejection fraction (EF) may not be able to detect subtle changes in cardiac function. Myocardial strain describes the relative change in length of myocardium through the cardiac cycle and is an objective measure of LV function. It can be measured during both systole and diastole and hence provides a reflection of both systolic and diastolic LV contractility. Acutely measured strain post-STEMI may help in predicting markers of poor prognosis [such as development of adverse LV remodelling or major adverse cardiac events (MACE)] at follow-up.

Research frontiers

Strain can be assessed using post-processing speckle-tracking echocardiography (STE) or cardiac magnetic resonance imaging (CMR)-based techniques [such as tagging or novel feature tracking (FT) software]. Such techniques can quantify strain at a segmental and global level and may provide additional information to LV volumes and EF.

Innovations and breakthroughs

This is the first paper to review the literature and present all the studies that have assessed acutely measured global strain parameters to predict markers of outcome post-STEMI. Three studies have shown that global longitudinal strain (GLS) measured by STE is a predictor of adverse remodelling following STEMI whilst four studies have shown that it can predict MACE at follow-up. Therefore, GLS may be a useful clinical measure of identifying patients at a "high risk" of developing poor outcomes. There were also no CMR-based studies assessing strain and its relation to prognosis following STEMI.

Applications

GLS may help improve risk stratification following STEMI but further studies are required to show that this improves outcome.

Terminology

Myocardial strain describes the relative change in length of myocardium through the cardiac cycle -GLS is a measure of LV contractility in the longitudinal vector; STE is an echocardiography-based post-processing software that analyses myocardial deformation parameters (such as global strain) by tracking the motion of "speckles" from one frame to another through the cardiac cycle; Tagging is a post-processing CMR-based software that evaluates strain on tagged sequences - examples of such sequences include spatial modulation of magnetisation (SPAMM) and complementary SPAMM; FT is a post-processing software that assesses strain on cine steady-state free precession images, a type of sequence that is routinely acquired during a clinical CMR scan.

Peer-review

The article is interesting, well-written and supported by updated references.

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