

# World Journal of *Cardiology*

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2014-2017

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## Omega 3 and atrial fibrillation: Where are we?

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### Abstract

Anti-arrhythmic properties of n-3 polyunsaturated

fatty acids, at least in part mediated by anti-oxidant, anti-inflammatory and anti-fibrotic power, have been widely proved. Effect of fish oil on atrial fibrillation, both in primary and in secondary prevention and after cardiac surgery, are controversial, mostly due to lack of homogeneity between studies but also due to individual variability in response to fatty acids administration. Inclusion of measurement of incorporation of fish oil into cell membranes, appears to be essential in future studies, to assess their antiarrhythmic effect.

**Key words:** N-3 polyunsaturated fatty acids; Atrial fibrillation; Upstream therapy; Omega-3 index; Cardiac surgery

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**Core tip:** Individual variability in response to fish oil administration, in terms of eicosapentaenoic and docosahexaenoic acids in corporation into cell membranes, is responsible for controversial results of n-3 polyunsaturated fatty acids administration in patients suffering atrial fibrillation.

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### INTRODUCTION

N-3 polyunsaturated fatty acids (PUFA) anti-arrhythmic effects have been debated for several years, since their electrophysiological properties have been recognized.

Through direct interaction with membrane bound proteins and thanks to incorporation into the phospholipid bilayer, n-3 PUFA are well known to influence ion channels and transmembrane pumps<sup>[1]</sup> to modulate signal transduction, protein trafficking and ion channels kinetic and to regulate gene expression<sup>[2]</sup>. N-3 PUFA can also exert anti-

**Table 1** Clinical studies investigating the effect of n-3 poly-unsaturated fatty acids on primary prevention for atrial fibrillation

Study design	Population	PUFA administration	PUFA quantification	AF diagnosis	Results
Prospective cohort <sup>[5]</sup>	4815 individuals; age 72.8 yr; United States	Broiled/backed fish assessment. FU: 12 yr	FFQ	Annual ECG; hospital discharge diagnoses	Lower AF risk of 31% with fish intake $\geq 5$ times/wk <i>vs</i> $< 1$ /mo. $P = 0.008$
Prospective cohort <sup>[12]</sup>	2174 subjects; mean age: 52.8 yr; Finland	Serum EPA and DHA and dosage. FU: 17.7 yr	DHA, EPA serum dosage	National computerized hospitalization registry	Lower AF risk of 38% for higher DHA levels. $P = 0.02$
Prospective cohort <sup>[6]</sup>	3326 subjects; age: 74.1 yr; United States	Serum EPA, DHA dosage	DHA, EPA serum dosage	Annual ECG; telephonic contact 2/yr; hospitalizations	Lower AF risk for top <i>vs</i> lowest quartile of PUFA/DHA levels
Population study <sup>[7]</sup>	3242 subjects affected by acute myocardial infarction; age: 54.1 yr; Italy	Previous PUFA intake <i>vs</i> not. FU: 360 d	FFQ	AF episodes during hospitalization	Lower risk of AF with fish oil
Prospective cohort <sup>[8]</sup>	47949 subjects; age: 46 yr; Denmark	Fish-oil intake assessment. FU: 5.7 yr	FFQ	Danish national hospitalization registry	Higher AF risk for top <i>vs</i> lowest quintiles of fish intake
Prospective cohort <sup>[9]</sup>	5184 subjects; age 67.4 yr; the Netherland	Fish-oil intake assessment. FU: 6.4 yr	FFQ	Two ECGs during FU; clinical data from general practitioners	No AF risk reduction in the highest tertile of fish intake
Prospective cohort <sup>[10]</sup>	44720 female; age: 63 yr; United States	Fish intake assessment. FU: 6 yr	FFQ	ECG at baseline and at the third and sixth years	No lower AF risk for higher fish intake
Prospective cohort <sup>[11]</sup>	4526 individuals; age: 62 yr; United States	Fish intake assessment. FU: 4 yr	FFQ	Two ECGs every 4 yr of FU; hospitalizations	No AF risk reduction in the top <i>vs</i> the lowest tertile of fish intake
Post-hoc analysis of a RCT (Aleksova) <sup>[13]</sup>	5835 systolic heart failure subjects	N-3 PUFAs 1 g/d <i>vs</i> placebo; FU 3.9 yr	No PUFA dosage	ECG during FU visits	No AF risk reduction with n-3 PUFA

FU: Follow-up; FFQ: Food frequency questionnaires; AF: Atrial fibrillation; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; RCT: Randomized controller trial; PUFA: Poly-unsaturated fatty acids.

inflammatory effects by antagonizing pro-inflammatory prostaglandin formation<sup>[2]</sup>, and exert anti-fibrotic effects<sup>[3]</sup>, as well as cardiac autonomic modulation<sup>[4]</sup>.

In particular, the influence of n-3 PUFA on atrial fibrillation (AF) primary and secondary prevention, including post-operative AF (POAF) has also been the object of numerous clinical studies.

### N-3 PUFA in primary and secondary prevention and in POAF

**Primary prevention:** With regard to primary prevention of AF (Table 1), two studies involving elderly subjects<sup>[5,6]</sup> and one focusing on patients affected by acute myocardial infarction<sup>[7]</sup> proved n-3 PUFA to be protective against AF, while other studies<sup>[8-12]</sup>, showed no benefit. The influence of various diet habits, including fish consumption<sup>[8,9]</sup>, can possibly explain different results, as well as different methodologies used for assessment of fish intake and for AF diagnosis. In particular, positive studies, generally included elderly individuals<sup>[5-7]</sup>, suggesting benefit from antifibrotic properties of fish-oil. However, a post-hoc analysis of the randomized controlled trial GISSI-HF<sup>[13]</sup> showed no effect of long-term PUFA administration on AF development in heart failure patients, thus allowing no conclusions for the role of n-3 PUFA in AF primary prevention.

**Post-operative AF:** The effect of n-3 PUFA in the context of POAF, that is characterized by inflammation,

electrolyte disturbances and hemodynamic instability secondary to cardiac surgery, have also been also widely investigated. An open label study<sup>[14]</sup> firstly observed a short-term n-3 PUFA administration-related decrease in POAF incidence after coronary artery bypass grafting. Two papers<sup>[15,16]</sup> also gained benefit from various fish-oil preparations and administration timings (Table 2). A recent randomized-controlled trial (RCT)<sup>[17]</sup> also observed reduction of POAF with n-3 PUFAs plus vitamins C and E administration in comparison to placebo, in 203 patients scheduled for cardiac surgery. Further studies however, failed to prove both prevention of AF<sup>[18,19]</sup> and decrease of inflammation<sup>[20]</sup> from higher serum levels of n-3 PUFA, eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA), and from higher n3-PUFA atrial content<sup>[21,22]</sup>. Recently, the multicenter double-blind RCT "OPERA"<sup>[23]</sup> showed no influence on POAF occurrence, from short-term n3-PUFA administration. The effect was unrelated to patients characteristics, kind of cardiac-surgery, antiarrhythmic drugs, fish intake and serum n-3 PUFA. In a substudy of this trial indeed<sup>[24]</sup>, including 564 subjects receiving short-term PUFA or placebo before surgery, the risk of POAF was unrelated to fish oil concentrations at enrollment and day of surgery. Interestingly, PUFA increase, was characterized by significant inter-individual variability (0.7%-7.5% after 5 d of supplementation). Finally, Metcalf *et al.*<sup>[25]</sup>, by using combined data from previous RCTs, demonstrated less incidence of POAF among subject within the fourth quintile of red blood cell

**Table 2** Principal clinical studies investigating the effect of n-3 poly-unsaturated fatty acids on post-operative atrial fibrillation

Study design	Population	PUFA administration	PUFA quantification	AF diagnosis	Results
Randomized, open label <sup>[14]</sup>	160 CABG pts; age: 66.2 yr; Italy; BB: approximately 57%; statins approximately 58%	N-3 PUFA 2 g/d (EPA/DHA: 1:2) $\geq$ 5 d before CS, until discharge <i>vs</i> not	No PUFA dosage	Continuous 5 d monitoring + daily ECG up to discharge. AF: $>$ 5 min/requiring therapy	Lower AF risk. $P = 0.013$
Prospective observational <sup>[15]</sup>	530 CS pts; age: 66.4 yr; Italy. BB: 53%; statins: 46%	N-3 PUFA 1 g/d (EPA/DHA: 0.9:1.5) 5 d pre-CS <i>vs</i> not	No PUFA dosage	Continuous monitoring during ICU-stay. AF: $\geq$ 5 min	Lower POAF during ICU stay. $P = 0.006$
Double blind-RCT <sup>[16]</sup>	102 CABG pts; age: 67 yr; Germany	Iv 100 mg fish oil/kg per day during ICU-stay <i>vs</i> soya oil	No PUFA dosage	Continuous monitoring during ICU-stay	Lower AF risk with PUFA. $P < 0.05$
Prospective cohort <sup>[19]</sup>	125 CABG pts; age: approximately 68 yr; Iceland. BB: 77.4%; statins: 84%	N3-PUFA (EPA/DHA: 1.2:1) 2.2 g/d 7 d pre-CABG <i>vs</i> placebo	PUFA dosage basally, before, 3 d after CS	Continuous monitoring during hospital stay. AF: $\geq$ 5 min	Positive DHA/POAF association (U-curve relationship)
Double blind-RCT <sup>[23]</sup>	1516 CS pts; age: 64 yr; Italy-United States-Argentina. BB: 76.9%; statins: 57.5%	N3-PUFA (EPA/DHA: 4.6:3.7) 2 g/d 5 d pre-CS up to discharge <i>vs</i> placebo	Serum PUFA dosage basally, before CS	Continuous 5 d monitoring. AF: $\geq$ 30 s	No lower AF despite 40% higher plasmatic PUFA
Double blind-RCT <sup>[18]</sup>	243 CS pts; age: 62.7 yr, United States. BB: 79%; statins: 73%	N-3 PUFA 2 g/d <i>vs</i> corn oil	Basal serum PUFA dosage, before, 3 d post CS	Continuous ECG during hospital stay; FU: 1 mo. AF: Episodes requiring treatment	No lower AF; plasma PUFA increase
Double blind-RCT <sup>[20]</sup>	170 CS pts; age: 67 yr; Iceland. BB: approximately 76%	N3-PUFA (EPA/DHA: 1.2:1) 2 g/d 1 wk before and 2 after CS <i>vs</i> olive oil	Serum DHA, EPA dosage basally, pre 3 d post CS	Continuous monitoring during hospital stay. AF: $\geq$ 5 min	No lower AF; plasma n-3 PUFA increase
Double blind-RCT <sup>[22]</sup>	200 CS pts; age: 64 yr; Australia, BB: 43%; statins: 73%	N-3 PUFA oil (EPA/DHA: 2.7:1.9) for 3 wk <i>vs</i> placebo	Dosage of serum PUFA basally, pre-CS; atrial PUFA	Continuous 72 h monitoring. AF/flutter $\geq$ 10 min/requiring treatment	No lower AF risk; increase in serum and atrial PUFA
Double blind RCT <sup>[21]</sup>	108 CABG pts; age: 64 yr; United Kingdom; BB: 88%; statins: 98%	N-3 PUFA (EPA/DHA: 1.2:1) 2 g/d for approximately 16 d <i>vs</i> olive oil	Dosage of serum PUFA basally, 3 d post CS; atrial PUFA	Continuous 5 d monitoring + daily ECG. AF: $>$ 30 s	No lower AF risk; higher serum and atrial PUFA

CABG: Coronary artery bypass grafting; pts: Patients; BB: Beta blockers; CS: Cardiac surgery; ICU: Intensive care unit; PUFA: Poly-unsaturated fatty acids; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; AF: Atrial fibrillation.

n-3 DHA, thus suggesting a U-shaped relation between n-3 PUFA intake and POAF. Four recent meta-analyses of the previously presented studies showed in turn, overall protective or neutral effect on POAF from n-3 PUFA<sup>[26-29]</sup> (Table 3). Of note, none of these meta-analyses has assessed n-3 PUFA treatment duration to surgery as a covariate in a meta-regression analysis.

Dissimilarities may be explained by various study designs and populations, AF definitions, cardiac surgery, co-administration of anti-arrhythmic or anti-inflammatory drugs, dietary PUFA intake, EPA/DHA ratios and fish oil-administration modes (*i.e.*, intravenous or through nasogastric tube) and fish-oil administration time courses. Conversely, no effects of n-3 PUFA administration on myocardial infarction and bleeding after cardiac surgery, eventually influencing POAF occurrence, have been demonstrated<sup>[27]</sup>.

Interestingly, all RCTs that failed to demonstrate a beneficial effect, used a formulation containing 1.24 EPA:DHA ratio<sup>[18,20,23]</sup>. In contrast, Rodorigo *et al.*<sup>[17]</sup> administered PUFA with an EPA:DHA ratio equal to 0.5.

**Secondary prevention:** Several studies have finally investigated the effect on n-3 PUFA on relapses of

paroxysmal and persistent AF. Two studies<sup>[30,31]</sup>, found fish oil administration (from 1 mo before, to 6 mo after cardioversion) helpful in AF prevention (Table 4). On the other hand, 4 further studies<sup>[32-35]</sup> failed to prove any effect.

A recent study<sup>[36]</sup> including 337 patients with symptomatic paroxysmal/persistent AF, randomized to receive fish oil (4 g/d) or placebo, showed no difference in time to first AF recurrence, as well as no significant decrease of inflammatory markers at 6 mo. Similarly, another RCT<sup>[37]</sup>, proved no effect from n-3 PUFA on the time to AF relapses, as well as on concentrations of biomarkers of oxidative stress and inflammation and at follow-up. In particular, a large RCT<sup>[34]</sup> involving 586 patients with symptomatic paroxysmal or persistent AF, randomized to n-3 PUFA (1 g/d) *vs* placebo for 1 year, also proved no significant differences between the two arms, in terms of symptomatic recurrence of AF.

Contrasting outcomes between studies may be related to differences in PUFA somministration and populations characteristics. Generally, papers including subjects with more evident cardiac disease<sup>[30]</sup>, more often co-administered with amiodarone<sup>[30]</sup> showed benefit. Of note, some unfavorable papers proved AF relapses to occur mostly within 3 wk, prior

**Table 3** Recent metaanalyses of studies of n-3 poly-unsaturated fatty acids in post-operative atrial fibrillation

Ref.	Clinical setting	NO. of studies and of patients	Results
Costanzo <i>et al</i> <sup>[26]</sup>	POAF	8 RCTs/2687 pts	AF reduction
Benedetto <i>et al</i> <sup>[27]</sup>	POAF	431 pts	No AF reduction; at meta-regression analysis: Trend toward a benefit from PUFA for administration of EPA/DHA ratio = 1:2
Zhang <i>et al</i> <sup>[28]</sup>	POAF	8 RCT/2687 pts	No AF reduction
Ali-Hassan-Sayegh <i>et al</i> <sup>[29]</sup>	POAF	23 RCTs/4278 pts	AF reduction

RCTs: Randomized controller trials; pts: Patients; PO: Post-operative; AF: Atrial fibrillation; PUFA: Poly-unsaturated fatty acids; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

**Table 4** Clinical studies investigating the effect of n-3 poly-unsaturated fatty acids on secondary prevention for atrial fibrillation

Study design	Population	PUFA administration	PUFA quantification	AF diagnosis	Results
Double blind-RCT <sup>[30]</sup>	109 pts, age: 70 yr; Italy; heart structural abnormality: 90%; Amiodarone + ACE-i/ARBs: 100%	N-3 PUFA (EPA/DHA 1.2:1) 2 g/d, 1 mo before and 12 after ECV <i>vs</i> olive oil	No PUFA dosage	Weekly ECG for the first 3 wk after ECV and ECG + Holter ECG after 1, 3, 6, 12 mo and at symptoms occurrence	Less AF relapses with PUFA
Open-label randomized <sup>[31]</sup>	178 pts, Australia. Concomitant amiodarone, sotalol, ACE-i/ARBs	N-3 PUFA (EPA/DHA 1.3:1) 1.8 g/d for approximately 56 d before ECV and 1 year thereafter <i>vs</i> not	Serum dosage of EPA, DHA basally, before ECV	ECG at week 2 and 6 and every 3 mo. AF: $\geq 1$ wk	Less AF relapses at 90 d and 1 yr with PUFA, $P < 0.001$ ; higher serum EPA, DHA
Double blind-RCT <sup>[33]</sup>	663 pts; paroxysmal AF: 18%; age: 60.5 yr; United States. No heart abnormality. Amiodarone: 0%, antiarrhythmic drugs: 13%; ACE-i/ARBs: 39%	N-3 PUFA (EPA/DHA 4.6:3.7; load: 8 g/d for 1 wk) 4 g/d for 24 wk <i>vs</i> oil	Serum DHA, EPA dosage basally, after 4 and 24 wk	Biweekly transtelephonic monitoring	No lower symptomatic AF recurrence in the paroxysmal and persistent
Prospective <sup>[35]</sup>	50 pts; $\geq 2$ previous AF episodes; age: 54 yr, Japan. IC antiarrhythmic drugs: 100%	Observational period: no PUFA for 6 mo. Interventional period: EPA 1.8 g/d for 6 mo	Serum EPA, DHA dosage basally and at study end	Daily ECG monitoring and at symptoms occurrence	No lower AF burden and time to first relapse
Double blind-RCT <sup>[32]</sup>	204 pts, age: 69.3 yr; Italy. LAs 45 mm. First ECV: 59%; IC antiarrhythmic drugs: 29.5%, sotalol: 12.6%, amiodarone: 27.4%	N-3 PUFA (EPA/DHA 1.2:1) 3 g/d $\geq 1$ wk before and 2 g/d after ECV for 6 mo <i>vs</i> olive oil	N-3 PUFA serum dosage basally, 6 mo after ECV	Transtelephonic monitoring: 2/first week after ECV and 3/wk for 3 mo + clinical visits after 7 d, 1, 3, 6 mo	No difference in ECV success, AF incidence, time to first relapse. Increase of EPA and DHA
Double blind RCT <sup>[36]</sup>	337 pts; symptomatic paroxysmal or persistent AF within 6 mo of enrollment	Fish oil (4 g/d) or placebo	Followed, on average, for $271 \pm 129$ d	Trans-telephonic event recorder, 12-lead ECG or Holter	No lower AF with PUFA
Double blind-RCT <sup>[37]</sup>	190 pts with paroxysmal or persistent AF	N-3 PUFAs (4 g/d; $n = 126$ ) or placebo ( $n = 64$ ) in a 2:1 ratio	No PUFA dosage	Not specified	No reduction of AF recurrence and inflammation markers
Double blind-RCT <sup>[34]</sup>	586 pts with symptomatic paroxysmal AF requiring ECV ( $n = 428$ ), at least 2 episodes of AF in the 6 mo before ( $n = 55$ ), or both (103)	N-3 PUFA (1 g/d) or placebo for 12 mo	No PUFA dosage	Not specified	No lower AF with PUFA

RCTs: Randomized controller trials; pts: Patients; PO: Post-operative; AF: Atrial fibrillation; PUFA: Poly-unsaturated fatty acids; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; ACE-I: Angiotensin converting enzyme inhibitor; ARB: Angiotensin receptor blockers.

to an eventual effect from n-3 PUFA.

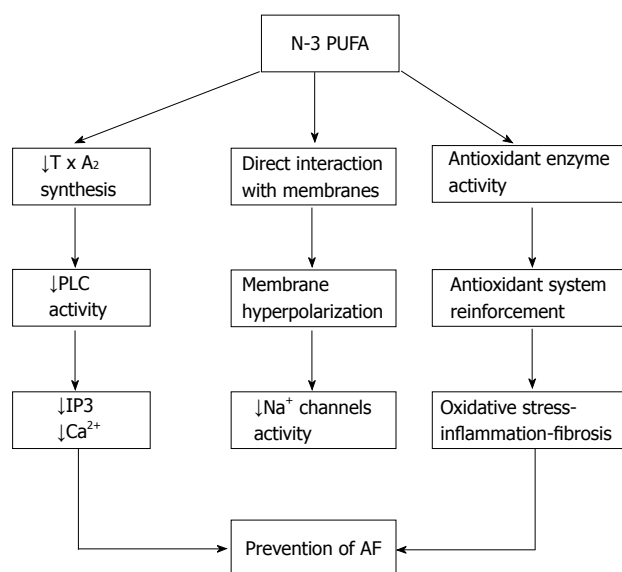
## DISCUSSION

The effect of n-3 PUFA on AF primary and secondary prevention and after cardiac surgery, remains controversial. A major reason for this uncertainty, is to be found in differences between studies, in particular regarding study designs, patients characteristics, AF definition and types (lone, vagally/adrenergically induced, secondary to structural disease), fish oil-administration modes,

formulations and time courses. Moreover, a great variability in n-3 PUFA serum concentrations between subjects, despite similar fish-oil administration, has been recently proved, likely secondary to genetic predisposition in PUFA metabolism.

Noteworthy, however, a recent RCT<sup>[38]</sup> examined the effects of high (6 g/d) or medium dose (3 g/d) fish oil supplementation, with or without multivitamin, on the inclusion of n-3 and n-6 PUFA within membranes of red blood cells after 16 wk. The authors found all treatments effective in increasing EPA composition of cell membranes





**Figure 1 Antiarrhythmic effects of n-3 poly-unsaturated fatty acids.** N-3 PUFA: N-3 poly-unsaturated fatty acids; TxA<sub>2</sub>: Thromboxane A<sub>2</sub>; PLC: Phospholipase C; IP<sub>3</sub>: Inositol triphosphate; AF: Atrial fibrillation.

in females, but not in males, for whom the higher dose n-3 PUFA plus multivitamin combination was necessary. As a consequence, discrepancies between trials could be partially related to individual capability of n-3 PUFA incorporation, which in turn, could be influenced by sex, age, vitamin and/or drug administration. To counteract the variability in response to fish oil administration, inclusion of blood measures of n-3 PUFA status appears therefore to be essential in future studies.

The “Omega-3 Index” is the percentage of PUFA composed of EPA + DHA in red blood cell membranes<sup>[39]</sup> may represent a measurement of clinical utility to assess individual response to fish oil intake. Moreover, it may contribute to better understand the pharmacokinetics and pharmacodynamics of PUFA. Considering the results of recent studies showing an U-curve relationship between PUFA concentrations and AF<sup>[19,25]</sup>, the greater protection from AF could be obtained from an individually-targeted approach for fish oil inclusion within membranes.

## CONCLUSION

The complexity of the biological interactions of n-3 PUFA, their incorporation into cell membranes and the variability of clinical contexts, likely justify why PUFA administration does not automatically lead to AF reduction. RCTs focusing on clinical contexts of AF, and characterized by more accurate follow-ups and definitions of PUFA incorporation into red blood cells (or hopefully, in atrial tissue in the setting of cardiac surgery), are required. The RCT NCT00692718, will hopefully add information regarding fish oil effect on AF prevention in the context of HF and/or AMI.

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## 2016 Nonalcoholic Fatty Liver Disease: Global view

# Psoriasis, non-alcoholic fatty liver disease, and cardiovascular disease: Three different diseases on a unique background

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## Abstract

Psoriasis is a chronic inflammatory immune-mediated skin disease, frequently associated with systemic

comorbidities. According to recent data, patients with psoriasis show a greater prevalence of metabolic syndrome, which confers a higher cardiovascular risk. The link between these pathological conditions appears to be a chronic low-grade inflammatory status. The aim of this review is to focus on the multiple epidemiological and physio-pathogenetic aspects linking non-alcoholic fatty liver disease, psoriasis, and cardiovascular disease.

**Key words:** Psoriasis; Non-alcoholic fatty liver disease; Cardiovascular risk

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**Core tip:** The review focuses on recent scientific data regarding the multiple physio-pathogenetic aspects of the possible link between psoriasis, non-alcoholic fatty liver disease, and cardiovascular disease. The multidisciplinary approach to psoriatic patients appears mandatory to treat concomitant psoriasis-related comorbidity, and the risk/benefit of both biologic and non-biologic therapies should be evaluated.

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## INTRODUCTION

Psoriasis is a chronic inflammatory relapsing disease affecting 1%-4% of the general population<sup>[1]</sup>.

Despite that psoriasis is a skin disorder clinically

characterized by red scaly plaques, it is no more limited to the skin surface and has been identified as a complex clinical entity with a systemic involvement. Many comorbidities have been associated with psoriatic disease, such as psoriatic arthritis (PsA), metabolic syndrome (MetS), cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), inflammatory bowel disease, uveitis, depression and malignancy<sup>[2-5]</sup>.

An higher prevalence of cardiovascular risk factors, such as dyslipidemia and obesity, has been reported in psoriatic patients<sup>[4,6]</sup>.

NAFLD is one of the most frequent cause of chronic liver disease with a prevalence of 10%-25% in the general population<sup>[7]</sup>.

NAFLD is now considered the hepatic manifestation of the MetS and a prospective cohort study has evidenced that MetS and its components may independently predict the risk of NAFLD<sup>[8,9]</sup>.

NAFLD itself represents a further independent cardiovascular risk factor for atherosclerosis which is likely linked to arterial stiffness<sup>[10,11]</sup>.

The aim of the present review is to focus on the association of psoriasis, NAFLD and CVD, focusing on epidemiologic data and the underlying common pathogenic process.

## NAFLD AND PSORIASIS

The prevalence of the MetS has been estimated to be about 15%-25% in the general population, appearing significantly higher (an increase by about 3-fold) in psoriatic patients, as documented by many case-controls studies<sup>[12-16]</sup>.

The association between psoriasis and MetS is directly correlated with the severity of psoriasis, independent from the presence of obesity in psoriatics<sup>[17-19]</sup>.

NAFLD is defined as a spectrum of hepatic pathologies ranging from fatty liver disease (steatosis) to steatohepatitis (NASH) with the risk of evolution to cirrhosis and hepatocellular carcinoma<sup>[20]</sup>.

NAFLD is more prevalent in psoriatic patients than in the general population. Roberts *et al.*<sup>[21]</sup> enrolled a cohort of 103 psoriatic patients and found that NAFLD affected about 47% of patients and one of five of them showed NASH.

A large prospective population-based cohort study had been conducted by van der Voort *et al.*<sup>[22]</sup> in patients older than 55 years. Among 2292 participants, 5.1% of the population were affected by psoriasis with a prevalence of NAFLD of about 46.2% in psoriatic patients vs 33.3% in subjects without psoriasis.

Furthermore, a recent meta-analysis has documented that patients with PsA and patients with moderate to severe psoriasis showed a significantly greater risk of NAFLD compared with those with mild psoriasis<sup>[23]</sup>.

Aspartate transaminase (AST)/alanine aminotransferase (ALT) ratio is considered an independent predictive factor for liver fibrosis in patients with NAFLD; significantly higher AST/ALT ratio and higher non-invasive fibrosis scores have

been detected in patients with both psoriasis and NAFLD compared to controls with only NAFLD<sup>[24]</sup>.

## CARDIOVASCULAR RISK FACTORS AND PSORIASIS

MetS confers an increased risk of cardiovascular events and mortality due to CVDs<sup>[25,26]</sup>. Psoriatic patients show a higher prevalence of cardiovascular risk factors, which are shared by NAFLD and CVD, thus representing the trade union between these pathologies. Obesity represents a great burden in global individual's health, significantly increasing morbidity and mortality<sup>[27]</sup>.

Data from large cohort studies have shown that among 163517 enrolled individuals, 17% were obese (11465 men and 16612 women). Thus, obesity represents a great public health problem reaching worrying proportions both in pediatric and adult populations<sup>[27]</sup>.

As demonstrated by recent observational studies, psoriatic population may have a higher risk of overweight and obesity with the consequent higher risk of components of MetS<sup>[28]</sup>.

Danielsen *et al.*<sup>[29]</sup> have conducted a recent population-based study confirming an increased prevalence of MetS in patients affected by psoriasis compared to controls. Interestingly, a different trend was emphasized between genders: A 3.8-times higher odds of MetS was found in young women (< 30 years) with an odds ratio reduction with increasing age. Conversely, men showed a 1.35-times higher odds ratio of MetS, independently from age.

Moreover, a direct correlation between severity of psoriasis and obesity has been evidenced in a recent meta-analysis: An odds ratio of 1.46 was found in mild psoriasis and an odds ratio of 2.23 in severe psoriasis<sup>[22,30]</sup>.

Dyslipidemia is a further risk factor, which is shared by NAFLD, psoriasis and CVD. Observational studies have detected a lipid metabolism alteration in psoriatic patients contributing to a dyslipidemic profile and conferring a significant cardiovascular risk<sup>[31]</sup>.

Psoriatic children present high plasma levels of total cholesterol, high content of total cholesterol and high cholesterol/protein ratio in LDL and in HDL<sup>[32]</sup>.

Moreover, an increased odds of hypertriglyceridemia, significantly reduced levels of HDL cholesterol (< 40 mg/dL), hyperlipoproteinemia and hypercholesterolemia have been identified in psoriatic populations<sup>[31,33,34]</sup>.

As for obesity, a positive correlation was found between dyslipidemia and severity of psoriasis with an increased odds of 1.10-3.38 in mild psoriasis and 1.36-5.55 in severe psoriasis<sup>[35,36]</sup>.

The dyslipidemic profile appears extremely relevant; in fact, it is known that hypercholesterolemia can lead to atherosclerosis and coronary heart disease. In animal models, adipocyte differentiation and maturation can be altered by cholesterol accumulation in preadipocytes, leading to adipocyte hypertrophy and adipose tissue inflammation. In humans, it has been demonstrated that hypercholesterolemia leads to an imbalance in the



pro- and anti-inflammatory adipocytokine production by adipose tissue<sup>[37]</sup>.

## CVDS AND PSORIASIS

CVDs include atherosclerosis, hypertension, ischemic heart disease, myocardial infarction, stroke and arrhythmias<sup>[4]</sup>.

An increased incidence of cardiovascular risk factors and major cardiovascular events has been found in psoriasis<sup>[4,5,15]</sup>.

Gelfand *et al.*<sup>[38]</sup> performed a cohort study on patients affected by severe psoriasis, evidencing a further 6.2% absolute risk of a 10-year rate of major cardiovascular events and suggesting the possible role of severity of the disease in the pathogenesis of CVD.

In particular, a 6-year reduction in life expectancy has been evidenced in patients with severe psoriasis<sup>[39]</sup>.

Although the role of the extent of psoriasis-involved body sites has not been completely elucidated, studies showed that a wide skin involvement and the presence of inter-gluteal lesions may represent independent predictor factors of CVD in psoriatics<sup>[40]</sup>.

A prospective, population-based cohort study had been conducted by Gelfand *et al.*<sup>[41]</sup> in 2006, evaluating the risk of myocardial infarction (MI) in psoriatic patients. The authors found that psoriatics had a higher incidence of MI which was positively correlated with disease severity: 4.04 per 1000 person-years (95%CI: 3.88-4.21) in mild psoriasis and 5.13 per 1000 person-years (95%CI: 4.22-6.17) in severe psoriasis. Moreover, the risk of MI was higher in young 30-year-old psoriatic patients, and this risk persisted higher after adjustment for major risk factors for MI, suggesting that psoriasis itself confers an independent risk of MI.

This aspect was also confirmed by Brauchli *et al.*<sup>[42]</sup>, who found the highest incidence rate of MI in psoriatic patients aged 30-39 years with severe skin disease.

The concomitant presence of PsA seems to lead to an increased risk of non-fatal MI; a risk up to 10% of CVD disease within 10 years of PsA incidence has been identified in most of newly diagnosed PsA patients<sup>[43,44]</sup>.

A retrospective study has shown that the concomitant presence of arterial hypertension (AH) and diabetes mellitus (DM) enhances the risk of CVD in PsA patients. The prevalence of AH and DM was significantly greater in PsA patients who have had CVD compared to those without CVD; the prevalence of AH was 95% vs 45% and the prevalence of DM was 60% vs 19%. These aspects have important repercussions on early recognition and targeted treatment of comorbidities in psoriatic patients in order to reduce morbidity and mortality<sup>[45]</sup>.

An association between psoriasis and atherosclerotic disease has been recognized. A cross-sectional study conducted by Yiu *et al.*<sup>[46]</sup> evaluated the prevalence and the extent of coronary and carotid atherosclerosis in 70 psoriatic patients compared to age- and gender-matched healthy controls. Psoriatic patients showed a 10-fold increased risk of subclinical coronary atherosclerosis

and premature diffuse coronary and carotid atherosclerosis.

The subclinical vascular atherosclerosis in psoriasis has been also studied by Balci *et al.*<sup>[47]</sup> on 43 psoriatic patients without cardiovascular risk factors and 43 healthy controls matched for sex and age. Significantly higher mean intima-media thickness values of the right, left and averaged common carotid arteries had been detected in psoriatics than in controls ( $0.607 \pm 0.144$  mm vs  $0.532 \pm 0.101$  mm,  $0.611 \pm 0.157$  mm vs  $0.521 \pm 0.117$  mm, and  $0.609 \pm 0.146$  mm vs  $0.526 \pm 0.104$  mm, respectively). Conversely, the mean flow-mediated dilatation and nitroglycerin-induced dilatation values were significantly lower in patients with psoriasis than in controls ( $13.36 \pm 6.39$  mm vs  $19.60 \pm 11.23$  mm and  $21.08 \pm 8.38$  mm vs  $26.85 \pm 12.38$  mm;  $P = 0.002$  and  $P = 0.013$ , respectively).

It is well documented that calcium exerts an important role in atherosclerosis, and it is an important index of subclinical atherosclerosis and greatly impacts on the atherosclerotic plaque burden<sup>[48]</sup>.

A recent case-control study was conducted on 40 patients with psoriasis and 42 controls matched for age, sex, and cardiovascular risk profile in order to examine the prevalence of coronary calcification. The same prevalence of calcified and non-calcified atherosclerotic coronary lesions was evidenced in both groups<sup>[49]</sup>. Conversely, emerging data show that patients with psoriasis have a higher coronary calcium score (CAC), which was directly correlated with psoriasis severity<sup>[50]</sup>.

A cross-sectional study was conducted on Mediterranean population, aiming to determine the prevalence of ischemic CAD in patients with psoriasis establishing a significant independent association between psoriasis and CAD<sup>[51]</sup>.

The coronary microvascular function has been evaluated in psoriatic patients by echocardiographic examination to emphasize the coronary flow reserve (CFR). A coronary impairment was shown with a reduction in CFR and with a positive inverse correlation between CFR and PASI score, disease duration and C-reactive protein<sup>[52]</sup>.

Interestingly, it has been recently documented that psoriasis and coronary artery disease share similarities in coronary function and myocardial deformation with a subclinical left ventricular deformation. This aspect may contribute to vascular dysfunction in psoriatic patients, increasing the risk of coronary artery disease<sup>[53]</sup>.

The early detection of specific inflammatory biomarkers implicated in CVDs and in subclinical atherosclerosis remains a fundamental item to promptly identify the cardiovascular risk in this population<sup>[54]</sup>.

New data have emerged from studies on this topic. In particular, N-terminal pro B-type natriuretic peptide (NT-proBNP) is a molecule secreted by the ventricular myocardium in response to increased ventricular stretch and it plays an important role as a predictor of cardiovascular mortality, of negative outcome in stroke and of left ventricular systolic dysfunction<sup>[54]</sup>.

Significantly higher serum levels of NT-proBNP were

found in 73 male psoriatic patients compared to controls with a direct correlation with disease duration<sup>[55]</sup>.

These results appear relevant in the light of echocardiographic abnormalities found by Biyik *et al.*<sup>[56]</sup>, who showed left ventricle hypertrophy, diastolic dysfunction and wall motion alterations in patients affected by psoriasis. Moreover, a higher frequency of mitral and tricuspid valve prolapse had been diagnosed in psoriatics.

Another useful biomarker is homocysteine, which is considered an independent risk factor for CVD by promoting oxidative stress, lipoperoxidation and endothelial cell dysfunction. Moreover, hyperhomocysteinemia is considered an independent risk factor for CVD, conferring an elevated risk of atherosclerosis, stroke and peripheral occlusive vascular diseases<sup>[57]</sup>.

Homocysteine plasma levels have been evaluated in psoriasis, and significantly higher levels were found in psoriasis patients compared to healthy subjects, with a positive correlation with disease severity. No correlation was found between homocysteine serum levels and disease duration or the presence of arthritis<sup>[57,58]</sup>.

Furthermore, high homocysteine plasma levels and reduced folic acid plasma levels in psoriatic patients seem to be implicated in a pro-thrombotic state<sup>[59]</sup>.

A new interesting biomarker of vascular damage, YKL-40, has been recently studied in psoriatic patients. YKL-40 belongs to the chitinase family and it has been detected in atherosclerotic plaque, contributing to endothelial dysfunction (ED) and predicting early vascular damage in diseases with high cardiovascular risk. Increased levels of YKL-40 have been found in inflammatory conditions, such as rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus and Crohn's disease<sup>[60,61]</sup>.

A case-control study on 48 psoriatic patients has emphasized a statistically significant elevation of YKL-40 levels. These data had been confirmed by Erfan *et al.*<sup>[60]</sup>, who had also performed ultrasonography in order to identify ED. Psoriatic patients with ED showed higher YKL-40 serum levels than healthy controls without ED; moreover, psoriatic patients with concomitant cardiovascular risk factors, such as smoking, obesity and diabetes, showed higher YKL-40 levels than those without<sup>[62]</sup>.

The higher cardiovascular risk in psoriasis appears also linked to the increased prevalence and incidence of hypertension. In fact, hypertension is a well-established risk factor for CVDs and cardiovascular mortality<sup>[63]</sup>.

The association between psoriasis and hypertension has been evaluated in a recent meta-analysis conducted by Armstrong *et al.*<sup>[63]</sup>, who documented a higher odds of hypertension of 1.58 times in psoriatics compared to the general population. Moreover, hypertension and psoriasis severity were positively correlated with a hazard ratio (HR) of 1.17 in patients with severe psoriasis and HR of 1.07 in those with mild psoriasis. PsA patients showed a higher odds ratio of 2.07 compared to patients with only psoriasis.

The increased detection of hypertension in psoriatic patients could explain the increased risk of atrial

fibrillation in this population. Atrial fibrillation is the most frequent cardiac arrhythmia, accounting for 0.4%-1% of the general population and strictly linking to cardiovascular morbidity and mortality<sup>[64]</sup>.

Emerging data have focused on the potential association between psoriasis and atrial fibrillation and documented that psoriasis may be independently associated with a higher risk of new onset atrial fibrillation<sup>[64]</sup>.

A Danish nationwide cohort study evaluated 36765 mild psoriasis patients and 2793 severe psoriasis patients vs 4478926 controls: An increased risk of atrial fibrillation was found in psoriatics with a direct correlation with skin disease severity. Furthermore, a strong association between atrial fibrillation and early onset psoriasis had emerged<sup>[65]</sup>.

Conversely, Armstrong *et al.*<sup>[66]</sup> had considered a cohort of 2078 psoriatic patients matched to 6234 healthy subjects and evidenced no statistically significant difference in a 5-year atrial fibrillation incidence between the two groups (2.5% vs 3.3%) and no association between incident atrial fibrillation and psoriasis severity.

## PSORIASIS, NAFLD AND CVD: A COMMON INFLAMMATORY PROCESS

Psoriasis, NAFLD and CVD are considered multifactorial and multi-step diseases with not completely fully elucidated interactions between genetic, immunological and environmental factors<sup>[67,68]</sup>.

Psoriasis is an immune-mediated disorder sustained and maintained by a Th1-Th17-Th22 cell immune response. The Th1-Th17-Th22 downstream pro-inflammatory cytokines contribute to creating a cytokine milieu participating in a systemic chronic inflammation process<sup>[69,70]</sup>.

In fact, the low-grade chronic inflammatory process seems to represent the major component linking psoriasis to its comorbidities and leading to insulin resistance, to dysmetabolic profile and to ED and thus predisposing psoriatic patients to atherosclerosis and higher cardiovascular risk<sup>[2,4]</sup>.

Both innate and adaptive immunity participates in physio-pathologic mechanism underlying psoriasis and atherosclerosis. Hansson *et al.*<sup>[71]</sup> in 2012 have interestingly proposed the concept of "two plaques for one syndrome". In fact, the development of both psoriatic and atherosclerotic plaques is strictly dependent on T cells, monocytes, macrophages and pro-inflammatory cytokines. It is known that Th1 hyperactivity and the overexpression of Th1-related cytokines represent the basis for ED being associated with atherosclerotic plaque instability and with an increased risk of athero-thrombotic events<sup>[71-74]</sup>.

Most of inflammatory cytokines are produced by the adipose tissue<sup>[75,76]</sup> (Table 1). It is known that the adipose tissue is a real endocrine organ able to synthesize adipocytokines, bioactive molecules deeply involved in the inflammation and in the development of MetS and its components, such as dyslipidemia and insulin resistance<sup>[75,76]</sup>.

**Table 1** Role of inflammatory biomarkers in psoriasis, non-alcoholic fatty liver disease and cardiovascular diseases

	Psoriasis	CVD	NAFLD
TNF- $\alpha$	<ul style="list-style-type: none"> <li>↑Keratinocyte proliferation</li> <li>↑Pro-inflammatory cytokine production</li> <li>↑Expression of vascular endothelial cell adhesion molecules</li> <li>↑Angiogenesis</li> </ul>	<ul style="list-style-type: none"> <li>↑LDL transcytosis</li> </ul>	<ul style="list-style-type: none"> <li>↓IRS-1 phosphorylation</li> <li>↑Insulin-resistance</li> <li>↑Hepatic fibrogenesis</li> </ul>
IL-1	<ul style="list-style-type: none"> <li>↑Keratinocyte proliferation</li> <li>↑Pro-inflammatory cytokine production</li> <li>↑Expression of vascular endothelial cell adhesion molecules</li> </ul>	<ul style="list-style-type: none"> <li>↑Synthesis of IL-6, fibrinogen, RCP</li> <li>↑Expression of adhesion molecules (ICAM, VCAM)</li> </ul>	<ul style="list-style-type: none"> <li>↑Activation of MAP and ERK pathways</li> </ul>
IL-6	<ul style="list-style-type: none"> <li>↑Pro-inflammatory cytokines (TNF-<math>\alpha</math>, IL-1, IL-17)</li> <li>↑Dermal and epidermal cell growth and differentiation</li> <li>↑T cell migration into the epidermis</li> </ul>	<ul style="list-style-type: none"> <li>↑Pro-inflammatory cytokine production</li> </ul>	<ul style="list-style-type: none"> <li>↑Insulin-resistance</li> <li>↓Hepatic cytokine signaling-3</li> </ul>
Leptin	<ul style="list-style-type: none"> <li>↑Keratinocyte proliferation</li> <li>↑Promotes Th1 responses</li> <li>↑Angiogenesis</li> </ul>	<ul style="list-style-type: none"> <li>↑Vascular smooth muscle cell migration and proliferation</li> <li>↑Synthesis of TNF-<math>\alpha</math></li> </ul>	<ul style="list-style-type: none"> <li>↑Activation of JAK-2/IRS-2/PI3-K/ Akt pathways</li> <li>↑Leptin resistance</li> <li>↑Hepatic fibrogenesis</li> </ul>
Adiponectin	<ul style="list-style-type: none"> <li>↑Anti-inflammatory cytokine production (Reduced levels in PsO)</li> </ul>	<ul style="list-style-type: none"> <li>↑Endothelial NO production</li> <li>↑Endothelial dysfunction (Reduced levels in CVD)</li> </ul>	<ul style="list-style-type: none"> <li>↑Insulin sensitivity (Reduced levels in NAFLD)</li> </ul>
Resistin	<ul style="list-style-type: none"> <li>↑Pro-inflammatory cytokine production</li> </ul>	<ul style="list-style-type: none"> <li>↑Arterial inflammation</li> <li>↑Vascular smooth muscle cell proliferation</li> <li>↑Endothelial dysfunction</li> </ul>	<ul style="list-style-type: none"> <li>↑Insulin resistance (controversial data on NAFLD)</li> </ul>
Visfatin	<ul style="list-style-type: none"> <li>↑</li> </ul>	<ul style="list-style-type: none"> <li>↑</li> </ul>	<ul style="list-style-type: none"> <li>↑Protection against liver injury (not altered in the early stage)</li> </ul>
IL-17	<ul style="list-style-type: none"> <li>↑Pro-inflammatory cytokine production</li> <li>↑Expression of vascular endothelial cell adhesion molecules</li> </ul>	<ul style="list-style-type: none"> <li>↑Atherosclerotic plaque vulnerability</li> </ul>	<ul style="list-style-type: none"> <li>↑Hepatic steatosis</li> <li>↑Synthesis of pro-inflammatory cytokines</li> </ul>
VEGF	<ul style="list-style-type: none"> <li>↑Keratinocyte proliferation</li> <li>↑Angiogenesis</li> </ul>	<ul style="list-style-type: none"> <li>↑</li> </ul>	<ul style="list-style-type: none"> <li>↑Microvascular changes implicated in the hepatic disease (fibrosis to cirrhosis)</li> </ul>

CVD: Cardiovascular disease; NAFLD: Non-alcoholic fatty liver disease; LDL: Low-density lipoprotein; IRS-1: Insulin receptor substrate 1; MAPK: Mitogen-activated protein; ERK: Extracellular signal-regulated kinase; JAK: Janus kinase-signal transducers; VEGF: Vascular endothelial growth factor; IL: Interleukin; TNF: Tumor necrosis factor.

Among Th1 pro-inflammatory cytokines, tumor necrosis factor (TNF)- $\alpha$  is considered one of the most representative cytokines in psoriasis; elevated serum levels of TNF- $\alpha$  have been detected in psoriatics with a positive correlation with disease severity<sup>[77]</sup> (Table 1).

In psoriasis, TNF- $\alpha$  promotes keratinocyte proliferation, pro-inflammatory cytokine production, expression of vascular endothelial cell adhesion molecules and angiogenesis<sup>[78]</sup>.

Although the role of TNF- $\alpha$  in the pathogenesis of atherosclerosis remains not completely elucidated, it seems to increase the LDL transcytosis across endothelial cells and to facilitate LDL retention in the vascular wall<sup>[79]</sup> (Table 1).

Furthermore, TNF- $\alpha$  interferes with insulin metabolism, thus reducing the auto-phosphorylation of tyrosine residues of insulin receptor and phosphorylation of insulin receptor substrate 1 (IRS-1) and contributing to the first hit of NAFLD<sup>[80]</sup> (Table 1).

Interleukin (IL)-1 is another important pro-inflammatory cytokine exerting both autocrine and paracrine effects on keratinocytes, lymphocytes and vascular endothelium. In particular, it stimulates the synthesis of inflammatory cardiovascular mediators such as IL-6, fibrinogen, C-reactive protein, and increases the expression of adhesion molecules ICAM and VCAM-1 by dermal endothelial cells, leading to the skin recruitment of immune cells<sup>[81]</sup> (Table 1).

Human atherosclerotic plaques show elevated levels of IL-1 $\beta$  mRNA. This element could suggest that the synthesis of growth factors and other cytokines leading to local inflammatory cascades may be activated by locally synthesized IL-1 protein<sup>[82]</sup> (Table 1).

IL-1 also participates in pancreatic  $\beta$ -cell activity by stimulating mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinase (ERK), by affecting the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and by activating the inducible nitric oxide synthase (iNOS)<sup>[83]</sup>.

IL-6 is an inflammatory cytokine, which amplifies inflammatory responses by synergizing with other pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1 and IL-17. IL-6 is responsible for dermal and epidermal cell growth and differentiation and for T cell migration into the epidermis<sup>[84]</sup> (Table 1).

Although the role of IL-6 is contradictory in NAFLD, recent data have evidenced that it may suppress hepatic cytokine signaling-3 leading to insulin resistance<sup>[85]</sup> (Table 1).

In addition to the above cytokines, adipose tissue produces leptin, adiponectin, resistin and visfatin, which are impaired in psoriasis and NAFLD and contribute to the ED<sup>[86]</sup>.

ED is considered an early manifestation of vascular

alterations which precede the development of hypertension and atherosclerosis in obese people<sup>[86]</sup>.

In psoriasis, increased serum levels of leptin, resistin and visfatin and reduced serum levels of adiponectin have been detected<sup>[7]</sup>.

Leptin is a pro-inflammatory adipocytokine which interacts with its specific receptor on endothelial cells, leading to the activation of JAK-2/IRS-2/PI3-K/Akt pathways and nuclear translocation of STAT (signal transducer and activator of transcription) proteins<sup>[87]</sup>.

Moreover, leptin is considered a pro-atherogenic factor by promoting vascular smooth muscle cell migration and proliferation and by stimulating the synthesis of TNF- $\alpha$  with the consequent amplification of inflammatory TNF- $\alpha$  related pathways. Recently, hyperleptinemia has been found as a possible risk factor for acute myocardial infarction<sup>[88,89]</sup> (Table 1).

Resistin seems to support atherosclerosis by favoring ED, vascular smooth muscle cell proliferation, arterial inflammation and foam cell formation. Serum levels of resistin were higher in patients with acute myocardial infarction compared to patients with stable angina. As another pro-inflammatory adipocytokine, resistin may be involved in the pathogenesis of MetS in psoriatic patients, despite its role in NAFLD remains uncertain<sup>[90,91]</sup> (Table 1).

Adiponectin is an anti-inflammatory adipocytokine that increases nitric oxide production in endothelial cells by the activation of phosphatidylinositol-3 (PI-3) kinase/Akt signalling pathway. Serum level of adiponectin appears reduced both in psoriasis and NAFLD and it may be associated to the decreased endothelial production of NO, which is in turn considered a marker of ED<sup>[87,91]</sup> (Table 1).

Visfatin is pro-inflammatory adipocytokine contributing to insulin resistance and to atherosclerotic plaque destabilization. Serum levels of visfatin had been found higher in patients with ischemic cerebrovascular disease and myocardial infarction<sup>[92,93]</sup> (Table 1).

Th17 is implied in the pathogenesis of psoriasis and of other immune-mediated inflammatory diseases by modulating immune cell trafficking and initiating inflammation and cytokine production<sup>[94]</sup>.

Th17 had been found overexpressed both in psoriatics' serum and plaque with a positive correlation with disease severity; IL-17A levels were significantly higher in moderate to severe psoriasis than in mild psoriasis<sup>[95,96]</sup> (Table 1).

Although the precise role of Th17 in atherosclerosis remains controversial, recent data have hypothesized a putative role in the atherosclerotic plaque vulnerability, which represents the initial step of plaque rupture leading to vessel occlusion, myocardial infarction and stroke. In fact, increased expression of IL-17A has been observed in human carotid artery plaques of symptomatic patients with stroke or transient ischemic attack<sup>[97]</sup> (Table 1).

In mice, Th17 and IL-17 may be implicated in the progression from steatosis to steatohepatitis<sup>[98]</sup> (Table 1).

Angiogenesis is a physio-pathologic process charac-

terized by the new blood vessel formation from the pre-existing vasculature and appears important in inflammatory, autoimmune and neoplastic diseases. Therefore, angiogenesis may represent a further link between psoriasis and psoriasis-related comorbidities<sup>[99]</sup>.

Vascular endothelial growth factor (VEGF) is the pivotal angiogenic factor participating in the regulation of metabolism, gene expression, cell proliferation, migration, and survival<sup>[100]</sup> (Table 1).

VEGF participates in the pathogenesis of psoriasis either in an autocrine manner by directly stimulating keratinocyte proliferation and in a paracrine manner by inducing angiogenesis and by providing the fundamental elements to support epidermal proliferation. VEGF is upregulated in serum and lesional psoriatic skin with a correlation with disease severity<sup>[101]</sup> (Table 1).

Coulon *et al.*<sup>[102]</sup> tested the TNF- $\alpha$ , IL-6 and VEGF serum concentrations in an obese population with NAFLD and found higher levels than those of controls, thus indicating the role of pro-inflammatory and pro-angiogenic factors in this pathology.

This aspect appears relevant; in fact, angiogenesis participates in the microvascular changes which are implicated in the hepatic disease progression from fibrosis to cirrhosis<sup>[103]</sup>.

A further mechanism shared by psoriasis, NAFLD and CVD may be oxidative stress. Oxidative stress results from disequilibrium between the reduced anti-oxidant systems and abnormal excessive production of reactive oxygen species (ROS) or reactive nitrogen species. ROS are produced mainly by mitochondria and their production is regulated by the redox state of the respiratory chain<sup>[104,105]</sup>.

The pathogenesis and progression of psoriasis are strictly linked to the redox sensitive cellular signaling pathways, such as mitogen-activated protein kinase/activator protein 1 (MAPK/AP1), NF- $\kappa$ B, and Janus kinase-signal transducers (JAK) and transcription activators<sup>[106]</sup>.

Many studies have been conducted to investigate the role of oxidative stress in psoriasis and have evidenced that psoriatics show an imbalance between biomarkers of oxidative stress and the antioxidant system. Ferretti *et al.*<sup>[107]</sup> have shown an impairment of oxidant/antioxidant system; significantly higher serum levels of lipoprotein a [Lp(a)] and lipid hydroperoxides have been found in psoriatics compared to controls. Conversely, paraoxonase-1 (PON1), an anti-inflammatory and antioxidant enzyme, was lower than in healthy subjects. A positive correlation was found between serum levels of Lp(a), markers of lipid peroxidation and the severity of the disease, whereas PON1 activity and Lp(a) were negatively correlated<sup>[107,108]</sup>.

Emre *et al.*<sup>[109]</sup> have investigated the relation between oxidative status and smoking in psoriasis, demonstrating the increased serum levels of triglycerides and reduced levels of HDL cholesterol and arylesterase activity in smoker compared to non-smoker psoriatic patients. Therefore, smoking could be considered a risk factor for psoriasis severity by increasing oxidative stress and



thus predisposing psoriatic patients to a higher risk of cardiovascular comorbidities.

A reduction in total antioxidant capacity and in antioxidant vitamins A and E has been found by Rocha-Pereira *et al.*<sup>[110]</sup>, who had also confirmed a pro-atherogenic lipid profile in psoriatic patients with an increase of cholesterol, triglycerides, low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL), apolipoprotein B (apo B), Lp(a) and lipoperoxidation products. These data tend to underline an increased cardiovascular risk in psoriatic patients, particularly in those with severe disease.

It is known that oxidative stress participates in the second hit of the pathogenesis of NAFLD and it may be implicated in the NAFLD progression by interfering with normal cell division. In murine models, alterations of the polyploidization process were found in fatty liver with a large proportion of highly polyploid mononuclear cells, which were only rarely observed in normal hepatic parenchyma. Moreover, in humans, alterations in hepatocyte ploidy have been documented in liver biopsies from patients with NASH<sup>[111]</sup>.

Oxidative stress participates in the mild chronic vascular inflammation in CVD. In fact, oxygen metabolites are able to interfere with LDL metabolism and promote the formation of oxidized low-density lipoprotein (Ox-LDL), which plays a representative role in atherosclerotic plaque development and in endothelial damage favoring inflammatory vascular cell infiltration<sup>[112,113]</sup> (Figure 1).

## EFFECTS OF TNF-ALPHA INHIBITORS AND CONVENTIONAL PSORIATIC THERAPIES ON NAFLD AND ON CARDIOVASCULAR RISK FACTORS

As seen above, the inflammatory process represents the mainstay linking the pathogenesis of psoriasis, NAFLD and CVD. Therefore, anti-inflammatory drugs may represent important therapeutic options in the treatment and prevention of these pathologies. Data in the literature on the effect of both conventional and biological psoriatic therapies have shown discordant results on their possible action on NAFLD and cardiovascular risk factors<sup>[114]</sup>.

Conventional treatments for moderate to severe psoriasis include cyclosporine A, methotrexate and retinoids. Although effective, their safety profile should be evaluated in their long-term use, with psoriasis-related comorbidities considered<sup>[115]</sup>.

In fact, it is well known that methotrexate can mediate liver toxicity and patients with liver dysfunction, such as NAFLD/NASH patients, could present impaired drug metabolism with consequent liver accumulation and increased susceptibility to liver toxicity<sup>[116]</sup>.

Methotrexate exerts opposite effects on cardiovascular risk in psoriatic patients. In 1989, Refsum *et al.*<sup>[117]</sup> investigated the effect of methotrexate 25 mg weekly on plasma homocysteine levels and found a significant and

transient increase within 48 h after administration.

Conversely, a lower risk of CVD has been found in psoriatic patient treated with MTX compared to patients without MTX<sup>[118]</sup>.

Elevated serum levels of cholesterol and triglycerides can occur during treatment with retinoids and cyclosporine, although no evidence of an increased cardiovascular risk has been stated with long-term use of etetrinate<sup>[118,119]</sup>.

Moreover, as demonstrated in a prospective non-randomized study on patients affected by PsA, cyclosporine has been associated with a significant elevation of blood pressure values<sup>[119]</sup>.

TNF- $\alpha$  inhibitors, IL12/23 inhibitors and IL-17 inhibitors represent three new classes of drugs used in moderate to severe psoriasis with a good efficacy and safety profile. Among biologics, metabolic effects of TNF- $\alpha$  blockers are most widely studied<sup>[120,121]</sup>.

A cross-sectional study evaluated epicardial fat thickness (EAT), an emerging marker of cardiometabolic risk, in patients with rheumatoid arthritis (RA) treated with TNF- $\alpha$  inhibitors compared to RA patients treated with non-biological disease-modifying anti-rheumatic drugs (DMARDs). A significantly lower EAT thickness was detected in patients treated with TNF- $\alpha$  inhibitors than in those treated with DMARDs ( $8.56 \pm 1.90$  mm and  $9.71 \pm 1.45$  mm, respectively)<sup>[122]</sup>.

Jókai *et al.*<sup>[123]</sup> evaluated the positive effect of TNF- $\alpha$  inhibitors on carotid and brachial intima-media thickness in patients with psoriasis.

Although data are few, TNF- $\alpha$  blockers seem to act on lipid and glucose metabolism by exerting a potential action on cardiovascular risk factors. An improvement of insulin-sensitivity in psoriatic patients treated with Etanercept and Infliximab has been evidenced<sup>[124,125]</sup>.

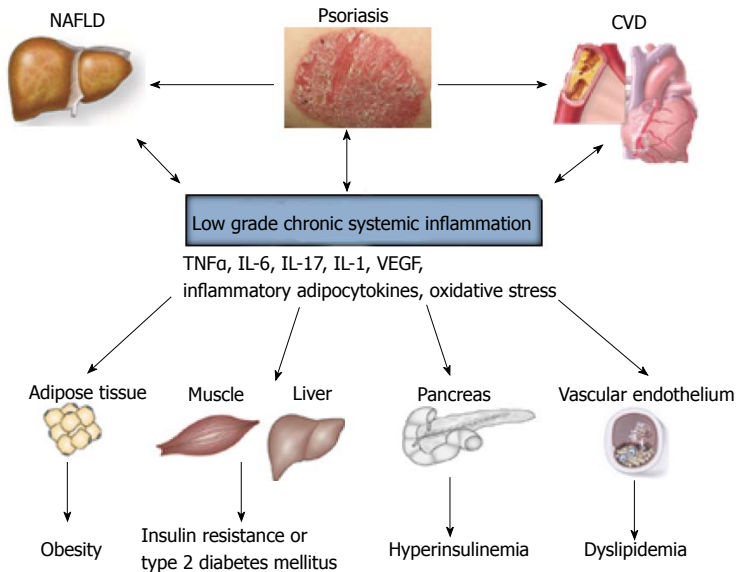
Conversely, long-term use of TNF- $\alpha$  inhibitors in patients with rheumatoid arthritis seem not to influence insulin resistance parameters<sup>[126]</sup>.

With regard to the lipid profile, although no statistically significant difference, raised values of total cholesterol, LDL-C and triglycerides were found after 24 wk of treatment with Etanercept in psoriatic patients<sup>[127]</sup>.

Adipocytokine levels and fat distribution have been assessed in patients with RA and ankylosing spondylitis during long-term treatment with TNF- $\alpha$  blockers. A fat mass gain with a tendency to visceral fat accumulation, a reduction of resistin serum levels and no significant modification in leptin, total adiponectin or visfatin serum levels have been evidenced<sup>[128]</sup>. Another recent study focused on the influence of TNF- $\alpha$  inhibitors on serum levels of adipocytokines, showing a partial rebalancing between pro- and anti-inflammatory adipocytokines after 24 wk of anti-TNF- $\alpha$  treatment with a reduction of leptin, visfatin and resistin and a mild adiponectin increase<sup>[76]</sup>.

A body weight increment has been identified after 6-mo treatment with Etanercept compared to psoriatic patients treated with methotrexate<sup>[129]</sup>.

These data have been confirmed by Campanati *et al.*<sup>[24]</sup> who showed an increase in waist-hip-ratio and



**Figure 1** Psoriasis, non-alcoholic fatty liver disease, cardiovascular diseases and cardiovascular risk factors: A unique inflammatory background. CVD: Cardiovascular disease; NAFLD: Non-alcoholic fatty liver disease; VEGF: Vascular endothelial growth factor; IL: Interleukin; TNF: Tumor necrosis factor.

BMI during treatment with Etanercept. The authors documented a possible preventive effect of Etanercept on liver fibrosis, evidencing a significant reduction of AST/ALT ratio and an improvement of insulin-sensitivity parameters. These elements confirm the strong relation between the alteration of glucose metabolism and NAFLD<sup>[24]</sup>.

Although further larger studies are needed to confirm these data, this hypothetic preventive role may be linked to anti-inflammatory properties of TNF- $\alpha$  inhibitors and their action on glucose homeostasis<sup>[24]</sup>.

The favorable effect of TNF- $\alpha$  blockers on the risk of MI has been identified in a retrospective study monitoring patients affected by only psoriasis, by only PsA and by both psoriasis and PsA. Patients with only psoriasis had a significant MI risk reduction (HR = 0.26; 95%CI: 0.12-0.56), whereas a non-significant MI risk reduction was detected in those with only PsA (HR = 0.86; 95%CI: 0.28-2.70) and in those with both psoriasis and PsA. The duration of TNF- $\alpha$  inhibitor treatment did not seem to influence the risk of MI<sup>[130,131]</sup>.

## CONCLUSION

Psoriasis is a complex and already partially unknown disease whose skin manifestations represent only the edge of an iceberg, which is widely submerged and unknown. Psoriasis and psoriasis-related comorbidities significantly impact on patient's health and quality of life and negatively interfere in physical-psychic well-being with important repercussion in working daily life. As a multi-organ pathology, psoriasis needs a multidisciplinary approach and clinicians should evaluate this holistic vision in order to promptly identify and manage psoriasis-related comorbidities influencing patients' morbidity and mortality. The underlying inflammatory process is the leitmotiv shared by psoriasis, NAFLD and CVD and overlaps both the common genetic predisposition and modifiable risk factors, such as sedentary lifestyle,

smoking and alcohol consumption.

Therefore, the therapeutic strategy for psoriasis should be multifaceted and should specifically tailor outcome tools and disease-related items by a patient-based evaluation and by selectively verifying the risk/benefit of each single therapeutic option.

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## Impact of cardiac magnetic resonance imaging in non-ischemic cardiomyopathies

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### Abstract

Non-ischemic cardiomyopathies include a wide spectrum of disease states afflicting the heart, whether a primary process or secondary to a systemic condition. Cardiac magnetic resonance imaging (CMR) has established itself as an important imaging modality in the evaluation of non-ischemic cardiomyopathies. CMR is useful in the diagnosis of cardiomyopathy, quantification of ventricular function, establishing etiology, determining prognosis and risk stratification. Technical advances and extensive research over the last decade have resulted in the accumulation of a tremendous amount of data with regards to the utility of CMR in these cardiomyopathies. In this article, we review CMR findings of various non-ischemic cardiomyopathies and focus on current literature investigating the clinical impact of CMR on risk stratification, treatment, and prognosis.

**Key words:** Cardiomyopathy; Magnetic resonance imaging; Heart; Cardiovascular imaging; Cardiology

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**Core tip:** Cardiac magnetic resonance imaging (CMR) has established itself as a vital modality in the evaluation of numerous aspects of non-ischemic cardiomyopathies, ranging from establishing a diagnosis to detailed analysis of cardiac function. Lately, increasing data has become available regarding the clinical utility of CMR in the evaluation of these patients, although few articles have consolidated these findings regarding CMR's impact in these pathologies. This review will summarize current literature investigating the clinical impact of CMR on risk stratification, treatment, and prognosis in the setting of non-ischemic cardiomyopathies.

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## INTRODUCTION

Non-ischemic cardiomyopathies (NICM) include a wide spectrum of disease states afflicting the heart, whether a primary process or secondary to a systemic condition<sup>[1,2]</sup>. Several imaging modalities are used in the evaluation of NICM, particularly echocardiography, nuclear medicine, and cardiac catheterization. Cardiac magnetic resonance imaging (CMR) has established itself as an important modality in the evaluation of cardiomyopathies. The last decade has seen tremendous technological advances in CMR, both in software and hardware<sup>[3]</sup>. CMR offers a number of advantages that makes it an ideal imaging modality in a number of clinical settings. CMR allows for the non-operator dependent acquisition of high spatial and temporal resolution images in any desired imaging plane and regardless of patient-specific factors such size and body composition. With these high resolution images, accurate assessments of various chamber and vessels functional parameters can be made. Additionally, CMR is free of ionizing radiation, which makes it an ideal modality for evaluation of young patients, and those who may require frequent or regular follow-up assessments.

The increased use of CMR has resulted in accumulation of a tremendous amount of data on the utility of CMR in the clinical management of these patients. CMR is moving from simply an initial diagnostic tool to one whose findings can also have for significant clinical impact, including those on therapy response, risk stratification, and prognosis determination.

In this article, we review CMR findings of various non-ischemic cardiomyopathies and focus on current literature investigating the clinical impact of CMR on risk stratification, treatment, and prognosis.

## MAGNETIC RESONANCE IMAGING OF NICM

In a patient with NICM, several dedicated CMR sequences are used as a part of the magnetic resonance imaging (MRI) protocol. Steady-state free precession (SSFP) is the most commonly used sequence, which helps in evaluating ventricular morphology and function. In addition, ventricular function can also be quantified by drawing endocardial and epicardial contours. Velocity-encoded phase contrast MR images enable flow and velocity quantification in vascular and valvular structures. Multi-echo gradient echo images are used for detecting and quantifying myocardial iron. T2-weighted images are useful in detection of myocardial edema, seen in acute

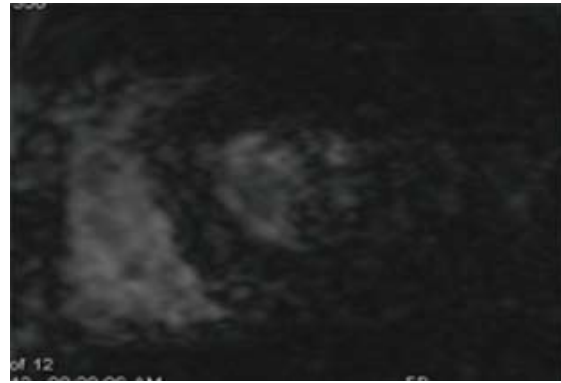


Figure 1 Iron overload cardiomyopathy. Short axis gradient echo image with long echo time (15 ms) shows dark signal in the left ventricular myocardium due to increased iron deposition.

myocardial infarction or myocarditis. T2-mapping is a more accurate technique of quantifying the myocardial fluid. Dynamic first-pass perfusion images are utilized for evaluation of perfusion defects or microvascular dysfunction. Delayed-enhancement images show scar and fibrosis, seen as different patterns of late gadolinium enhancement (LGE), which is useful in the characterization of cardiomyopathies. T1-mapping techniques can quantify the T1 values of myocardium, either before (native) or after administration of contrast and can measure extracellular volume (ECV), which is a biomarker of fibrosis. MR angiography is useful in evaluation of vascular anatomy. 3D-whole heart navigator gated SSFP sequence is useful for evaluation of coronary artery anatomy as well as vascular anatomy without administration of contrast.

A summary of main diagnostic CMR findings as well as the commonly evaluated CMR parameters and their clinical implications, discussed in greater in the following sections, are included in Table 1.

### Iron overload cardiomyopathy

Myocardial iron deposition is shown on gradient-echo images, with lower signal at higher Echo time (TE) values (Figure 1). Utilizing gradient echo images at different TE levels (Multi-echo GRE), the absolute myocardial T2\* can be measured and this has shown to be a more reliable indicator of true myocardial iron content as compared to serum ferritin levels or liver iron<sup>[4,5]</sup>. Myocardial T2\* < 20 ms is considered to be significant iron deposition and < 10 ms is considered to be advanced iron deposition.

Myocardial T2\* values have also been shown to detect myocardial changes of iron overload, significantly earlier than changes in left ventricular ejection fraction (LVEF)<sup>[4]</sup>. Myocardial T2\* has been shown to be a strong independent predictor of adverse clinical outcomes such as development of heart failure, arrhythmias, and sudden cardiac death. A study by Anderson *et al*<sup>[4]</sup> showed that patients with a T2\* < 20 ms were at significantly increased risk for arrhythmias, and this risk was also shown to be increased further at lower T2\*

**Table 1** Summary of diagnostic findings and prognostic parameters at cardiac magnetic resonance

Cardiomyopathy	Key diagnostic CMR findings	Prognostic CMR parameters	Clinical outcomes evaluated
Iron overload cardiomyopathy	Myocardial T2* < 20 ms	Myocardial T2*	Adverse cardiac events, sudden cardiac death, treatment monitoring
Idiopathic dilated cardiomyopathy	LV dilatation, global systolic dysfunction, mid-myocardial septal LGE	LGE, longitudinal myocardial strain	Adverse cardiac events, transplant status, sudden cardiac death, treatment monitoring
Hypertrophic cardiomyopathy	Asymmetric septal hypertrophy, patchy LGE (RV insertion points), mitral valve systolic anterior motion	LGE	Adverse cardiac events, sudden cardiac death
Sarcoidosis	Mid-myocardial or sub-epicardial LGE with (acute) or without (chronic) edema	LGE	Adverse cardiac events, treatment monitoring
Myocarditis	Myocardial edema, high T2 in T2 mapping, early gadolinium enhancement, mid-myocardial or subepicardial distribution LGE	LGE	Adverse cardiac events, sudden cardiac death, cardiac function recovery
Amyloidosis	Diffuse subendocardial-transmural enhancement, early myocardial nulling on T1 mapping	LGE, ECV estimation, T2 ratio	Mortality, disease subtype differentiation
Left ventricular non-compaction	Non-compacted to compacted myocardium ratio (end diastole) > 2.3	Non-compacted to compacted thickness ratio, LGE	Functional status, adverse cardiac events, sudden cardiac death
Arrhythmogenic right ventricular dysplasia	Major wall motion abnormality, low ejection fraction, dilated RV (major criteria)	RV and LV abnormalities, LGE	Adverse cardiac events, sudden cardiac death, treatment planning
Takotsubo cardiomyopathy	Reduced global systolic function, abnormal apical wall motion with normal/hyperkinetic basal segments	Type of segmental involvement, LGE	Cardiac dysfunction severity and recovery
Fabry disease	Concentric LV thickening, basal inferolateral segment mid myocardial-subepicardial LGE	LGE, T1 mapping	Adverse cardiac events, sudden cardiac death, treatment monitoring
Muscular dystrophy	Ventricular dilation, systolic dysfunction, mid myocardial-subepicardial LGE	LGE, T1 mapping, ECV estimation, myocardial strain	Adverse cardiac events

CMR: Cardiac magnetic resonance; RV: Right ventricle; LV: Left ventricle; LGE: Late gadolinium enhancement; ECV: Extracellular volume.

levels. T2\* value < 10 ms had a substantially higher risk of developing heart failure at the time of follow-up with risk increasing further for patients with T2\* < 6 ms. As with the level of myocardial iron content, these outcomes predictors did not correlate with parameters such as serum ferritin or liver iron content. Similar findings were also seen in data from Patton *et al*<sup>[5]</sup>, which also included sudden cardiac death as a part of their composite outcome. Data from this study also demonstrated worsening outcomes measured at lower T2\* levels, leading them to propose a three-tiered risk stratification model based on T2\* values - low risk: T2\* > 20 ms; intermediate risk: T2\* between 10 ms and 20 ms; and higher risk: T2\* < 10 ms.

In addition to predicting outcomes, CMR has also shown to be an invaluable tool in the monitoring of treatment response to chelation therapies, which comprises a crucial element of the treatment of iron-overload cardiomyopathy. Multiple published studies have shown improvements in T2\*<sup>[6-13]</sup> and LVEF<sup>[6-11]</sup> when evaluating treatment responses to several different chelating agents over variable treatment durations. The longest studied follow-up time was performed by Ambati *et al*<sup>[11]</sup>, which demonstrated continued improvement in both T2\* and LVEF extending to five years after treatment initiation. Although most studies evaluating cardiac response of chelation therapies have focused on objective parameters such as T2\* and LVEF, Pennell *et al*<sup>[14]</sup> demonstrated that improvements in myocardial T2\* and LVEF were also associated with significantly reduced risk of developing

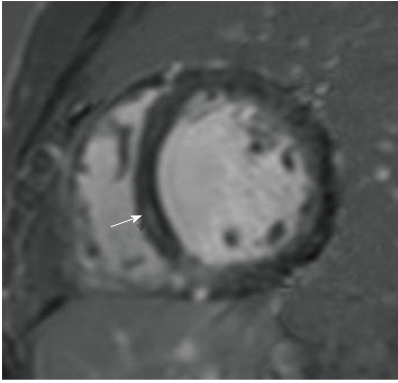
heart failure. It should be noted that this observed risk reduction was seen in the setting of only minimally improved LVEF, suggesting that, in the setting the chelation treatment of iron overload cardiomyopathy, conventional functional parameters such as LVEF may underestimate the clinical impact of therapies.

Given the evidence for the use of CMR in the diagnosis, risk stratification, and treatment monitoring in iron overload cardiomyopathy, CMR is recognized in the most current American Heart Association (AHA) Consensus Statement<sup>[15]</sup> as a critical tool in the diagnosis and clinical management of patients with iron overload cardiomyopathy. Additionally, the widespread adoption of CMR in management of these patients has correlated with the reduction in mortality from cardiac iron overload in patients in the United Kingdom<sup>[16,17]</sup>, which has been largely attributed to clinical guidance by CMR findings in these patients. For example, Modell *et al*<sup>[16]</sup> showed that the death rate from iron overload between 2000 and 2003 was 2.3 per 1000 patients, significantly decreased from 7.9 per 1000 prior to the initiation of CMR screening in thalassemia patients. Additionally, Chouliaras *et al*<sup>[17]</sup> estimated that the risk of cardiac death before CMR screening of United Kingdom thalassemia patients was 82% higher compared to the risk observed after CMR screening.

#### **Idiopathic dilated cardiomyopathy**

Idiopathic dilated cardiomyopathy is characterized by dilation of the left ventricular left ventricle (LV) with global





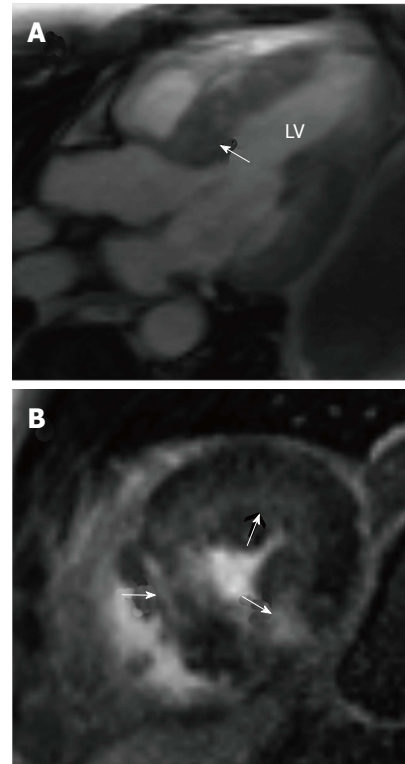
**Figure 2 Idiopathic dilated cardiomyopathy.** Short axis delayed-enhancement image shows linear mid myocardial enhancement (arrow) in the basal septum, and dilated left ventricle, which is indicative of idiopathic dilated cardiomyopathy.

systolic dysfunction. A linear mid-myocardial pattern of LGE in the septum (Figure 2) has been reported in these patients<sup>[18]</sup>, due to presence of fibrosis. A study by McCrohon *et al.*<sup>[19]</sup> showed that in a population with dilated cardiomyopathy, this linear mid-myocardial pattern was seen in 28% of patients, with no particular enhancement in 59% of patient. In 13% of these patients, a subendocardial pattern was seen in spite of normal coronary arteries in catheterization<sup>[19]</sup>.

Buss *et al.*<sup>[20]</sup> demonstrated the association of various strain parameters with cardiac outcomes including cardiac death and transplantation. In their analysis, longitudinal strain was shown to be a superior predictor of outcome compared to not only conventional parameters such as LVEF and New York Heart Association functional class, but the presence of LGE as well. Additionally, preserved longitudinal strain was associated with better outcomes, even in the presence of LGE or depressed LVEF<sup>[20]</sup>.

Several published studies have shown the presence of LGE in these patients to be a significant risk factor for the development of arrhythmic events, including sudden cardiac death<sup>[18,21-24]</sup>. A pair of studies<sup>[18,24]</sup> have shown specifically the presence of mid-wall fibrosis to be associated with increased risk of adverse cardiac events and sudden death<sup>[18,24]</sup>. Furthermore, a study by Perazzolo Marra *et al.*<sup>[21]</sup> demonstrated that the presence of LGE was a superior predictor to traditional parameters including depressed LVEF (less than 35%) in predicting arrhythmic events and sudden cardiac events. The presence of LGE has also been shown to be a useful predictor of adverse cardiac events in cohorts of asymptomatic and minimally symptomatic patients<sup>[25]</sup>.

Prospective data is limited regarding the impact on screening dilated cardiomyopathy patients on management or treatment outcomes. However, in an analysis by Gulati *et al.*<sup>[24]</sup>, assuming a 15% threshold for sudden cardiac death risk for implantable cardioverter defibrillator (ICD) implantation, the addition of LGE to their risk assessment model would have resulted in nearly 19% of studied patients would have undergone ICD implantation, and 11% would have avoided ICD implantation. Although



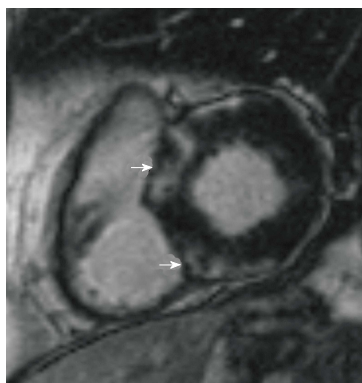
**Figure 3 Hypertrophic cardiomyopathy.** A: Three-chamber steady state free precession image shows severe hypertrophy of the basal anteroseptum (arrow), which causes LVOT obstruction; B: Short-axis delayed enhancement image shows patchy mid myocardial enhancement in hypertrophied segments, suggestive of interstitial fibrosis in a pattern specific for hypertrophic cardiomyopathy. LV: Left ventricular.

long-term clinical outcome data is lacking, this suggests that measurement of LGE at CMR may be an effective way to guide ICD therapies in these patients.

### **Hypertrophic cardiomyopathy**

Hypertrophic cardiomyopathy (HCM) is a genetic disorder with a heterogeneous phenotypic expression. MRI can diagnose HCM and also characterize the morphology. The most common morphological type is asymmetric septal hypertrophy (ASH), and other forms include apical, mid-ventricular, concentric, spiral and mass-like forms. In ASH, there is hypertrophy of the basal septum (Figure 3A). MRI can detect and quantify LVOT flow obstruction and the flow velocity/gradient. Systolic anterior motion of the mitral valve and mitral regurgitation can also be detected and quantified. MRI is also useful in detection of papillary muscle abnormalities such as anomalous insertion, double bifid morphology, anteroapical displacement and hypermobile papillary muscles, which can cause obstruction without significant myocardial hypertrophy. Delayed enhancement is seen in 60% of patients<sup>[26]</sup> with HCM due to interstitial fibrosis, microfibrillar disarray or microvascular obstruction. This is typically seen in a mid-myocardial, patchy pattern at the RV insertion points, but is also seen in the rest of the hypertrophied (Figure 3B) and non-hypertrophied myocardium.





**Figure 4 Sarcoidosis.** Short axis delayed enhancement image shows patchy areas of mid myocardial enhancement in the basal septum and basal inferior segments.

The presence of LGE at CMR plays an important role in risk stratification and estimating prognosis in HCM. Several studies have demonstrated the independent predictive ability of the presence of LGE for cardiac outcomes including worsening heart failure symptoms, ventricular arrhythmias, ICD discharge, and sudden cardiac death<sup>[27-30]</sup>. Furthermore, the absence of LGE has shown to have useful negative predictive value in that the absence of LGE was associated with a lower, but not absent, risk for adverse cardiac outcomes<sup>[31]</sup>. However, unlike in dilated cardiomyopathy, several larger studies in HCM patients have noted that the extent of LGE, rather than its presence alone, is a significant predictor of adverse cardiac outcomes<sup>[31-34]</sup>. This observation may be in part due these larger studies being better powered to evaluate the full range of adverse outcomes. For example, a study by Ismail *et al*<sup>[35]</sup>, the largest published to date evaluating CMR findings and clinical outcomes in over four hundred patients, demonstrated that only the extent of myocardial LGE was a strong predictor of cardiac events and mortality. However, contrary to other studies, LGE was not shown to be the strongest predictor (behind LVEF) of adverse events in this patient cohort.

To date, limited studies are available regarding the use of CMR in monitoring of treatment for hypertrophic HCM, whether pharmacologic, minimally invasive, or surgical. A study by Yuan *et al*<sup>[36]</sup> demonstrated the utility of CMR in characterizing the infarct size from septal ablations as well as decreased LV mass followed up to one year, although clinical outcome data was not included.

Although CMR remains an important modality in the diagnosis of hypertrophic cardiomyopathy, particularly in the setting of equivocal echocardiogram findings, it is yet to be formally recommended for all patients<sup>[37,38]</sup>. According to the most recent consensus AHA guidelines from 2011<sup>[37]</sup>, the use of LGE with CMR for risk stratification received at a class II a recommendation and may be considered when risk stratification with conventional risk factors (*i.e.*, prior history of ventricular arrhythmias, family history of sudden cardiac death, and personal history of syncopal episode) are

inconclusive.

### Sarcoidosis

Cardiac sarcoidosis is characterized by the presence of necrotizing granulomas in the myocardium. In the acute phase, myocardial thickening and edema may be seen. LGE is seen in a mid-myocardial (Figure 4) or sub-epicardial distribution. In chronic phase, wall thickening and LGE is seen, but edema is absent. In burnt out sarcoidosis, transmural enhancement may be seen<sup>[39]</sup>.

The presence of LGE in sarcoidosis has been shown to be associated with adverse outcomes<sup>[40-42]</sup>. For example, Greulich *et al*<sup>[40]</sup> demonstrated that the presence of LGE as the strongest independent predictor death as well as other adverse events such as aborted sudden death, appropriate ICD discharge, and ventricular arrhythmias. The presence of LGE was also shown to be a stronger predictor of adverse outcomes relative to other functional and clinical parameters such as LVEF and clinical symptoms at presentation. Additionally, no included patients without LGE in this study died at the time of follow-up suggesting the potential high negative predictability of LGE in this patient population.

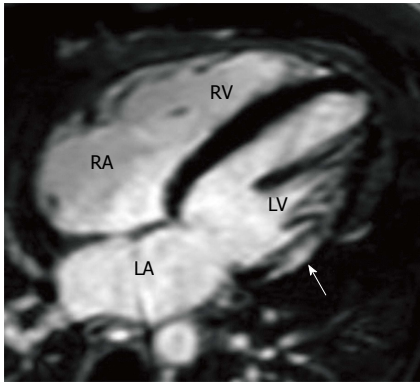
CMR has also been shown in several small studies to be effective in monitoring cardiac improvement in response to steroid therapy<sup>[42-44]</sup>. Overall, steroid therapy has been shown to be associated with not only improved functional parameters such as LVEF and LV end diastolic volume (EDV) index, but also decrease in LGE. However, data from Ise *et al*<sup>[42]</sup> suggest that CMR response to steroid therapy may depend on the extent of LGE upon treatment initiation. In the studied population, treated patients with a lower amount of LGE had significantly decreased LVEF and LV EDV after treatment. However, patients with more severe disease as indicated disease as evidenced by a larger extent of LGE were noted to not only have no significant change in LVEF or LV EDV, but also had worse clinical outcomes.

Similar to the assessment of dilated cardiomyopathy, current appropriate use guidelines from the AHA<sup>[3,45]</sup> still do not specifically recommend CMR exclusively for the purposes of risk stratification or prognostication with its use reserved for diagnosis and differentiation from other cardiomyopathies as well as functional assessment.

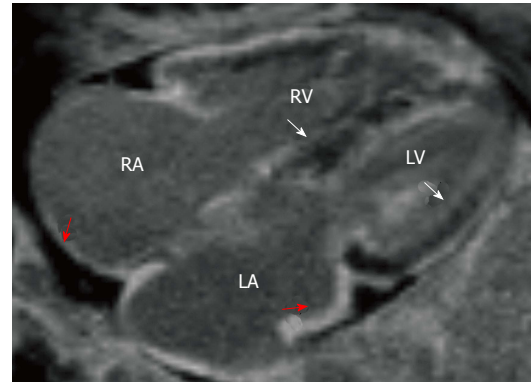
### Myocarditis

Acute myocarditis seen in MRI as high signal in T2-weighted images and elevated values in T2 mapping due to myocardial edema, early gadolinium enhancement and LGE in a mid-myocardial or subepicardial distribution (Figure 5). Different patterns of enhancement have been described based on the etiological agent. Parvovirus B19 infection often involves the basal inferolateral segment, in a mid-myocardial/subepicardial pattern and usually recovers without lasting damage, whereas human herpesvirus-6 more commonly involves the septum, in a linear mid-myocardial pattern and rapidly progresses to heart failure<sup>[46]</sup>.

As in other cardiomyopathies, the presence and per-



**Figure 5 Myocarditis.** Four-chamber delayed enhancement image shows mid myocardial enhancement in the basal lateral segment, consistent with acute myocarditis. RA: Right atrium; LA: Left atrium; RV: Right ventricular; LV: Left ventricular.



**Figure 6 Amyloidosis.** Four-chamber delayed enhancement image shows diffuse subendocardial enhancement of the ventricles (arrows) and atrial walls (red arrows). Note that the blood has lower signal than normal. RA: Right atrium; LA: Left atrium; RV: Right ventricular; LV: Left ventricular.

sistence of LGE in the setting the myocarditis reflects the presence of irreversible myocardial injury<sup>[47]</sup>. The presence, amount, and distribution of LGE at the time of diagnosis has shown to have important implication in cardiac functional parameters at follow-up after recovery from acute illness. For example, Mahrholdt *et al.*<sup>[46]</sup> showed total amount of LGE (%LGE) was a significant independent predictor of impaired ventricular function and ventricular dilatation at follow-up. Additionally, the presence of LGE in the ventricular septum was shown to be the strongest CMR predictor for chronic ventricular dysfunction as well as ventricular dilatation.

CMR has shown promise in predicting clinical outcomes and adverse events in patients with myocarditis. Schumm *et al.*<sup>[48]</sup> demonstrated that in the setting of suspected myocarditis, patients with abnormal CMR (defined at abnormalities in either LVEF, LV volume, or presence of LGE) had significantly more major adverse cardiac events including cardiac death, sudden cardiac death, ICD discharge, and aborted SCD. Additionally, no patients with a normal CMR suffered death or any major adverse cardiac events, suggesting a much more favorable recovery and long term course in patients with normal CMR findings. Similar to the other aforementioned non-ischemic cardiomyopathies, the presence of LGE on the diagnostic CMR was associated with increased of all-cause and cardiac mortality, independent of clinical presentation at diagnosis<sup>[49]</sup>. The absence of LGE was also associated with a more favorable clinical outcome with no sudden cardiac death events seen at a median follow-up of nearly five years in the study population.

Although typically regarded clinically as an acute, self-limiting illness<sup>[50]</sup>, abnormal CMR findings may persist after the resolution of the acute phase of illness. Specifically, several studies have followed the presence of CMR abnormalities in various groups of myocarditis over their clinical course<sup>[46,51,52]</sup>. Specifically, LGE has been shown in anywhere between 24%-40% at the time of follow-up, with the relatively wide range of values likely reflective of heterogeneity of the studied patient populations<sup>[51]</sup>.

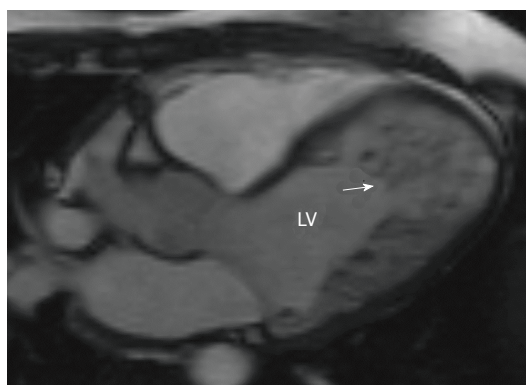
Additionally, Wagner *et al.*<sup>[53]</sup> showed in a small cohort

of patients that the presence of CMR inflammatory markers at four weeks post-diagnosis was associated with poorer long-term LVEF and symptom score. Thus, given the impact of CMR findings at initial diagnosis on long-term cardiac functional parameters and clinical outcomes as well as potential prognostic implication of persistent abnormal CMR findings, a follow-up CMR exam at least 4 wk after the onset of disease can be considered to differentiate uncomplicated involvement of the myocardium in a systemic viral illness from a more complicated, persistent course<sup>[47]</sup>.

### Amyloidosis

Cardiac amyloidosis is characterized by diffuse sub-endocardial to transmural enhancement of not only the left ventricle, but also the right ventricle, interatrial septa and atrial walls (Figure 6). The T1 kinetics are altered, with the myocardium nulling before the blood pool (normal - the myocardium always nulls after the blood pull). The blood pool also appears darker on cardiac amyloidosis, due to high ECV and rapid redistribution of gadolinium from the blood pool. There is also concentric myocardial thickening, along with thickening of the interatrial septa and atrial walls.

Unlike many other non-ischemic cardiomyopathies, the use of LGE in risk stratification and evaluation of prognosis has seen mixed results. While several studies<sup>[54,55]</sup> have shown a significant association between the presence of LGE in cardiac amyloidosis patients after adjustment for other clinical parameters, data in other studies have not shown this trend. For example, Migrino *et al.*<sup>[56]</sup> demonstrated a significantly higher one-year mortality rate for those patients with LGE, although LGE failed to remain predictive of mortality when observation carried out to five years. However, instead of presence or absence of LGE in amyloidosis patients, gadolinium kinetics may prove to be more useful in assessing prognosis. In a study by Maceira *et al.*<sup>[57]</sup>, presence of LGE in itself was not predictive of mortality; however, post-gadolinium intra-myocardial T1 difference between the subepicardial and subendocardial greater than 23 ms was instead shown to predict mortality



**Figure 7 Left ventricular non compaction.** Three-chamber steady state free precession image shows excessive trabeculations in the left ventricle, with the ratio of trabeculated to non trabeculated myocardium of 8, consistent with left ventricular non compaction. LV: Left ventricular.

with 85% accuracy. Lastly, as a modification of the more conventional CMR LGE analysis, White *et al.*<sup>[58]</sup> showed that the presence of diffuse hyperenhancement by a visual T1 assessment is not only able to identify patients with cardiac involvement among patients with high clinical suspicion, but is also a strong predictor of mortality.

Additionally, the emerging techniques of T1 mapping and ECV estimation have shown promise in correlating with cardiac function and risk stratification<sup>[59,60]</sup>. For example, ECV measured at contrast equilibrium greater than 0.45 and pre-contrast T1 > 1044 ms have shown to be predictors of mortality. ECV was also shown to be predictive of mortality even when corrected for markers of ventricular function and serum proBNP values<sup>[59]</sup>. Furthermore, T2 weighted imaging has also shown prognostic implication in cardiac amyloidosis in that low T2 signal (*i.e.*, T2 ratio < 1.5) at triple-inverted fast spin echo imaging was associated with decreased survival<sup>[61]</sup>.

In addition to its role in identifying cardiac involvement in amyloidosis, CMR has also shown promise in differentiating among subtypes of cardiac amyloidosis, namely between light chain amyloid (AL) and transthyretin-related amyloidosis (ATTR) based on parameters such as LV mass as well as location and extent of LGE. Distinguishing among cardiac amyloidosis subtypes is of critical importance given the marked difference of treatment strategies<sup>[62]</sup>. Additionally, cardiac amyloidosis subtype also impacts prognosis, with survival worse in AL as compared to ATTR subtype<sup>[62]</sup>.

### LV non-compaction

LV non-compaction is caused by persistence of embryonal sinusoids, resulting in an exaggerated presence of non-compacted myocardium compared to compacted myocardium. On MRI, a ratio of > 2.3 between non-compacted and compacted myocardium in end-diastole is considered diagnostic of non-compaction (Figure 7)<sup>[63]</sup>. Thrombosis, arrhythmia and LV dysfunction are complications.

The degree of LV non-compaction assessed at CMR

has shown to correlate with not only cardiac function but risk assessment as well<sup>[64,65]</sup>. For example, Ashrith *et al.*<sup>[64]</sup> showed that patients with a maximum non-compacted to compacted thickness ratio less than three were shown to have significantly greater improvement in NYHA functional class at follow up than those with ratio greater than three. Additionally, in patients with reduced LVEF, change in LVEF at follow up was also shown inversely correlated with non-compaction-compaction ratio. Furthermore, data from Stacey *et al.*<sup>[65]</sup> suggest that measurement of non-compaction to compacted ratio measured at end-systole had a higher calculated higher odds ratio for combined cardiovascular events, including death than calculated at end-diastole.

Assessment of late gadolinium enhancement, both trabecular and myocardial, has also shown value in the clinical assessment of LV non-compaction<sup>[64,66-68]</sup>. The degree of trabecular LGE has shown to be an independent predictor of LVEF as well as correlate with severity of clinical stage of disease<sup>[66]</sup>. Additionally, both the presence and extent of myocardial LGE were shown to be significantly related to symptomatic status and electrocardiographic abnormalities as well as a significant predictor of LVEF, suggesting non-compaction as a marker of an underlying diffuse cardiomyopathy<sup>[67]</sup>.

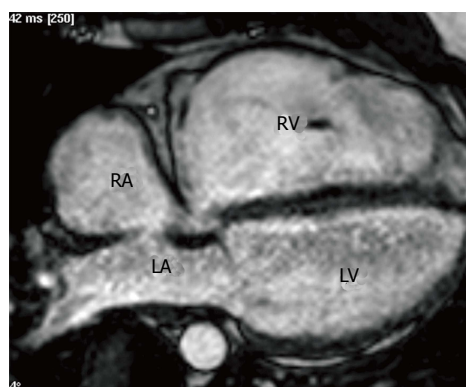
### Arrhythmogenic right ventricular dysplasia/ cardiomyopathy

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is characterized by fibrofatty replacement of the right ventricular myocardium. The diagnosis is based on Task Force criteria. On MRI, the presence of a major wall motion abnormality (aneurysm, akinesis, dyskinesis, asynchronous contraction) along with either low ejection fraction (EF) (< 40%) or dilated RV (EDVi > 110 mL/m<sup>2</sup> in men, > 100 mL/m<sup>2</sup> in women) is considered a major criteria (Figure 8). Major wall motion abnormality along with low EF (40%-45%) or dilated RV (EDVi 100-110 mL/m<sup>2</sup> in men, 90-100 mL/m<sup>2</sup> in women) is considered minor criteria. Other criteria include family history, tissue characterization, repolarization, depolarization and arrhythmia. Two major or one major and two minor or four minor criteria are required for a diagnosis of ARVD. Fat may be seen in the RV myocardium, but this is not critical for diagnosis. LGE may be seen in the RV free wall. Furthermore, if myocardial biopsy is warranted to help confirm the diagnosis of ARVD, CMR findings can be used to help select an appropriate target for biopsy<sup>[69]</sup>.

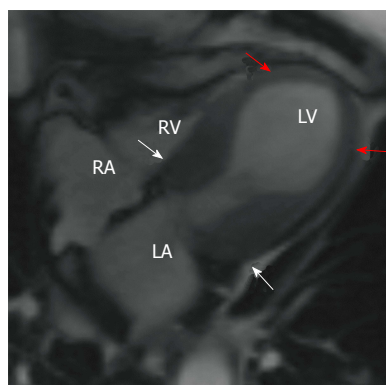
In the setting of clinically diagnosed ARVD, the presence of abnormalities at CMR has been shown to be associated with adverse cardiac outcomes<sup>[70-72]</sup>. Patients with right ventricular abnormalities at CMR experienced higher rates of cardiac death, ICD discharge, and ventricular arrhythmias. Furthermore, the presence of multiple abnormalities at CMR was shown to carry a higher clinical risk, while a normal CMR in patients meeting clinical criteria for ARVC was associated with a significantly better prognosis<sup>[70]</sup>.

Although LGE assessment in the right ventricle can





**Figure 8 Arrhythmogenic right ventricular dysplasia.** Four-chamber cine steady state free precession image shows wall shows aneurysmal dilation of the right ventricle. There was also low ejection fraction (ejection fraction-35%) and severe right ventricle dilation (end diastolic volume index-130 mL/m<sup>2</sup>). These magnetic resonance imaging features satisfy one major criterion for arrhythmogenic right ventricular dysplasia. RA: Right atrium; LA: Left atrium; RV: Right ventricular; LV: Left ventricular.



**Figure 9 Takotsubo cardiomyopathy.** Four-chamber cine steady state free precession image shows classical appearances of Takotsubo cardiomyopathy, with hyperkinesis of the basal segments (arrows) and akinesis of the apical segments (red arrows), which resembles a Japanese octopus pot. RA: Right atrium; LA: Left atrium; RV: Right ventricular; LV: Left ventricular.

be somewhat limited as compared to that within the left ventricle<sup>[73]</sup>, LGE has shown to be useful in risk stratification in ARVD patients. In patients meeting diagnostic criteria for ARVD, up to 88% of patients demonstrated areas of LGE at CMR<sup>[74]</sup>. The presence of LGE has also shown to play a role in ARVD risk assessment with right ventricular LGE predicting the induction of ventricular tachycardia at electrophysiological testing<sup>[75]</sup>.

Despite the emphasis placed on right ventricular findings, LV changes are also frequently seen in the setting of ARVD with CMR allowing assessment of LV changes not seen at other modalities<sup>[76]</sup>. Additionally, LV changes may also be more pronounced than those seen within the right ventricle ("left-dominant" disease). LV involvement at CMR was associated with a higher prevalence of ventricular arrhythmias, even in the setting of normal right ventricular size and function<sup>[76,77]</sup>.

Lastly, CMR is an emerging as a tool in guiding ablation therapies in ARVD patients. For example, in a recent study by Wijnmaalen *et al.*<sup>[78]</sup> CMR has been proposed as a useful adjunct in combination with voltage mapping in guidance of techniques in providing a potential roadmap for myocardial ablation. Specifically, CMR was shown to identify areas of non-transmural scar and infarct grey zones not detected by traditional voltage mapping.

### **Stress-induced (Takotsubo) cardiomyopathy**

Stress-induced cardiomyopathy is classically seen on MRI as decreased global systolic function and abnormal wall motion of the apical segments with normal/hyperkinetic basal segments (Figure 9). There may be myocardial edema, but LGE is not typically seen. Variants include a reverse Takotsubo cardiomyopathy, with akinesis of the basal segments and hyperkinesis of the apical segments. These functional abnormalities are transient and recover with treatment of cardiac failure.

Takotsubo variants can readily be distinguished at CMR<sup>[79]</sup>. Accurate characterization of the particular

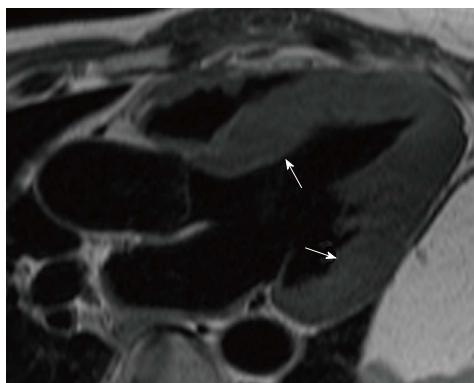
segmental involvement is important as certain variants, namely typical and mid-ventricular types, have been associated worse worsened LV function<sup>[80]</sup>. Furthermore, CMR can readily detect associated valvular complications such as mitral regurgitation, which can complicate certain takotsubo subtypes<sup>[79]</sup>. Additionally, CMR can more easily detect right ventricular involvement, which can be seen in approximately one-third of cases<sup>[79]</sup>. Detection of right ventricular involvement, if present, has been associated with longer hospitalization and worse LV function<sup>[81]</sup>.

While not a prominent feature in Takotsubo cardiomyopathy, LGE can be present to varying degrees, as shown in several small studies<sup>[82-85]</sup>. However, its implications for adverse events and recovery are mixed. For example, a pair of studies<sup>[82,83]</sup> showed that the presence of LGE on CMR performed in the acute or subacute phase (*i.e.*, within one week of presentation) was associated with increased risk of cardiogenic shock, longer duration for ECG normalization, and longer duration of wall motion abnormality recovery. Conversely, multiple studies<sup>[84,85]</sup> have shown no association with worsened LVEF or development of adverse outcomes as compared to patients without LGE.

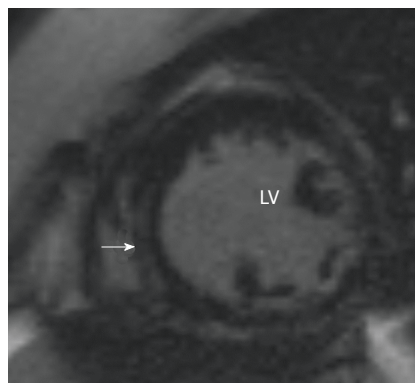
### **Fabry disease**

Fabry disease is seen on MRI as concentric LV thickening (Figure 10), which is not infrequently confused with HCM. There may be mid myocardial or subepicardial pattern of LGE, typically in the basal inferolateral segment<sup>[86]</sup>.

The presence of LGE in Fabry's patients has shown to be associated with development of ventricular arrhythmias as well as sudden cardiac death<sup>[87]</sup>. However, a patient's annual increase in fibrosis as determined of LGE findings, rather than presence or absence of LGE, was the only independent predictor ventricular arrhythmias. Additionally, CMR findings of fibrosis were found to poorly correlate with blood serum markers of fibrosis<sup>[87]</sup>. T1 mapping techniques have also been applied to the characterization of Fabry's cardiomyopathy. Prior to the onset of LV hypertrophy, reduction in T1 values was associated with reduced longitudinal strain as well as early



**Figure 10 Fabry disease.** Three-chamber black blood image shows moderate to severe concentric hypertrophy in a patient with Fabry's disease.



**Figure 11 Duchenne muscular dystrophy.** Short-axis delayed enhancement image shows dilated left ventricle, with mid myocardial septal enhancement (arrow) in a patient with Duchenne muscular dystrophy. LV: Left ventricular.

diastolic dysfunction, suggesting that T1 mapping may be useful in detecting early systolic and diastolic dysfunction before onset of cardiac structural abnormalities<sup>[88]</sup>.

CMR has also been used in monitoring cardiac treatment response to enzyme replacement therapies<sup>[87,89-91]</sup>. While no significant changes in LVEF were seen at follow-up, reductions in LV mass at CMR with corresponding improvement in symptoms were noted<sup>[89-91]</sup>. Furthermore, a study by Krämer *et al.*<sup>[87]</sup> showed that of a limited number of patients who underwent enzyme replacement therapy, LGE actually progressed despite therapy suggesting that patients undergoing treatment are still prone to developing worsening fibrosis. However, no clinical outcomes at follow-up were noted for these patients.

### Muscular dystrophy

On MRI, muscular dystrophy may present with ventricular dilation, systolic dysfunction and mid myocardial/sub-epicardial pattern of LGE (Figure 11).

The significance of the presence of LGE with arrhythmic events has shown mixed results. While a pair of studies<sup>[92,93]</sup> have demonstrated significant association between LGE and the development of arrhythmias, Tandon *et al.*<sup>[94]</sup> demonstrated no significant increased risk in arrhythmia seen in patients with at least one LGE-positive segments. Additionally, in the same study, greater number of LGE positive cardiac segments was predictive of decreases in LVEF, while decreases in LVEF were not seen at follow-up in patients without LGE. T1 mapping and ECV estimation have also been evaluated in muscular dystrophy. Calculated global ECV have been shown to correlate to LVEF and to the number of LGE-positive segments with global ECV significantly associated with occurrence of arrhythmic events<sup>[93]</sup>. Lastly, myocardial strain analysis has also been applied in this patient population with several studies<sup>[95,96]</sup> demonstrating that changes in myocardial strain precede changes in LVEF. However, data regarding association with clinical outcomes is lacking.

Limited data is available regarding CMR changes in response to steroid therapy. In a single study<sup>[94]</sup>, longer steroid treatment durations were associated with lower

age-related increases in LGE-positive segments, although its impact on clinical outcome is unknown.

### Limitation of CMR

Although CMR has been shown to be a powerful tool in diagnosis and clinical assessment and offers a number of distinct advantages over other modalities, certain limitations and challenges are still present. Specific areas in which data is still lacking or contradictory for particular clinical outcomes was discussed in greater detail in the preceding sections. As a whole, although data on the utility of CMR has grown substantially, formal recommendations regarding the specific use of CMR in various clinical settings is lacking for most non-ischemic cardiomyopathies, which may limit its utilization. Furthermore, various technical and logistical aspects of CMR may also limit its usefulness. General contraindications to MRI such as the presence of metallic devices, particularly pacemakers and implantable defibrillators, may limit the usefulness in some cardiac patients. Furthermore, due to the risk of nephrogenic systemic fibrosis, the use gadolinium-based contrast agents, and therefore the assessment of LGE, is limited in patients with renal disease. Lastly, other factors such as the lack of widespread availability and intensive post-processing may further limit the use of CMR in some settings.

## CONCLUSION

MRI is a valuable tool in the evaluation of non-ischemic cardiomyopathies, not only in the diagnosis, but also in risk stratification and prognostic determination. The results of several large scale studies show that there is a good correlation between MRI findings and clinical outcomes, which demonstrate the impact of cardiac MRI on the management of these patients.

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## Mechanical dyssynchrony and deformation imaging in patients with functional mitral regurgitation

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### Abstract

Chronic functional mitral regurgitation (FMR) is a frequent finding of ischemic heart disease and dilated

cardiomyopathy (DCM), associated with unfavourable prognosis. Several pathophysiologic mechanisms are involved in FMR, such as annular dilatation and dysfunction, left ventricle (LV) remodeling, dysfunction and dyssynchrony, papillary muscles displacement and dyssynchrony. The best therapeutic choice for FMR is still debated. When optimal medical treatment has already been set, a further option for cardiac resynchronization therapy (CRT) and/or surgical correction should be considered. CRT is able to contrast most of the pathophysiologic determinants of FMR by minimizing LV dyssynchrony through different mechanisms: Increasing closing forces, reducing tethering forces, reshaping annular geometry and function, correcting diastolic MR. Deformation imaging in terms of two-dimensional speckle tracking has been validated for LV dyssynchrony assessment. Radial speckle tracking and three-dimensional strain analysis appear to be the best methods to quantify intraventricular delay and to predict CRT-responders. Speckle-tracking echocardiography in patients with mitral valve regurgitation has been usually proposed for the assessment of LV and left atrial function. However it has also revealed a fundamental role of intraventricular dyssynchrony in determining FMR especially in DCM, rather than in ischemic cardiomyopathy in which MR severity seems to be more related to mitral valve deformation indexes. Furthermore speckle tracking allows the assessment of papillary muscle dyssynchrony. Therefore this technique can help to identify optimal candidates to CRT that will probably demonstrate a reduction in FMR degree and thus will experience a better outcome.

**Key words:** Mitral regurgitation; Deformation imaging; 3D echocardiography; Mechanical dyssynchrony; Speckle tracking

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**Core tip:** The epidemiologic and prognostic impact

of chronic functional mitral regurgitation (FMR) is fully acknowledged. Multiple factors are involved in the pathophysiology of FMR, such as mitral valve remodeling, left ventricle (LV) remodeling and mechanical dyssynchrony. Deformation imaging by 2 dimensional speckle tracking and 3 dimensional echocardiography are the echocardiographic techniques currently used to better characterize LV dyssynchrony. Pharmacologic and cardiac resynchronization therapy is the first line-therapeutic approach to treat FMR. In case of failure of this first therapeutic approach, surgery and percutaneous treatment in high risk patients represent an alternative option.

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## INTRODUCTION

Chronic functional mitral regurgitation (FMR) is a frequent complication of ischemic heart disease or less frequently dilated cardiomyopathy (DCM), following left ventricular (LV) dysfunction and remodeling. Various degrees of severity of FMR are commonly described in patients with LV dysfunction despite a structurally normal valve. Indeed, according to Carpentier's functional classification, FMR can be due to dilated mitral annulus (type I) or more often to a systolic restriction of leaflet motion (type III b).

The exact occurrence of FMR is difficult to assess because of different diagnostic approaches and timing of evaluation. The prevalence of FMR is nevertheless considerable, varying from 20% to 50% after myocardial infarction (MI) as assessed by echocardiographic studies<sup>[1]</sup>. This has been further confirmed by recent studies assessing long-term outcome of patients affected by heart failure associated with FMR treated with standard medical therapy<sup>[2]</sup>.

Both ischemic and non-ischemic FMR are related to an unfavourable outcome in DCM<sup>[3,4]</sup>, independently of the degree of ventricular dysfunction. Additionally the degree of FMR relates directly to the mortality and heart failure events. Actually, FMR is related to a decreased survival rate even if of mild degree, as MR severity positively correlates to increased mortality. An effective regurgitant orifice area > 20 mm<sup>2</sup> has been shown to double all-cause mortality and the risk of admission for acute decompensated heart failure. Furthermore, the presence of even moderate MR increased the risk of heart failure and death by more than 3-fold and 2-fold at 5 years respectively<sup>[5]</sup>.

## PATHOPHYSIOLOGY

Several pathophysiologic mechanisms are involved

in determining FMR. Kaul *et al*<sup>[6]</sup> speculated that MR resulted from global LV dysfunction, rejecting the role of dysfunction of papillary muscles and the adjacent LV myocardium in determining FMR. Further studies failed to demonstrate that LV systolic dysfunction in the absence of LV dilatation and remodeling produced significant MR, whereas leaflets tethering was the only independent predictor of MR and LV sphericity was correlated to MR grade. Certainly an imbalance between closing and tethering forces is responsible for FMR due to LV dilatation and reduction of contractility, global LV dyssynchrony, papillary muscles displacement and dyssynchrony, altered systolic mitral annular contraction<sup>[7]</sup>.

Tethering is the principal determinant of FMR, because of LV remodeling associated to apical and posterior papillary muscle displacement, that lead to a reduction in closing forces.

Depending on the type of global or local LV remodeling, two tethering patterns have been described<sup>[6]</sup>: The asymmetric and symmetric ones, depending on mitral leaflets position and their point of coaptation<sup>[8]</sup> (Figure 1).

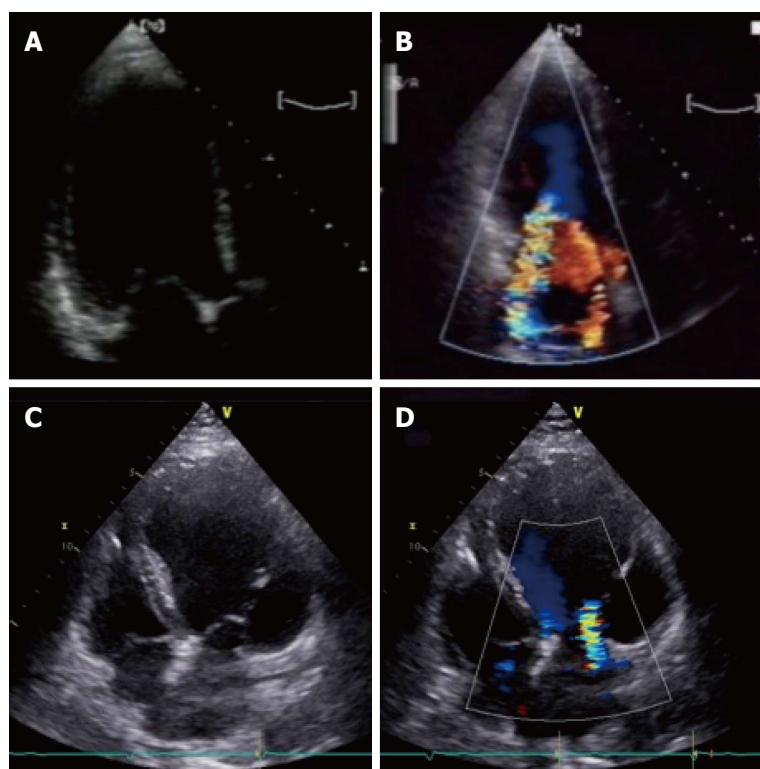
The asymmetric pattern is caused by an asymmetric shift of the posterior papillary muscle, determining a greater tenting of the posterior leaflet compared to the anterior one. Papillary muscle tethers the body of the anterior leaflet generating a "hockey stick" or "bent knee" configuration (Figure 1A and B). MV coaptation point is moved posteriorly and the anterior leaflet coapts creating a "pseudo-prolapse" appearance. The associated MR jet is typically eccentric, directed posteriorly in the left atrium (LA). Conversely in the symmetric pattern MV leaflet coaptation point is displaced towards the apex and both leaflets are tethered, generating a typically central MR jet (Figure 1C and D). This usually occurs in the context of a large anterior myocardial infarction, multiple infarcted area or idiopathic DCM.

Chronic FMR cause progressive LV dilation and papillary muscles displacement, leading to a further increase of tethering forces acting on mitral leaflets and, therefore, to a worsening of MR in vicious cycle.

Also conduction abnormalities, caused either by right ventricular pacing or bundle branch block, predispose to FMR. In fact the presence of intraventricular conduction determines mechanical dyssynchrony and mitral valve deformation<sup>[9]</sup>.

Cardiac mechanical dyssynchrony can be distinguished in atrioventricular, inter- and intraventricular. Prolongation of the atrioventricular conduction time delays systolic ventricular contraction, hampering early diastolic filling when atrial suddenly decrease. Accordingly LV diastolic pressure exceeds atrial pressure causing diastolic mitral regurgitation. The reduction in LV preload determines a decrease in its contractility, according to Starling law.

As for inter- and intraventricular dyssynchrony, the former refers to delayed activation of LV relative to the right one, whereas the latter indicates differences in the timing of contraction of distinct myocardial segments. Both types of conduction delays cause an asynchronous



**Figure 1 Asymmetric and symmetric tethering pattern.** A, B:: Asymmetric tethering pattern. Typical “hockey stick” or “bent knee” configuration. MV coaptation point is moved posteriorly and the anterior leaflet coapts creating a “pseudo-prolapse” appearance with a large regurgitant jet oriented along the posterior wall of the left atrium; C: Symmetric tethering pattern. Both leaflets are apically dislocated and coapt at the same level into the ventricle; D: Color-Doppler shows large central jet.

contraction of LV wall (ventricular dyssynchrony), reducing stroke volume.

Mauer *et al*<sup>[10]</sup> first proved that significant differences in MR existed depending on the site of cardiac pacing. In particular they demonstrated in dogs that artificial stimulation through a right ventricular apical pacemaker generated a severe MR compared to a basal LV pacing within the coronary sinus.

Mechanical dyssynchrony may contribute to FMR as follows. First a decrease in MV closing forces can be determined by LV global dyssynchrony that may decrease the efficacy of LV systolic contraction<sup>[11,12]</sup>. Secondly, a geometric distortion of mitral valve apparatus may be induced by dyssynchronous contraction of the papillary muscle insertion sites<sup>[13]</sup>. Third, impaired leaflet coaptation can be enhanced by dyssynchronous contraction of LV basal segments, that may cause a papillary muscles asynchronous contraction<sup>[14]</sup> (Figure 2). The prolonged QRS duration correlates with both FMR severity and duration in patients with DCM<sup>[15,16]</sup>. Supporting this, several studies have shown that one of the positive effects of cardiac resynchronization therapy (CRT) is a decrease in FMR grade<sup>[17-20]</sup>. Soyama *et al*<sup>[21]</sup> analysed 32 patients affected by DCM with Tissue Doppler echocardiography showing that a dyssynchronous activation of myocardial segments adjacent to the papillary muscles could cause MR determining a non-synchronized closure of mitral leaflets. Donal *et al*<sup>[22]</sup> reported that MR in patients with DCM is a multifactorial and complex phenomenon, thus its accurate description should take into account LV contraction abnormalities and dyssynchrony, LV geometry and mitral orifice.

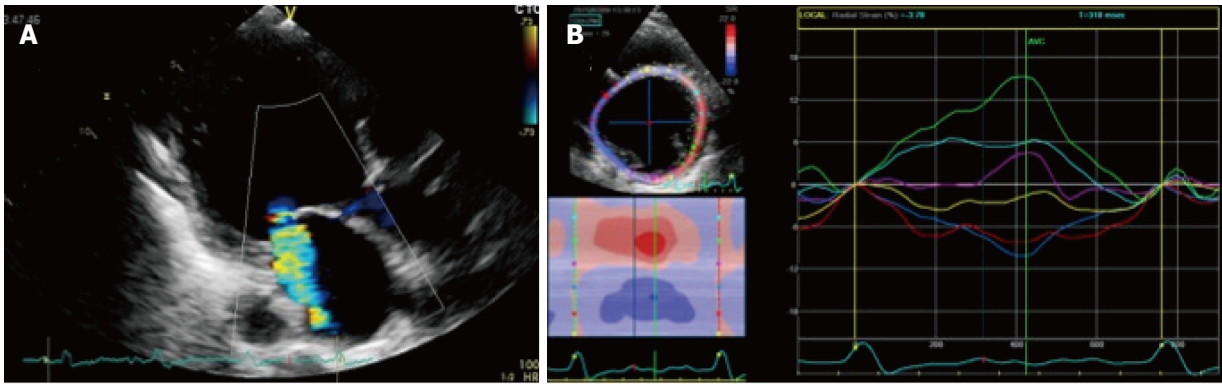
Considering LV reverse remodeling after CRT, certainly

the changes in MV apparatus influence the improvement of FMR. Konstantinou *et al*<sup>[23]</sup> studied FMR secondary to ischemic ( $n = 55$ ) and non-ischemic DCM ( $n = 48$ ) and found that FMR severity is mainly determined by the degree of mitral apparatus distortion; furthermore the authors found that in these patients, a quick estimation of FMR severity could be obtained observing coaptation height. Finally, additional determinants of FMR included the presence of global LV dyssynchrony and reduced myocardial systolic velocities of the posteromedial papillary muscle insertion site.

FMR is a dynamic condition, changing dramatically with loading conditions, because of phasic fluctuations in the balance between tethering and closing forces. The increase in afterload (*i.e.*, hypertension, exercise) worsens MR, further deforming the infarcted papillary muscles bearing segments because they promptly deforms in response to increased intraventricular pressure. On the contrary diuretic therapy, afterload decrease by vasodilators or general anaesthesia reduce FMR severity. FMR is therefore a dynamic lesion, varying through the cardiac cycle, as the regurgitant volume is greater in the early and late systolic phases, lower in the mid systole, having a beat to beat variation<sup>[24]</sup>. In addition, Ennezat *et al*<sup>[25]</sup> showed that rest LV dyssynchrony is associated with worsening of FMR during exertion. In this study, 20% of patients with significant LV dyssynchrony developed an exercise-induced EROA increase, whereas the rest did not have a decrease in mitral EROA during exercise<sup>[25]</sup>.

## DEFORMATION IMAGING

Myocardial deformation imaging is a novel echocardiographic



**Figure 2** Impaired leaflet coaptation can be enhanced by dyssynchronous contraction of left ventricle basal segments, that may cause a papillary muscles asynchronous contraction. A: 2D radial strain of a papillary muscles short axis in a patient with functional MR; B: During systole, myocardial segments adjacent to postero-medial papillary muscles (red and blue segments) show negative radial strain values, whereas antero-lateral papillary muscles (light blue and green segments) show a positive strain values, resulting in a significant papillary dyssynchrony. 2D: 2 dimensional; MR: Magnetic resonance.

tool that can be used to evaluate global and regional myocardial function.

The evaluation of contractile function with echocardiography has traditionally been limited to volume-based assessment of global systolic function with ejection fraction (EF) and of segmental wall motion or visual estimation of regional thickening. These methods have suffered from lack of reproducibility and standardization and are generally considered to be extremely sensitive to loading conditions. These limitations have led to an interest in techniques that provide more objective and reproducible measures of contractile function.

During systolic phase, ventricular myocardium shortens in the longitudinal and circumferential planes, while getting thicker in the radial plane. Deformation imaging allows for a more direct evaluation of myocardial changes through the cardiac cycle by speckle tracking analysis.

Myocardial deformation imaging with echocardiography can be performed with the use of either tissue Doppler-based or 2-dimensional (2D) speckle tracking-based methods. Doppler methods suffer from limitations similar to those of traditional Doppler because it can only accurately assess deformation in the plane incident with the ultrasound beam and requires prospective acquisition of dedicated images at high frame rate.

Speckle tracking analysis is obtained assessing the spatial dislocation (tracking) of speckles (spots created by the interplay between ultrasounds and myocardial fibers) on bidimensional echo. This tool offers the advantage of an objective quantitative assessment of regional and global myocardial function, not affected by insonation angle, cardiac translational movements<sup>[26-28]</sup>, with a good interobserver and intraobserver reproducibility, because of its semi-automated feature<sup>[29]</sup>. Furthermore, although initially proposed only for the LV functional assessment, many authors have showed its utility for the evaluation of other cardiac structures, in particular of the LA. A recent comparison between speckle tracking derived measures and jagged MRI showed feasibility and reproducibility of this echocardiographic tool<sup>[30]</sup>.

Speckle tracking analysis evaluates strain, that

can be described as the systolic change in length of a myocardial segment relatively to its length at rest, so expressed as a percentage. Strain rate is also calculated as the rate of this deformation<sup>[31]</sup>.

This technique has some limitations, based on the need of a good definition of the endocardial borders, therefore being highly dependent on the quality of 2D images and frame rate.

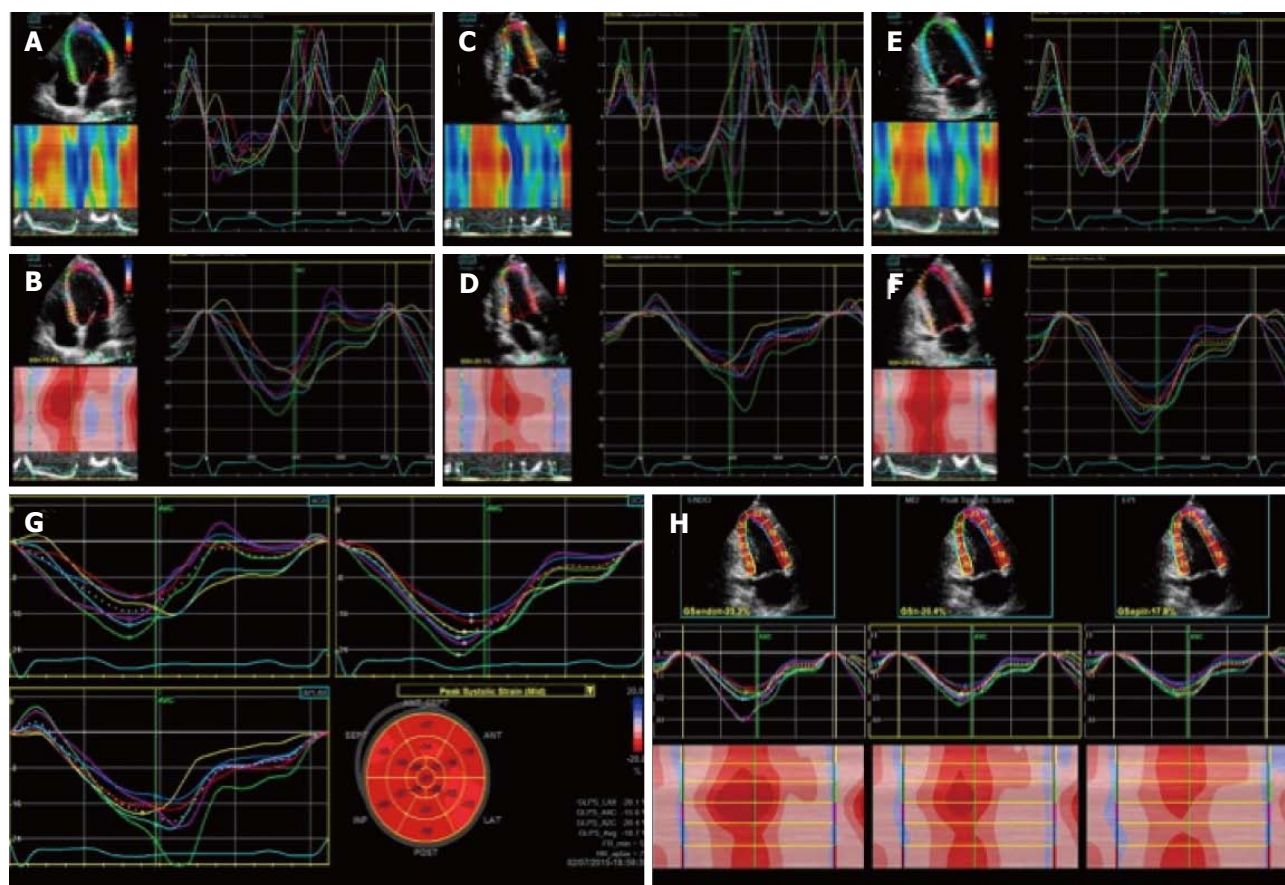
Myocardial strain can be assessed in four principal planes of deformation: Radial strain (myocardial thickening) and circumferential strain (myocardial shortening) from short-axis views; transverse (myocardial thickening) and longitudinal strains (myocardial shortening) assessed from apical views. Furthermore, speckle tracking analysis allows a complete characterization of LV rotation (occurrence, direction and velocity) during cardiac cycle<sup>[32]</sup>.

Longitudinal strain describes the systolic myocardial fibers shortening from the base to the apex. This deformation is expressed in negative trend curves, obtained analysing the myocardial shortening in apical 4-chamber, 2-chamber and long axis view (Figure 3). Both regional, so for each of the 17 LV myocardial segments, and global values are computed. Global longitudinal strain value has been shown to be a quantitative index of LV systolic performance<sup>[33]</sup>. Longitudinal strain can also be applied to LA<sup>[34]</sup> and right ventricle (RV) analysis strain<sup>[35]</sup>, respectively assessing the peak atrial longitudinal strain and RV longitudinal strain values.

Radial strain describes myocardial deformation directed radially towards the centre of LV cavity, represented by systolic thickening and diastolic thinning (Figure 4). Therefore, during the systolic phase radial strain curve will have positive values. Radial strain can be obtained through the analysis of parasternal short axis view, both from the basal and the apical cut<sup>[36]</sup>.

Circumferential strain is also obtained from speckle tracking analysis of the parasternal short axis view<sup>[37]</sup>. It represents the LV myocardial systolic shortening along its circumference and is expressed by systolic negative curves (Figure 5). A global circumferential strain value can also be calculated.





**Figure 3 Two dimensional longitudinal strain.** 2D longitudinal strain rate (A, C, E) and strain (B, D, F) analysis. Longitudinal strain is obtained from the 3 LV apical views (4C view, 2C view and 3C view). Strain values are then displayed in a bulls eye reconstruction (G). Longitudinal strain values for endocardium, mesocardium and epicardium can also be obtained (H). LV: Left ventricle; 2D: 2 dimensional.

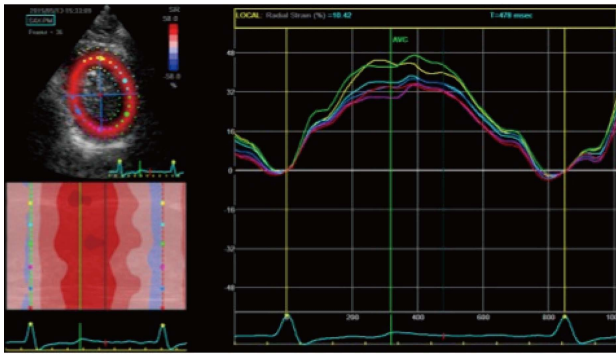
Finally LV twisting<sup>[37]</sup>, a fundamental component of LV systolic contraction, can be studied with speckle tracking analysis in terms of systolic reciprocal rotation of LV base and apex. LV twisting is computed as the difference between the mean rotation of the basal and the apical levels respectively, that can be normalized for the apex-to-base distance, obtaining a "LV torsion" value<sup>[38]</sup>.

Speckle tracking allows an early identification of global and segmental myocardial dysfunction, analysing the percentage of myocardial deformation that reflects the changes occurring in myocardial ultrastructure. Therefore lots of potential clinical application of this technique can be proposed, including the possibility to detect LV subclinical systolic impairment, if an alteration of longitudinal strain is discovered, for example in the setting of diabetes, coronary artery disease or valvulopathies. Several authors contributed to confirm this clinical application. Choi *et al.*<sup>[39]</sup> studied asymptomatic patients without wall abnormalities and found that the presence of lower longitudinal strain values was a strong predictor of stable ischemic disease. Recently Voight *et al.*<sup>[40]</sup> identified post systolic motion, after aortic valve closure, as a significant quantitative marker of the ischaemic myocardium. Further, it has been showed that with New York Heart Association (NYHA) functional class

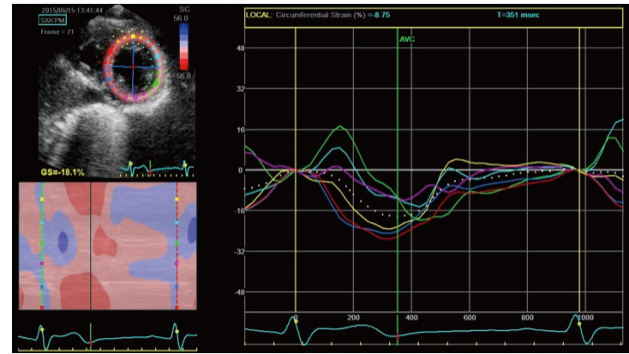
worsening from I to IV, progressively lower longitudinal strain values are observed in HF patients; in addition, in NYHA class III and IV, a systolic impairment of LV circumferential and radial strain become evident<sup>[41,42]</sup>. Stanton *et al.*<sup>[43]</sup> studied HF patients with low EF and found global circumferential strain to be a strong predictor of cardiovascular adverse events. Furthermore, global longitudinal strain was also found to be a stronger predictor of outcome than EF in Mele *et al.*<sup>[44]</sup> study. In low EF patients with indication to CRT, strain parameters have recently been shown to identify CRT responders with good reproducibility and accuracy<sup>[45]</sup>. In particular, dyssynchrony analysis by radial strain has been shown to effectively predict CRT efficacy<sup>[46,47]</sup>.

Three-dimensional speckle tracking echocardiography (3D-STE) is the newest tool among deformation imaging and dyssynchrony analysis<sup>[48,49]</sup>. Differently from 2D speckle tracking, that analyses only a single plane and may oversimplify the complexities of LV mechanics, 3D speckle tracking takes advantage of pyramidal and strain data that include the whole LV, acquired with a matrix arrays transducer, therefore tracking speckles moving through a 3D space (Figure 6).

Acquisition of a full-volume dataset requires smaller wedge-shaped sub-volumes from at least four consecutive heart beats (asking the patient to hold the breath),



**Figure 4** Radial strain analysis with 2 dimensional speckle tracking. Synchronous strain pattern in a normal patient. During systole, radial strain values are represented by positive curves.



**Figure 5** Two dimensional circumferential strain in a normal patient. It represents left ventricle myocardial fiber shortening along the circular perimeter on a short-axis view and during systole, it is represented by synchronous negative curves.

automatically combined in a single larger pyramidal volume. 3D datasets are displayed in different cross-sections including standard three short-axis views and apical four- and two-chamber views that could be modified interactively. Regions of interest are placed on the endocardium and epicardium from apical views, and the software automatically divides data into 16 standard segments to generate corresponding time-strain curves<sup>[50]</sup>. 3D-STE offers the advantage of simultaneously calculating radial, circumferential and longitudinal strain values in the whole LV myocardium. Furthermore, 3D-STE could help in selection of patients who may be CRT responders as it offers an accurate mechanical dyssynchrony map, and potentially it could guide the electrophysiologists during CRT implantation, precisely localizing the site of latest myocardial activation.

## ROLE OF DEFORMATION IMAGING IN MITRAL VALVE DISEASE

STE in patients with mitral valve disease has been usually performed for LV and LA functional assessment. Asymptomatic patients with severe organic MR might develop latent LV systolic impairment even if EF appears to be normal. In asymptomatic patients affected by severe MR, preoperative evidence of LV dysfunction is associated with post-operative lower long term survival and worsening of systolic function. In fact, these patients usually have lower post-operative EF, higher incidence of heart failure and mortality, as compared to patients with severe MR without LV impairment before surgery<sup>[51]</sup>. Agricola *et al.*<sup>[52]</sup> studied patients with MR and normal EF using TDI of the mitral annulus. They found out that longitudinal function can be altered despite normal EF and that systolic TDI value can predict post-operative LV impairment. Thus TDI has been proposed as a simple, available and immediate method to early recognize LV dysfunction due to volume overload in patients with significant MR. More recently, Lancellotti *et al.*<sup>[53]</sup> underlined that limited exercise increase of global longitudinal strain in patients with degenerative MR,

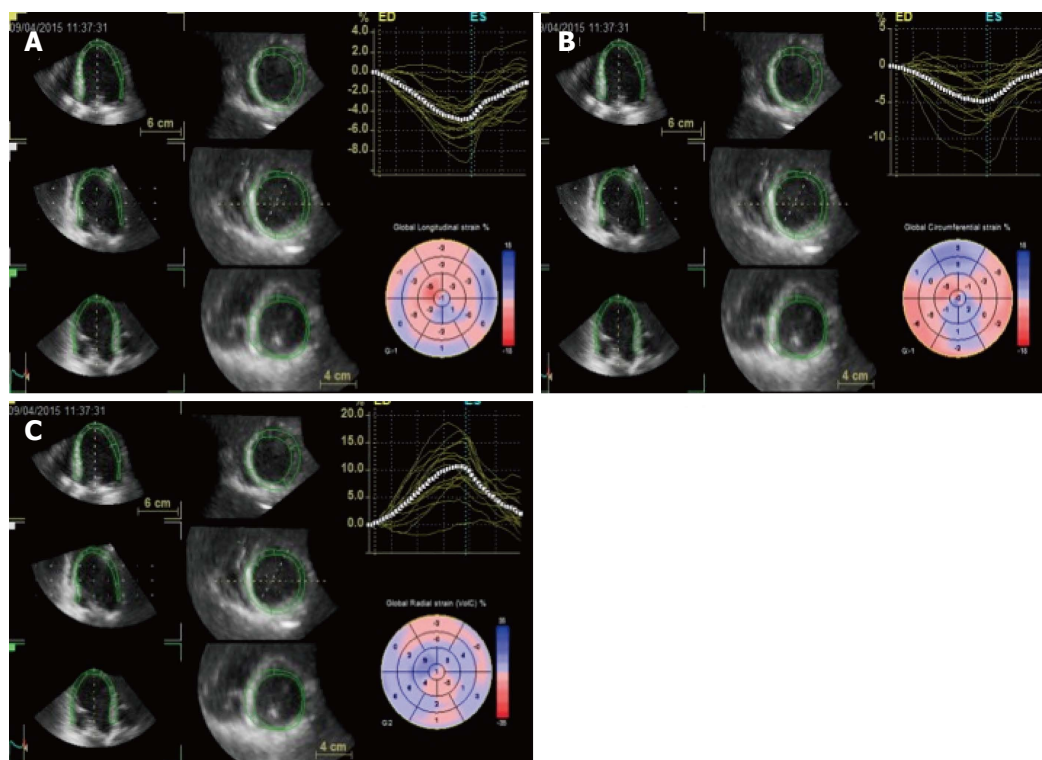
candidates to cardiac surgery, predicted post-operative LV dysfunction development.

Moonen *et al.*<sup>[54]</sup> studied patients with MR and matched healthy controls using longitudinal strain analysis with 2D speckle tracking, both at rest and during exercise. At rest global longitudinal strain was significantly lower in MR patients. During exercise, a lower increase of this value was observed in MR patients compared to control group. In addition, up a small increase of global longitudinal strain at peak exercise was shown to be a predictor LV dysfunction during follow up<sup>[54]</sup>.

MR generally progresses insidiously. Patients can be asymptomatic for a long time and, as the heart compensate to the regurgitant volume with LA enlargement, interpretation of LV EF can be challenging in presence of significant MR. Later, chronic volume overload will progressive LV dysfunction, subsequently worsening outcome.

Left atrial remodeling and dilation is associated with myocardiocyte hypertrophy and interstitial fibrosis, bringing with it the risk of atrial fibrillation (AF)<sup>[55]</sup>. Furthermore, the presence of LA remodeling predicts cardiovascular events, in particular stroke, death and heart failure. Transthoracic echocardiography permits only the evaluation of LA dimensions LA that has prognostic implication<sup>[56]</sup>. However the study of regional LA function may add information about atrial electromechanical remodeling, being useful for prognostic stratification, AF risk and management<sup>[55]</sup>.

LA longitudinal deformation dynamics can be assessed by speckle tracking analysis. Peak atrial longitudinal strain allows the quantification of the reservoir phase of LA, that depends on atrial compliance. In fact, during this phase, LA longitudinal strain increases, reaching a peak at the end of LA filling, just before mitral valve opening (Figure 7). Cameli *et al.*<sup>[57]</sup> demonstrated an inverse correlation between global peak longitudinal strain (PALS) and MR degree, as lower values of PALS were observed in patients with moderate and severe MR, compared to patients with mild MR. In this study, LA myocardial reservoir function impairment was associated with an higher incidence of



**Figure 6** Three dimensional speckle tracking: data sets are displayed in different cross-sections including standard three short-axis views and apical four- and two-chamber views. The software automatically divides data into 16 standard segments to generate corresponding time-strain curves. Wall motion parameters are simultaneously displayed in particular, the 3 orthogonal strain values (longitudinal strain in A, circumferential strain in B and radial strain in C).

paroxysmal AF.

## DEFORMATION IMAGING IN THE EVALUATION OF MECHANICAL DYSSYNCHRONY

Mechanical dyssynchrony can be assessed using different imaging modalities: Conventional M-Mode, Doppler echocardiography, tissue Doppler imaging (TDI) and newer modalities such as strain rate imaging (SRI) and 3D STE.

Echocardiographic evaluation of mechanical dyssynchrony has been of great interest for the identification of potential responders to CRT. Even though the largest body of publications on LV dyssynchrony and CRT response prediction is based on TDI<sup>[58,59]</sup>. However, in the PROSPECT (Predictors of Responders to Cardiac Resynchronization Therapy) trial time to peak time-to-peak dyssynchrony evaluation did not have enough predictive value to replace standard selection criteria for resynchronization therapy<sup>[60]</sup>. Also pulsed-Doppler evaluation of interventricular dyssynchrony may predict the response to CRT, but more solid evidence supports intraventricular dyssynchrony assessment by speckle tracking as a mean to identify CRT responder.

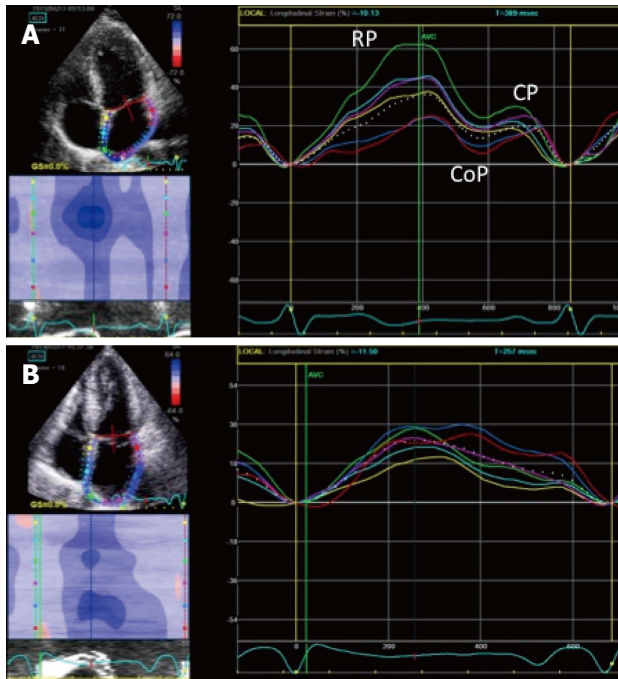
LV dyssynchrony study by speckle tracking was firstly proposed by Suffoletto *et al*<sup>[61]</sup>. In this study radial strain dyssynchrony analysis was performed in a cohort of 50 HF patients with standard indications to CRT.

The authors found the presence of baseline significant radial dyssynchrony to be associated with a significant increase in EF at 5 to 8 mo after CRT. Furthermore a greater increase in EF was observed in patients with lead position concordant to the latest site of activation identified at the radial strain study, compared to patients with discordant lead position.

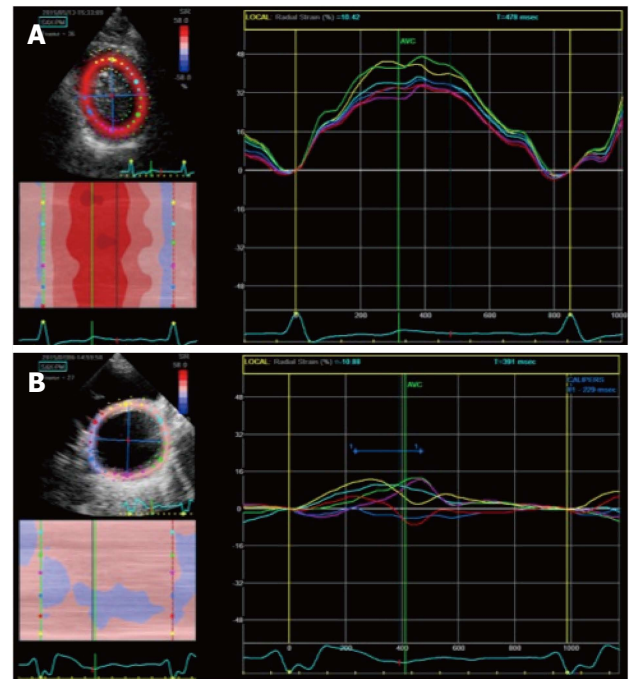
Gorcsan *et al*<sup>[62]</sup> studied 176 HF patients candidates to CRT with both 12-site TDI time to peak dyssynchrony analysis and radial strain. They found that 95% of patients with significant dyssynchrony both at TDI (> 60 msec) and radial strain (> 130 msec) studies showed an EF improvement, while only the 21% of patients without dyssynchrony at both tests had an EF response<sup>[62]</sup>. Based on several trials, actually a value of antero-septal to posterior wall peak delay > 130 msec is considered indicative of significant radial dyssynchrony (Figure 8). Bank *et al*<sup>[63]</sup> in the PROMISE-CRT trial studied HF patients with radial strain analysis and concluded that the presence of radial dyssynchrony predicted reverse remodeling after CRT. However the sample was small and so placebo effect could not be overcome. Gorcsan *et al*<sup>[64]</sup> then studied 197 candidates to CRT with radial strain, considering a delay > 130 msec significant for radial dyssynchrony. They found that patients with significant radial dyssynchrony before CRT had a lower incidence of adverse events [heart transplant, need for a LV assistance device (LVAD), death] at 4-year follow up, compared to patients without baseline dyssynchrony.

Most recently, in the STAR trial by Tanaka *et al*<sup>[65]</sup>,





**Figure 7** Two dimensional longitudinal atrial strain. A: PALS in a normal patient. Triphasic strain pattern is evident: Reservoir phase (RP), conduit (CoP) and contractile phase (CP); B: Reduced PALS in a patient with a large MV flail and severe MR, without triphasic strain pattern. PALS: Peak atrial longitudinal strain; MV: Mitral valve; MR: Mitral regurgitation.



**Figure 8** Radial strain analysis with 2 dimensional speckle tracking. A: Synchronous strain pattern in a normal patients; B: Significant intraventricular dyssynchrony in a patient with dilated cardiomyopathy. A significant delay ( $\geq 130$  msec) between anteroapical peak strain (yellow segment) and posterior peak strain (pink segment) is evident.

baseline dyssynchrony assessed with both radial and transverse strain was found to be a predictor of response to CRT, in terms of EF improvement and better long term survival, as only 11%–13% of these patients died or underwent heart transplant or LVAD implantation. Patients without either radial or transverse dyssynchrony prior to CRT had a worse prognosis, as in 50% of these cases an unfavourable event occurred. On the other hand, in one third of patients responders to CRT, both longitudinal and circumferential strain failed to detect significant dyssynchrony. Thus, the authors concluded that radial and transverse strain are the most reliable methods to assess dyssynchrony and predict response to CRT. Furthermore, radial strain dyssynchrony evaluation permitted the identification of the most delayed site of LV activation. Ypenburg *et al*<sup>[66]</sup> studied 244 patients before CRT with 2D radial strain analysis and found that the latest site of LV activation was most frequently represented by the posterior (36%) and the lateral segments (33%). They also evidenced that if the LV lead position was concordant to the identified latest site of activation, better echocardiographic response and long term outcome could be expected after CRT.

As for longitudinal dyssynchrony, Lim *et al*<sup>[67]</sup> studied 100 HF patients before CRT using longitudinal strain and derived a strain delay index, that resulted to be a marker of dyssynchrony and viability or scar. They reported a strain delay index  $> 25\%$  to be consistently associated with LV reverse remodeling after CRT.

Shi *et al*<sup>[68]</sup> studied 53 HF patients with 2D speckle tracking obtaining standard deviation of time to PALS in

12 LV segments (Tstrain-SD) and standard deviation of time to the end of longitudinal systolic strain rate in six basal LV segments (Tsr-SD). No significant difference was observed in baseline Tsr-SD, and Tstrain-SD between non-responders and responders to CRT. However, the Tsr-SD was significantly higher in responders than non-responders. Ma *et al*<sup>[69]</sup> found that both global and regional longitudinal strain can be predictors of long-term response to CRT in patient with ischemic cardiomyopathy. Further, baseline longitudinal strain values in the site of LV leads were consistently higher in responder patients. Finally, also baseline global longitudinal strain was found to be higher in CRT responder patients than in non-responders, with a global longitudinal strain of  $-13\%$  predicting response to CRT, thus suggesting that patients with better global LV function had less scar tissue and are likely to benefit more significantly from CRT. Becker *et al*<sup>[70]</sup> applied circumferential strain analysis to HF patients before CRT, showing that dyssynchrony assessed by circumferential strain did not differ between CRT responders and non-responders. Of note, this trial was more focused on the effect of LV lead position on CRT response than on the role of dyssynchrony. Delgado *et al*<sup>[71]</sup> compared circumferential, longitudinal and radial strain and found that only dyssynchrony assessed with radial strain was able to predict CRT responders in a study group of 161 patients, while circumferential and longitudinal strain were not. As a consequence of these several trials, we can assume that LV dyssynchrony analysed with radial strain is more informative than longitudinal or circumferential strain analysis and can predict CRT responders. Furthermore,



also magnetic resonance imaging studies confirmed that radial dynamics analysis can be more sensitive in identifying the presence of dyssynchrony, compared to longitudinal myocardial deformation study<sup>[72,73]</sup>. However higher global longitudinal strain and regional longitudinal strain at the site of LV lead positioning can also predict the response to CRT<sup>[74]</sup>. The dyssynchrony strain pattern evidenced by 2D speckle tracking echo is also very important to predict the response to CRT. In patients with heart failure, the presence of intraventricular dyssynchrony is usually evidenced by left bundle branch block (LBBB) at EKG. In presence of a true LBBB or right ventricular pacing, the contraction pattern is characterized by early contraction in early activated walls (septum) and pre-stretch followed by late contraction in late activated walls (lateral wall). The strain-pattern can reflect a complete LBBB in the so called "classical" pattern, that is defined by three components: (1) early activation of at least one basal or midventricular segment in the septal or antero-septal wall and early stretching in at least one basal or midventricular segment in the opposite wall; (2) early peak contraction not exceeding 70% of the ejection phase; and (3) early stretching wall showing peak contraction after aortic valve closure. Patients who do not fulfil all these three criteria are considered having a heterogeneous strain-pattern. Risum *et al.*<sup>[74]</sup> showed that contraction patterns reflective of a consistently delayed LV activation are predictive of response. The presence of a "classic" strain pattern strongly predicted the CRT efficacy, while other patients with wall motion patterns inconsistent with a LV activation delay were less likely to benefit. Importantly, the presence of a classical pattern significantly added to other predictors of response (etiology and QRS > 150 ms) further emphasizing a valuable role for pre-implantation assessment of mechanical dyssynchrony by speckle tracking approach.

Also apical transverse motion (ATM), to quantify "apical rocking", has been introduced as a new and integrative parameter for for LV dyssynchrony assessment and as a promising predictor of CRT efficacy<sup>[75]</sup>. ATM was proposed by Voigt *et al.*<sup>[76]</sup>, who suggested that ATM integrated information about temporal and regional inhomogeneities of LV function and exhibited a significant correlation with the difference between tissue Doppler-derived average strains of the septal and lateral wall. Gürel *et al.*<sup>[77]</sup> showed that ATM is closely associated with radial dyssynchrony assessed by 2D speckle tracking analysis. Of note, a cut-off value of 2.5 mm for ATM loop could clearly differentiate between patients with and without radial dyssynchrony<sup>[77]</sup>.

Previous studies showed that LV rotational mechanics are altered in patients with advanced HF and prolonged QRS<sup>[78]</sup>. In those with significant dyssynchrony, not only torsion is reduced but the basal and apical rotation sometimes follows the same direction of rotation<sup>[79]</sup>. Further, Sade *et al.*<sup>[80]</sup> showed that LV altered rotational mechanics can be restored by resynchronization therapy. These authors then suggested the use of LV rotational parameters (LV torsion and twist)<sup>[80]</sup> for predicting CRT responders.

Potential important technical challenges encountered with 2D STE include interpretation of biphasic or multiple peaks in one segment at strain analysis. Seo *et al.*<sup>[81]</sup> suggested to consider the earliest peak of the segmental strain curve, when more than one peak is evident, as it appears to be the most predictive of response to CRT. Another important limitation of strain analysis has to be faced in presence of akinetic or scar regions, as it could be difficult to get reliable strain curves of these segments. However, LV dyssynchrony is also a 3D phenomenon. Therefore 3D speckle tracking dyssynchrony analysis has been recently introduced and validated. Valuable studies in several studies, 3D-echocardiography has been used to study LV dyssynchrony, assessing volumetric changes in endocardial movement and regional blood displacement<sup>[82,83]</sup>. Tanaka *et al.*<sup>[84]</sup> enrolled 54 candidates to CRT and used a 3D speckle tracking system to assess LV dyssynchrony. Radial dyssynchrony was analysed using a 16-segments scheme, expressed as maximal opposing wall delay in time-to-peak strain and standard deviation of time-to-peak strain. The authors found that both parameters significantly correlated with 2D radial strain antero-septal to posterior delay. Furthermore, 3D speckle tracking offers the advantage of identifying the most delayed myocardial region in 3D, in contrast to 2D speckle tracking and TDI analysis.

In fact radial strain 3D speckle tracking analysis offers a complete 3D mechanical activation map with the color-code 3D cine-loop map that provide an immediate visual assessment of the latest activation site among the 16 segments. In another study, Tanaka *et al.*<sup>[85]</sup> used 3D speckle tracking to study 57 HF patients with prolonged QRS due to either LBBB (group 1) or RV pacing (group 2) with a need to undergo CRT upgrading. They found that the site of earliest mechanical activation was consistently different between the 2 groups (apex 6% in group 1 vs 28% in group 2 respectively), but the most delayed site of activation was similar in the both kind of LV conduction delay. These data supported the use of resynchronization therapy in patients with low EF and a need for pacing.

According to these data, we can conclude that deformation imaging can help to define the presence of intraventricular dyssynchrony better than TDI. Radial speckle tracking and 3D strain analysis appear to be the best method to quantify intraventricular delay and to predict CRT responders.

## DEFORMATION IMAGING IN PATIENTS WITH MECHANICAL DYSSYNCHRONY AND FMR

LV dyssynchrony also plays a role in the pathophysiology of FMR. In fact it has been described that intraventricular mechanical dyssynchrony is an important contributor to functional MR<sup>[86,87]</sup>.

Liang *et al.*<sup>[11]</sup> enrolled patients with EF < 50% and at least mild MR, using TDI to assess global systolic

dyssynchrony (maximal difference in time to peak systolic velocity among the 12 LV segments) and regional dyssynchrony (delay between anterolateral and posteromedial papillary muscles insertion regions). These authors concluded that only global dyssynchrony could be considered a FMR determinant, with an incremental value to valve remodeling parameters (tenting area) that play the main role in FMR pathophysiology.

Soyama *et al.*<sup>[21]</sup> analysed 32 patients with non-ischaemic DCM using TDI derived strain of the papillary muscles; they found FMR to be more frequent in patients with a significant delayed activation of the papillary muscles adjacent LV segments, and concluded that regional dyssynchrony and LV sphericity were independent predictors of FMR.

Agricola *et al.*<sup>[88]</sup> evaluated 74 patients with chronic LV dysfunction (53% ischemic patients) with varying degrees of MR and suggested that systolic tenting was the main determinant of FMR, as a consequence of global and regional LV remodeling. The authors reported that regional dyssynchrony was independently associated with MR severity, with a minor influence, only in patients with DCM and not in those with ischaemic cardiomyopathy. They also found that the QRS duration had no effect on the severity of FMR. Of note, in this study intraventricular dyssynchrony was expressed as the SD of time-to-peak systolic velocity of 8 (not 12) LV segments. Donal *et al.*<sup>[22]</sup>, using regional strain analysis in 87 patients with DCM, demonstrated that the degree of FMR was determined by mitral orifice morphology, LV features, especially longitudinal contractility (strain of LV mid-lateral wall) and dyssynchrony defined as the delay between the septal and lateral mid-portion strain divided by RR squared root. In addition the authors found that MR was not correlated with interventricular mechanical delay. Also Sardari *et al.*<sup>[89]</sup> demonstrated that the severity of MR was not correlated with the QRS duration nor with the echocardiographic interventricular dyssynchrony indices in the patients with ischemic or DCM. Moreover, in this study also intraventricular dyssynchrony was not correlated with MR severity. However in this study only Doppler imaging was applied to evaluate LV synchronicity, as neither strain nor time to peak systolic strain analysis were performed.

As for ischemic cardiomyopathy, inferior wall myocardial infarction is known to be associated with more severe MR degree, while anterior myocardial infarction should theoretically be characterized by a higher dyssynchrony index, due to larger infarct dimensions. Patients with anterior acute myocardial infarction (AMI), but not inferior AMI have worse prognosis, and either a larger dyssynchrony index or increased MR severity determine LV remodeling and outcome. Hung *et al.*<sup>[90]</sup> found that both global and regional dyssynchrony in patients with anterior MI were independently associated with FMR degree. Dyssynchronized myocardial segments were assessed by 3D echo showing an independent impact on FMR grade in a narrow QRS population.

The dyssynchronous contraction of LV papillary mus-

cles is a leading cause of FMR in HF patients, as inferior, posterior and lateral regions are usually identified as the most delayed sites and papillary muscles are regularly located adjacent to lateral and inferior walls. As the majority of the studies regarding papillary muscles dyssynchrony reported different cut off values, the optimal delay for cardiac resynchronization has not been established yet. Thus, there is the need for defined cut off values in order to clearly identify the presence of papillary muscles dyssynchrony before CRT in patients with FMR. Tigen *et al.*<sup>[91]</sup> reported that papillary muscle dyssynchrony with > 60 msec delay (assessed by TDI-derived longitudinal strain) was able to predict a regurgitant volume > 20 mL in DCM patients. Kjorbybach *et al.*<sup>[92]</sup> studied 31 patients with EF lower than 35% of both ischemic and non-ischaemic aetiology and evaluated papillary dyssynchrony by TDI derived time to peak strain. They showed that papillary muscles dyssynchrony was associated with the deformation of mitral apparatus (tenting area), but the haemodynamic consequences of MR (in particular left atrial area) could be better characterized by papillary dyssynchrony only in DCM. Ypenburg *et al.*<sup>[13]</sup> and Golland *et al.*<sup>[93]</sup> both assessed dyssynchrony at the papillary muscles insertion sites using radial strain analysis. They reported that MR improvement after CRT was significantly more frequent in patients with baseline dyssynchrony. In 2010, Tigen *et al.*<sup>[94]</sup> firstly investigated both papillary muscles with 2D speckle tracking from the longitudinal axis in patients with DCM. They found that FMR was significantly correlated with intraventricular dyssynchrony and mitral valve remodeling parameters.

In addition, in this study significant papillary muscles dyssynchrony was found to be the only independent predictor of more than moderate MR. The proposed cut-off value for papillary muscles dyssynchrony (30 ms) predicted a mitral regurgitant volume > 20 mL or EROA > 0.20 cm<sup>2</sup> with high sensitivity and specificity.

STE has shown a fundamental role of intraventricular dyssynchrony in determining FMR especially in DCM, rather than in ischemic cardiomyopathy, in which MR severity seems to be more related to mitral valve deformation indexes. Finally the assessment of papillary muscle dyssynchrony can help to identify optimal candidates to CRT, especially among patients with DCM-associated FMR.

## THERAPEUTIC CONSIDERATIONS

### Medical therapy

The currently accepted optimal pharmacological therapy for HF embraces ACE-inhibitors, diuretics, aldosterone antagonists and beta-blockers<sup>[95]</sup>, and its beneficial effects on HF symptoms in subjects with FMR and LV dysfunction may be remarkable. This combination therapy acts on both neurohormonal activation and the underlying maladaptive pathways, leading to a favourable myocardial remodeling. Several combinations of the above-mentioned drugs are commonly used aiming at reducing the severity of MR and reversing or at least delaying the LV remodeling

progression. Afterload-reducing drugs, *i.e.*, ACE-inhibitors, decrease MR regurgitant volume and increase forward output by reducing the pressure gradient between LV and LA. Vasodilators decrease MR regurgitant volume through a systolic unloading on the EROA. Likewise a reduction in MR might be achieved with preload reduction agents, *i.e.*, diuretics, through LV unloading and accordingly a decrease in leaflet tethering. The administration of ACE-inhibitors and beta-blockers is an independent predictor of better long-term survival in subjects with ischemic MR and LV dysfunction since they reduce the progression of LV remodeling and prevent sudden death. Beta-blocker therapy in HF patients reduces all cause mortality, cardiovascular mortality and mortality due to LV systolic dysfunction and sudden death by roughly 31%-39%<sup>[96]</sup>. In addition, it has been demonstrated that a combined therapy of carvedilol plus ACE-inhibitors decreases FMR by reducing LV dilation<sup>[97]</sup>.

### Indications for intervention

FMR surgery is indicated in patients with severe MR and LVEF > 30% undergoing coronary artery bypass grafting (CABG) (recommendation class I, level of evidence C)<sup>[98]</sup>. It should be considered in patients with moderate MR undergoing CABG (II a, C) and in symptomatic patients with severe MR, LVEF < 30%, option for revascularization and evidence of myocardial viability (II a, C). Furthermore FMR surgery may be considered in patients with severe MR and LVEF > 30% with persisting symptoms despite optimal medical management and with low comorbidity, when revascularization is not indicated (II b, C). In the other patients, optimal medical treatment and extended HF treatment is currently the best option.

Percutaneous mitral valve repair is feasible at low procedural risk in patients with secondary MR and may provide short-term improvement in functional condition and LV function. The percutaneous MitraClip procedure may be considered in patients with symptomatic severe secondary MR despite optimal medical therapy who fulfil the echocardiographic criteria of eligibility, are judged at high surgical risk by a team of cardiologists and cardiac surgeons, and who have a life expectancy greater than one year (II b, C). A recent metanalysis showed that MitraClip represents an efficacious strategy for patients with HF and severe MR, improving functional class and cardiac remodeling<sup>[99]</sup>.

The management of moderate ischaemic MR in patients undergoing CABG is still unclear. In this circumstance, valve repair is preferable. In patients with low EF, mitral valve surgery should be considered if there is evidence of myocardial viability and if comorbidity is low. Exercise echocardiography should be considered in patients capable of exercising, since exercise-induced dyspnoea and a substantial increase in MR severity and systolic pulmonary artery pressure support mitral surgery in addition to myocardial revascularization.

### CRT in patients with FMR

It has been widely demonstrated that CRT decreases

mortality and hospitalization rate, improving cardiac function and structure in symptomatic chronic HF patients managed with optimal medical treatment<sup>[100]</sup>, who present severely depressed LVEF ( $\leq 35\%$ ) and complete LBBB (class I recommendation, level of evidence A). In these patients, CRT is superior either to optimal medical therapy or to ICD alone. Efficacy tends to be lower in patients with NYHA class I and IV and in case of non-LBBB morphology with QRS duration < 150 ms. Therefore, in HF patients without LBBB and QRS  $\geq 150$  ms or LBBB and QRS duration 120-149 ms, CRT is still recommended but considered class II a or II b indication<sup>[101]</sup>.

However the improvement in HF symptoms and survival profile after CRT is proportionate to the extent of improvement in LV systolic function. CRT reduces MR severity in patients with chronic HF and FMR. As showed by Upadhyay *et al.*<sup>[102]</sup>, reduction in MR after CRT is considerably related to lesser HF hospitalization and improved survival. In this study baseline MR degree and longer surface QRS to LV lead time were significant predictors of MR change. Furthermore mitral valve was less remodelled in patients with evidence of MR reduction after CRT. Indeed these patients exhibited a lower tenting area and coaptation height than those with stable or worsening MR, suggesting that ventricular geometry improvement could be a mechanism for MR change.

CRT is then responsible for immediate and late reduction in FMR contrasting its pathophysiologic determinants by reducing or virtually eliminating LV dyssynchrony through different mechanisms: (1) increasing "closing forces" (global synchronization); (2) reducing "tethering forces" (local synchronization); (3) reshaping annular geometry and function (local synchronization); and (4) correcting diastolic MR [atrio-ventricular (AV) synchronization].

As for global synchronization, CRT can restore AV and LV synchrony, increasing global LV contraction efficiency and therefore MV coaptation forces. In fact CRT generates a higher pressure-gradient through MV with a consequent rise in trans-mitral closing forces counteracting the tethering forces. Breithardt *et al.*<sup>[17]</sup> studied 24 HF patients with LBBB and FMR after CRT implantation and confirmed that FMR reduction is directly related to the increased closing force (expressed as LV dP/dt max) that aid mitral valve closure. In addition CRT reduces FMR not only by increasing closing forces but also through "local" synchronization<sup>[36]</sup>. It was noticed that in CRT responders with FMR reduction, resynchronization was induced at the level of basal and mid-LV segments. At the multivariate analysis mid-LV segments synchrony was the most significant predictor of FMR reduction, suggesting that a more "local" synchronous contraction involving the segments adjacent to papillary muscles could determine FMR improvement. Kanzaki's *et al.*<sup>[38]</sup> firstly correlated the immediate reduction in MR after CRT with a more synchronized mechanical activation of papillary muscle insertion points. Further, Golland *et al.*<sup>[93]</sup>, using through 2D Speckle Tracking Radial Strain (2D-RS), showed that a significant delay of time-to-peak 2D-RS in the mid-posterior and inferior segments prior to CRT, along

with preserved radial strain in the posterior and inferior segments, were strong predictors of FMR improvement after CRT. Most recently three echocardiographic aspects have been independently associated with FMR change after CRT<sup>[103]</sup>: Antero-septal to posterior wall radial strain dyssynchrony > 200 ms, non-severe LV dilatation (LV end-systolic diameter index < 29 mm/m<sup>2</sup>), absence of scar at papillary muscle insertion sites. In the same study the importance of myocardial viability in predicting FMR response was stressed, because CRT can be effective only when responsive viable segments are present. Sénéchal *et al.*<sup>[104]</sup> evaluated the presence of viability using dobutamine-stress echocardiography before CRT and confirmed that local viability was able to predict acute response to CRT with a proper sensitivity, making local viability an essential precondition for response to CRT. CRT is also thought to improve contraction of the posterior mitral annulus, coordinating the contraction of the segments at the base of the LV. However data are discordant as some studies demonstrate no immediate changes in mitral annular dimensions after CRT and other studies<sup>[14]</sup> show that annular contraction is correlated to FMR reduction after CRT, although as a minor determinant.

Interestingly all these described effects are pacing dependent as the interruption of CRT causes an immediate recurrence of MR.

According to the timing of response to CRT, there is clear distinction between two phases of MR reduction: (1) immediate MR reduction, occurring suddenly after CRT implantation; and (2) long-term MR reduction, occurring from weeks to months after CRT.

MR may show an immediate improvement after CRT, but the underlying mechanism is not completely clear. It is probably more likely to occur when LV dyssynchrony is mainly related to papillary muscles dyssynchrony. Ypenburg *et al.*<sup>[105]</sup> showed that CRT may lead to an acute reduction in MR in LV dyssynchrony involving the posterior papillary muscle, as opposed to a late response when the lateral wall is involved. Long-term reduction is the consequence of LV reverse remodeling. In addition, CRT can be associated with acute decrease in resting MR but not in exercise-induced MR. In fact, after CRT only late reversed LV remodeling, restoring mitral apparatus geometry, is associated with a reduction in both resting and exercise-induced MR<sup>[106]</sup>.

Between the two phases of FMR reduction, immediate MR reduction is the major determinant of favourable response to CRT, as it contributes to the acute reduction of volume overload, determining a rapid reverse remodeling. Therefore, immediate MR reduction is a major prognostic determinant after CRT<sup>[107]</sup>.

### Surgery

The ideal surgical strategy for the management of ischemic MR is still debated. Peri-operative mortality is higher compared to primary MR, and the long-term prognosis is worse mainly due to the more severe associated comorbidities. Moreover, there is a significant persistence and recurrence rate of MR after valve repair,

as McGee demonstrated on a cohort of 585 patients with FMR<sup>[108]</sup>, as well as the absence of prognostic evidences. Severe ischaemic MR is not usually improved by sole revascularization. At the same time the impact of valve surgery on survival remains uncertain because randomized trials are missing and the available observational studies do not draw definite conclusions because of study limitations<sup>[109]</sup>. With regard to prognosis, most studies failed to demonstrate improved long-term clinical outcome following surgical correction of secondary MR<sup>[110,111]</sup>. Fattouch *et al.*<sup>[112]</sup> compared CABG vs CABG plus valve repair in patients with moderate ischemic MR, showing that the addition of MR repair improved functional class, EF, pulmonary artery pressure and LV diameter in the short-term. The study though was not designed to analyse the effect on survival of the addition of valve repair to CABG. When surgery is indicated, valve repair using undersized rigid ring annuloplasty is the first option, offering a low operative risk although it is associated with a significant rate of MR recurrence<sup>[113]</sup>. Preoperative predictors of recurrent secondary MR after undersized annuloplasty, associated with a worse prognosis, are left ventricular end diastolic diameter, posterior mitral leaflet angle, distal anterior mitral leaflet angle, systolic tenting area, coaptation distance, end-systolic inter-papillary muscle distance, and systolic sphericity index<sup>[114]</sup>. A meta-analysis of retrospective studies by Vassileva *et al.*<sup>[115]</sup> suggested better short-term and long-term survival following valve repair compared to its replacement. A recent study on 251 patients with severe ischemic mitral regurgitation randomized to either mitral-valve repair or chordal-sparing replacement revealed no significant difference in LV reverse remodeling or survival at 12 mo; replacement provided a more durable correction of mitral regurgitation, but there was no significant difference in clinical outcomes between-group<sup>[116]</sup>.

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## Micromanaging cardiac regeneration: Targeted delivery of microRNAs for cardiac repair and regeneration

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### Abstract

The loss of cardiomyocytes during injury and disease can result in heart failure and sudden death, while the adult heart has a limited capacity for endogenous regeneration and repair. Current stem cell-based regenerative medicine approaches modestly improve cardiomyocyte survival, but offer neglectable cardiomyogenesis. This has prompted the need for methodological developments that create *de novo* cardiomyocytes. Current insights in cardiac development on the processes and regulatory mechanisms in embryonic cardiomyocyte differentiation provide a basis to therapeutically induce these pathways to generate new cardiomyocytes. Here, we discuss the current knowledge on embryonic cardiomyocyte differentiation and the implementation of this knowledge in state-of-the-art protocols to the direct reprogramming of cardiac fibroblasts into *de novo* cardiomyocytes *in vitro* and *in vivo* with an emphasis on microRNA-mediated reprogramming. Additionally, we discuss current advances on state-of-the-art targeted drug delivery systems that can be employed to deliver these microRNAs to the damaged cardiac tissue. Together, the advances in our understanding of cardiac development, recent advances in microRNA-based therapeutics, and innovative drug delivery systems, highlight exciting opportunities for effective therapies for myocardial infarction and heart failure.

**Key words:** Cardiac repair; Cellular plasticity; Targeted drug delivery; MicroRNA; Reprogramming

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**Core tip:** Cardiac fibroblast reprogramming into cardiomyocytes holds great promise for future cardiac regenerative medicine therapies. Here, we discuss current advances in the state-of-the-art protocols for the direct reprogramming of cardiac fibroblasts into *de novo* cardiomyocytes *in vitro* and *in vivo* with an emphasis on

microRNA-mediated reprogramming. Additionally, we discuss current advances on the state-of-the-art targeted drug delivery systems that can be employed to deliver these microRNAs to the damaged cardiac tissue.

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## INTRODUCTION

Ischemic cardiac disease is characterized by a chronic or acute reduction in myocardial perfusion and affects over 120 million people globally of which approximately 4% suffer from myocardial infarction (MI) annually<sup>[1,2]</sup>. MI is the process of cell death occurring after occlusion of a coronary vessel that supplies blood to a specific area of the heart and results in a massive loss (up to 11 billion cells) of viable muscle cells<sup>[3]</sup>. This loss of cardiac tissue may in turn lead to functional cardiac impairments and, if large enough, severe contractile dysfunction with an inability of the heart to maintain organ perfusion resulting in sudden death.

Although the recognition of MI and the success rates of primary angioplasty have greatly improved in the past decades, treatment of MI is commenced after the cardiac damage response has already started. Cell death, either by apoptosis or necrosis, is the initial response of cardiomyocytes to the decreased oxygen supply and commences already 4 h after MI<sup>[4,5]</sup>. Cardiomyocyte cell death is followed by the influx of inflammatory cells that phagocytize the dead cells, resulting in thinning of the ventricle wall. Cytokines secreted by these inflammatory cells recruit myofibroblasts that secrete collagens and replace the lost cardiomyocytes<sup>[6,7]</sup>. This remodeling process culminates in the formation of a scar tissue that preserves the ventricle integrity, but possesses little contractile function which hampers cardiac function. At this stage, chronic heart failure is likely to develop as the cardiac tissue is unable to regain its normal function<sup>[8,9]</sup>. Current treatment options consist of appropriate diet and lifestyle changes and medicinal in the use of diuretics, ACE inhibitors and AT receptor blockers, in an attempt to alleviate the heart from the warring strains it encounters. However, although these interventions have a pronounced effect on increasing the patients lifespan, they do not treat the underlying pathology, which is the loss of cardiomyocyte mass<sup>[10-12]</sup>.

So, if the morbidity following MI is due to the massive loss of cardiomyocytes, would it not be logical to therapeutically induce cardiomyocyte proliferation to compensate for the lost myocytes?

Although most cardiomyocytes form terminally

differentiated binucleated cells that withdraw from the cell cycle<sup>[13,14]</sup>, limiting the myocardial regenerative capacity, some evidence exists for postnatal cardiomyocyte proliferation. Retrospective birth dating of human cardiomyocytes using carbon-14 in the DNA of cardiomyocytes demonstrated that human cardiomyocytes have a turnover rate of approximately 0.45%-1% per year<sup>[15]</sup>. During normal human wound healing, cell cycle activation occurs which compensates for the loss of tissue<sup>[16,17]</sup>. Indeed, a small number of cardiomyocytes enters the cell division cycle following myocardial infarction<sup>[18]</sup>, however the level of proliferation is insufficient to regenerate the lost tissue.

The observation that the postnatal heart retains some proliferative capacity has inspired therapeutic approaches that aim to enhance the endogenous cardiomyocyte proliferation for regeneration. Indeed, forced expression of cell cycle activators such as Cyclin A2 and D2 promotes the proliferation of postnatal cardiomyocytes and limits damage following MI<sup>[19,20]</sup>. Additionally, regenerative medicine approaches using a wide variety of growth factors (*i.e.*, ERBB2<sup>[21]</sup>, FGF1<sup>[22,23]</sup>, HGF<sup>[24,25]</sup>, IGF1<sup>[25]</sup>, NRG1<sup>[22,26,27]</sup>, MYDGF<sup>[28]</sup>, and POSTN<sup>[29]</sup>, reviewed in<sup>[30,31]</sup>) induce cardiomyocyte proliferation after MI, albeit relatively ineffectively.

The relative ineffectiveness of cardiomitogenic therapies using growth factors in restoring cardiomyocyte numbers following myocardial infarction warrants the need to increase cardiomyocyte numbers from exogenous sources. The effectiveness of adult stem and progenitor cells of various origins (*i.e.*, bone marrow-derived cells [Mesenchymal stem cells (MSC)<sup>[32]</sup> and endothelial progenitor cells (EPC/ECFC)<sup>[33]</sup>], adipose tissue-derived regenerative cells (ADRC)<sup>[34]</sup> and cardiac-derived progenitor cells (CPC)<sup>[35]</sup> to induce cardiac regeneration has been assessed in numerous clinical studies (reviewed in<sup>[36-39]</sup>). In general, intramyocardial transplantation of adult stem and progenitor cells in the post-infarct myocardium induces neoangiogenesis and promotes cardiomyocyte survival<sup>[40]</sup> and thereby reduces the infarct size and improves cardiac function long term<sup>[39]</sup>. Although these effects are beneficial to the survival of the myocardium, retention of therapeutic cells at the site of cardiomyocyte death is highly limited<sup>[41,42]</sup> and their cardiomyogenic effects are neglectable<sup>[43,44]</sup>. Hence, the regenerative effectiveness of transplantation of adult stem and progenitor cells is under debate<sup>[43,45]</sup>.

Thus, MI results in a massive loss of cardiomyocytes that are replaced by scar tissue. Endogenous repair mechanisms, such as cardiomyocyte proliferation, are insufficient to efficiently regenerate the lost myocardial tissue and therapeutic approaches to induce cardiomyocyte proliferation using growth factors are ineffective. Current regenerative medicine therapies using stem and progenitor cells improve cardiomyocyte survival, but pose neglectable cardiomyogenesis. This warrants the development of new therapeutic strategies that focus on increasing the number of viable cardiomyocytes at the infarct site, reviewed below.

## CELLULAR PLASTICITY AS THE NEW THERAPEUTIC OPPORTUNITY

### ***Induced pluripotent stem cells and cardiomyogenesis***

In 2006, Takahashi *et al.*<sup>[46]</sup> challenged the dogma of terminal cell differentiation. Probing the effects of transcription factors that are pivotal to embryonic stem cell maintenance in terminally differentiated skin fibroblasts, four transcription factors (*i.e.*, Oct4, Sox2, Klf4 and c-Myc) were identified that could convert skin fibroblasts into a more primitive pluripotent stem cell resembling embryonic stem cells<sup>[46,47]</sup>. These data exemplify that cell fate is not fixed, but is determined by the available transcription factors and can be altered by the addition of alternative transcription factors. The obtained induced-pluripotent stem cells (iPSC) introduced a new era in regenerative medicine wherein cellular reprogramming is used to treat disease.

iPSC have been used in preclinical models of MI repair<sup>[48-51]</sup>. Transplantation of iPSC directly into the infarcted myocardium improves cardiac function [*e.g.*, left ventricle ejection fraction (LVEF), fractional shortening, and contractility] and reduces infarct size<sup>[48-50]</sup>. Although transplanted iPSC contribute to cardiac repair, a major impediment to their clinical use in human patients lies in the inefficiency of transplanted iPSC to form cardiomyocytes (0.5%-2%)<sup>[49]</sup>, their tumorigenicity<sup>[52]</sup>, and their limited retention in the infarcted tissue. Yet, proof-of-concept that iPSC can differentiate into functional cardiomyocytes has tantalized researchers in studying cardiac embryology as iPSC differentiation into functional cardiomyocytes is merely a reiteration of embryology.

Embryonic cardiogenesis (Figure 1A) begins from the mesoderm that arises from the primitive streak during gastrulation. Gene regulation and cell movement that control cardiogenesis are spatially and temporally stringently regulated (reviewed in<sup>[53]</sup>). Bone morphogenetic protein (BMP)-4, activin A and fibroblast growth factor (FGF)-2 induce mesoderm specification<sup>[54-56]</sup> from pluripotent progenitors in the primitive streak by inducing Wnt3a expression, whereas Notch signaling inhibits the transition from mesodermal precursors into cardiac mesoderm<sup>[57]</sup>. MESP1, the most early expressed marker of the cardiac lineage<sup>[58,59]</sup>, is expressed by all cardiac precursors that arise from the cardiac mesoderm and drives further cardiac specification by the Dkk1-mediated repression of Wnt signaling<sup>[60]</sup>, resulting in the formation of specialized cardiac progenitor cells. This pool of cardiac precursors gives rise to the endocardium, the first heart field (from which the atria, left ventricle and nodal conduction system are formed) and the second heart field (from which the right ventricle and outflow tract are formed)<sup>[61]</sup>. Specification of cardiac precursors into cells of the first and second heart field is regulated by the complex interplay of transcription factors downstream of MESP1<sup>[62,63]</sup>. Herein, GATA4, MEF2c, HAND2 and NKX2.5 represent common transcription factors to all cardiac precursors, whereas the expression of TBX5 is restricted to the first heart field<sup>[64]</sup> and ISL1 and TBX1

are restricted to the second heart field<sup>[65,66]</sup>. Once formed, cardiac cells of the first and second heart field proliferate in response to endocardial-derived Neuregulin (NRG1) and epicardial-derived retinoic acid and FGF2<sup>[67,68]</sup>.

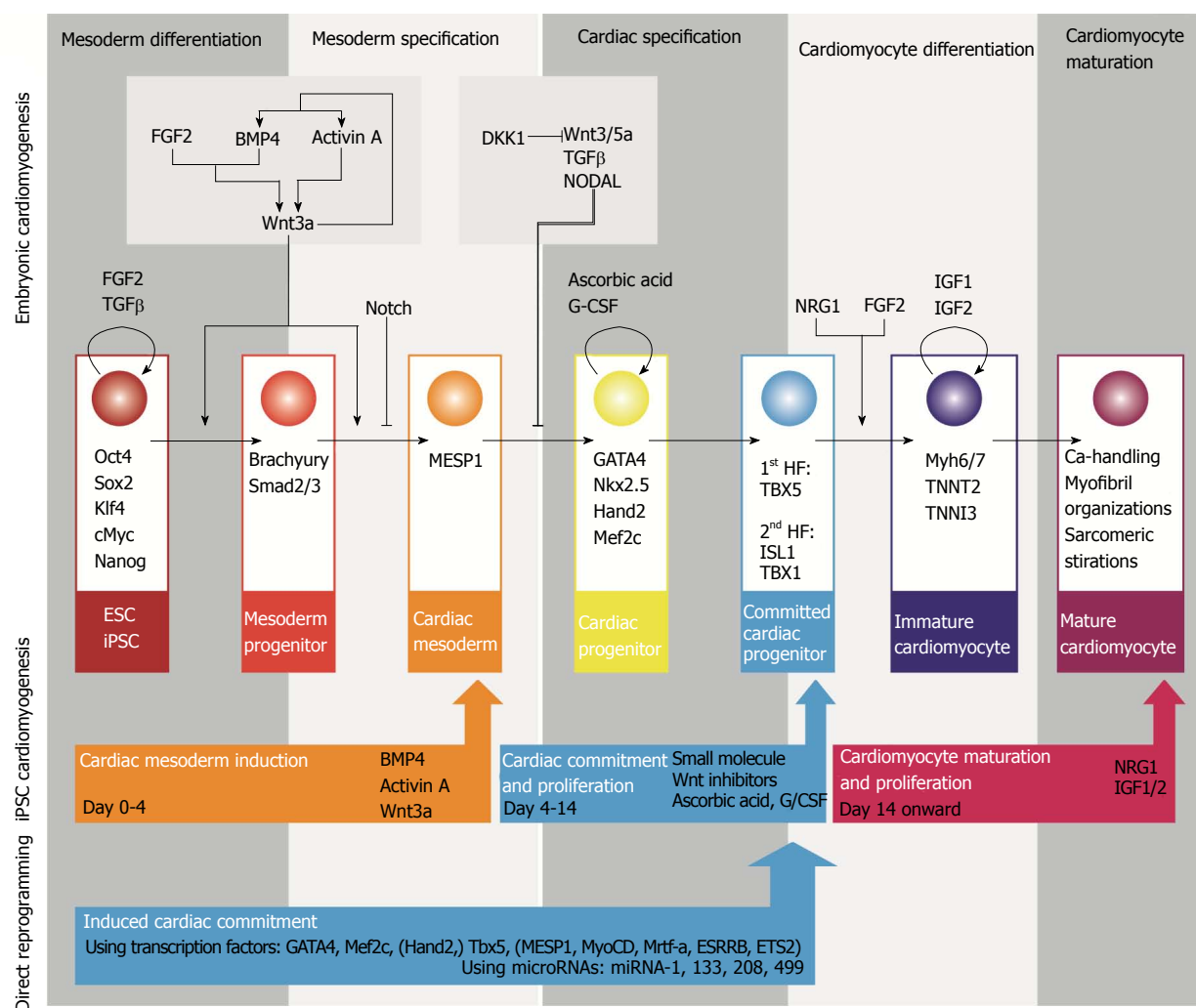
Indeed, reiteration of key steps in cardiogenesis by supplying iPSC with stage-specific pivotal signaling molecules efficiently differentiates iPSC into the cardiac lineage. Differentiation protocols rely on progressive sequential inductive signals using growth factors (Figure 1B). Monolayers of iPSC are stimulated with BMP4, Activin A and Wnt3a in the first 4 d of differentiation to induce cardiac mesoderm formation<sup>[69-72]</sup>. Inhibition of Wnt signaling using small molecule inhibitors after day 4 of differentiation advances mesodermal precursors to cardiac progenitors and reiterates the actions of Dkk1-mediated inhibition of Wnt signaling during embryology<sup>[69,70]</sup>. The addition of ascorbic acid<sup>[73]</sup> or G-CSF<sup>[74]</sup> at this stage enhances cardiomyocyte formation by stimulating proliferation of cardiac progenitor cells (Figure 1B). Culture of the obtained cardiac progenitor cells in the presence of NRG1 or IGF1 allows further maturation of cardiac progenitor cells into immature cardiac cells from the first and second heart field<sup>[75]</sup>. Modifications to this general protocol include embedding in extracellular matrix<sup>[76]</sup>, mechanical<sup>[77]</sup> and electrical<sup>[78]</sup> stimulation of the immature cardiomyocytes. These modifications may influence the maturity of the iPSC-derived cardiomyocytes but do not increase the differentiation efficiency.

### ***Direct reprogramming of cardiac fibroblasts into cardiomyocytes***

In equivalence to the iPSC generation, where pluripotency-associated transcription factors are expressed in terminally differentiated cells, direct conversion of fibroblasts into the cardiac lineage has been attempted<sup>[79-83]</sup>. Although no single master regulator of cardiomyogenesis has been identified to date, in analogy to the pioneering iPSC work of Yamanaka, Ieda *et al.*<sup>[79]</sup> used a reductionist approach to test fourteen different transcription factors to induce cardiomyogenic gene expression in fibroblasts, and found that the combination of cardiac-specific transcription factors GATA4, Mef2c and Tbx5 successfully reprograms murine cardiac fibroblasts directly into immature cardiomyocytes (Figure 1C)<sup>[79]</sup>. Although the efficiency of fibroblast reprogramming is rather low, with only about 30% of transduced cells display spontaneous contraction (about 6% of the total fibroblast population)<sup>[79,84]</sup>, the proof-of-concept that cardiac fibroblasts can be converted into cardiomyocytes by retroviral expression of GATA4, Mef2c and Tbx5 paved the way for *in vivo* delivery of these transcription factors.

Cardiac fibroblasts account for the majority of cells in the heart<sup>[85]</sup> and are therefore considered a viable cell population for reprogramming and restoration of cardiac function. Lineage tracing models<sup>[86,87]</sup>, wherein the cardiac fibroblasts are genetically tagged with a marker protein, were subjected to cardiac damage (either coronary ligation<sup>[86,87]</sup> or cryoinjury<sup>[84]</sup>) and treated with GATA4, Mef2c and TBX5 retroviruses. Up to three months





**Figure 1 Schematic of factors involved in cardiomyocyte differentiation in embryology, embryonic stem cell/induced-pluripotent stem cells and cardiac fibroblast reprogramming.** Factors that influence the progression through the five steps in cardiomyocyte differentiation and maturation: mesoderm differentiation, mesoderm specification, cardiac specification, cardiomyocyte differentiation and cardiomyocyte maturation in: A: Embryonic cardiomyocyte differentiation; B: Cardiomyocyte differentiation from ESC and iPSC using exogenous (growth) factors; C: In direct reprogramming of cardiac fibroblasts into cardiomyocytes. Transcription factors associated with each of the seven cell types during cardiomyocyte differentiation are presented in the boxes below. ESC: Embryonic stem cell; iPSC: Induced-pluripotent stem cells.

after treatment, cardiac transcription factor delivery to the heart reduces infarct sizes and attenuates cardiac dysfunction<sup>[84,86,87]</sup>, providing therapeutic proof-of-concept for *in vivo* cellular reprogramming, although efficiencies differ widely (1%-30%) between studies. Surprisingly, *in vivo* reprogrammed cardiomyocytes develop more characteristics (e.g., binucleation, assembled sarcomeres) of native cardiomyocytes as compared to their *in vitro* counterparts<sup>[87]</sup>. This improvement in reprogramming may be derived from microenvironmental clues, exposure to native extracellular matrix or mechanical forces during reprogramming and could provide clues for further improvements to the reprogramming protocols.

Additionally, it must be noted that reprogramming of cardiac fibroblasts into cardiomyocytes is efficient in mice, however the conversion of human fibroblasts into the cardiac lineage proves more difficult<sup>[80-83]</sup>. The expression of GATA4, Mef2c and TBX5 in human cardiac fibroblasts is insufficient for cardiac induction. The addition of MESP1 and Myocardin (MyoCD)<sup>[80]</sup>, MyoCD and MyoCD-related

transcription factor-A (Mrtf-a)<sup>[81]</sup>, MESP1 and estrogen-related receptor beta (ESRRB)<sup>[82]</sup>, or MESP1 and ETS2 (Figure 1C)<sup>[83]</sup> all increase reprogramming efficiency of human cardiac myocytes and underscore the need for further research in this area before a definite transcription factor cocktail can be put to the test in human trials.

Moreover, additional major impediments need to be addressed prior to clinical translation. Although issues such as tumorigenicity and retention encountered with iPSC and stem cell therapeutics, may be minimized by the direct conversion of cardiac fibroblasts into cardiomyocytes, heterogeneity in reprogramming efficacy, leading to the formation of immature cardiomyocytes that do not properly couple to adjacent cardiomyocytes, may cause fatal arrhythmias. Furthermore, current strategies rely on the use of viruses integrating randomly in the genome of cells that undergo reprogramming, which may elicit tumorigenic events. It is evident that *in vivo* reprogramming protocols without the use of viruses are essential before clinical translation can commence.

### MicroRNAs in cardiomyocytes reprogramming

The use of microRNAs in reprogramming strategies may overcome some of the limitations encountered in reprogramming fibroblasts into cardiomyocytes using viruses, since chemically synthesized microRNA mimics are easily transfected into cells and exhibit low toxicity in animal models<sup>[88]</sup>. MicroRNAs are endogenous small (about 21-23 nucleotides in length) non-coding RNAs that function as repressors of gene translation<sup>[89,90]</sup>. Endogenously, microRNAs are encoded in the genome either in extronic regions that form microRNA gene clusters or intronically in both protein-coding and non-coding genes. Regardless of their genomic location, microRNA transcription is initiated by the RNA Polymerase II, resulting in the generation of a pri-microRNA<sup>[91]</sup>. Pri-microRNAs are processed into pre-microRNAs by the RNA-processing complex formed by Drosha and DGCR8 and exported from the nucleus by Exportin 5<sup>[92-94]</sup>. In the cytosol, pre-microRNAs undergo a second processing step, performed by the cytoplasmic endonuclease Dicer, which forms the mature microRNA duplex<sup>[95]</sup>. Next, one strand of the microRNA duplex is loaded into the RNAi-induced silencing complex (RISC)<sup>[96]</sup> that utilizes the microRNA to identify and silence its target genes<sup>[97,98]</sup> (extensively reviewed in<sup>[90,99]</sup>). The effects of microRNAs on cardiomyogenesis might be powerful, as a single microRNA may target multiple signaling pathways simultaneously, a phenomenon known as multiplicity of microRNA targets<sup>[100]</sup>. Indeed, mice lacking the enzyme Dicer, which is essential to process microRNA precursors into their mature form<sup>[90]</sup>, die at day E12.5 from cardiac failure<sup>[101]</sup>.

Advances on iPSC and embryonic stem cell (ESC) differentiation into cardiomyocytes (described in sections "*Induced pluripotent stem cells and cardiomyogenesis*" and "*Direct reprogramming of cardiac fibroblasts into cardiomyocytes*") allowed Fu *et al.*<sup>[102]</sup> and Wilson *et al.*<sup>[103]</sup> to identify microRNAs essential to cardiomyogenesis. ESCs were differentiated using exogenous growth factors into beating cardiomyocytes and their "microRNA-ome" were analyzed on array platforms. Next, these microRNA signatures were compared to genuine fetal and adult cardiomyocytes and adult cardiac fibroblasts. MicroRNAs that are differentially expressed in ESC-derived cardiomyocytes and native ESC and that are not expressed by cardiac fibroblasts were identified as cardiomyogenic microRNAs or "cardiomiRs". Although the two "cardiomiR" screens show limited overlap (46%) when considering all differentially expressed microRNAs between native ESC and ESC-derived cardiomyocytes, the overlap is greatly increased when only microRNAs with increased abundance are compared (85%). This comparison allowed the identification of 7 "cardiomiRs" whose expression is increased during cardiomyogenesis (Table 1)<sup>[102,103]</sup>.

MicroRNA-1 and microRNA-133 are pivotal regulators of muscle differentiation<sup>[104]</sup> and loss of microRNA-1 or microRNA-133 results in embryonic lethality due

to several cardiac failures, including defective morphogenesis, electrical conduction and cardiomyocyte proliferation<sup>[101,105]</sup>. MicroRNA-1 and microRNA-133 are polycistronically transcribed from a duplicated locus in the human genome on chromosomes 18 and 20. MicroRNA-1 and microRNA-133 expression is under control of SRF and promotes cardiac mesoderm formation from naive ESCs<sup>[101,106]</sup>.

MicroRNA-1 is highly conserved among mammals and its expression in ESC shifts their gene expression profile toward that of cardiomyocytes<sup>[107,108]</sup>. The induction of the cardiomyogenic phenotype is mediated through several cooperative actions of microRNA-1. Inhibition of Notch signaling by microRNA-1-mediated direct repression of Dll1<sup>[106]</sup> and its downstream effector Hes1<sup>[109]</sup>, liberates the expression of the cardiac transcription factors GATA4, Nkx2.5 and Myogenin, whereas repression of the histone deacetylase HDAC4<sup>[104]</sup> liberates the cardiac transcription factor Mef2c (Figure 2). Additionally, repression of Hand2<sup>[110]</sup> and the smooth muscle transcription factor Myocardin<sup>[111]</sup> by microRNA-1 facilitate cardiomyocyte maturation through the repression of proliferation of mesenchymal progenitors and smooth muscle gene expression, respectively. Interestingly, the sole expression of microRNA-1 in cardiac fibroblasts is sufficient to induce cardiac reprogramming<sup>[112]</sup>.

MicroRNA-133 aids in cardiomyogenesis, however, in contrast to microRNA-1, its sole expression is insufficient to differentiate ESC into spontaneously contracting cells<sup>[106]</sup>. MicroRNA-133 promotes the actions of microRNA-1 through the suppression of smooth muscle specific genes in the myogenic precursors, thereby facilitating cardiomyocyte maturation. The direct repression of SRF<sup>[104,105]</sup> and the mesenchymal transcription factor Snai1<sup>[113]</sup> during cardiac differentiation of ESC or reprogramming of cardiac fibroblasts into cardiomyocytes reduces smooth muscle and fibroblast associated genes, which allows for the maturation of cardiomyocytes (Figure 2).

The cardiac myosin genes, which facilitate cardiac contraction, house three additional cardiomiRs, namely microRNA-499 and the microRNAs-208a and b that are encoded by the Myh7b and Myh6/7, respectively<sup>[114]</sup>. MicroRNA-499 facilitates expression of the cardiogenic transcription factor Mef2c<sup>[103]</sup> through a Wnt/ $\beta$ -Catenin-mediated mechanism (Figure 2)<sup>[115]</sup>, which remains to be elucidated but appears to involve repression of the transcription factor Sox6 and the transcription inhibitor Regulator of differentiation (Rod)-1<sup>[116]</sup>.

MicroRNA-208a and microRNA-208b are involved in cardiomyocyte maturation and orchestrate the expression of myosin fibers in the heart. In the adult heart, the abundance of myosin fibers are alpha fibers (or fast fibers) whereas in the developing heart the majority of myosin fibers are beta fibers (or slow fibers). The gene encoding alpha-MHC encodes a cardiac-specific microRNA (microRNA-208a) that targets the repressors of beta-MHC Sox6, Pur $\beta$  and SP3<sup>[114,117]</sup>. MicroRNA-208a-mediated repression of these inhibitors thus facilitates the expression

**Table 1** MicroRNAs involved in cardiomyocytes differentiation

microRNA	Targets	Effect on cardiomyogenesis (mechanism)	Used in reprogramming	Ref.
Increased during cardiomyogenesis				
1	Dll1 (Notch)	↑ CM Differentiation (↑ Nkx2.5 and Myogenin)	+	[102-104,106,109-111]
	Hes1 (Notch)	↑ CM Differentiation (↑ Nkx2.5 and GATA4)		
	Hand2	↓ CM Proliferation		
	HDAC4	↑ CM Differentiation (↑ Mef2c)		
30a-e	Myocardin	↑ CM Maturation (↓ SMC phenotype)	-	[102,103,120]
	Snai2	↑ CM Differentiation (↓ mesenchymal genes)		
	Smarcd2	↑ CM Differentiation (↓ mesenchymal genes)		
	Tnrc6a	↑ CM Maturation (↓ miR-206: ↓ SMC Phenotype)		
133a-b	Snai1	↑ CM Differentiation (↓ mesenchymal genes)	+	[102-105,113]
	SRF	↓ CM Proliferation		
	Cyclin D2	↓ CM Proliferation		
181a-d	?	↑ CM Proliferation	-	[103,175]
195	Cyclin D1	↓ CM Proliferation	-	[102,103,119,176]
	HMGA	↓ CM Differentiation (↓ Nkx2.5)		
208b	Myostatin	↑ CM Proliferation	+	[103,114,117,118]
	Sox6, Purβ	↑ CM Maturation (↑ beta-Myosin Heavy Chain)		
	THRAP1	↑ CM Maturation (↑ beta-Myosin Heavy Chain)		
	?	↑ CM Differentiation (↑ Nkx2.5, Mef2c and GATA4)		
499-5p	?	↑ CM Differentiation (↑ Nkx2.5, Mef2c and GATA4)	+	[102,103,115]
Decreased during cardiomyogenesis				
31	?	?	-	[103]
34c-3p	?	?	-	[103]
151-3p	ATP2a2	↓ CM Maturation (↓ beta-Myosin Heavy Chain)	-	[103,177]
221	?	?	-	[103]
222	?	?	-	[103]

ATP2a2: Sarcoplasmic reticulum  $\text{Ca}^{2+}$  ATPase 2; CM: Cardiomyocyte; Dll1: Delta-like 1; GATA4: GATA Binding Protein 4; Hand2: Heart and neural crest derivatives expressed 2; HDAC4: Histone deacetylase 4; Mef: Myocyte enhancer factor; miR: MicroRNA; Nkx2.5: NK2 homeobox 5; Purβ: Purine-rich element binding protein beta; Smarcd2: SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily d member 2; SMC: Smooth muscle cell; Snai: Snail family zinc finger; Sox6: Sex determining region Y-box 6; SRF: Serum response factor; THRAP1: Thyroid hormone receptor associated protein 1; Tnrc6a: Trinucleotide repeat-containing gene 6A; Wnt: Wingless-type MMTV integration site family.

of beta-MHC by the developing cardiomyocyte. Moreover, the beta-MHC gene (encoded by *Myh7*) contains the related microRNA-208b. Expression of beta-MHC, induced by microRNA-208a, thus induces the expression of microRNA-208b that provides a feed forward mechanism that maintains the expression of beta-MHC<sup>[114,117]</sup>. Additionally, microRNA-208 targets myostatin<sup>[118]</sup>, a known inhibitor of cardiac progenitor cell proliferation, which reduces the inhibitory effect of myostatin on cardiac progenitor cell propagation.

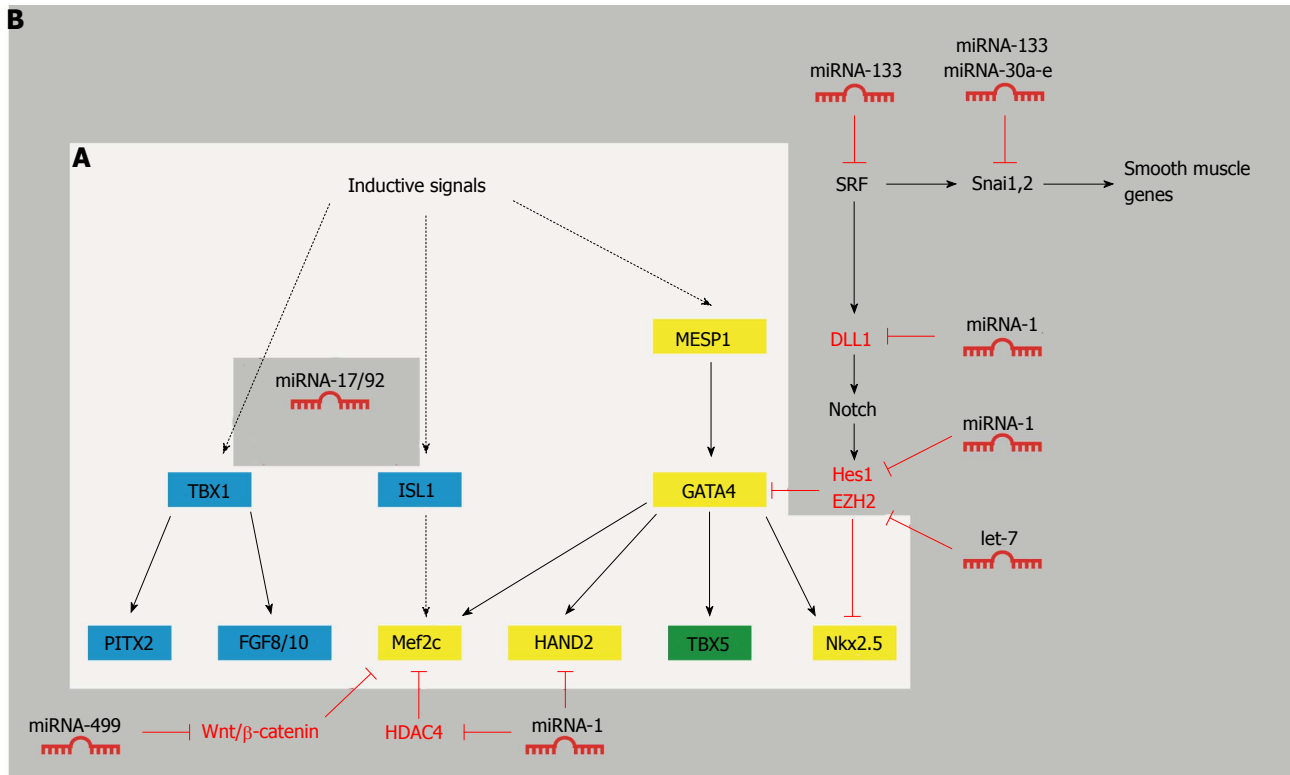
The other cardiomiRs, microRNA-30a-e, microRNA-181a and microRNA-195, are less well characterized. Overexpression of microRNA181a in ESC increased proliferation of differentiated cardiomyocytes through unidentified mechanisms<sup>[103]</sup>, whereas the expression of microRNA-195 decreases cardiomyocyte proliferation through the inhibition of cell cycle regulator cyclin D1<sup>[119]</sup>. MicroRNA-30a-e regulate cardiomyogenesis by targeting *Snai2* and *Smarcd2*<sup>[120]</sup>, two known inducers of mesenchymal gene expression. Their inhibition by microRNA-30a-e thus favors maturation of the cardiac phenotype over the maintenance of the mesenchymal phenotype (Figure 2).

The non-cardiac restricted microRNAs let-7, microRNA-99, and the microRNA-17/92 cluster also facilitate cardiomyogenesis<sup>[121,122]</sup>. MicroRNA-99 facilitates the transition from mesenchymal precursor to cardiac pro-

genitor cells by the Smarca5-mediated repression of TGFβ signaling<sup>[121]</sup>. Additionally, let-7 induces the expression of cardiogenic transcription factors GATA4, Mef2c, Nkx2.5 and Tbx5 by the repression of EZH2, a histone methyltransferase that epigenetically silences these genes in mesenchymal precursors<sup>[121]</sup>. The microRNA-17/92 cluster subsequently facilitates ventricular myocyte generation from the first heart field. The microRNA-17/92 cluster targets Tbx1 and ISL1, the master transcription factors for second heart field development, thereby favoring differentiation of the first heart field (Figure 2)<sup>[122]</sup>.

Notably, Jayawardena *et al.*<sup>[112]</sup> used the most abundantly expressed cardiomiRs, *i.e.*, microRNA-1, 133, 208 and 499, to reprogram cardiac fibroblasts directly into cardiomyocytes. Transient expression of these four microRNAs *in vitro* generated mature cardiomyocytes that spontaneous beat, albeit at low efficiency (1.5%-7.7% of all fibroblasts). The reprogramming efficiency could be increased to about 28% by the addition of a Janus Kinase inhibitor. Moreover, the four microRNAs reprogram cardiac fibroblasts *in vivo* in a mouse model of MI, providing therapeutic proof-of-concept for the microRNA-mediated reprogramming of fibroblasts to ameliorate damage following MI<sup>[112]</sup>.

Thus, advances in iPSC biology and cardiac reprogramming have identified exogenous growth factors and endogenous transcription factors that drive cardio-



**Figure 2** The complex web of transcription factors in cardiac specification and their regulation by microRNAs. A: Crosstalk between transcription factors involved in the formation of the first and second heart field (light grey box). MESP1, GATA4, Mef2c, HAND2 and Nkx2.5 are central transcription factors in the first and second heart field (yellow). TBX5 is only expressed in the first heart field (green). ISL1 and TBX1 are expressed in the second heart field (blue); B: MicroRNA-mediated regulation of cardiac transcription factors during cardiomyocyte differentiation (dark grey box).

myogenesis, and have provided novel therapeutic approaches for the amelioration of damage from MI by the therapeutic expression of cardiac transcription factors. Moreover, these recent advances have provided a platform to study cardiogenesis in more detail. MicroRNAs can similarly induce fibroblast reprogramming into cardiomyocytes and can be delivered to the cardiac tissue without the use of randomly integrating viruses, and may thus improve safety of reprogramming in a clinical context. The question that remains is how to deliver these microRNAs safely and efficiently to the site of damage and cell type of choice to perform their function. This question is addressed in the next section.

## TARGETING MICRORNAS FOR CARDIAC REGENERATION

MicroRNA-mediated reprogramming of cardiac fibroblasts *in vivo* requires advanced delivery strategies. In the section below, we will describe general and targeted drug delivery strategies and discuss possibilities to specifically target microRNAs to cardiac fibroblasts.

A range of chemical modifications to enhance cellular uptake of microRNAs have been developed recently. Additionally, particulate drug delivery systems, including liposomes, polymeric micelles, polymeric vesicles, polymeric nanoparticles (NPs), and dendrimers have been investigated

for targeted delivery of drugs<sup>[123]</sup> including microRNAs in a variety of disease models outside the cardiac field and with varying degrees of success. Current advances in targeted drug delivery from these fields provide a solid basis for the burgeoning field of cardiac drug delivery.

In general, the prime reasons for targeted drug delivery is the modulation of the drug's pharmacokinetics, the avoidance of toxicity of the drug in non-diseased tissue or cells and to alter the apparent physicochemical characteristics of a drug by making use of a carrier. An ideal drug delivery vehicle needs to be non-toxic, biocompatible, non-immunogenic and biodegradable<sup>[123]</sup>. Particle sizes of the drug delivery system have a preferred size between 10 and 200 nm. The lower limit is determined by the glomerular permselectivity in the kidney that captures particles below 10 nm and rapidly clears them through renal filtration<sup>[124]</sup>, whereas the upper limit is set by clearance through the reticuloendothelial system and uptake by the spleen and liver<sup>[125]</sup>. Additionally, surface charge and chemistry are key parameters in the design of drug delivery systems. Systems with a positive surface charge may electrostatically interact with the cell membrane or its associated negatively-charged proteoglycans and subsequently internalized through endocytosis<sup>[126,127]</sup>. Negatively charged systems are preferentially recognized by monocytes/macrophages and internalized *via* the caveolar or clathrin endocytic



pathways<sup>[128-130]</sup>.

### Classes of drug targeting systems

Cardiac microRNA delivery poses huge challenges as unmodified microRNAs are rapidly degraded by systemic nucleases, secreted through renal filtration and phagocytosed by monocytes/macrophages, limiting their ability to reach their target cell<sup>[131,132]</sup>. A range of chemical modifications to enhance microRNA stability and cell permeability, including 2'-O-methyl modifications, locked nucleic acid chemistry, the conjunction of small molecules or cell penetrating peptides (Figure 3)<sup>[133]</sup> and peptide nucleic acids have been developed that increase therapeutic efficacy of microRNA therapies (reviewed in<sup>[131,132,134]</sup>), albeit they do not add cell or organ specificity. Hence, the development of targeted delivery systems for myocardial microRNA delivery is of the utmost importance.

As described above, various particulate drug delivery systems have been developed for cell and organ specific targeted delivery of drugs (Table 2). Liposomes<sup>[135]</sup>, the related polymerosomes<sup>[136]</sup> and polymeric micelles<sup>[137]</sup> are a system of lipids or polymers that self-assemble into spherical structures with an aqueous core that can hold the microRNA payload<sup>[123,138,139]</sup>. Single or multiple types of lipids and polymers can be combined to generate liposomes, polymerosomes and polymeric micelles, which allows for additional flexibility in designing the physical and chemical properties of the drug delivery vehicle<sup>[140]</sup>. Liposomes and polymerosomes are internalized *via* endocytosis and destined for lysosomal degradation<sup>[141]</sup>. Endosomal escape from the liposomal content occurs through pH-sensitive fusion of the liposome and the endosomal membrane, resulting in drug release in the cytoplasm<sup>[142]</sup>. Although liposomes have a long history in drug delivery in basic and clinical medicine with FDA approval, some concerns regarding their clinical applicability are reported, such as the immunogenicity and toxicity of certain cationic lipid particles<sup>[143,144]</sup>. Regardless, liposomes and polymerosomes are highly promising for future clinical microRNA delivery.

Microbubbles (Table 2) are a second class of drug delivery systems that can be used for microRNA delivery *in vivo* and represent a specialized form of liposome that is sensitive to external clues, such as high powered ultrasound (described below). Microbubbles are gas-filled lipid spheres of various diameters (10-1000 nm)<sup>[145,146]</sup>. Cationic microbubbles can form complexes with anionic drugs, such as microRNAs, by electrostatic interaction<sup>[147,148]</sup>. The sensitivity of microbubbles to ultrasound, which destroys the microbubble, delivers the payload directly to its environment<sup>[145,147]</sup>. Hence, for efficient targeting of microRNAs into the tissue, additional modifications to the microRNA (described above) may be necessary to increase cellular uptake by the target cells<sup>[131,132]</sup>.

Nanoparticles and nanospheres (Table 2) are a third class of drug delivery vehicles that consist of lipids or block co-polymers, respectively<sup>[149,150]</sup>. Nanoparticles and nanospheres are commonly produced using emulsion or

precipitation techniques which form solid structures typically 10-100 nm in size<sup>[139,151]</sup>. Changing the composition of the block co-polymers that build up the nanoparticle allows tuning drug delivery rates<sup>[128]</sup>, as drug delivery occurs through diffusion of the drug through the solid nanoparticle or *via* biodegradation of the particle<sup>[139,150,151]</sup>. The solid nature of nanoparticles confers great stability advantages *in vivo* and provides slow-release properties. Therefore, nanoparticles are more efficient in delivering proteinaceous and small molecule drugs than microRNAs, as cellular uptake and degradation properties are inferior to the delivery efficiency of liposomes and polymeric micelles.

Dendrimers (Table 2), represent the last class of drug delivery systems are highly branched macromolecules with a controlled repeated branching around a central core that forms a small (1-10 nm), spherical and highly dense nanocarrier that holds many cavities that may contain drugs<sup>[152-155]</sup>. Targeting efficacy and extravasation of dendrimers can be controlled by their size, molecular weight and the functional groups present on their surface<sup>[153,156]</sup>.

### Passive drug targeting

Targeting of drug delivery systems can be achieved *via* two general concepts, namely passive or active targeting. Passive targeting is based on the so-called enhanced permeability and retention effect (EPR)<sup>[157]</sup>. At sites of inflammation, the integrity of the endothelial lining is often compromised, resulting in a defective or leaky vasculature. Circulating drug delivery systems are able to pass these leaky vessels and can thus enter the inflamed tissue. Hence, colloidal drug delivery systems passively accumulate at sites of inflammation, such as the infarcted heart<sup>[158,159]</sup>. An important prerequisite for passive targeting is a relatively long (hours-days) circulation time of the drug delivery system since extravasation occurs only by chance. Additionally, if passive drug delivery is to be used to target cardiac fibroblasts, detection by monocytes/macrophages needs to be avoided in order to reduce rapid clearance of the drug carriers from the cardiac tissue by these phagocytic cells.

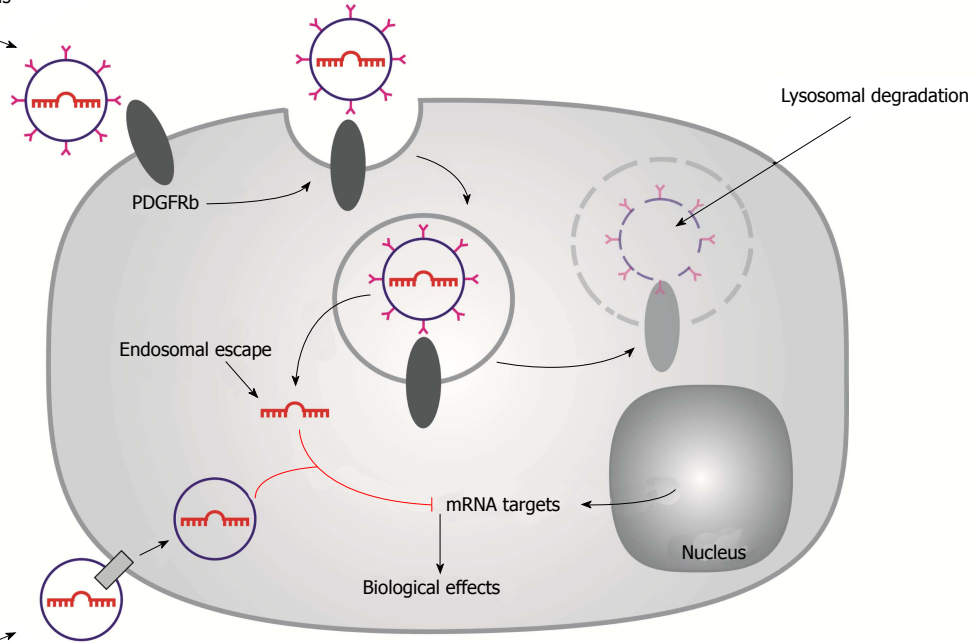
### Active drug targeting

Active targeting drug delivery systems are equipped with specific targeting devices that recognize or have affinity for certain cells. Although the recent identification of biomarkers that are differentially expressed in the diseased cardiac tissue has advanced the development of experimental therapies that can be employed for the targeted delivery of microRNAs, there is a huge challenge for active-targeting strategies to find specific target molecules for a certain disease process and to test its effectiveness in drug delivery therapies.

Active drug targeting of microRNAs to cardiac fibroblasts may be achieved in two distinct manners, depending on the interaction of the targeting device and the cell. Either the drug delivery system can be internalized by the cell where it releases the microRNAs subsequently (epitope targeted drug delivery, Figure 3), or the drug delivery system can

**B**

Epitope-targeted intracellular delivery *via* antibody/peptide-mediated binding and receptor-mediated endocytosis

**A**

CPP-mediated intracellular delivery *via* electrostatic interactions or by macropinocytosis

**Figure 3 Schematic of passive and active targeted drug delivery systems for microRNA delivery.** A: Passive targeting by cell-penetrating peptide-coated nanoparticles are internalized by receptor-mediated endocytosis; B: Active targeting by PDGFRb-targeted liposomes. Liposomes interact with cell surface receptors (PDGFRb) and internalized via receptor-mediated endocytosis. The endocytotic vesicles fuse to form early endosomes which ultimately become part of the lysosomes, where proteins and nucleic acids are degraded by acid hydrolases. To achieve target gene silencing, microRNAs need to be released from the liposome and escape from the endosomes into the cytoplasm, where the microRNA directs the cleavage of target mRNAs.

bind to the cell and act as a drug release depot that can be activated at the diseased site (inducible targeted drug delivery). Although targeted drug delivery approaches have been pursued cardiovascular disease, data on the delivery of microRNA to fibroblasts are scarce<sup>[160]</sup>.

Epitope targeting of drug delivery systems is a rapidly evolving field in cardiac drug delivery and was shown by Dasa *et al.*<sup>[161]</sup>, who used *in vivo* phage display methods to identify peptide sequences specific for cardiac endothelial cells, cardiomyocytes and myofibroblasts<sup>[161]</sup>. These peptide sequences were conjugated to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) liposomes using polyethylene glycol (PEG). The obtained peptide-PEG-DSPE was loaded with the small molecule inhibitor of PARP-1 activation AZ7379. Although the publication only shows proof-of-concept data in efficiently (> 90%) reducing PARP-1 activation in cardiomyocytes<sup>[161]</sup>, it is tempting to assume that the targeted delivery of small molecule inhibitors or microRNAs to cardiac fibroblasts would be equally efficient as antibody-functionalized liposomes are highly efficient in delivering non-coding RNAs to vascular cells<sup>[162]</sup>.

Inducible targeted drug delivery uses drug delivery systems that are sensitive to their environment, *e.g.*,

heat<sup>[163]</sup>, light<sup>[164]</sup>, pH<sup>[165]</sup> or ultrasound<sup>[145]</sup>, that will release their payload by the indicated external trigger if present at the disease site. Ultrasound-sensitive microbubbles (described in section "Classes of drug targeting systems") have been used for cardiac microRNA delivery with high efficiency, although reports on targeting of cardiac fibroblast remain scarce. Gill *et al.*<sup>[166]</sup> used liposomal ultrasound-sensitive microbubbles to deliver microRNA-133 into HL1 cardiomyocytes *in vitro*. Both encapsulated (inside the microbubble) and complexed (on the outer shell of the microbubble) microRNA formulations efficiently delivered the microRNA-133 mimic, without affecting cardiomyocyte viability, indicating that, although encapsulation increases the microRNA-carrying capacity of microRNAs, complexation strategies do not affect the ability of microbubbles to deliver microRNAs<sup>[166]</sup>. Using a similar approach, Liu *et al.*<sup>[167]</sup> delivered microRNA-21 mimics into the hearts of swine without inflicting cardiac damage. Myocardial microRNA-21 expression levels were efficiently elevated in hearts treated with the microRNA-microbubble complex that received ultrasound activation compared to control conditions. Interestingly, the transfection efficiency of microRNA-microbubble complexes that were administered by intracoronary

**Table 2** Characteristics of particulate drug delivery systems

Carrier	Size range (nm)	Preparation method	Advantages for drug delivery	Disadvantages for drug delivery	Ref.
Liposomes and polymerosomes	10-2000	Self-assembly in aqueous solutions	High drug-carrying capacity Good for hydrophobic and hydrophilic drugs Surface functionalization possible Simple preparation	Batch-to-batch variability Difficulties in sterilization	[123,135,138,141,143,150,161,178]
Microbubbles	10-1000	Various depending on type	Surface functionalization possible	Not good for hydrophobic drugs Low drug-carrying capacity	[145-148,166,168,179]
Polymeric micelles	10-100	Direct organization or controlled aggregation in solvent	Long blood circulation time Surface functionalization possible Simple preparation	Not good for hydrophobic drugs Low drug-carrying capacity	[123,136,137,155,158]
Nanoparticles and nanospheres	10-100	Nanoparticles: Polymerization of monomers by emulsion Nanospheres: Interfacial polymerization and phase inversion with polymeric emulsions	Shape, size and mechanical properties tunable Possibility for controlled release	Toxicity of residual chemicals from preparation process Limited cellular uptake and degradation	[123,126,128,139,150,151,155,180]
Dendrimeres	1-10	Convergent or divergent synthesis	High functionalized surface	Difficult preparation process Toxicity	[123,154,156]

injection was higher compared to systemic administration. These results indicate that the application site may affect therapeutic outcome and should be considered in clinical translation<sup>[167]</sup>. Kwekkeboom *et al.*<sup>[168]</sup> delivered microRNA mimics and anti-miRs to the cardiac endothelium using a combination of microbubbles and ultrasound activation. Notably, delivery of anti-miRs (cholesterol-conjugates anti-miRs<sup>[169]</sup>) had a higher transfection efficacy compared to control anti-miRs implying that cellular uptake of delivered microRNAs is still highly dependent on their physicochemical properties<sup>[168]</sup>.

The concept of cardiac fibroblast reprogramming into cardiomyocytes holds great therapeutic value for the treatment of MI and its associated cardiac failure. However, fibroblast reprogramming is a recent concept and although current studies have provided proof-of-concept, focus on its clinical translation is limited. A range of drug delivery systems are reported for the delivery of microRNAs outside the cardiac field (reviewed in<sup>[149,170]</sup>) that can easily be transposed onto the reprogramming paradigm. As this field evolves, clinically relevant delivery approaches and suitable targeting epitopes for fibroblast-specific drug delivery will be explored as will their clinical effectiveness.

## SUMMARY AND FUTURE PERSPECTIVES

Deciphering the signaling pathways that underlie cardiac development has led to new therapeutic strategies that trigger cardiac regeneration. Vast progress is made in promoting cardiomyocyte proliferation and in direct reprogramming of cardiac fibroblasts into cardiomyocytes, which offer new perspectives on the possibility to advance

from treating cardiac disease to curing cardiac disease. Additionally, advances in drug delivery have yielded a plethora of drug delivery systems that can selectively deliver therapeutic agents to relevant cell populations at the site of damage. However, many challenges remain to be addressed before clinical translation can commence.

During a MI, billions of cardiomyocytes are lost and although current reprogramming strategies using exogenous transcription factors or microRNAs have emerged as potential therapeutic strategies, they are vastly inefficient. Thus, to enhance cardiac regeneration it will be pivotal to develop procedures that increase the yield and efficiency of generating *de novo* cardiomyocytes. Advancing our mechanistic understanding of the reprogramming process, including the directed differentiation of subtypes of cardiomyocyte (*i.e.*, ventricular, atrial or nodal), is key to the success of this promising therapy, however when subtype specification occurs during development and how these processes are regulated remain elusive. Moreover, *in vivo* efficacy and safety in large animals needs to be addressed before clinical translation can commence.

Additionally, it has been reported that the delivery of immature or heterogeneous populations of cardiomyocyte derived from progenitor cells or iPSC can lead to arrhythmias<sup>[171,172]</sup>. Currently, reprogrammed cardiomyocytes are immature and phenotypical heterogeneous, which could contribute to arrhythmogenesis. Hence, it is crucial to promote maturation and integration of reprogrammed cardiomyocytes. Yet, our current understanding of these processes is limited and further research into these processes is highly warranted.

While an intense research focus has been on the

development of new drug delivery systems, efforts to identify epitopes that are differentially expressed in diseased cardiac tissue has received little attention, as the field of cardiac drug delivery is still in its infancy. The identification of target epitopes that discriminate between fibroblasts in the affected vs the healthy tissue is pivotal to clinical translation of targeted delivery of microRNAs using liposomes, polymeric micelles or microbubbles. In addition, the heart contains a large population of fibroblasts that are necessary for its normal function<sup>[173,174]</sup>. Therefore, it may be detrimental to the cardiac function to target all fibroblasts for reprogramming. Drug delivery systems may need to be comprised of multiple targeting mechanisms, *e.g.*, ultrasound sensitive and fibroblast targeted, if a sufficiently selective molecular targeting epitope cannot be identified that distinguishes fibroblasts in the scar tissue from those elsewhere in the heart.

In summary, MI results in a massive loss of cardiomyocytes that are replaced by scar tissue. Endogenous repair mechanisms are insufficient to efficiently regenerate the lost myocardial tissue and therapeutic approaches to induce cardiomyocyte proliferation using growth factors are relatively ineffective. Advances in our basic understanding of cardiomyogenesis obtained from embryology and iPSC biology has led to the identification of factors that drive cardiomyogenesis, and have provided a novel therapeutic approach for the amelioration of damage from MI through the therapeutic delivery of microRNAs that reprogram cardiac fibroblasts into cardiomyocytes. These microRNAs can be delivered to the cardiac fibroblasts using advanced drug delivery systems. Although there are many challenges ahead in advancing this emerging technology, the opportunities and potential clinical benefits are substantial and we are confident that the field will continue to push this technology further in the years to come.

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## Genetic testing in congenital heart disease: A clinical approach

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### Abstract

Congenital heart disease (CHD) is the most common type of birth defect. Traditionally, a polygenic model defined by the interaction of multiple genes and environmental factors was hypothesized to account for different forms of CHD. It is now understood that the contribution of genetics to CHD extends beyond a single unified paradigm. For example, monogenic models and chromosomal abnormalities have been associated with various syndromic and non-syndromic forms of CHD. In such instances, genetic investigation and testing may potentially play an important role in clinical care. A family tree with a detailed phenotypic description serves as the initial screening tool to identify potentially inherited defects and to guide further genetic investigation. The selection of a genetic test is contingent upon the particular diagnostic hypothesis generated by clinical examination. Genetic investigation in CHD may carry the potential to improve prognosis by yielding valuable information with regards to personalized medical care, confidence in the clinical diagnosis, and/or targeted patient follow-up. Moreover, genetic assessment may serve as a tool to predict recurrence risk, define the pattern of inheritance within a family, and evaluate the need for further family screening. In some circumstances, prenatal or preimplantation genetic screening could identify fetuses or embryos at high risk for CHD. Although genetics may appear to constitute a highly specialized sector of cardiology, basic knowledge regarding inheritance patterns, recurrence risks, and available screening and diagnostic tools, including their strengths and limitations, could assist the treating physician in providing sound counsel.

**Key words:** Congenital heart disease; Genetics; Genetic screening; Genetic testing

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**Core tip:** Monogenic models and chromosomal abnormalities have been associated with syndromic and non-

syndromic forms of congenital heart disease (CHD), paving the way for genetic investigation and testing to shoulder an important role in patient management. Herein, we present an overview of the role of genetics in CHD, propose various clinical scenarios in which genetic testing may be appropriate, and discuss practical implications with regards to when and how to order genetic tests. Summary tables are provided regarding the various genes implicated in syndromic and non-syndromic forms of CHD and recurrence risks in siblings and offspring.

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## INTRODUCTION

Congenital heart disease (CHD) afflicts 2 to 3 children per 100 live births<sup>[1,2]</sup>. It is the most common type of birth defect and encompasses a wide range of malformations. The spectrum of severity ranges from insignificant and even self-resolving lesions, such as ventricular septal defects that spontaneously close, to highly complex and multiorgan manifestations that are incompatible with natural survival. While much progress has been made regarding the management of children and adults with CHD, a greater understanding of underlying etiologies could potentially lead to further advances in preventive care and therapeutic strategies<sup>[3]</sup>.

The complexity and heterogeneity of CHD has traditionally been attributed to multifactorial etiologies arising from interactions between multiple genes and environmental factors (so-called "polygenic model")<sup>[4]</sup>. Early investigations into environmental factors spawned recommendations for maternal multivitamin supplementation containing folic acid to reduce risks of developing CHD<sup>[5-7]</sup>. Other implicated maternal factors include pregestational diabetes, pollakiuria, febrile illnesses, rubeola, influenza, alcohol consumption, cigarette smoking, and teratogenic pharmacological agents such as thalidomide, warfarin, angiotensin converting enzyme inhibitors, and certain anticonvulsant and anti-inflammatory drugs<sup>[8]</sup>.

Technological advances have permitted the confirmation of clinically suspected monogenetic subtypes of CHD, with dominant or recessive inheritance patterns. However, some forms of CHD could not be explained by a polygenic model<sup>[9]</sup>, with much higher recurrence risks in first-degree relatives than predicted<sup>[3,10]</sup>. Chromosomal abnormalities have been associated with cardiac defects, particularly in the setting of syndromic phenotypes (e.g., trisomy 21, DiGeorge, and Williams-Beuren syndromes). In so-called multiplex families with several affected members, identified candidate genes have been consistent with monogenetic models with Mendelian inheritance.

Furthermore, the rate of CHD increases with consanguinity, as described in Arabic countries<sup>[11]</sup>.

The fact that monogenic and chromosomal abnormality models account for a substantial proportion of CHD enhances the potential value of genetic investigation and testing<sup>[12]</sup>. Genetics carries the potential to unravel etiological mysteries that underpin CHD, provide pathophysiological insights, assist in risk assessment, inform clinical management, and counsel families regarding future offspring. The focus of this review is on the genetics of structural CHD, as opposed to other disease categories such as inherited channelopathies. Our review known implicated genes and chromosomal abnormalities, discussed when and how to perform genetic testing, and shared our perspective regarding clinical applications.

## GENETICS IN STRUCTURAL CONGENITAL HEART DISEASES

Approximately 30% of patients diagnosed with CHD have syndromic phenotypes with extracardiac manifestations. The influence of genetics is well established for chromosomal aneuploidies such as Down, Turner, and DiGeorge syndromes. Other syndromes are linked to a mutation or deletion in one gene, such as Noonan, Alagille, and Holt-Oram syndromes<sup>[3]</sup>. For the 70% of CHD cases that are non-syndromic, new genes with Mendelian inheritance (dominant or recessive) have been identified, particularly in families with several affected members. Table 1 summarizes current knowledge regarding genetic etiology for several forms of CHD with syndromic or non-syndromic phenotypes.

Genes etiologically linked to CHD directly impact embryologic development. For example, defects in genes responsible for the embryonic formation of the atrial septum (e.g., MYH6, TBX20) can result in atrial septal defects (ASD)<sup>[13,14]</sup>. In addition to their function in embryologic cardiac development, implicated genes may also play a role in heart regulation throughout life<sup>[15]</sup>. The critical purpose of these genes, which are primarily transcription factors, explains the possibility of dominant heritability. A mutation that modifies the protein function in one of these genes may have a major effect on cardiac development and regulation. Furthermore, interactions between transcription factors explain the diverse consequences associated with individual mutations. For example, NKX2.5 mutations may result in ASD, atrioventricular block, ventricular septal defect (VSD), Ebstein anomaly, and tetralogy of fallot (TOF). GATA4, a transcription factor, has been associated with ASD, VSD, and pulmonary stenosis. TBX1 has been implicated in TOF, patent ductus arteriosus, and interrupted aortic arch; and TBX20 in ASD, VSD, valve defects, and impaired chamber growth. In addition to these transcription factors, other genes with varied roles have been implicated in CHD, such as MYH6, which codes for an alpha myosin heavy chain (ASD) and Notch 1, which is implicated in valve formation (bicuspid aortic valve and aortic stenosis)<sup>[15,16]</sup>.



**Table 1 Genes described in syndromic and non-syndromic forms of congenital heart disease**

Syndromic: Syndrome name Non syndromic: Gene implicated	Phenotype associated with structural heart disease	Syndromic: Chromosomal aneuploidie, microdeletion or gene/locus/inheritance Non syndromic: Locus/inheritance	Ref.
Atrioventricular septal defect (AVSD)			
Down syndrome	MR, facial dysmorphia	Trisomy 21	[71-73]
Edward syndrome	IUGR, facial dysmorphia, clenched fingers	Trisomy 18	[74]
Patau syndrome	Cleft lip and palate, microphthalmia, polydactyly	Trisomy 13	[74]
Holt-Oram syndrome	Preaxial limb defects, absent or dysmorphic thumbs, cardiac conduction disease	TBX5/12q24.1/AD	[75]
Noonan syndrome	Hypertrophic cardiomyopathy, short stature, broad neck, unusual chest shape, facial dysmorphia, developmental delay	PTPN11/12q24/AD, <i>de novo</i> ; SOS1/2p21/AD, <i>de novo</i> ; KRAS/12p12.1/AD, <i>de novo</i>	[76]
Ellis-van Creveld syndrome	Common atrium, polydactyly, deformity of upper lip, dwarfism with narrow thorax, ASVD partial to complete	EVC and EVC2/4p16/AR	[77]
Locus 1p31-p21	AVSD partial to complete	Gene no yet found/AD	[78]
CRELD1	partial AVSD, heterotaxy syndrome	3p25/AD	[79-81]
GATA4	Family with ASD, VSD and one member with AVSD	8p23.1/AD, <i>de novo</i>	[82]
Atrial septal defect (ASD)			
Holt Oram syndrome	See AVSD above	See above AVSD	[83,84]
Noonan syndrome	See AVSD above	See above AVSD	[85,86]
Ellis-van Creveld syndrome	See AVSD above	See above AVSD	[77]
Cardiofaciocutaneous syndrome	Hypertrophic cardiomyopathy, facial dysmorphia, skin abnormalities: keratosis pilaris, nevi	MAP2K1/15q22.31/AD, <i>de novo</i> ; MAP2K2/19p13.3/AD, <i>de novo</i> ; KRAS/7q34/AD, <i>de novo</i> ; BRAF/12p12.1/AD, <i>de novo</i>	[87]
Cri du Chat	Sound of cry similar to cat's cry, facial dysmorphia, MR	CTNND2/5p15.2/ <i>de novo</i>	[88]
NK2X-5	+/- Atrioventricular block	5q35.1/AD	[89]
GATA4	+/- Pulmonary stenosis	8p23.1/AD, <i>de novo</i>	[82,90]
MYH6		14q11.2/AD	[13]
TBX20		7p14.2/AD	[14,91]
Ventricular septal defect (VSD)			
Holt Oram syndrome	See AVSD above	See AVSD above	[92]
Ellis-van Creveld syndrome	See AVSD above	See AVSD above	[77]
Cri du chat	See AVSD above	See AVSD above	[88]
Down syndrome	See AVSD above	See AVSD above	[93,94]
Edward/patau syndrome	See AVSD above	See AVSD above	[95,96]
Digeorges syndrome	Facial dysmorphia, speech delay, learning delay, psychiatric disorder, cleft palate, immune deficiency, hypoplastic/aplastic thymus, hypocalcaemia.	Deletion 22q11.21/ <i>de novo</i> , AD	[97]
NK2X-5	Atrioventricular block	5q35.1/AD	[89]
GATA4		8p23.1/AD, <i>de novo</i>	[82,98]
Ebstein anomaly			
Down syndrome	See AVSD above	See AVSD above	[99]
NKX2-5		5q35.1/AD	[100]
Pulmonary stenosis			
Noonan syndrome	See ASVD above	See AVSD above	[101, 102]
Costello syndrome	Hypertrophic cardiomyopathy, MR, loose skin, facial dysmorphia large mouth	HRAS/11p15.5/AD	[103]
Leopard syndrome	Lentigines, short stature, hearing loss, closed to Noonan syndrome	PTPN11/12q24/AD, <i>de novo</i> ; RAF1/3p25.2/AD, <i>de novo</i> ; BRAF/7q34/AD, <i>de novo</i>	[104]
Alagille syndrome	Pulmonary branch stenosis, bile duct paucity, cholestasis, facial dysmorphia, deep-set eyes, butterfly vertebrae	JAG1/20p12/AD NOTCH2/1p12/AD Deletion/20p12/AD	[105, 106]
Cardiofaciocutaneous syndrome	See ASD above	See ASD above	[107]
GATA4	+/- Atrial septal defect	8p23.1/AD, <i>de novo</i>	[90, 108]
Aortic valve stenosis			
Turner syndrome	Female, webbed neck, widely spaced nipples, short stature, streaked ovaries	Monosomy X or mosaics (45,X/46,XX)	[109]
Noonan syndrome	See above AVSD	See above AVSD	[76]
NOTCH1		9q34.3/AD	[16, 110]
SMAD6		15q22.31/?	[111]
Supravalvular aortic stenosis			
Williams-Beuren syndrome	Elfin facies, cocktail personality, hypercalcaemia, developmental delay, thyroid disorder, renal and connective tissue abnormalities.	Deletion/7q11.23/ <i>de novo</i> , AD	[112]
Aortic coarctation			
Turner syndrome	See aortic valve stenosis above	See aortic valve stenosis above	[109]

Down/Edward/Patau syndrome	See AVSD above	See AVSD above	[113]
NOTCH1		9q34.3/AD	[110]
Bicuspid aortic valve			
Turner syndrome	See aortic valve stenosis above	See aortic valve stenosis above	[109]
Anderson syndrome	Long QT syndrome, ventricular arrhythmias, sudden cardiac death, facial dysmorphism, short stature	KCNJ2/17q24.3/AD	[114]
NOTCH1		9q34.3/AD	[16,110]
SMAD6		15q22.31/?	[111]
Tetralogy of fallot			
DiGeorges syndrome	See VSD above	See VSD above	[97]
Alagille syndrome			
Cat-Eye syndrome	See pulmonary stenosis above	See pulmonary stenosis above	[105,106]
	Dysmorphic ears, microphthalmia, anal atresia, renal abnormalities, coloboma, cleft palate	Duplication/22q11/ <i>de novo</i>	[115]
NKX2.5		5q35.1/AD	[100]
GATA4		8p23.1/AD, <i>de novo</i>	[116]
NOTCH1		9q34.3/AD	[16]
FOG2		8q23.1/?	[117,118]
Truncus arteriosus			
Digeorges syndrome	See VSD above	See VSD above	[97]
Hypoplastic left heart syndrome			
NKX2.5		5q35.1/AD	[119]
NOTCH1		9q34.3/AD	[16,110]

The phenotype syndrome most commonly associated with the particular CHD is indicated in bold. AVSD: Atrioventricular septal defect; ASD: Atrial septal defect; VSD: Ventricular septal defect; MR: Mental retardation; IUGR: Intrauterine growth retardation; AD: Autosomal dominant; AR: Autosomic recessive; CHD: Congenital heart disease.

## WHEN AND HOW TO PERFORM A GENETIC INVESTIGATION?

### When to consider genetic testing

The first clinical situation to consider genetic testing in CHD is the presence of a syndromic phenotype. A comprehensive clinical examination is paramount in recognizing extracardiac involvement. Common physical findings include facial dysmorphism (eye, ear, mouth, nose abnormalities), limb dysmorphism (atrophy, length reduction), hand and feet dysmorphism (polydactyly, short fingers, clinodactyly), and other skeletal abnormalities such as scoliosis<sup>[17]</sup>. Growth delays may be identified by monitoring height and weight and neurological status must be assessed to diagnose mental impairment and learning disabilities. Other organs must be screened to exclude associated gastrointestinal, urologic, and genital defects. Thus, a thorough investigation often involves a multidisciplinary approach including a neurologist, ophthalmologist, otolaryngologist, gastrointestinal specialist, and orthopedic surgeon. Additional paraclinical testing may be guided by the clinical examination: radiography, abdominal ultrasound, cerebral imaging, and laboratory testing (liver and renal function, and others depending on the clinical examination). While investigating newborns in the intensive care unit can be particularly difficult, it is important to identify defects that may benefit from early surgical intervention. Variable expressivity adds a further layer of complexity justifying a broader screening approach. For example, it has been recommended to screen all children with supra-valvular aortic stenosis or pulmonary stenosis for Williams-Beuren syndrome and those with an interrupted aortic arch, truncus arteriosus, TOF, VSD with aortic arch anomaly, isolated aortic arch anomaly, or discontinuous branch pulmonary arteries for

DiGeorge syndrome<sup>[17]</sup>. In general, genetic consultation is recommended when a probable syndromic phenotype is identified.

The second clinical situation to consider genetic testing is in the context of a multiplex family, *i.e.*, a family in which a person diagnosed with CHD has an afflicted first- or second-degree relative. A comprehensive clinical investigation includes a detailed assessment of past medical, surgical, and family histories. A family history can point to a genetically transmitted disease and is important in understanding inheritance patterns (autosomal recessive, dominant, X-linked, and mitochondrial), penetrance, and expressivity of genetic variations. While some have advocated exhaustive family history questionnaires<sup>[18]</sup>, basic themes include screening for cardiac diseases within families, particular phenotypes such as dysmorphias, aborted pregnancies, other birth defects, infertility, and early deaths. Importantly, in some families with CHD, different phenotypes may be expressed such as a bicuspid aortic valve in one family member and hypoplastic left heart syndrome in another. The origin of all four grandparents may provide relevant information, such as the potential for consanguinity. If positive elements are detected, a detailed family tree should be performed that includes each proband's first- and second-degree relatives. The family tree may be further expanded, depending of which side of the family has diseased members. Supportive documents, such as surgical and autopsy reports, should be sought. It is also important to update family pedigrees to include new events over time.

In summary, the phenotypic description associated with the family tree is an essential tool in guiding further genetic investigation. Identification of a clinical feature related to an established syndrome associated with CHD should prompt syndrome-specific investigation. Wider scale screening is recommended on the basis of variable expressivity

for syndromes such as Williams-Beuren and DiGeorge. For non-syndromic CHD, the family tree may orient the clinician towards a genetic etiology and a specific pattern of inheritance. Nevertheless, in the majority of cases, there are no known karyotype abnormalities to investigate. Currently, genetic testing of known cardiac candidate genes is not routinely recommended in the clinical setting. However, genetic testing of multiplex families in the context of research studies may identify novel mutations in known genes or entirely new causal genes.

### **Choosing a genetic test**

An individualized approach to genetic testing begins with the diagnostic hypotheses elicited by a thorough clinical assessment. In general, chromosomal abnormalities represent changes in the structure or number of chromosomes and are diagnosed by cytogenetic methods. The standard metaphase karyotype analysis detects numerical and structural chromosomal aberrations with a resolution of 5 megabases. It is indicated to search for such anomalies as trisomy 21, 18, 13, or monosomy X. Fluorescence in situ hybridization (FISH) is a method to detect deletion or duplication of specific regions of DNA using targeted probes. It provides a higher resolution than karyotype and is the predominant technique used to identify Williams-Beuren, DiGeorge, and Alagille syndromes. Subtelomere FISH analyses, while less commonly used today, provide a high resolution to detect abnormalities in subtelomere (*i.e.*, DNA segments between telomeric caps and chromatin) and telomere (*i.e.*, regions of repetitive nucleotide sequences at each end of a chromatid) DNA regions<sup>[17]</sup>. Subtelomeric anomalies have been reported in patients with a syndromic phenotype associated with facial dysmorphism and mental retardation combined with CHD such as VSD, ASD, pulmonary stenosis, and right sided aortic arch<sup>[19,20]</sup>.

Array-based comparative genomic hybridization (aCGH) is used to detect unbalanced structural and numerical chromosomal abnormalities with a resolution inferior to 5 megabases, such as copy number variants (CNV), *i.e.*, number of copies of a particular gene that deviate from normal (two for autosomes, one X chromosome for males (XY), and two X chromosomes for females (XX)). This molecular karyotype provides rapid identification of duplications/deletions, unbalanced translocations, and aneuploidies. This method analyzes the entire genome and compares it to controls, in contrast to FISH techniques that target specific DNA regions. It may be particularly useful when a probable chromosomal syndrome is identified but the karyotype is normal and there is no known specific region to test<sup>[21]</sup>. Furthermore, this method is of additional value in detecting CNVs such as in screening for DiGeorge syndrome when the karyotype and 22q11 microdeletion analyses by FISH are unrevealing<sup>[20]</sup>. Cytogenetic testing has been recommended for all children with CHD associated with mental retardation, developmental delay, dysmorphic features, or other organ involvement and for establishing a prenatal diagnosis when CHD is identified by fetal echocardiography<sup>[17]</sup>. Most CNV studies in CHD

report 10%-25% of abnormal findings across the disease spectrum.

Gene mutations represent a second category of genetic abnormalities. Mutations can affect the coding portion of a gene, a case in which interpretation is usually straightforward. They can also affect the non-coding portion of the genome, in which case they are more difficult to interpret. With the advent of NextGeneration sequencing technologies, large gene panels, which specifically target genes that are known or suspected to play a role in cardiac biology, can be more readily screened than previously possible by Sanger sequencing<sup>[22,23]</sup>. This approach affords a high quality diagnosis. Gene sequencing can be helpful in conditions such as Noonan syndrome, Alagille syndrome with a normal FISH analysis, Holt-Oram syndrome, and several other diseases.

### **Interpretation of a genetic test**

When a genetic variation is diagnosed, the clinician must determine its relation to the phenotype. Although genetic variants are identified with increasing frequency by high throughput sequencing, not all variants are pathogenic<sup>[22,23]</sup>. Determination of pathogenic potential is based on the following three questions: (1) Has this genetic variant already been described in association with the particular phenotype? (2) Is the genetic variant predicted to alter gene function or regulation, gene coding, or the gene splice site, and does it occur in an evolutionarily conserved nucleotide? and (3) Does the genetic variant segregate with the affected family members and not unaffected members or controls? This assessment is not foolproof. For example, genetic variants may be identified in unaffected family members because of variable penetrance and expressivity. Each genetic result must, therefore, be placed in context of the clinical and family evaluation.

### **Genetic counseling**

Genetic counseling is important before and after genetic testing<sup>[24]</sup>. Prior to testing, the patient or guarantor should be informed of the risks of a negative result arising from the fact that all genes implicated in a given phenotype have not been identified. Second, the pathogenic potential of a genetic variant may be difficult to determine. Third, if a genetic familial disorder is identified, the patient is responsible for informing the family. After genetic testing, counseling is important to review the results, explain the genetic variant, and discuss implications with the patient and family<sup>[25]</sup>.

## **OBJECTIVES OF GENETIC TESTING IN CLINICAL PRACTICE**

### **Confidence in the diagnosis**

Objectives of genetic testing may vary according to the clinical scenario. One objective is to establish confidence in the diagnosis. An accurate diagnosis could allow the clinician to explain causes and mechanisms of disease, provide more precise prognostic information, and elucidate implications for future offspring. Genetic counseling is of

paramount importance in relaying such information<sup>[26]</sup>.

### Appropriate management

**Non-cardiac organ involvement:** An accurate diagnosis could alert the clinician to the possibility of associated non-cardiac organ involvement. Down, Patau, Edward, DiGeorge, Turner, Williams-Beuren, Noonan, and Alagille syndromes all involve extracardiac abnormalities<sup>[27]</sup>.

Craniofacial anomalies have been associated with endocardial cushion defect, truncus arteriosus, and aortic arch anomalies; respiratory disease with endocardial cushion defect and pulmonary valve disease; genitourinary malformations with septal defects, pulmonary valve disease, aortic valve disease, and truncus arteriosus; and situs inversus with heterotaxy and endocardial cushion defect<sup>[27]</sup>.

Establishing a genetic diagnosis could help orient clinical and paraclinical investigations and subspecialty referral for all potential organs involved. Unrecognized and untreated interactions between various organ pathologies could worsen the cardiac prognosis. Identification of a genetic syndrome may also prove useful in the event of an emergency, when a frequent complication associated with a given syndrome occurs. Moreover, recognition of a syndrome provides a more defined guide for follow-up, including surveillance and screening for reported complications.

**Other associated cardiac complications:** In addition to the genetic origins of CHD, genetic variations can modulate the propensity to develop associated cardiac complications, such as arrhythmias<sup>[28]</sup> and heart failure<sup>[29,30]</sup>. Transcription factors play a key role in the formation of cardiac structures and maintenance of cardiac function and, conversely, their dysregulation can have multifaceted manifestations. For example, in the setting of an ASD, those with an NKX2.5 syndrome are more likely to develop atrioventricular block and progressive ventricular dysfunction<sup>[28]</sup>. Interestingly, patients with NKX2.5 mutations can also develop dilated cardiomyopathy<sup>[31]</sup>. *TBX5*, a gene implicated in Holt-Oram syndrome (septation defects, atrioventricular node disease, and upper limb defects) also modulates diastolic function<sup>[32]</sup>. Genes implicated in RASopathy syndromes responsible for Noonan, Leopard, cardiofaciocutaneous, and Costello syndromes are also responsible for cardiac hypertrophy in later development<sup>[33]</sup>. Thus, the genetic environment could modulate the prognosis of various forms of CHD, help to elucidate risks of developing conduction defects and systolic and diastolic dysfunction, and provide a basis to adapt follow-up accordingly.

### Overlap of CHD with muscular heart disease:

Structural CHD and cardiomyopathy may be modulated by the same mutations that give rise to varied phenotypes within the same family. For example, some family members with a *TBX20* mutation may have an underlying ASD, VSD, or mitral valve disease or may present exclusively with pulmonary hypertension or cardiomyopathy<sup>[14]</sup>. Mutations in *MYH6* (alpha-cardiac myosin heavy chain)

are associated with various forms of CHD but also dilated and hypertrophic cardiomyopathy<sup>[34]</sup>. Moreover, mutations in *MYH7* have been reported in patients with Ebstein anomaly and left ventricular noncompaction<sup>[35,36]</sup>. Some family members may have CHD whereas others could develop progressive cardiomyopathy or electrophysiologic disorders. Thus, if a mutation is discovered in a family with a discordant phenotype, clinical screening and genetic testing can identify seemingly phenotypically normal individuals who are at risk of developing cardiomyopathy or electrophysiologic manifestations.

**Prognosis:** In addition to the prognostic implications of genetic factors discussed above, certain gene defects have been associated with post-operative survival and long-term outcomes. For example, endothelin-1 G5665T has been associated with transplant-free survival in patients with single ventricles, primarily hypoplastic left heart syndrome<sup>[37]</sup>. This variant is linked to increased vascular reactivity and hypertension. Similarly, in a study of genetic variants involved in vascular response and oxidative stress, two major alleles of two single nucleotide polymorphisms (SNPs; *i.e.*, *VEGFA* rs833069 and *SOD2* rs2758331) were associated with worse transplant-free survival in patients with non-syndromic CHD<sup>[38]</sup>. The higher number of copies of deleterious alleles, the worse the prognosis<sup>[38]</sup>. Genotype has also been associated with early postoperative outcomes. For example, in patients with TOF, 22q11.2 deletion (DiGeorge syndrome) predicts a longer cardiopulmonary bypass time and a greater length of stay in intensive care<sup>[39]</sup>. While several explanations have been proposed, potential factors include a higher prevalence of aortopulmonary shunts and respiratory problems prior to surgical repair in patients with 22q11.2 deletion, resulting in longer mechanical ventilatory support. Conceivably, a SNP profile may one day prove to be of value in pre-operative risk assessment.

**Therapeutic potential:** Ultimately, the holy grail of genetically diagnosing CHD is to provide targeted curative therapy. While such interventions are currently beyond our reach, provocative studies support its potential. For example, a knock-out model of *Wnt2* in null mutants results in a phenotype resembling complete atrioventricular septal defect<sup>[40,41]</sup>. The phenotype could be rescued *in vivo* by pharmacological activation of *Wnt* signalling.

### Genetics and recurrence risk

With a Mendelian pattern of inheritance, recurrence risks are 50% and 25% for autosomally dominant and recessive genes, respectively. However, variable penetrance complicates these predictions, even for syndromic CHD. In the majority of cases with CHD, difficulties in estimating recurrence risks are compounded by the absence of a clear genetic diagnosis<sup>[42,43]</sup>. Estimates are, therefore, largely based on a detailed family tree and the published literature<sup>[18]</sup>.

In patients with atrial septal defects, the recurrence



**Table 2** Recurrence risks for non-syndromic congenital heart disease in first-degree relatives

Type of non-syndromic CHD	Recurrence risk of same CHD in first-degree relatives (%)	Recurrence risk of discordant CHD in first-degree relatives (%)	Recurrence risk of any CHD in first-degree relatives (%)
ASVD	1.10	2.2	3.30
ASD	0.88	2.4	3.28
VSD	0.67	1.9	2.57
ASD and VSD	0.24	2.2	2.44
Conotruncal defect <sup>1</sup>	1.30	2.4	3.70
Right ventricular outflow tract obstruction <sup>2</sup>	1.70	3.0	4.70
Left sided obstructions <sup>3</sup>	0.79	2.4	3.19

The recurrence risks for non-syndromic CHD in first-degree relatives are derived from a Danish cohort study<sup>[10,56]</sup>. <sup>1</sup>Tetralogy of fallot, truncus arteriosus, interrupted aortic arch, double outlet ventricle, transposition of the arteries; <sup>2</sup>Pulmonary valve stenosis, infundibular or subvalvular stenosis, double chambered right ventricle; <sup>3</sup>Bicuspid aortic valve, aortic coarctation, aortic stenosis, hypoplastic left heart, shone complex. First-degree relatives include parents, siblings and twins; CHD: Congenital heart disease; ASVD: Atrioventricular septal defect; ASD: Atrial septal defect; VSD: Ventricular septal defect.

risk has been estimated to be 3% in first-degree relatives, although a dominant inheritance pattern has been described in some families. A CHD recurrence risk of 1.2% was reported for first-degree relatives with an isolated septal defect<sup>[10]</sup>. For probands with atrioventricular septal defects, the prevalence of any CHD in a family member appears to be in the order of 12%-15% overall, 1%-2% of parents, 2%-4% of siblings, and 10%-14% of offspring<sup>[44-46]</sup>. Risk of recurrence is greater if the mother rather than the father has the atrioventricular septal defect (*i.e.*, 14% vs 10%). Nevertheless, exact figures remain debated with some studies reporting considerably lower risks<sup>[10]</sup>. In TOF, the recurrence risk has been estimated to be 2.5%-3% overall, with a phenotype that is often concordant<sup>[44,47]</sup>. However, the recurrence risk in offspring is higher when the mother is affected<sup>[9]</sup>. Moreover, some families without a 22q11 deletion syndrome have been suspected of having a recessive inheritance pattern<sup>[48]</sup>. In complete transposition of the great arteries, a very low recurrence risk has been described with no offspring affected in a British collaborative study, suggesting a sporadic model<sup>[9]</sup>. Other studies have reported a recurrence risk of 1.8% in siblings<sup>[49]</sup> and 2.7% in first-degree relatives (siblings and parents)<sup>[50]</sup>, which includes varied forms of CHD such as aortic valve stenosis and double outlet right ventricle<sup>[50]</sup>. In patients with congenitally corrected transposition of the great arteries, a 5.2% recurrence risk was reported in siblings, with concordant and discordant phenotypes, including complete transposition of the great arteries, suggesting that some genes may be common to both types of transposition<sup>[51]</sup>.

Left-sided obstructive lesions (*e.g.*, aortic coarctation, hypoplastic left heart syndrome, aortic stenosis, bicuspid aortic valve, and hypoplastic aortic arch) may segregate within families, suggesting a common genetic basis<sup>[52,53]</sup>.

Overall recurrence risks have ranged from 1.8% to 3.2% of siblings, 3% of offspring of affected fathers, and 8% to 13% of offspring of affected mothers<sup>[52]</sup>. However, much higher recurrence risks have been described in certain geographic locations, such as 37% of first-degree relatives in Texas<sup>[52]</sup>. Moreover, some defects appear to have higher recurrence risks, such as aortic coarctation (13% of siblings)<sup>[50]</sup>, hypoplastic left heart syndrome (31% of siblings)<sup>[50]</sup>, and bicuspid aortic valves (> 10% of siblings)<sup>[54,55]</sup>. However, considerably lower recurrence risks for left-sided obstructive lesions in first degree relatives have also been reported (*e.g.*, 0.79% with a relative risk of 12.9)<sup>[10]</sup>.

As noted by the examples above, estimating recurrence risks is an imperfect science. Empiric estimates consider the mathematical prediction of recurrence in a polygenic model of inheritance combined with the type of CHD, current knowledge base, and relationship to proband. As a general rule of thumb, recurrence risks are in the order of 1% to 6% for siblings of affected probands with unaffected parents and increase to approximately 10% when two siblings are affected. Recurrence risks in offspring are greater than siblings, higher if the proband is the mother<sup>[3]</sup>, and generally higher for left-sided obstructive lesions (8%-10%). A recent population-based study from Denmark challenges these statistics and provides far lower estimates for first-degree relatives than previously reported, as summarized in Table 2<sup>[10]</sup>. These disparate results could be explained, in part, by differences in the study designs and methodologies employed, and underscore the difficulties in accurately quantifying recurrence risks. Estimating recurrence risks must consider an in depth analysis of the family history to identify specific patterns of inheritance. If the pedigree is not informative and estimates are based on a polygenic model of inheritance, limitations of empiric estimates should be discussed with the patients, including the possibility of under- or overestimation. The notion of concordant or discordant recurrent phenotypes should also be conveyed. Overall, exact concordance is low for left-sided obstructive defects (26%), intermediate for outflow tract defects (37%), and higher for septal defects (48%)<sup>[43]</sup>. Conceptually, CHD may be grouped into constellations of malformations such as septal defects, conotruncal anomalies, and left-sided obstructive lesions that share implicated genes, although such a concept is not universally supported<sup>[56]</sup>.

### Assessing family members

A strong case has been made for screening first-degree relatives of patients with left-sided obstructive lesions and bicuspid aortic valves. As previously noted, recurrent phenotypes in first-degree relatives are relatively common and frequently discordant such that a bicuspid aortic valve, aortic coarctation, and/or aortic dilation may be identified in asymptomatic family members. Echocardiographic screening has been recommended for first-degree relatives of patients with bicuspid aortic valve or supra-aortic stenosis, since a physical examination alone lacks sensitivity<sup>[57]</sup>. The rationale for family screening is that early

detection may help avert complications related to aortic dilatation (e.g., 6-fold higher risk of aortic dissection), aortic stenosis, aortic insufficiency, endocarditis, and aortic coarctation (e.g., arterial hypertension). Early detection may lead to lifestyle recommendations (e.g., limit isometric exercises), enhanced monitoring (e.g., for progressive aortic dilatation), or preventive surgery (e.g., prior to aortic dissection). Age at screening remains controversial. It should generally be proposed to adults if not previously performed during childhood.

At present, systematic screening of first-degree relatives is not recommended for other forms of non-syndromic CHD. However, fetal echocardiographic screening is indicated if either parent is afflicted with any form of CHD. It should be performed in a specialized center at 18-20 wk of gestation<sup>[58]</sup>. Early detection of complex CHD can drastically improve outcomes by planning delivery in a specialized (level 3) tertiary care center with appropriate monitoring and early catheter-based or surgical interventions when indicated<sup>[58-60]</sup>. Furthermore, prenatal diagnosis may lead to a parental decision to terminate the pregnancy.

Finally, identification of a specific mutation in a multiplex family with CHD may allow for targeted screening of additional family members. While there is no clear-cut indication for genetic screening to identify CHD in family members with structurally normal hearts, there may be a rationale to screen seemingly normal family members for entities that include CHD as one aspect of a multiple constellation phenotype.

### **Prenatal diagnosis**

The impact of a prenatal diagnosis of CHD on the pregnancy termination rate varies by region. For example, reported pregnancy termination rates for severe CHD identified by prenatal screening were 45% in the Netherlands<sup>[58]</sup>, 49% in Boston, MA<sup>[61]</sup>, and 86% in Switzerland<sup>[62]</sup>. In a study from France, factors associated with pregnancy termination included severity of CHD, gestational age at diagnosis, presence of chromosomal abnormalities, and parental ethnicity<sup>[63]</sup>.

Fetal genetic screening for CHD is also possible, including genome-wide high-resolution SNP arrays to identify CNVs<sup>[64]</sup> and competitive genomic hybridization to detect submicroscopic chromosomal aberrations<sup>[65]</sup>. A prenatal diagnostic test can be performed after chorionic villus sampling before 14 wk of gestation. Thus far, such testing has been limited to specific disease entities such as trisomy 21, 18, and 13, cystic fibrosis, and microdeletion syndromes (e.g., DiGeorge). It could also be performed for any severe monogenetic disease if the result could influence the decision to terminate pregnancy<sup>[66]</sup>. Preimplantation diagnostic testing could be proposed in selected cases, particularly for women with a history of multiple therapeutic abortions. It has already been used for Holt Oram and Marfan syndromes<sup>[67]</sup>. Beyond syndromes such as trisomy 21, 18 or 13, prenatal or preimplantation genetic screening remains controversial. Ethical dilemmas may arise as a result of uncertainties in interpreting tests, potential for false positives, and

the inability to predict disease severity, penetrance and expressivity of a mutation, and concordant or discordant phenotypes.

### **Limitation of genetics in CHD**

Despite the fact that CHD is the most common birth defect, the genetic etiology remains unknown in the majority of cases, with slower progress than for other forms of heart disease such as inherited arrhythmia syndromes and hypertrophic and dilated cardiomyopathy. Genetic studies in CHD were traditionally restricted to multiplex families with strong phenotypic penetrance, which represent the minority of cases<sup>[40]</sup>. The relatively low familial recurrence risk is not fully understood but may be due, in part, to *de novo* mutations, incomplete penetrance, and other etiological factors such as environmental influences. Patterns of inheritance may be difficult to sort out in the presence of environmental interactions, age-dependent or incomplete penetrance, and variable expressivity. In addition, genetic analysis based on individual families requires a large number of members or consanguinity<sup>[11]</sup>. Moreover, mutations may involve non-exonic DNA, such as regulatory regions, the functional validation of which is more difficult and resource consuming. Establishing genotype-phenotype correlations may be further complicated by mutations that are rare and unique to individual families<sup>[2]</sup>. In fact, most CHD mutations identified to date appear to be private or do not recur. Despite these numerous limitations, genetics has and will hopefully continue to provide insights into the etiology of CHD, embryonic heart development, potential therapeutic targets, risk assessment, and patterns of inheritance.

### **Future perspective**

Objectives of genetic testing for clinical reasons differ from research goals. From a clinical perspective, a genetic test should be directly relevant to a patient by serving the purpose of establishing or confirming a diagnosis, providing prognostic information, informing therapeutic decisions, and/or assisting with family planning. In contrast, genetic testing for research purposes may provide pathophysiological insights into a disease entity and identify potential therapeutic targets, thereby carrying the potential to impact care at a longer-term horizon. Nevertheless, genetic results derived from research studies are generally communicated to the clinical team and may directly contribute to the care of a given family<sup>[68]</sup>. It is important, therefore, for the clinical team to be well versed in the domain in order to effectively communicate with the patient, explain results, and establish an appropriate surveillance plan. In parallel, genetic testing within clinical laboratories may discover new mutations in known genes and novel implicated genes, particularly when modern technologies that sequence a broad array of genes are applied. In the future, therefore, enhanced partnerships between clinical and research teams could maximize the potential for progress. Resources available for research, including highly qualified personnel, informatics infrastructures, laboratory equipment, novel platforms, and more rapid time to analyses could complement the

clinical laboratory setting in enhancing clinical care. Greater integration between clinical and research teams could also contribute to ensuring that discoveries are progressive and clinically meaningful, with direct applications to patient care. Along these lines, the multicenter prospective “CHD GENES” study was initiated in December 2010 to explore relationships between genetic factors, clinical features, and outcomes in patients with CHD<sup>[2]</sup>.

## CONCLUSION

Despite major inroads over the past few decades in genetics related to CHD, the majority of patients with CHD are without a genetic diagnosis such that the etiology of their CHD remains incompletely understood. In this article, we discussed the multifaceted implications of genetics in CHD including the potential for personalized care, confidence in the clinical diagnosis, prognostic implications, early identification of non-cardiac organ involvement and associated complications, and tailoring clinical follow-up. Genetic testing could also provide valuable information in predicting recurrence risk, defining the pattern of inheritance, screening family members, and family planning. Various methodologies are available to diagnose chromosomal abnormalities and gene mutations. The challenge lies in first identifying potential genetic etiologies, selecting the appropriate test, and interpreting the test within the context of available knowledge. Collaboration between clinicians and genetics researchers offers the best opportunity for progress in clinical care and innovative breakthroughs<sup>[69,70]</sup>. Much remains to be discovered in tapping the potential of genetics in CHD.

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## Diagnosis and management of patients with asymptomatic severe aortic stenosis

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### Abstract

Aortic stenosis (AS) is a disease that progresses slowly for years without symptoms, so patients need to be carefully managed with appropriate follow up and referred for aortic valve replacement in a timely manner. Development of symptoms is a clear indication for aortic valve intervention

in patients with severe AS. The decision for early surgery in patients with asymptomatic severe AS is more complex. In this review, we discuss how to identify high-risk patients with asymptomatic severe AS who may benefit from early surgery.

**Key words:** Aortic stenosis; Asymptomatic; Diagnosis; Management; Treatment

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**Core tip:** We focused on how to identify high-risk patients in asymptomatic aortic stenosis. Revised American Heart Association/American College of Cardiology guidelines and diagnostic testing for appropriate clinical decision making are discussed in this article.

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### INTRODUCTION

As a result of the aging population, aortic stenosis (AS) is currently one of the most common valvular heart diseases to need surgical intervention. AS is a slowly progressive chronic condition, but once a patient becomes symptomatic, the prognosis is dismal. Although percutaneous valve technology is now approved for high-risk patients with symptomatic AS, clinical management of asymptomatic patients with severe AS is still difficult. Assessment of symptoms in sedentary elderly patients with severe AS is often challenging. It is common for patients to have nonspecific symptoms such as shortness of breath or general feelings of weakness that can be



explained by many reasons other than cardiac diseases. Advances in multiple modality imaging provide additional objective information about subtle functional deterioration of the left ventricle (LV), myocardial tissue damage, and the amount of the valve calcification. Existing and new parameters are investigated to improve the clinical decision-making process.

In this review, we focus on recent advances in diagnostic methods for assessment of AS and discuss how to implement these methods in current clinical practice as it relates to the management of patients with asymptomatic severe AS.

## PATHOPHYSIOLOGY AND HEMODYNAMICS OF AS

A normal aortic valve is tricuspid and a normal valve opening area is 3 to 4 cm<sup>2</sup>. Progression from aortic valve sclerosis to AS is reported to be 9% per 5 years<sup>[1]</sup>. There is some evidence to suggest that NOTCH 1 genetic mutations and specific lipoprotein polymorphism is associated with congenital AS and valve calcification<sup>[2]</sup>. In AS, it has been reported that an average rate of increase in mean gradient is 7 to 8 mmHg/year; in maximum velocity, 0.2 to 0.4 m/s per year; and in a decrease in valve area, 0.1 to 0.15 cm<sup>2</sup>/year<sup>[3-6]</sup>. Hemodynamic progression of AS is gradual and linear, though there is variability and some patients present with rapid progression. Presence of aortic valve calcification, coronary artery disease, advanced age, renal impairment, and baseline AS severity are risk factors for rapid progression<sup>[4,7-9]</sup>.

The hemodynamic progression of AS lead to LV hypertrophy (LVH) as a compensation mechanism of the heart. Morphological changes such as increasing muscle fiber thickness, collagen volume, and interstitial fibrosis occur in AS patients<sup>[10]</sup>. These changes result in LV diastolic and systolic dysfunction<sup>[11]</sup>. LV mass regression starts soon after aortic valve replacement (AVR) and may continue through another 8 years postoperatively, while diastolic dysfunction persists up to 2 years due to the relatively increased amount of fibrotic tissue in the myocardium<sup>[12-14]</sup>. These results may encourage AVR before the fibrotic change becomes too substantial or irreversible to advance postoperative recovery. Thus, the amount of myocardial structural change in AS can be a good parameter to define the severity of AS, and the new imaging technique gains greater prominence to determine the timing of surgical intervention in asymptomatic severe AS<sup>[15]</sup>.

When the valve area is decreased to one-fourth of the normal valve area (0.75-1.00 cm<sup>2</sup>), in general, patients develop symptoms, although there is high inter-individual variability. A fundamental principle of fluid dynamics is that flow velocity within the conduit depends on volumetric flow rate. Patients with normal LVEF (LV ejection fraction) and normal flow will generally have a mean gradient > 40 mmHg in the setting of severe AS. However, recent studies have identified a

new entity, termed "paradoxical low-gradient severe AS", where the stroke volume is reduced in the setting of increased afterload and concentric LVH, resulting in a low gradient despite severe AS in the setting of normal LVEF. Recent American College of Cardiology/American Heart Association (ACC/AHA) guidelines have recognized this entity and have developed guidelines for management of this new group of AS patients.

## DEFINITION OF AS

AHA/ACC guidelines for the management of patients with valvular heart disease, which was revised in 2014, has a major change for the staging of AS (Table 1)<sup>[16]</sup>.

It should be noted that the stage D3 definition is based on a relatively new concept related to the progression of AS. Specifically, low-flow/low-gradient AS with preserved EF represents a more advanced stage of AS with severe concentric hypertrophy, high peripheral arterial pressure, and low systemic arterial compliance<sup>[17-19]</sup>. Valvulo-arterial impedance (Zva) calculated as shown below was introduced as a global hemodynamic load on the LV in AS<sup>[17,20,21]</sup>.  $Zva = (\text{systolic blood pressure} + \text{mean gradient of aortic valve})/\text{stroke volume index}$ .

More importantly, low-flow/low-gradient severe AS is reported to have a poorer prognosis without surgical intervention in some studies<sup>[17,22,23]</sup>, while other studies report a better prognosis-similar to the prognosis of patients with moderate AS<sup>[24-26]</sup>. Nevertheless, the majority of evidence is in favor of the new entity termed "paradoxical low-gradient AS", in which LVEF is preserved yet the mean aortic valve gradient is low due to low stroke volume.

This condition must be diagnosed with utmost caution, avoiding measurement errors and, in some cases, establishing additional diagnostic methods such as cardiac catheterization or other imaging studies, including magnetic resonance imaging (MRI) and computed tomographic (CT) scans.

Current ACC/AHA guidelines put much more focus on velocity/pressure gradient findings than on aortic valve area (AVA) given that prior natural history studies show their prognostic importance. Namely, aortic velocity (> 4 m/s) is reported to be one of the most important factors associated with a higher event rate in AS<sup>[4,5,27]</sup>. Asymptomatic patients with very severe AS with a  $V_{max} \geq 5$  m/s or mean gradient  $\geq 60$  mmHg have an even worse event-free survival<sup>[28]</sup>.

## DIAGNOSTIC TESTING

### Echocardiography for diagnosis of severe AS

Two-dimensional/Doppler echocardiography plays a fundamental role in the diagnosis of AS. It is important to examine the etiology of AS, visual severity of valve calcification, position of the coronary artery orifice, concomitant myocardial disease, wall motion asynergy, and other valvular heart diseases with echocardiography.



**Table 1 Stages of aortic stenosis on the basis of American College of Cardiology/American Heart Association recommendations**

		Hemodynamics	LV Function	AVA	Aortic valve
A	At risk of AS	$V_{\max} < 2$ m/s	Normal EF	-	Bicuspid, sclerosis
B	Progressive AS	Mild AS: $V_{\max} < 2.0$ - $2.9$ m/s or mean $\Delta P < 20$ mmHg Moderate AS: $V_{\max} > 3.0$ - $3.9$ m/s or mean $\Delta P > 20$ - $39$ mmHg	Normal EF Early diastolic dysfunction	-	Mild to moderate calcification Reduction in motion Commissural fusion
C1	Asymptomatic severe AS	$V_{\max} \geq 4$ m/s or mean $\Delta P \geq 40$ mmHg	Normal EF Diastolic dysfunction	$\leq 1.0$ cm <sup>2</sup> or $\leq 0.6$ cm <sup>2</sup> /m <sup>2</sup>	Severe calcification Severely reduced opening
C2	Asymptomatic severe AS with LV dysfunction	$V_{\max} \geq 4$ m/s or mean $\Delta P \geq 40$ mmHg	EF < 50%	$\leq 1.0$ cm <sup>2</sup> or $\leq 0.6$ cm <sup>2</sup> /m <sup>2</sup>	Severe calcification Severely reduced opening
D1	Symptomatic severe high-gradient AS	$V_{\max} \geq 4$ m/s or mean $\Delta P \geq 40$ mmHg	EF normal or decreased diastolic dysfunction	$\leq 1.0$ cm <sup>2</sup> or $\leq 0.6$ cm <sup>2</sup> /m <sup>2</sup> Larger with AR/MR	Severe calcification Severely reduced opening
D2	Symptomatic severe low-flow/low-gradient AS with reduced LVEF	$V_{\max} < 4$ m/s or mean $\Delta P < 40$ mmHg DOB stress shows $V_{\max} > 4$ m/s and AVA $\leq 1.0$ cm <sup>2</sup>	EF < 50% diastolic dysfunction	$\leq 1.0$ cm <sup>2</sup>	Severe calcification Severely reduced opening
D3	Symptomatic severe low-gradient AS with normal LVEF or paradoxical low-flow severe AS	$V_{\max} < 4$ m/s or mean $\Delta P < 40$ mmHg Stroke volume index < 35 mL/m <sup>2</sup>	EF $\geq 50\%$ Small LV chamber Restrictive diastolic filling	$\leq 1.0$ cm <sup>2</sup> or $\leq 0.6$ cm <sup>2</sup> /m <sup>2</sup>	Severe calcification Severely reduced opening

Modified from Nishimura *et al*<sup>[16]</sup> with permission. ACC/AHA: American College of Cardiology/American Heart Association; AR: Aortic regurgitation; AS: Aortic stenosis; AVA: Aortic valve area; EF: Ejection fraction; LV: Left ventricular; MR: Mitral regurgitation;  $\Delta P$ : Pressure gradient;  $V_{\max}$ : Maximum aortic velocity.

Echocardiography can provide systolic and diastolic functions. All parameters referred to in guidelines are available by echocardiography, which sometimes needs careful data interpretation while recognizing limitations.

An important consideration in echocardiography is to detect the highest peak aortic flow velocity using multiple transducer positions (the suprasternal window and right parasternal window with right decubitus position should be used in addition to the apical window). The Pedoff probe, which has a high signal to noise ratio, is ideal to detect the highest velocity. This requires advanced operator skill and, therefore, missing the highest velocity in AS is one of the causes of underestimation of the gradient.

The pressure gradient is calculated according to the modified Bernoulli equation; the pressure gradient =  $4 \times v^2$ . However, if there is an increased velocity ( $> 1.5$  m/s) at the LV outflow tract (LVOT) by septal thickening or by systolic anterior motion of the mitral valve, this simplified equation is less reliable. In those cases, it is recommended that the corrected peak to peak gradient should be used<sup>[29]</sup>.

AVA is calculated by a continuity equation. Measurement of the LVOT size for stroke volume calculation is the second possible error for diagnostic severity. American Society of Echocardiography guidelines recommend the measurement at the same position of the pulse wave sample volume, specifically 0.5 to 1 cm below the aortic annulus<sup>[29]</sup>. Accurate measurement of LVOT diameter is critical, as the continuity method requires squaring of this measurement. Even an error of a few millimeters in this measurement can lead to large differences in the

calculated valve area.

Additionally, appropriate position of the pulse-wave Doppler signal to avoid flow acceleration by calcified valve or outflow obstruction is important. Overestimation or underestimation of stroke volume can lead to an unreliable calculation of AVA. In some patients, one may also encounter dynamic LVOT obstruction with flow acceleration in the LVOT. In these patients, one must calculate stroke volume either by two-dimensional or three-dimensional volumetric methods. One can also use RVOT diameter and Doppler signals at the right ventricular outflow tract to calculate stroke volume.

AVA can also be measured by planimetry, both by transthoracic echocardiography and transesophageal echocardiography<sup>[30]</sup>. The planimetry method has its own limitations. Shadowing by calcification interferes with the visualization of the valve edge. The anatomical orifice area can be measured larger than the effective orifice area. Nonplanar structures of the valve may cause difficulty in reliable measurement, which is improved by real-time three-dimensional echocardiography<sup>[31]</sup>. With careful attention to these limitations, planimetry can be considered an alternative/complimentary measure when Doppler measurements are not appropriate.

A low-dose dobutamine stress echocardiography is performed to diagnose true or pseudo AS in low-gradient, reduced EF patients (though usually symptomatic, patients rarely present with low LVEF and gradient without any symptom). In addition, low-dose dobutamine echocardiography can identify high-risk patients who do not have contractile reserve, *i.e.*, an increase in stroke volume  $\geq 20\%$ . Loss of contractile reserve suggests a

patient may have other myocardial disease or advanced stages of severe AS. A maximum velocity  $\geq 4.0$  m/s with AVA  $\leq 1.0$  cm<sup>2</sup> at any flow rate during dobutamine stress echocardiography is diagnosed as true severe AS<sup>[16,29]</sup>. Pseudo AS would show an increase of valve area to  $> 1.0$  cm<sup>2</sup>. Although suggested, evidence for the use of dobutamine stress evaluation of low-flow/low-gradient AS with preserved EF ("paradoxical low-gradient severe AS") to diagnose true/pseudo AS is limited.

Diastolic dysfunction is an important parameter in the evaluation of AS. Worsening of diastolic function is related to age and other comorbidities, such as hypertension, that are not uncommon in the elderly. It has been reported by Park *et al.*<sup>[32]</sup> that echocardiographic markers of diastolic dysfunction, such as increased E/e' and left atrial volume index, are associated with dyspnea in severe AS patients. Increased E/e' ( $> 15$ ) has been shown to predict survival in both asymptomatic and symptomatic patients with AS (adjusted mortality risk = 2.34; 95%CI: 1.27-4.33)<sup>[33]</sup>. Although echocardiographic measures of diastolic dysfunction are markers of worse AS, current guidelines do not support its use in surgical decision making in patients with either symptomatic or asymptomatic AS.

Recent advances in echocardiography led to the development of newer methods to detect subtle changes in LV function beyond EF. Specifically, two-dimensional speckle-tracking echocardiography has been used in numerous research studies to detect early systolic functional deterioration in cardiomyopathies, including amyloidosis and hypertrophic cardiomyopathy. Global longitudinal strain (GLS) by two-dimensional speckle-tracking echocardiography is decreased in severe AS and can be used as a prognostic measure. Kearney *et al.*<sup>[34]</sup> reported that decreased GLS ( $> -15\%$ ) in asymptomatic severe AS with preserved EF had poor survival when compared to patients with GLS  $\leq -15\%$ , and GLS was a predictor of all-cause mortality (HR = 1.42; 95%CI: 1.27-1.59). Using echocardiography, van Dalen *et al.*<sup>[35]</sup> and Staron *et al.*<sup>[36]</sup> reported that increased apical rotation is more common in AS patients than control patients. In general, worsening systolic longitudinal motion, apical rotation, and diastolic untwisting working in concert are manifestations of progressive AS. Further research studies and standardization of analyzing software are necessary to incorporate these measurements into current clinical practice, specifically their role in surgical decision making for patients with asymptomatic severe AS.

### CT calcification score

Aortic valve calcification (AVC) is a prognostic factor in asymptomatic AS. Rosenhek *et al.*<sup>[27]</sup> evaluated the degree of AVC using echocardiography and showed moderate and severe AVC related to future death or development of symptoms (RR = 5.2; 95%CI: 2.4-13.5). However, the definition of the degree of AVC by echocardiography has not been established. Therefore, evaluation of AVC by echocardiography is still a qualitative and subjective measure that is dependent on the echocardiographer's experience. A cardiac CT scan can provide quantitative

measure of AVC, and  $> 1000$  Agaston units can be considered severe calcification<sup>[16,37]</sup>. Currently, a cardiac CT scan can be used as a complementary method for the diagnosis and management of AS<sup>[16]</sup>. When it is difficult to judge the severity of AS due to discordant measurements in echocardiography or a possible paradoxical low-flow/low-gradient AS, CT imaging can help to provide the calcium score, which relates to stenosis severity and prognosis<sup>[38,39]</sup>. Due to the recent rapid development of transcatheter aortic valve replacement (TAVR) procedures, the cardiac CT scan has emerged as a key imaging modality not only to assess aortic valve and root calcification, but also for precise measurement of the aortic annulus and peripheral arteries<sup>[40-42]</sup>. Whether or not three-dimensional LVOT measurements by CT imaging should replace echocardiography in order to resolve the measurement error issue is still uncertain, and warrants further research<sup>[43,44]</sup>.

### Magnetic resonance imaging

Magnetic resonance imaging (MRI) has the advantage of providing more accurate anatomical and hemodynamic information, LV mass, and stroke volume than echocardiography. In addition, cardiac MRI with gadolinium contrast can provide information about fibrosis or collagen deposition of the myocardium, which is a consequence of long-term exposure to substantial afterload. The presence or absence of myocardial fibrosis in any cardiac disease is an important prognostic factor<sup>[45-48]</sup>. Dweck *et al.*<sup>[49]</sup> performed contrast-enhanced cardiac MRI in 143 AS patients, and the reported late gadolinium enhancement in the mid wall was a predictor of all-cause mortality (HR = 8.59; 95%CI: 1.97-37.38). Further research using more sensitive methods, *i.e.*, T1 mapping, to detect myocardial fibrosis in AS patients, as well as prognostic studies linking fibrosis to better outcomes, are warranted before MRI can be used in routine clinical practice for the management of patients with AS<sup>[50]</sup>.

### Biomarkers in AS

Brain natriuretic peptide (BNP) is thought to be a good marker of increased wall stress in the myocardium, thus BNP increases with age, the presence of hypertension, valvular heart disease, and other myocardial diseases. It has been reported that BNP increases along with the severity of AS, but considerable overlap between the groups has also been observed. Bergler-Klein *et al.*<sup>[51]</sup> reported that asymptomatic severe AS patients whose plasma BNP was  $< 130$  pg/mL rarely developed symptoms for 6 to 9 mo. Another study showed that a BNP  $\geq 300$  pg/mL was a poor prognostic factor in medically followed severe AS patients who were both symptomatic and asymptomatic<sup>[33]</sup>. More recently, Clavel *et al.*<sup>[52]</sup> reported that moderate/severe asymptomatic AS patients with BNP clinical activation and an elevated BNP greater than the upper normal range of the same age/sex have a higher rate of mortality (HR = 2.35; 95%CI: 1.57-3.56). Recently published research is summarized in Table 2. Disadvantages of BNP include

**Table 2** High-risk patients predicted from brain natriuretic peptide level

Source	BNP cut-off value	Results	Enrolled patients
Bergler-Klein <i>et al</i> <sup>[51]</sup>	BNP 130 pg/mL	BNP < 130 pg/mL ( <i>n</i> = 25) had better symptom-free survival ( <i>P</i> < 0.001)	Asymptomatic severe AS, EF ≥ 50% ( <i>n</i> = 43)
Biner <i>et al</i> <sup>[33]</sup>	BNP 300 pg/mL	Combined use of BNP > 300 pg/mL and E/e' > 15 predicted 1-yr mortality (hazard ratio = 2.59; 95%CI: 1.21-5.55, <i>P</i> = 0.014)	Severe AS, symptomatic and asymptomatic, any EF included ( <i>n</i> = 79)
Berger-Klein <i>et al</i> <sup>[54]</sup>	BNP 550 pg/mL	BNP ≥ 550 pg/mL showed poorer survival both in medically and surgically treated groups	Indexed effective orifice area ≤ 0.6 cm <sup>2</sup> /m <sup>2</sup> with low-flow/low-gradient AS; symptomatic and asymptomatic, with EF ≤ 40% ( <i>n</i> = 69)
Clavel <i>et al</i> <sup>[52]</sup>	BNP ratio: Measured BNP/maximal-normal-BNP for age and sex	Higher BNP ratio showed worse mortality in asymptomatic patients with preserved EF (hazard ratio = 2.35; 95%CI: 1.57-3.56, <i>P</i> < 0.0001)	Total, moderate or severe AS, any EF ( <i>n</i> = 1953) Asymptomatic, with EF > 50% ( <i>n</i> = 565)

AS: Aortic stenosis; BNP: Brain natriuretic peptide; EF: Ejection fraction.

that fact that it is not disease-specific, and BNP levels vary even in the same patient according to physical activities and loading conditions. Therefore, a single value of BNP may not be helpful in surgical decision making in asymptomatic severe AS patients. However, serial measures and rising levels of BNP can be used for surgical decision making in asymptomatic severe AS patients, as proposed by European Society of Cardiology (ESC) guidelines<sup>[53]</sup>.

### Stress testing in AS

Symptom onset is the key to referring severe AS patients for AVR because of a poor prognosis without AVR. However, it is challenging in some patients who claim to be asymptomatic yet have severe AS. In order to risk-stratify high-risk asymptomatic patients, an exercise test, such as the standard treadmill test, without imaging is reasonable according to recently published guidelines<sup>[16,55-57]</sup>. Development of symptoms early on in exercise treadmill testing or an abnormal blood pressure response (below baseline or an inadequate increase of blood pressure < 20 mmHg) are considered indications for surgery in patients with severe AS; however, exercise testing is contraindicated in patients with symptomatic severe AS (Class III)<sup>[16]</sup>. Although ESC guidelines<sup>[53]</sup> have suggested the use of an increased mean gradient during exercise testing (> 20 mmHg) as an indication for surgery in asymptomatic patients (Class II b), it was not supported in the more recent ACC/AHA guidelines<sup>[16]</sup>.

### Cardiac catheterization

Catheterization has the risk of a small cerebral emboli when the wire crosses the valve<sup>[58]</sup>; thus, catheterization is recommended only when there is discrepancy between noninvasive testing, clinical examination, and clinical presentation.

## MANAGEMENT

### Indications for AVR

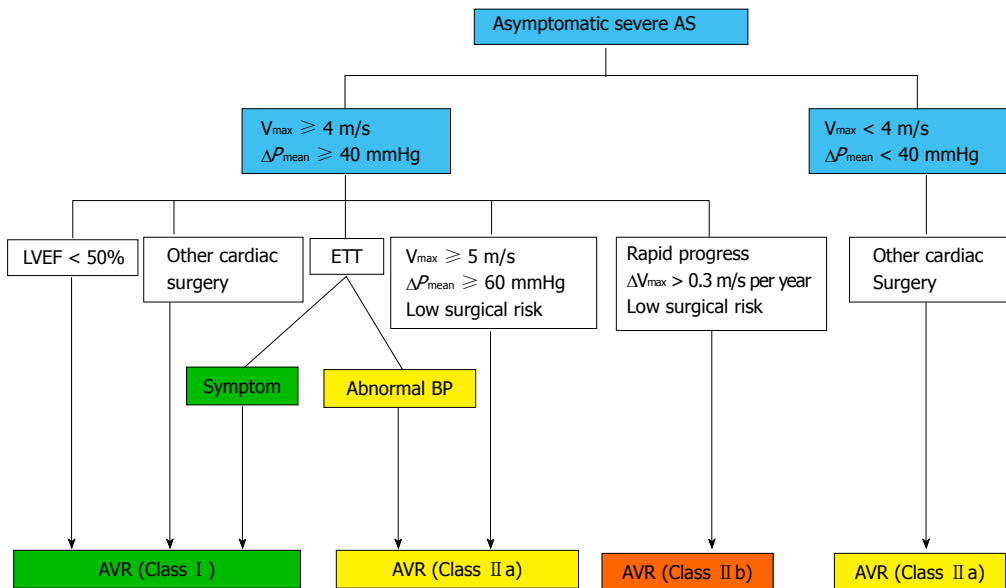
It is clear that AVR is recommended in symptomatic

patients with severe AS; however, the decision to recommend early surgery in asymptomatic severe AS patients is still challenging. Indications for AVR in asymptomatic patients are shown in Figure 1, which is based on 2014 AHA/ACC guidelines. Indications for AVR have been consistent between AHA/ACC guidelines and ESC guidelines, though there are slight differences. Asymptomatic patients with severe calcification and a rapid increase in aortic peak transvalvular velocity should be considered for AVR in ESC guidelines with a Class II a indication, but that is a Class II b indication according to AHA/ACC guidelines. Patients with elevated BNP levels, an increase in the Doppler mean pressure gradient with exercise, and excessive LVH may be considered for AVR by ESC guidelines (Class II b), but these are not employed in AHA/ACC guidelines.

Based on the current evidence and guidelines, it is reasonable to consider AVR in severe AS patients when (1) systolic function is decreased (EF < 50%); (2) it is very severe AS ( $V_{\max} \geq 5$  m/s,  $\Delta P \geq 60$  mmHg); (3) results of the exercise test are abnormal; or (4) there is rapid progression in AS severity ( $\Delta V_{\max} > 0.3$  m/s per year) (Figure 1). One must follow patients more closely despite asymptomatic severe AS when there is (1) severe aortic valve calcification; (2) end-stage renal disease; (3) worsening diastolic dysfunction; (4) increased left atrial volume; (5) high brain natriuretic peptide, especially during serial measurements; and (6) new onset of atrial fibrillation or frequent episodes of paroxysmal atrial fibrillation.

### Possible beneficial medications

Coronary artery disease and AS have similar risk factors. Additionally, AS has an active inflammation that causes valve calcification. Positive results in experimental and clinical studies on the effectiveness of statins to decrease hemodynamic progression have been published<sup>[59,60]</sup>, while randomized clinical trials were performed to validate the effect of statins on AS progression<sup>[61]</sup>. Although there was the benefit of fewer ischemic cardiovascular events in the treatment groups, no considerable difference in



**Figure 1** Indications for aortic valve replacement in patients with asymptomatic severe aortic stenosis on the basis of American College of Cardiology/American Heart Association recommendations. Modified from Nishimura *et al*<sup>[16]</sup> with permission. ACC/AHA: American College of Cardiology/American Heart Association; AS: Aortic stenosis; AVR: Aortic valve replacement; ETT: Exercise treadmill test; LVEF: Left ventricular ejection fraction.

hemodynamic progression was observed between the treatment and placebo groups<sup>[6]</sup>. However, many patients with AS have known concomitant coronary artery disease or risk factors and hyperlipidemia. Guideline-based statin therapy should be considered in these patients regardless of presence of AS.

For AS, the only effective treatment is valve replacement, but it is important to properly manage comorbidities, especially hypertension. Calcific AS is commonly found in the elderly, thus many patients have already been on antihypertensive medication at the time of diagnosis, including diuretics and vasodilators, though diuretics and vasodilators have been thought to be avoided. The current guidelines recommend following guideline-directed medical therapy for hypertension, starting at a low dose and gradually increasing to achieve appropriate blood pressure control. Effectiveness of angiotensin-converting enzyme inhibitors has been investigated on AS in terms of potential benefit on reducing LV fibrosis<sup>[62-64]</sup>. Patients with LVOT obstruction caused by discrete upper septal thickening or mid-ventricular obstruction by severely concentric hypertrophy in the setting of AS and hypertension pose a clinical challenge. A  $\beta$ -blocker and appropriate hydration is recommended, and diuretics/vasodilators should be avoided in these patients.

## CONCLUSION

Currently, due to an aging population, AS is one of the most common valvular heart diseases. Recent ACC/AHA guidelines provide a new classification system of categorizing valve diseases in patients, including those with AS, that is similar to the classification used in patients with heart failure. In addition, diagnostic strategies and treatment options for the new entity

termed "paradoxical low-gradient severe AS", despite preserved EF, are given.

Decisions for AVR are based on the presence or absence of symptoms, but proactive investigation with multimodality testing for risk assessment is recommended in patients who are asymptomatic or who have indeterminate symptoms. Exercise stress testing is recommended for asymptomatic severe AS patients in addition to two-dimensional/Doppler echocardiographic testing at rest for risk stratification. If a patient is not physically appropriate for exercise testing, use of a biomarker and multiple imaging modalities, such as CT and MRI with contrast, can complement the risk stratification of asymptomatic severe AS.

Based on the available evidence, it is now reasonable to consider AVR in asymptomatic severe AS patients with (1) decreased EF (< 50%); (2) very severe AS ( $V_{\max} > 5$  m/s or  $\Delta P \geq 60$  mmHg); (3) an abnormal exercise test; (4) rapid progression of AS ( $\Delta V_{\max} > 0.3$  m/s per year); and (5) progressively rising BNP.

Careful attention with frequent follow up is necessary in patients with (1) heavy calcification of the aortic valve (especially end-stage renal disease patients); (2) advanced stage of diastolic dysfunction ( $\geq$  stage 2); (3) elevated BNP compared to same age/sex; and (4) new onset of atrial fibrillation.

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## Dyslipidemia management in primary prevention of cardiovascular disease: Current guidelines and strategies

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### Abstract

Cardiovascular disease is the leading cause of death in the United States. In 2010, the Centers for Disease Control and Prevention estimated that \$444 billion was spent on cardiovascular diseases alone, about \$1 of every \$6 spent on health care. As life expectancy continues to increase, this annual cost will also increase, making cost-effective primary prevention of cardiovascular disease highly desirable. Because of its role in development of atherosclerosis and clinical events, dyslipidemia management is a high priority in cardiovascular prevention. Multiple major dyslipidemia guidelines have been published around the world recently, four of them by independent organizations in the United States alone. They share the goal of providing clinical guidance on optimal dyslipidemia management, but guidelines differ in their emphasis on pharmacotherapy, stratification of groups, emphasis on lifestyle modification, and use of a fixed target or percentage reduction in low density lipoprotein cholesterol. This review summarizes eight major guidelines for dyslipidemia management and considers the basis for their recommendations. Our primary aim is to enhance understanding of dyslipidemia management guidelines in patient care for primary prevention of future cardiovascular risk.

**Key words:** Dyslipidemia; Guidelines; Cardiovascular diseases

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**Core tip:** Guidelines for dyslipidemia management have been developed by independent organizations internationally for the purpose of improving patient care and reducing costs related to cardiovascular disease. In



this review article, we briefly summarize the key strategies suggested by each of eight major dyslipidemia guidelines, and the evidence that forms the foundation of the recommendations. We attempt to present a balanced view, commenting on potential strengths and weaknesses of each approach. Overall, we aim to enhance understanding of dyslipidemia management guidelines for primary prevention of future cardiovascular events.

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## CLINICAL CASE

A 59-year-old African American man with a history of chronic kidney disease, type 2 diabetes mellitus, and long-standing hypertension presents for a follow-up visit. His blood pressure is 135/80 mmHg, and hemoglobin A1c (HbA1c) is 7.2%. He denies any chest pain or shortness of breath and has no exercise intolerance. His recent fasting lipid panel shows: Total cholesterol 159 mg/dL, triglycerides 190 mg/dL, high density lipoprotein cholesterol (HDL-C) 45 mg/dL, and low density lipoprotein cholesterol (LDL-C) 76 mg/dL. He is currently on atorvastatin 10 mg daily, carvedilol 25 mg twice a day, lisinopril 40 mg daily, and aspirin 81 mg daily. Should he receive a higher dose of atorvastatin?

## INTRODUCTION

Cardiovascular disease (CVD) has been recognized as the number one killer in the United States and in the world for decades. Even though there was a 31% decline in CVD deaths from 2001 to 2011 in the United States, CVD still accounted for 1 of every 3 deaths in 2011<sup>[1]</sup>. With the decline of cigarette smoking, dyslipidemia has become the number one modifiable risk factor for vascular disease. In the INTERHEART case-control study with 27098 participants in 52 countries, dyslipidemia [elevated apolipoprotein (Apo) ApoB/ApoA1] had the highest mortality odds ratio (3.25), followed by smoking (2.87), psychosocial factors (2.67), and history of diabetes (2.37), and hypertension (1.91)<sup>[2]</sup>. In a prospective study of 27673 women, in addition to Apos, CVD risk was also strongly related to nuclear magnetic resonance measures of dyslipidemia and standard lipids (TC/HDL-C)<sup>[3]</sup>.

Multiple lines of evidence have shown the central role of dyslipidemia in development of atherosclerosis and major CVD events. Traditional management of dyslipidemia includes lifestyle modification and pharmacotherapy based on identification of groups considered at high, medium, or low risk of major cardiovascular events. Guidelines for dyslipidemia management have been developed by

independent organizations internationally for the purpose of improving patient care and reducing costs related to cardiovascular disease. However, many busy clinicians may have difficulty finding the time to read them. Moreover, the existence of multiple different guideline recommendations from different societies can present an added challenge.

In this review article, we briefly summarize the key strategies suggested by each of eight major dyslipidemia guidelines, and the evidence that forms the foundation of the recommendations. We attempt to present a balanced view, commenting on potential strengths and weaknesses of each approach. Overall, we aim to enhance understanding of dyslipidemia management guidelines for primary prevention of future cardiovascular events.

## GUIDELINES

### *American College of Cardiology/American Heart Association 2013*

The American College of Cardiology/American Heart Association (ACC/AHA) 2013 guideline recognizes four "statin benefit groups" in whom the risk reduction benefits clearly outweigh the risk of adverse events<sup>[4]</sup> (Table 1). Follow-up monitoring includes assessment for the anticipated LDL-C reduction (30%-49% and  $\geq 50\%$  with moderate- and high-intensity statin therapy, respectively) from baseline after starting the maximal tolerable dose of statin therapy. When such a percentage reduction is not seen, adherence to lifestyle modification and medication should be reinforced, along with evaluation for a secondary cause of dyslipidemia. Non-statin therapy can be considered in high-risk groups if the response to statin therapy is not acceptable. The ACC/AHA guidelines removed fixed target LDL-C levels, although when the baseline LDL-C is not known, the guideline notes that "an LDL-C < 100 mg/dL was observed in most individuals receiving high-intensity statin therapy in RCTs".

The new Pooled Cohort Equations (PCE) are used to calculate 10-year risk of atherosclerotic cardiovascular disease (ASCVD) in this guideline. In contrast to the Framingham Risk Score (FRS) used in adult treatment panel III (ATP III), the PCE use separate equations based on sex and race. Stroke is now included with coronary events in an ASCVD endpoint, whereas the ATP III FRS only predicted coronary events. Along with the ASCVD endpoint, a new cut-point of 7.5% is featured to guide statin decision making. The use of this cut-point is not intended to lead to automatic prescription of a statin, but instead, to serve as the starting point for a clinician-patient risk/benefit discussion and consideration of statin therapy as one management option<sup>[5]</sup>.

The 7.5% cut-point is derived from three exclusively primary prevention clinical trials: Air Force Coronary Atherosclerosis Prevention Study, Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese, and Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trials. It is felt that this new cut-point

**Table 1** Fixed-dose strategies

Strategy	ACC/AHA 2013	NICE 2014	VA/DoD 2014
Risk score	PCE to determine 10-yr risk of non-fatal and fatal hard ASCVD events (CHD and CVA)	QRISK2 to determine 10-yr risk of non-fatal and fatal CVD events (CHD, CVA, PAD)	FRS or PCE to determine 10-yr risk of non-fatal and fatal CVD events
Step 1: Identify statin-benefit group	<p>Statin benefit groups: (moderate to high-intensity statin)</p> <p>History of ASCVD;</p> <p>LDL-C <math>\geq 190</math>, age <math>\geq 21</math>;</p> <p>DM at age 40-75 with LDL-C <math>\geq 70</math>;</p> <p><math>\geq 7.5\%</math> of ASCVD risk at age 40-75 with LDL-C; <math>\geq 70</math> (in some individuals, not all; discussion required)</p> <p>Consider moderate intensity statin as initial dose for:</p> <p>DM with <math>\leq 7.5\%</math> ASCVD risk;</p> <p><math>\geq 7.5\%</math> of ASCVD risk without DM</p> <p>Inadequate data to make recommendation (weigh risk, benefit and patient preference)</p> <p>DM at age <math>&lt; 40</math> or <math>&gt; 75</math> with LDL-C <math>&gt; 70</math>;</p> <p>Age <math>&lt; 40</math> or <math>&gt; 75</math> with LDL-C <math>&gt; 70</math>;</p> <p>5%-7.4% of ASCVD risk at age 40-75 with LDL-C <math>&gt; 70</math>;</p> <p><math>&lt; 5\%</math> of ASCVD risk at age 40-75 with LDL-C <math>&gt; 70</math>;</p> <p>Age <math>&lt; 40</math> with low 10 yr ASCVD risk but high lifetime risk based on 1 strong or multiple risk factors;</p> <p>Those with serious co-morbidities and increased ASCVD risk (<i>e.g.</i>, HIV, rheumatologic or inflammatory diseases, or solid organ transplantation)</p> <p>Other factors for consideration: family history of premature CVD, hsCRP <math>\geq 2</math>, elevated CAC, ABI <math>&lt; 0.9</math>, LDL-C <math>\geq 160</math></p>	<p>Statin benefit groups: (initial dose: Atorvastatin 20 mg/d)</p> <p>Type 1 DM;</p> <p>CKD st. III;</p> <p>Risk score <math>&gt; 10\%</math>;</p> <p>Age <math>&gt; 85</math>;</p> <p>Familial hypercholesterolemia</p> <p>Elevated risk groups that are underestimated by or not included in QRISK2: Possible benefit with statin</p> <p>HIV;</p> <p>Serious mental problem;</p> <p>On medication that cause dyslipidemia (antipsychotic, corticosteroid, immunosuppressant);</p> <p>Autoimmune disorder and systemic inflammatory disorder;</p> <p>TG <math>&gt; 175</math>;</p> <p>On anti-hypertension or lipid modification therapy;</p> <p>Recently stopped smoking</p>	<p>Statin benefit group: (initial dose: Atorvastatin 10-20 mg/d)</p> <p>Risk score <math>&gt; 12\%</math></p> <p>Moderate dose statin initiation can be considered in patient with 6%-12% risk score after discussion of benefit, risk, and patients' preference</p>
Step 2: Determine adequacy of treatment effect	<p>For group treated with high intensity statin:</p> <p><math>&gt; 50\%</math> <math>\downarrow</math> of LDL-C</p> <p>For group treated with moderate intensity statin:</p> <p>30%-50% <math>\downarrow</math> of LDL-C</p> <p>If patients are already on statin and baseline LDL-C is unknown, an LDL-C <math>&lt; 100</math> was observed in most individuals receiving high-intensity statin therapy in RCTs</p>	$> 40\%$ $\downarrow$ of non-HDL-C	No objective parameters recommended
Step 3: Follow-up lipids	<p>1-3 mo after initiation therapy</p> <p>Every 3-12 mo as clinically indicated thereafter</p>	<p>3 mo after initiation of therapy</p> <p>Annually when target achieved</p>	<p>Not recommended</p> <p>Lipid measurement can be utilized for compliance monitoring</p>
Step 4: Options if treatment effect judged not adequate	<p>Reinforce lifestyle change and adherence to medication</p> <p>Exclude secondary cause of dyslipidemia</p> <p>Add non-statin agent in those with LDL-C <math>\geq 190</math> or DM at age 40-75 with LDL-C <math>\geq 70</math></p>	<p>Discuss adherence to lifestyle and medication</p> <p>Up-titrate statin dose; may go up to a atorvastatin 80 mg/d</p>	No recommendation

ACC/AHA: American College of Cardiology/American Heart Association; NICE: National Institute for Health and Care Excellence; PCE: Pooled Cohort Equations; ASCVD: Atherosclerotic cardiovascular disease; CHD: Coronary heart disease; CVA: Cerebrovascular accident; CVD: Cardiovascular disease; PAD: Peripheral artery disease; FRS: Framingham Risk Score; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; DM: Diabetes mellitus; CKD: Chronic kidney disease; HIV: Human immunodeficiency virus; TG: Triglyceride; hsCRP: High sensitivity C-reactive protein; ABI: Ankle-brachial index; RCT: Randomized controlled trials.

builds in some room for potential overestimation of risk<sup>[6]</sup>. A recent study showed that these new guidelines significantly increase the number of potentially eligible adults for statin therapy (12.8 million people), especially in older age groups<sup>[7]</sup>.

A 2013 Cochrane review on use of statins in primary prevention of ASCVD reported that, for patients with estimated 5% to 10% 5-year ASCVD risk, 15 major vascular events would be avoided per 1000 people treated for five years, which correlates with a number needed to treat (NNT) of 67<sup>[8]</sup>. In comparison, a study based on 5 trials with a total of 18564 participants (mean age 46 years)<sup>[9]</sup> showed an estimated 5-year NNT of 120 for CVD events when treating patients with mild hypertension (BP 140-160/90-100 mmHg) with anti-

hypertensive medications for primary prevention.

The ACC/AHA guidelines rely on the highest quality randomized control trials (RCTs) and meta-analyses to date to form the foundation of evidence-based guidelines. The fixed-dose strategy promotes the appropriate use of high-intensity statin therapy and avoids overutilization of non-statin drugs, for which evidence is weaker and net benefit is less clear than evidence for statins. Under the "traditional" fixed target level strategy of combining statin and non-statin medications, a patient might receive a lower statin dose because of potential drug interaction with a second agent<sup>[10]</sup>. However, on-treatment lipid levels can still be used to motivate additional lifestyle change when statin therapy has been appropriately maximized, and can guide the selective addition of non-

statin therapy. Observational studies have consistently shown a log-linear association of LDL-C level and CVD morbidity<sup>[11]</sup>.

### **European Society of Cardiology/European Atherosclerosis Society 2011**

The European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) 2011 guideline uses a target level strategy<sup>[12]</sup> combined with risk stratification based on estimated 10-year risk of a fatal CVD event by the Systemic Coronary Risk Evaluation (SCORE)<sup>[13]</sup>. After stratification, ESC/EAS advises group-specific intervention. The initial statin dose is determined by calculating the percentage reduction needed to achieve the target level, and then choosing the intensity of statin accordingly. ApoB and non-high-density lipoprotein cholesterol (non-HDL-C) are alternatives to LDL-C as targets. Up-titration of the statin dose or addition of a non-statin agent may be considered if the target is not attained with the initial statin regimen.

SCORE is based on large European cohorts and can be calibrated to each European country. The rationale for focusing on fatal CVD events is that variation in the definition of non-fatal events makes that parameter less reliable. A 5% risk of fatal CVD events is approximately equal to 15% risk of total (fatal and non-fatal) CVD events<sup>[13]</sup>. Recognizing that risk must be interpreted in light of clinical judgment and the pretest probability of CVD, the guideline lists some conditions that are often associated with risk score underestimation, such as elevated high sensitivity C-reactive protein (hsCRP), elevated homocysteine, low HDL-C, family history of premature coronary artery disease, and asymptomatic atherosclerotic disease. High HDL-C and family history of longevity are associated with overestimation of risk.

Overall, the guideline uses an individualized strategy for management, accounting for specific conditions, such as heart failure, diabetes, autoimmune diseases, metabolic syndrome, and HIV. An extensive section of the guideline focuses on management of hypertriglyceridemia and low HDL-C, although the panelists acknowledge that the evidence for these variables impacting future CVD incidence is still weak.

### **Canadian Cardiovascular Society 2009 Guideline and 2012 Updates**

The Canadian Cardiovascular Society (CCS) guideline adopts the traditional approach of risk stratification and group-specific target treatment using LDL-C<sup>[14]</sup> (Table 2). Patients are stratified into low, intermediate, or high risk categories using comorbidities in addition to a modified FRS, which includes an additional rule of multiplying the calculated risk by 2 if there is a family history of premature coronary heart disease (CHD)<sup>[15]</sup>. The LDL-C level and percentage reduction in LDL-C are the recommended primary targets.

The high-risk and low-risk groups receive interventions according to their respective risk. The intermediate risk group is further refined using LDL-C and, if indicated,

ApoB and/or non-HDL-C to identify candidates for more aggressive intervention. Secondary tests, such as a coronary calcium scan and high-sensitivity C-reactive protein (hsCRP), are optional secondary tests to refine risk assessment in the low- and intermediate-risk groups.

When communicating risk to a patient, a unique aspect of this guideline is the suggestion to use "cardiovascular age" as an easier-to-understand explanation of a patient's ASCVD risk, with the potential to improve awareness and adherence. Cardiovascular age is calculated by age minus the difference between estimated life expectancy and average life expectancy, based on age and sex.

### **International Atherosclerosis Society 2013**

International Atherosclerosis Society (IAS) 2013 makes evidence-based recommendations based on numerous studies from the 1970s to 2013<sup>[16]</sup> (Table 2). The risk estimator used is the Lifetime Framingham risk score<sup>[17]</sup>, which may help call attention to risk in young people and motivate them to improve their lifestyle habits. This score can be recalibrated by nationality.

For those in high and moderately-high risk groups, it is suggested to aim for "optimal" lipid levels (LDL-C < 100 mg/dL or non-HDL-C < 130 mg/dL). "Near optimal" levels (LDL-C < 130 mg/dL or non HDL-C < 160 mg/dL) are considered acceptable for the lower risk group. Statins are the first-line drug when pharmacotherapy is indicated. The initial dose is tailored according to the group-specific lipid target.

### **National Lipid Association 2014**

The National Lipid Association (NLA) guideline uses a multilevel stratification approach to identify patients with a higher CVD risk factor who require more intensive management<sup>[18]</sup> (Table 2). First, "very-high" and "high risk" groups are identified based on specified parameters. The remaining patients are then further risk-stratified based on the number of major ASCVD risk factors. People with two major risk factors are deemed to be intermediate risk, but the presence of any secondary risk indicator or a high-risk score places them in the higher risk group. Similar to other guidelines, the goal of treatment in this guideline is group-specific. Non-HDL-C is favored over LDL-C as the therapeutic target, but both are viewed as reasonable.

The NLA guideline is thorough in categorizing groups for whom aggressive intervention is either necessary or optional. The guideline emphasizes the potential for risk score estimation to overestimate or underestimate the risk in certain settings. A general LDL-C goal of < 100 mg/dL or non-HDL-C goal of < 130 mg/dL is recommended for low to high risk groups. LDL-C targets are used to motivate lifestyle change in addition to drug therapy.

### **National Institute for Health and Care Excellence 2014**

The National Institute for Health and Care Excellence (NICE) guideline uses a fixed dose approach similar to ACC/AHA. All people aged  $\geq 40$  years are screened formally with the QRISK2 score<sup>[19]</sup> (Table 1). This

**Table 2 Target-level strategies**

Strategy	EAS/ESC 2011	CCS 2012	IAS 2013	NLA 2014	AACE 2012
Risk score	SCORE chart to estimate 10-yr risk of fatal CVD	Modified FRS to estimate 10-yr risk of non-fatal and fatal CVD	Lifetime FRS to estimate lifetime risk of non-fatal and fatal CVD	PCE or FRS or lifetime FRS	FRS to determine 10-yr risk of non-fatal and fatal CVD
Step 1: Stratify CVD risk	Very-high: $\geq 10\%$ of fatal CVD risk; CHD equivalent risk; DM with microalbuminuria; CKD st. III High: 5%-9% of fatal CVD risk; DM; 1 markedly abnormal risk factor Moderate: 1%-4% of fatal CVD risk Low: $< 1\%$ of fatal CVD risk	High: $\geq 20\%$ risk of CVD; CHD risk equivalent; DM, age $> 40$ or $> 30$ with 15 yr DM history; CKD st. III b or III a with microalbuminuria; HTN with $\geq 3$ CVD risk factors Intermediate: 10%-19% risk of CVD Low: $< 10\%$ risk of CVD (CVD risk factor: age $> 55$ , smoker, TC/HDL-C $> 6$ , LVH, abnormal ECG, microalbuminuria)	High: $\geq 45\%$ lifetime risk of CVD; DM with major risk factor; Familial hyperlipidemia; CKD Moderately-high: 30%-44% lifetime risk of CVD; DM alone; Metabolic syndrome; CKD Moderate: 15%-29% lifetime risk of CVD Low: $< 15\%$ lifetime risk of CVD [Major risk factor: high LDL-C, HDL-C $< 40$ , HTN, smoker, family history of premature CAD, age (men $> 55$ , women $> 65$ )]	Very-high: CHD risk equivalent; DM with $\geq 2$ major risk factors or evidence of end organ damage High: DM with 0-1 major risk factor; CKD st. III b; LDL-C $\geq 190$ ; $\geq 3$ major risk factors; $\geq 1$ secondary risk (marked major CVD risk, LDL-C $> 160$ or non-HDL-C $> 190$ , CAC $> 300$ , hsCRP $> 2$ , Lp(a) $> 50$ , microalbuminuria); High risk score (PCE $> 15\%$ , FRS $> 10\%$ , lifetime FRS $> 45\%$ ) Intermediate: 2 major risk factors Low: 0-1 risk factor	Very-high: CHD risk equivalent + $\geq 1$ major risk factor High: CAD risk equivalent; $\geq 2$ major risk factor + $\geq 20\%$ risk of CVD Moderately-high: $\geq 2$ major risk factor + 10%-19% risk of CVD Moderate: $\geq 2$ major risk factor + $< 10\%$ risk of CVD Low: $\geq 1$ major risk factor
Step 2: Determine target	Very-high: LDL-C $< 70$ ; Alt: ApoB $< 80$ , non-HDL-C $< 100$ High: LDL-C $< 100$ ; Alt: ApoB $< 80$ , non-HDL-C $< 130$ Moderate-Low: LDL-C $< 100$ -115	High: LDL-C $< 77$ or $\geq 50\%$ $\downarrow$ ; Alt: ApoB $< 80$ , Non-HDL-C $< 100$ Intermediate: LDL-C $< 77$ or $\geq 50\%$ $\downarrow$ Alt: ApoB $< 80$ , Non-HDL-C $< 100$ Low: $\geq 50\%$ $\downarrow$ of LDL-C	High to moderately-high: LDL-C $< 100$ or non-HDL-C $< 130$ (goal may be lower for very-high risk) Moderate to low: LDL-C $< 130$ or non-HDL-C $< 160$	Very-high: LDL-C $< 70$ , non-HDL-C $< 100$ Alt: ApoB $< 80$ High-Moderate-Low: LDL-C $< 100$ , non-HDL-C $< 130$	Very-high: LDL-C $< 70$ , ApoB $< 80$ High: LDL-C $< 100$ , ApoB $< 90$ Moderately-high: LDL-C $< 130$ Moderate: LDL-C $< 130$ ; Low: LDL-C $< 160$ ; All category: HDL-C $> 40$ , TG $< 150$
Step 3: Treat according to risk	Very-high or High: Lifestyle intervention + drug intervention Moderate: Lifestyle intervention; consider drug if uncontrolled with lifestyle Low: Life style intervention only	High: Statin and lifestyle change Intermediate: LDL-C $> 135$ : Statin if lifestyle change insufficient; LDL-C $< 135$ : Get ApoB or non-HDL-C: # Apo B $> 120$ or Non-HDL-C $> 165$ : Start statin if lifestyle change insufficient # Apo B $< 120$ or Non-HDL-C $< 165$ : Lifestyle change Optional use of secondary test for further stratification Low: LDL-C $> 190$ : Lifestyle change and statin; 5%-9% risk of CVD: Lifestyle change only optional use of secondary test for further stratification; $< 5\%$ risk of CVD: Lifestyle change only	High: Statin and lifestyle change Moderately-high: Lifestyle change; Initiation of statin may be considered Moderate: Lifestyle change; initiation of statin may be considered if LDL-C $> 160$ Low: Lifestyle change only	Very-high: Statin and lifestyle change; statin optional if baseline LDL-C, non-HDL-C and ApoB below target High: Concurrent statin and lifestyle change or statin after insufficient lifestyle change Moderate: Lifestyle change only; statin may be considered after 3 mo of optimal lifestyle change and LDL-C $> 130$ Low: Lifestyle change only; statin may be considered after 3 mo of optimal lifestyle change and LDL-C $> 160$	Exclude secondary cause of hyperlipidemia; Lifestyle change; Lipid lowering agent; Combination lipid lowering agent
Step 4: Follow-up lipids	1-12 wk after initiation; 1-3 mo after every change of dose or change of medication; Annually when target is achieved			Every 3 mo until target is achieved; Every 4-12 mo when target is achieved	6 wk after initiation; Every 6-12 mo when target is achieved
Step 5: Options if target not reached	Up-titration of statin dose; Add non-statin agent			Add non-statin agent; Referral to lipid specialist	

ESC: European Society of Cardiology; EAS: European Atherosclerosis Society; CCS: Canadian Cardiovascular Society; IAS: International Atherosclerosis Society; NLA: National Lipid Association; AACE: American Association of Clinical Endocrinologists; FRS: Framingham risk score; PCE: Pooled Cohort Equations; CVD: Cardiovascular disease; CHD: Coronary heart disease; CKD: Chronic kidney disease; DM: Diabetes mellitus; CAD: Coronary artery disease; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; hsCRP: High sensitivity C-reactive protein; TG: Triglyceride; ApoB: Apolipoprotein B.



estimates 10-year risk of CVD using validated population data in England, taking into account ethnicity and geographical location<sup>[20]</sup>. QRISK2 used the same main outcomes as PCE with addition of transient ischemic attack and angina; hence, a 10% risk estimation by the QRISK2 score is approximately equivalent to a 7.5% ASCVD risk estimation by PCE. Both scores are best used in the populations for which they were intended to be implemented. People with a QRISK2 score of  $\geq 10\%$  along with those who have other selected risk factors are categorized into a "statin-benefit group" wherein atorvastatin 20 mg is recommended.

The guideline lists conditions that are known to increase risk of cardiovascular disease which are not included in QRISK2, suggesting that risk may be underestimated in people with these conditions. Reducing non-HDL-C  $> 40\%$  is used as the target for people who initiate statin therapy. For people who do not attain the target with atorvastatin 20 mg/d, up-titration of atorvastatin to 80 mg/d and/or reinforcement of lifestyle and medication adherence are recommended.

NICE is the first guideline to endorse non-HDL-C as the sole target. The justification is based on epidemiologic evidence supporting non-HDL-C as a cardiovascular risk predictor and the greater practicality for testing because both fasting and non-fasting results are considered reasonable. In targeting non-HDL-C initially, the NICE guideline recommends 20 mg/d of atorvastatin rather than a higher dose for several reasons, including considerations of cost and net clinical benefits<sup>[21]</sup>.

### **American Association of Clinical Endocrinologists 2012**

The American Association of Clinical Endocrinologists (AACE) guideline uses conventional risk stratification and a group-specific target level strategy<sup>[22]</sup> (Table 2). Using a combination of the FRS and presence of major ASCVD risk, the guideline stratifies patients into 5 groups. The entire standard lipid panel is used as the target and for the highest risk population, ApoB can be used as an alternative. AACE 2012 endorses a comprehensive approach to managing dyslipidemia without giving specific criteria for when to initiate pharmacotherapy.

The guideline also does not specify an initial dose for statin therapy. For patients who fail to meet their target after initial management, a non-statin lipid lowering agent can be added. Ezetimibe is recommended as the non-statin agent of choice based on the SHARP (*Study of Heart and Renal Protection*) trial<sup>[23]</sup>. The guideline also endorses possible combination therapy with a fibrate, specifically when triglyceride levels are  $>200$  mg/dL and the HDL-C is  $< 40$  mg/dL, due to evidence of non-fatal CVD event reduction in the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) and ACCORD (Action to Control Cardiovascular Risk in Diabetes) trials<sup>[24]</sup>.

### **United States Department of Veteran Affairs and United States Department of Defense 2014**

Using similar rationale to 2013 ACC/AHA guideline, the

recent United States Department of Veteran Affairs and United States Department of Defense (VA/DoD) guideline advocates the use of a fixed-dose strategy<sup>[25]</sup> (Table 1). Men older than 35 years, and women older than 45 years are screened using a 10-year CVD risk calculator (either Framingham or PCE). Patients who have  $> 12\%$  estimated 10-year CVD risk are recommended to be started on a moderate dose of statin therapy based on evidence supporting that benefit clearly outweighs risk in this group. For people with intermediate risk (6%-12%), the recommendation for statin initiation is less clear. The guideline's distinct feature is its recommendation against the routine measurement of lipid panel after statin initiation. Thus, neither a target level nor a percentage change from baseline is utilized as a parameter of treatment adequacy. Combination with a non-statin agent is avoided, but a non-statin agent (gemfibrozil or bile acid sequestrant) may be used in patients who cannot tolerate statin.

## **DISCUSSION**

These guidelines approach primary prevention with similar overarching aims. Several adopt traditional risk stratification with group-specific management. ACC/AHA, NICE, and NLA (partially) recommend identifying groups in which benefits of statin therapy clearly outweigh adverse effects. Risk estimation using traditional risk factors to estimate an absolute risk score, secondary testing (including hsCRP, CAC, and ApoB), and secondary risk factors (such as HIV, autoimmune diseases, and medications) are tools that are commonly used to further stratify those in the intermediate risk group to guide management.

### **Critical role of risk scores**

In primary prevention, risk estimators/calculators may have a major impact in determining how many people will be treated with pharmacotherapy. The decision to use one over another could affect treatment of millions of people, and it is worth noting that when a calculator is applied to a given individual, the population from which the calculator was derived may not be representative of that specific individual. For example, a calculator developed from and valid for Asian Americans might not be as well suited to Asian people in general. When using PCE, it is specifically noted that underestimation of ASCVD risk is expected in American Indians, some Asian Americans (*e.g.*, of South Asian ancestry), and some Hispanics (*e.g.*, Puerto Ricans). On the other hand, the overestimation tends to occur in Asian Americans (*e.g.*, of East Asian ancestry) and some Hispanics (*e.g.*, Mexican Americans)<sup>[25]</sup>.

Ideally, every distinct population would have its own risk calculator; however, this is not practical at this time because of the lack of national representative cohorts in most countries. It is important to realize that the accuracy of a risk calculator in estimating "true" future risk is difficult to ascertain. A risk score is an estimate based

on a population average and the information needs to be contextualized through discussion with a patient and consideration of unique aspects of their case. Concerns with the potential inaccuracies of risk calculators support, in our view, a less calculator-reliant approach<sup>[5]</sup>.

### **Pharmacotherapy threshold**

Choosing a cut-point of ASCVD risk for stratification can be challenging, and while it can be data driven, it also requires panelist consensus to some extent. In the ACC/AHA guidelines, the cut-point of 7.5% was selected based on a balancing of the estimated NNT and number needed to harm (NNH). By extrapolation from trial data showing the NNT to avoid an ASCVD event with statin therapy vs the NNH for diabetes<sup>[4]</sup>, comparisons were made for moderate- and high-intensity statin therapy. Again, the cut-point is not intended to automatically trigger a statin prescription, but rather to start a clinician-patient risk discussion.

RCTs are attractive because they allow an unbiased comparison of the NNT and NNH in defined populations. Since there have been over 25 statin trials embracing various populations, guidelines based on high-quality RCTs have merit. But the NNT and NNH have potential shortcomings as the NNT is dependent on the time frame of the trials. In WOSCOPS, there was a significant difference in the NNT at 5 years vs 20 years of follow-up<sup>[26]</sup>. The NNH obtained from RCTs may also not reflect the true incidence of adverse effects in a particular case of interest. For example, statin-related diabetes appears to occur in persons with risk factors for diabetes (components of the metabolic syndrome) and, therefore, the NNT vs NNH assessment may not be as relevant to someone without these diabetes risk factors.

Moreover, many patients seen in routine clinical practice may differ from the patients who participated in RCTs. In a recent retrospective cohort study of 107835 statin-treated participants<sup>[27]</sup>, 17% of patients (18778) reported having a statin-related adverse effect, 40% of which were musculoskeletal. Of these individuals, 6579 subjects were re-challenged with statin. Eventually, over 90% of those previously intolerant patients continued on statin therapy suggesting that many adverse effects were incorrectly attributed to statins. In contrast, in RCTs, people with a history of statin intolerance and those who develop muscle symptoms or elevated CK during run-in phases may be excluded from trials. This selection process limits the ability to generalize such studies to the general population<sup>[28]</sup>.

### **Target treatment**

Arguments can be made to support a focus on the percentage LDL-C reduction (as in ACC/AHA, NICE) or target LDL-C level (as in EAS/ESC, CCS, IAS, NLA, and AACE). Both approaches inherently acknowledge that the benefit is through LDL-C lowering. Focus on the anticipated response to statin therapy, as reflected by the percentage LDL-C reduction, is felt to be more aligned with evidence from RCTs and high quality meta-

analyses. On the other hand, lack of RCT evidence for efficacy is not the same as RCT evidence for lack of efficacy<sup>[29]</sup>.

The fixed target LDL-C level could be easier for patients to understand, which theoretically could help maximize adherence to treatment and motivate lifestyle change. Having a target LDL-C level could also be helpful in assessing the success of treatment, particularly when baseline LDL-C is unknown, such as in patients already on a statin. Moreover, some high risk patients with high baseline LDL-C levels may not achieve what would be considered an optimal LDL-C level even with a large percentage change, and without a fixed target LDL-C, the role or timing of the addition of non-statin medications such as ezetimibe becomes less clear. Importantly, patient counseling about the primary goal of LDL-C reduction, which is prevention of future heart attacks and strokes, is critical.

As noted in three guidelines, on-treatment non-HDL-C levels can be a stronger predictor of future cardiovascular events than LDL-C<sup>[30,31]</sup>. One contributing factor is that non-HDL-C captures information on triglyceride-rich remnant lipoprotein cholesterol that LDL-C does not. In addition, calculated LDL-C can be inaccurate in the setting of elevated triglyceride levels or low LDL-C levels (particularly levels < 70 mg/dL) as it is derived from Friedewald estimation<sup>[32,33]</sup>. Avoiding the issues with such estimation, non-HDL-C is simply a subtraction of total and HDL cholesterol.

### **Follow-up**

Most guidelines advise follow-up at 6 to 12 wk after initiation of treatment and/or dose change and thereafter every 6 to 12 mo when the target is achieved. Reinforcement of lifestyle modification and medical adherence can be done at each follow-up visit. If inadequate time is given to observe the effect of lifestyle changes, this may lead to premature conclusions about the ineffectiveness of lifestyle modification and unnecessary medication changes.

### **Options for management after maximum statin therapy**

In addition to reinforcing intensive lifestyle modification, drug adherence and the possible role of adding a non-statin agent are relevant considerations. Effort to determine a possible secondary cause of dyslipidemia is reasonable when the expected response or target is not achieved (as in ACC/AHA and AACE). This management step may often be overlooked but can be important for treatment. Secondary causes of dyslipidemia include drugs, such as diuretics, steroid, amiodarone, cyclosporine, and protease inhibitors; and diseases, such as nephrotic syndrome, hypothyroidism, biliary obstruction, and anorexia.

Regarding combination therapy, recent evidence showing no overall benefit from the addition of niacin in AIM-HIGH and HPS2-THRIVE to patients with well-controlled LDL-C and fenofibrate in ACCORD<sup>[34-36]</sup> has led to less emphasis on routine non-statin therapy. This approach is articulated clearly by ACC/AHA and

NICE. The other guidelines also note the shortage of evidence for the additional use of non-statin agents to background statin therapy, although use of these agents appears to be more of a routine option in their recommendations.

However, the above trials did not test use of a second agent in people who were not at target despite statin therapy. The participants had generally well-controlled LDL-C levels on background therapy. Moreover, pre-specified subgroups with high triglycerides and low HDL-C showed benefit of added therapy. Thus, it may be an overgeneralized conclusion to say that combination therapy has no role in management of adults with mixed hyperlipidemia. Rather, it may be that selective use is reasonable, and indeed the guidelines would generally tend to support such a strategy.

For additional LDL-C lowering, the preferred agent at this time is likely ezetimibe, which showed additional benefit in combination with statin therapy in preliminary reporting of the secondary prevention IMPROVE-IT trial<sup>[37]</sup>. Of note, the relative risk reduction was fairly modest, consistent with the fairly modest 20% LDL-C lowering from ezetimibe. Therefore, the additional benefit is most justified in those with high enough absolute risk where such a reduction would be clinically significant.

## CASE DISCUSSION

Going back to our case, the patient has three important cardiovascular risk factors: Type 2 diabetes mellitus, chronic kidney disease, and hypertension. By all guidelines, he will be categorized as either high risk or in a statin-benefit group. He has been on chronic statin therapy, and determining a percentage reduction is not possible because baseline LDL-C and non-HDL-C are not known. Per the five guidelines that use a fixed target LDL-C goal (EAS/ESC, CCS, IAS, NLA, and AACE), the most aggressive LDL-C goal is < 70 mg/dL and non-HDL-C goal is < 100 mg/dL. With an LDL-C of 76 mg/dL and non-HDL of 119 mg/dL, the patient's on-treatment lipids are probably not optimal and this should be discussed with the patient. In addition, the on-treatment triglycerides level is elevated and LDL-C may be underestimated by the Friedewald equation. Options include improving medication adherence if there is a need, consideration of up-titrating drug therapy, further addressing lifestyle modification, and addressing a possible secondary cause of dyslipidemia. In this case, after clinician-patient discussion, the patient elected to work even harder on lifestyle modification and increase atorvastatin to 40 mg/d.

## CONCLUSION

There is no perfect guideline. Each guideline has advantages and limitations. We hope that, by gathering and elaborating upon current guidelines, important concepts were highlighted about dyslipidemia management to prevent ASCVD. We anticipate that, in the future, having

more congruent guidelines will help avoid confusion among clinicians throughout the world.

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## Novel epigenetic-based therapies useful in cardiovascular medicine

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### Abstract

Epigenetic modifications include DNA methylation, histone modifications, and microRNA. Gene alterations have been found to be associated with cardiovascular diseases, and epigenetic mechanisms are continuously being studied to find new useful strategies for the clinical management of afflicted patients. Numerous cardiovascular disorders are characterized by the abnormal methylation of CpG islands and so specific drugs that could inhibit DNA methyltransferase directly or by reducing its gene expression (*e.g.*, hydralazine and procainamide) are currently under investigation. The anti-proliferative and anti-inflammatory properties of histone deacetylase inhibitors and their cardio-protective effects have been confirmed in preclinical studies. Furthermore, the regulation of the expression of microRNA targets through pharmacological tools is still under development. Indeed, large controlled trials are required to establish whether current possible candidate antisense microRNAs could offer better therapeutic benefits in clinical practice. Here, we updated therapeutic properties, side effects, and feasibility of emerging epigenetic-based strategies in cardiovascular diseases by highlighting specific problematic issues that still affect the development of large scale novel therapeutic protocols.

**Key words:** Epigenetics; Cardiovascular diseases; Heart failure; Inhibitors of histone deacetylases; Antisense microRNAs

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**Core tip:** Recent evidence suggests that specific epigenetic regulatory mechanisms play key roles in cardiac differentiation, homeostasis, injury response, and disease development. Drug therapies that work *via* epigenetic mechanisms are currently limited to antineoplastic agents; large controlled trials are required to establish whether

current possible candidate antisense microRNAs or histone deacetylase inhibitors could offer better therapeutic benefits in cardiovascular disease. We review recent findings on the epigenetic control of several cardiovascular diseases and the new challenges for therapeutic strategies in cardiovascular diseases.

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## INTRODUCTION

Cardiovascular diseases (CVDs) are the primary cause of death worldwide, with 17.5 million deaths from CVD in 2012 representing 31% of all global deaths that year. CVDs include a number of alterations affecting heart and vascular structures, such as heart valves, heart muscle (e.g., cardiomyopathy), and pericardial and coronary artery diseases. All these conditions may result in cardiomyocyte loss, cardiac-remodeling with consequent heart failure (HF), and an increased risk of arrhythmias and death. Cardiac fibroblasts also have a pivotal role in HF<sup>[1]</sup>. Indeed, endothelial cell activation and inflammation promotes the transdifferentiation of fibroblasts to myofibroblasts which, after extensive collagen production, results in the release of chemokines and the activation of inflammatory cells, which in turn causes cardiomyocyte stiffness by contributing to HF pathogenesis<sup>[1]</sup>. Increasing evidence has shown that epigenetic mechanisms control and influence the expression of cell cycle central genes involved in human disease progression<sup>[2]</sup>. Toward this context, significant epigenetic and epigenomic findings have opened a new area of research by exploring the role of genetic heritability and environmental interaction in CVDs<sup>[3]</sup>. Some deregulated epigenetic steps are involved in the pathophysiology of CVDs<sup>[4]</sup>. Specific epigenetic regulatory mechanisms could impact on the endothelium, cardiac muscle, smooth muscle, and fibroblasts<sup>[5]</sup>. Thus, the pharmacological setting of these pathways might represent a specific target for CVDs. Due to the reversible nature of these modifications, researchers are continuously engaged in the development of novel epigenetic-based drugs (epidrugs) for CVD treatment<sup>[6,7]</sup>. The primary goal of future studies will be to allow for the identification of selective therapeutic molecules that have been conceived in order to act on specific epigenetic-related pathogenic events.

Here, we summarize the current knowledge concerning epigenetic-based strategies in CVDs by outlining novel therapeutic steps in clinical practice.

## EPIGENETICS INVOLVEMENT IN CVDs

### DNA methylation as therapeutic target

DNA methylation is the most studied epigenetic

modification and mainly involves methylation of CpG islands in the promoter genes. It has good long-term stability and is the most common modification involving the regulation of gene expression in the mammalian genome. All changes in methylation are modulated by specific catalytically-active enzymes, including "maintenance" methyltransferase (DNMT1) and "de novo" methyltransferase (DNMT3a and DNMT3b). DNMTs act by adding methyl groups to CpG residues, thereby modifying the accessibility of DNA to the transcriptional machinery. Altered regulation of cytosine methylation has been linked to CVD development and progression<sup>[8]</sup>, as well as to cancer cell development<sup>[9]</sup>. In addition, DNA methylation has been shown to regulate biological processes underlying CVDs, such as atherosclerosis, inflammation, hypertension, and diabetes<sup>[10,11]</sup>. DNA methylation is also involved in essential arterial hypertension<sup>[12,13]</sup>. To date, DNA methylation remains an attractive target for CVD interventions, owing to its reversible nature. Dietary compounds, including polyphenols and catechins, act on DNA methylation processes<sup>[14,15]</sup>. In particular, some interesting clinical studies have shown that elevated consumption of polyphenols decreases global DNA methylation of peripheral leukocytes in humans with cardiovascular risk factors (NCT00511420 and NCT00502047)<sup>[16]</sup>. However, the role of nutrients in the evolution of CVDs through the epigenetic link remains as yet studied. Conversely, the cardiovascular implication of pharmacological epigenetic compounds appears to be more direct and far-reaching. Indeed, some drugs are known to affect DNA methylation. Hydralazine, a vasodilator used to treat hypertension<sup>[17]</sup>, is an example of compound that has been shown to inhibit DNA methyltransferase directly or by reducing its gene expression<sup>[18]</sup>. There are several clinical trials focusing on the use of hydralazine to combat hypertensive conditions (Table 1). Many of these completed trials have highlighted the beneficial effect of hydralazine on both hypertension and other cardiovascular conditions compared to other compounds. Several studies have shown that hydralazine might function by modulating the effect of purine-like compounds released from sympathetic nerve endings and/or by inducing an altered Ca<sup>2+</sup> balance in vascular smooth muscle cells<sup>[19,20]</sup>; unfortunately, there are fundamental as-yet unresolved issues concerning this area of research that remain unclarified.

Procainamide is another drug that inhibits DNA methyltransferase I. It is a sodium channel blocker that belongs to the class of benzamides used against arrhythmias<sup>[21]</sup>. Clinical trials have evaluated this anti-arrhythmic drug in the acute treatment of monomorphic ventricular tachycardia with positive effects (Table 1). Nevertheless, recent evidence has shown toxic effects of procainamide on the lung after orthotopic cardiac transplantation<sup>[22]</sup>.

Despite the use of the aforementioned drugs in cancer treatment appearing to have promising results<sup>[23]</sup>, the implication of their epigenetic effects in CVDs requires further investigation in future studies.

**Table 1** Interventional and randomized ongoing clinical trials on the use of hydralazine and procainamide in cardiovascular diseases

No.	Status	Condition	No. of enrolled patients	Intervention
NCT00684489	Completed	Hypertension	52	Hydralazine and other drugs
NCT02305095	Not open for participant recruitment	Heart failure	500 (estimated enrollment)	Hydralazine in combination with isosorbide dinitrate
NCT00661895	Completed	Hypertension	99	Hydralazine and other drugs
NCT00599235	Completed	Hypertension	30	Hydralazine, sildenafil, and placebo
NCT00223717	Recruiting participants	Hypertension	160 (estimated enrollment)	Hydralazine and other drugs
NCT01255475	Completed	Heart failure, cardiac failure, and congestive heart failure	21	Hydralazine/amlodipine and placebo
NCT01516346	Recruiting participants	Heart failure and congestive heart failure	54 (estimated enrollment)	Hydralazine, isosorbide dinitrate, and placebo
NCT01822808	Recruiting participants	Acute heart failure and left ventricular dysfunction	500 (estimated enrollment)	Hydralazine, isosorbide dinitrate, and placebo
NCT00000499	Completed	Cardiovascular diseases, heart diseases, hypertension, and vascular diseases	Not provided	Hydralazine, reserpine, chlorthalidone, and metoprolol
NCT02050529	Recruiting participants	Hypertension, Pregnancy induced	180 (estimated enrollment)	Hydralazine, labetalol
NCT01538875	Completed	Hypertension, Pregnancy induced	261	Hydralazine, labetalol
NCT00383799	Unknown	Ventricular tachycardia	302 (estimated enrollment)	Procainamide, amiodarone
NCT00000464	Completed	Arrhythmia, Cardiovascular diseases	115	Procainamide, quinidine, disopyramide, and other drugs
NCT00702117	Completed	Atrial fibrillation, tachycardia	123	Procainamide, ajmaline, flecainide
NCT00589303	Terminated	Atrial fibrillation, heart failure	27	Rhythm control drugs: Procainamide and other drugs
NCT00000556	Completed	Arrhythmia, atrial fibrillation, cardiovascular diseases	4060	Procainamide and other drugs
NCT01205529	Recruiting	Atrial fibrillation	750 (estimated enrollment)	Procainamide

### Histone modifications as therapeutic target

Epigenetic alterations occur in the histone code, and so can modulate histone-DNA interactions and significantly influence chromatin structure by modifying the accessibility of transcriptional regulators to DNA-binding elements<sup>[24]</sup>. The most common modifications are lysine acetylation and methylation, arginine methylation, and serine phosphorylation. Histone acetylation is catalyzed by histone acetyltransferases (HATs), while histone deacetylation is carried out by histone deacetylases (HDACs)<sup>[25]</sup>.

Inhibitors of histone deacetylases (HDACi) represent a significant group of epidrugs that could be highly relevant to the treatment of CVDs. Indeed, HDACi exert anti-proliferative and anti-inflammatory effects, and their cardio-protective therapeutic use has been recently confirmed in preclinical studies<sup>[26,27]</sup>.

According to their chemistry, HDACi can be divided into four main groups: Hydroxamates, aliphatic acids, benzamides, and cyclic peptides. Hydroxamates like trichostatin A (TSA) and vorinostat (suberoylanilide hydroxamic acid, SAHA) serve as pan-HDACi and are generally most often used for preclinical studies<sup>[28-30]</sup>.

Principal histone modifications and therapeutic targets involved in CVDs are reported in Table 2. Animal studies *in vivo* showed that TSA treatment improved functional

myocardial recovery after myocardial infarction (MI) *via* a reduction in myocardial and serum tumor necrosis factor- $\alpha$ . Neo-angiogenesis was demonstrated in MI animals after receiving TSA treatment<sup>[31]</sup>. Taken together, these results indicate that HDACi could preserve cardiac performance and mitigate myocardial remodeling by stimulating endogenous cardiac regeneration<sup>[31]</sup>. HDAC inhibition was also shown to attenuate ischemic injury in the heart and other tissues. Pre-treatment with TSA resulted in improvements in post-ischemic ventricular function, with a reduction in infarct size in both early and delayed preconditioning models<sup>[32]</sup>. Despite the high activity of TSA, it was disqualified as a clinical drug due to its many side effects, such as non-transformed cell apoptosis and increased DNA damage<sup>[33]</sup>.

Vorinostat was approved by the United States Food and Drug Administration (FDA) for the treatment of advanced cutaneous T cell lymphoma<sup>[34]</sup>. Suberoylanilide hydroxamic acid (SAHA/vorinostat) reduced myocardial infarct size in a large animal model, even when delivered in the clinically relevant context of reperfusion<sup>[35,36]</sup>.

Aliphatic acids like valproic acid (VPA, 2-propylpentanoic acid) inhibits class I HDACs, causing accumulation of hyperacetylated histone tails (H3 and H4 histones) and other protein targets such as p53. VPA has anti-proliferative and pro-apoptotic activities. Lee *et al.*<sup>[37]</sup> demonstrated the



**Table 2** Histone modifications and therapeutic targets involved in cardiovascular diseases

Target	Epigenetic mechanisms	Condition	Organism/ <i>in vitro</i> , <i>in vivo</i>	Effects	Ref.
TSA	Inhibition of HDAC4	Ischemic injury	Mouse, <i>in vitro</i> and <i>in vivo</i>	HDACi would be predicted to have a beneficial effect in the context of active ischemia	Granger <i>et al</i> <sup>[28]</sup> (2008)
TSA/VPA	Class I HDACs	Cardiac hypertrophy	Mouse, <i>in vitro</i> and <i>in vivo</i>	Therapeutic target for preventing or reversing cardiac hypertrophy and subsequent heart failure	Kee <i>et al</i> <sup>[29]</sup> (2006)
TSA	Inhibition of HDACs	Atrial fibrosis and arrhythmias	Mouse, <i>in vitro</i> and <i>in vivo</i>	Reversed myocardial fibrosis	Liu <i>et al</i> <sup>[30]</sup> (2008)
TSA	Inhibition of HDACs	Acute myocardial ischemia and reperfusion injury	Mouse, <i>in vitro</i> and <i>in vivo</i>	Improved cardiac functional recovery and antagonized myocardial remodeling in chronic myocardial infarction	Zhang <i>et al</i> <sup>[31]</sup> (2012)
TSA/SAHA	HDAC inhibitor	Myocardial infarct	Mouse, rabbit, <i>in vivo</i>	Reduced infarct size in a large animal model	Xie <i>et al</i> <sup>[35]</sup> (2014)
SAHA/sodium valproate	Inhibition of HDACs	Ischemic injury	Mouse, <i>in vitro</i> and <i>in vivo</i>	Potential therapeutic strategy for restoring compromised cardiac proteostasis	Wang <i>et al</i> <sup>[36]</sup> (2011)
VPA or tributyrin	Inhibition of HDACs	Infarct	Rat, <i>in vitro</i>	Attenuated ventricular remodeling after infarction	Lee <i>et al</i> <sup>[37]</sup> (2007)
MS-275A	Inhibition of class I/II HDACs	Infarct	Rat, <i>in vivo</i>	Significant reduction of infarct area observed	Aune <i>et al</i> <sup>[39]</sup> (2014)
Apicidin	Inhibition of class I HDACs	Cardiac hypertrophy and heart failure	Rat pups, <i>in vitro</i>	Preserved cardiac function in the long-term	Gallo <i>et al</i> <sup>[42]</sup> (2008)
Curcumin	p300 HAT inhibitor	Heart failure	Rat, <i>in vitro</i>	Prevented deterioration of systolic function and heart failure	Morimoto <i>et al</i> <sup>[45]</sup> (2008)

TSA: Trichostatin A; HDAC: Histone deacetylases; HDACi: Inhibitors of histone deacetylases; SAHA: Suberoylanilide hydroxamic acid; VPA: Valproic acid.

attenuation of ventricular remodeling following MI *in vivo* when VPA or tributyrin was administered to rats 24 h after ligation of the left anterior descending artery. However, these short chain fatty acids are known to weakly inhibit HDAC activity with a number of off-target effects<sup>[38]</sup>.

Benzamides are small molecules that are mostly active against class I HDACs. Class I HDACi is entinostat (MS-275A) and prompts protective effects against ischemia reperfusion injury in isolated rat heart. MS-275A is not effective against class II b HDAC6<sup>[39]</sup>. Entinostat might be more advantageous than first-generation examples such as TSA, vorinostat, romidepsin, and VPA, as less profound side effects are observed<sup>[40]</sup>. Some studies have suggested that tranilast also has cardiovascular-protective effects<sup>[41]</sup>. Depsipeptide is a natural cyclic peptide that inhibits HDAC 1 and 2, and selectively modulates the expression levels of different genes such as c-myc, Hsp90, and p53. The cyclic peptide family includes other HDACi, such as apicidin. The apicidin derivative API-D is capable of reducing hypertrophy and, consequently, the transition to HF in mice subjected to thoracic aortic constriction. Treatment with this substance therefore establishes a relevant therapeutic approach for HF<sup>[42]</sup>.

The cardiovascular protective effects of p300 HAT inhibitor curcumin have been demonstrated<sup>[43,44]</sup>. In a rat model of HF and primary cultured rat cardiac myocytes and fibroblasts, curcumin prevented ventricular hypertrophy and preserved systolic function<sup>[45]</sup>.

### RNA-based mechanisms as novel biomarkers

MicroRNAs are key regulators of gene expression acting at

the post-transcriptional level. MiRNAs are implicated in the pathogenesis of several CVDs<sup>[46]</sup>. The modulation of miRNA expression could represent an innovative therapeutic approach to the treatment of cardiovascular conditions by targeting a single cell type or specific pathways, as demonstrated in an animal model<sup>[47,48]</sup>. Recently, several study population have investigated the involvement of transcriptionally regulated miRNAs as an attractive target for the treatment of several cardiovascular conditions (Table 3). Preclinical studies using antisense oligonucleotide (antagomir) -mediated knockdown have demonstrated the role of specific miRNAs in HF<sup>[47,49,50]</sup>. Indeed, it was shown that a single treatment with the infusion of a miR133 antagomir induced cardiac hypertrophy in mice<sup>[49]</sup>. Recently, Wahlquist *et al*<sup>[47]</sup> demonstrated that high levels of miR25 can depress cardiac function, although the inhibition of this miRNA by anti-miR25 effectively restores cardiac function in an HF mouse model. Interestingly, it was demonstrated that miRNAs secreted by cardiac fibroblasts may also act as mediators of cardiomyocyte hypertrophy *via* a paracrine mechanism<sup>[50]</sup>. During hypertension or pathological cardiac hypertrophy, reactivation of fetal cardiac genes such as atrial natriuretic peptide, (ANP)/B-type natriuretic peptide (BNP), and beta-myosin heavy chain ( $\beta$ -MHC) can occur. In a hypertensive mouse model, aldosterone-dependent inhibition of miR-208a can occur, resulting in  $\beta$ -MHC inhibition and an increase of cardiac hypertrophy<sup>[51]</sup>. It was also shown that therapeutic inhibition of miR-208a led to a reduction in cardiac remodeling, which coincided with a significant improvement in survival and cardiac function during heart disease<sup>[48]</sup>. Additionally, in hypertensive rat models, changes in  $\beta$ -MHC expression were observed

**Table 3** Recent evidence investigating the role of circulating miRNAs as biomarkers in several cardiovascular diseases

miRNAs	Sources	Conditions	Ref.
↑miR-339-5p, miR-483-3p ↓miR-139-5b	Plasma	LVI	Saddic <i>et al</i> <sup>[64]</sup> (2015)
↓miR-145	Plasma	AMI	Gao <i>et al</i> <sup>[65]</sup> (2015)
↑miR-122, miR-140-3p, miR-720, miR-2861, miR-3149	Plasma	ACS, AMI	Li <i>et al</i> <sup>[66]</sup> (2015)
↑Let-7e, miR-15a, miR-196b ↓miR-411	Plasma	AAA, Atherosclerosis	Stather <i>et al</i> <sup>[67]</sup> (2015)
↓miR-125b, miR-320b ↓miR-21	Plasma	AMI, CAD	Huang <i>et al</i> <sup>[68]</sup> (2014)
↓miR-31	Serum	CAD	Fan <i>et al</i> <sup>[69]</sup> (2014)
↑miR-146a, miR-186, miR-208b, miR-499	Plasma	CAD	Wang <i>et al</i> <sup>[70]</sup> (2014)
↑miR-210	Serum	ACS, Stable CAD, CV risk	Wu <i>et al</i> <sup>[71]</sup> (2014)
↑miR-21, miR-25, miR-92a, miR-106b, miR-126, miR-451, miR-590-5p	PBMC	HF	Endo <i>et al</i> <sup>[72]</sup> (2013)
↔ miR-1, miR-208a, miR-423-5p	Plasma	AP, UA	Ren <i>et al</i> <sup>[73]</sup> (2013)
↑miR-30a, miR-210	Plasma	AMI, CAD	Nabialek <i>et al</i> <sup>[74]</sup> (2013)
↑miR-337-5p, miR-433, miR-485-3p, miR-1, miR-122, miR-126, miR-133a/b, miR-199a	Serum	HF	Zhao <i>et al</i> <sup>[75]</sup> (2013)
↔miR-17-5p, miR-92a, miR-145, miR-155, miR-208a, miR-375, miR-799-5p	Plasma	AP, UA	D'Alessandra <i>et al</i> <sup>[76]</sup> (2013)
↓miR-103, miR-142-3p, miR-30b, miR-342-3p	Plasma	HF	Ellis <i>et al</i> <sup>[77]</sup> (2013)
↑miR-122, miR-200b, miR-520d-5p, miR-622	WB and	HF	Vogel <i>et al</i> <sup>[78]</sup> (2013)
↓miR-558	serum		
↑miR-21, miR-133a, miR-423-5p, miR-499-5p	Plasma	HF, NSTEMI	Olivieri <i>et al</i> <sup>[79]</sup> (2013)
↔miR-1, miR-208a			
↑miR-133a	Plasma	AMI, AP	Wang <i>et al</i> <sup>[80]</sup> (2013)
↓miR-214	Plasma	AMI, AP, UA	Lu <i>et al</i> <sup>[81]</sup> (2013)

AAA: Abdominal aortic aneurysm; ACS: Acute coronary syndrome; AMI: Acute myocardial infarction; AP: Angina pectoris; CAD: Coronary artery disease; CV: Cardiovascular; HF: Heart failure; LVI: Left ventricular ischemia; NSTEMI: Non-ST-elevation myocardial infarction; PBMC: Peripheral blood mononuclear cells; UA: Unstable angina; WB: Whole blood.

after treatment with anti-miR-208a that acted by reverting the levels of several miRNAs, including miR-16, -19b, and -20b<sup>[52]</sup>. Recently, the regulation of miR-208a and endoglin in AMI were investigated, with the authors demonstrating that the overexpression of antagomir-208a significantly inhibited the increase of myocardial endoglin and  $\beta$ -MHC protein expression induced by infarction<sup>[53]</sup>. In addition, pre-treatment with atorvastatin and valsartan, members of a drug class known as statins that are primarily used for the prevention of events associated with cardiovascular disease, can decrease myocardial fibrosis induced by AMI by attenuating miR-208a and endoglin expression<sup>[53]</sup>. Clinical evidence supports the different levels of miR-143, miR-145, miR-21, miR-133, and miR-1 expression in patients with essential hypertension, suggesting that these miRNAs can act in vascular smooth muscle cell phenotypic modulation and could represent potential therapeutic targets in essential hypertension<sup>[54]</sup>. It was found that the chronic restoration of miR-1 gene expression in an animal model reverted pressure-induced cardiac hypertrophy and prevented the adverse cardiac remodeling induced by pressure overload<sup>[55]</sup>. Recently, Han *et al*<sup>[56]</sup> found higher levels of miR-29a in patients with hypertension and left ventricular (LV) hypertrophy compared to patients with hypertension alone. MiR-29a levels were significantly associated with collagen type I and III and MMP-9 expression. The same authors, employing a mouse model of pressure overload, have shown that antagomir-29a significantly suppressed the hypertrophy of cardiomyocytes and reduced the expression of ANP

and  $\beta$ -MHC, suggesting a possible role of miR-29a as a therapeutic target<sup>[56]</sup>. Several preclinical studies showed the beneficial effects of antagomir-92a administration on small and large animal models before MI<sup>[57-59]</sup>. Inhibition of miR-92a by repeated intravenous injections of antagomir-92a induced angiogenesis and improved recovery of ventricular function in MI mouse model<sup>[57]</sup>. In MI large animal models, antagomir-92a treatment revealed cardio-protection against ischemia/reperfusion<sup>[58]</sup>. Recent evidence has demonstrated favorable post-ischemic myocardial repair after intravenous administration of antagomir-92a in adult large animal models<sup>[59]</sup>. Indeed, neovasculation and the prevention of adverse ventricular remodeling, the major cause of contractile dysfunction and HF after MI, were observed after intravenous administration of antagomir-92a<sup>[59]</sup>. These results reveal a promising therapeutic approach for patients affected by MI. Progression of post-infarction LV remodeling in mice was studied by Tolonen *et al*<sup>[60]</sup>, who observed that the inhibition of Let-7c was associated with decreased apoptosis, reduced fibrosis, and a reduction in the number of discoidin domain receptor 2-positive fibroblasts, while the number of c-kit<sup>+</sup> cardiac stem cells and Ki-67<sup>+</sup> proliferating cells remained unaltered<sup>[60]</sup>. Although Let-7c inhibitor injection improved cardiac function after MI, the safety of Let-7c inhibition has yet to be clarified due to its dualistic function that appears to have a causative role in various cancer diseases.

Circulating miRNA patterns are analyzed as potential disease specific biomarkers in CVDs in two observational

prospective studies on aortic aneurism in hereditary aortopathy syndromes (NCT02213484), coronary artery diseases, and myocardial infarction (NCT02076153). Three interventional randomized studies are focusing on the association between miRNA profile modifications and the administration of specific molecules like anti-platelet agents (NCT02071966) (NCT02447809) in coronary syndromes and anti-diabetics drugs in diabetic stable and unstable angina (NCT01331967).

## CONCLUSION

To date, several epidrugs (such as vorinostat and panobinostat) have been approved for the treatment of cancer and myelodysplastic syndromes, and are therefore commercially available. However, no epigenetic drugs for CVDs have yet been actually approved by the FDA. Nevertheless, the opportunity to control genetic and epigenetic processes could be considered a promising and attractive tool in cardiovascular medicine. For this reason, the investigation of epigenetic-related mechanisms might help to explain how environmental and lifestyle factors can influence aberrant gene expression patterns over a lifetime that can result in increased cardiovascular risk. Preclinical experiments have identified some HDACi that could have future implications in the treatment of several cardiovascular conditions, including atrial fibrillation, cardiac hypertrophy, and HF. Ongoing human clinical controlled studies are emphasizing the ability of some drugs such as hydralazine and procainamide to act on DNA methylation in CVDs. However, the clinical experience with HDACi in CVDs is limited due to the observed toxic cardiac side effects in oncologic patients.

The study of the human genome will find biomarkers that might affect CVDs. It is likely that only epigenetic profiles obtained from large cohorts of patients with the same genetic mutations will be able to promote the development of surveillance programs and novel effective drugs for the transition of *in vitro* to *in vivo* treatments for the early stage of CVDs<sup>[61-63]</sup>.

To date, few clinical trials have investigated the link between drugs and specific miRNA profiles, which might be considered as biomarkers for the classification of CVDs with scarce compliance to standard therapy and affected by the incidence of more aggressive clinical phenotypes. Unfortunately, antagomir in the area of cardiovascular disease has not yet been tested in clinical trials. However, the promising studies covered here reflect the open debate for possible future applications of miRNA therapeutics in CVDs.

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Retrospective Study

## Red cell distribution width in anemic patients undergoing transcatheter aortic valve implantation

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**Informed consent statement:** Patients were not required to give informed. To the study because the analysis used anonymous clinical data that was obtained after each patient agreed to treatment by written consent.

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### Abstract

**AIM:** To determine the impact of red blood cell distribution width on outcome in anemic patients undergoing transcatheter aortic valve implantation (TAVI).

**METHODS:** In a retrospective single center cohort study we determined the impact of baseline red cell distribution width (RDW) and anemia on outcome in 376 patients with aortic stenosis undergoing TAVI. All patients were discussed in the institutional heart team and declined for surgical aortic valve replacement due to high operative risk. Collected data included patient characteristics, imaging findings, periprocedural in hospital data, laboratory results and follow up data. Blood samples for hematology and biochemistry analysis were taken from every patient before and at fixed intervals up to 72 h after TAVI including blood count and creatinine. Descriptive statistics were used for patient's characteristics. Kaplan-Meier survival curves were used for time to event outcomes. A recursive partitioning regression and classification was used to investigate the association between potential risk factors and outcome variables.

**RESULTS:** Mean age in our study population was  $81 \pm 6.1$

years. Anemia was prevalent in 63.6% ( $n = 239$ ) of our patients. Age and creatinine were identified as risk factors for anemia. In our study population, anemia per se did influence 30-d mortality but did not predict longterm mortality. In contrast, a RDW  $> 14\%$  showed to be highly predictable for a reduced short- and longterm survival in patients with aortic valve disease after TAVI procedure.

**CONCLUSION:** Age and kidney function determine the degree of anemia. The anisocytosis of red blood cells in anemic patients supplements prognostic information in addition to that derived from the WHO-based definition of anemia.

**Key words:** Anemia; Red cell distribution width; Red blood cells; Transcatheter aortic valve implantation; Aortic stenosis

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**Core tip:** This is a retrospective study to evaluate the impact of prevalent anemia and the importance of red cell distribution width (RDW) on the outcome in patients undergoing transcatheter aortic valve replacement. Anemia was prevalent 63.6% of the patients and did influence 30-d mortality but did not predict longterm mortality. In contrast, a RDW  $> 14\%$  showed to be highly predictable for a reduced short- and long-term survival in patients with aortic valve disease after transcatheter aortic valve implantation procedure. Age and creatinine were identified as risk factors for anemia.

Hellhammer K, Zeus T, Verde PE, Veulemanns V, Kahlstadt L, Wolff G, Erkens R, Westenfeld R, Navarese EP, Merx MW, Rassaf T, Kelm M. Red cell distribution width in anemic patients undergoing transcatheter aortic valve implantation. *World J Cardiol* 2016; 8(2): 220-230 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v8/i2/220.htm> DOI: <http://dx.doi.org/10.4330/wjc.v8.i2.220>

## INTRODUCTION

Anemia is common in elderly patients with cardiovascular disease. An association of increased mortality with decreasing levels of hemoglobin has been shown in patients with coronary artery disease (CAD), acute myocardial infarction, cardiac heart failure (CHF) and structural heart disease<sup>[1-4]</sup>. Anemia also affects outcome after percutaneous coronary artery intervention (PCI), coronary artery bypass graft (CABG), and transcatheter aortic valve replacement (TAVI)<sup>[5-7]</sup>. In patients with aortic valve disease anemia often occurs in combination with occult bleeding within the gastro-intestinal tract.

According to the WHO, anemia is defined by a level of hemoglobin  $< 13$  g/dL in men and  $< 12$  g/dL in women<sup>[8]</sup>. Studies correcting anemia by either erythropoiesis stimulating agents (ESA) or by transfusion of packed red

blood cells (RBC) yielded conflicting results<sup>[9]</sup>. ESA failed to improve outcome in acute myocardial infarction<sup>[10]</sup>, chronic kidney disease<sup>[11]</sup>, and heart failure<sup>[12]</sup>. RBC transfusions to patients undergoing primary PCI<sup>[13,14]</sup>, CABG<sup>[15]</sup>, and TAVI<sup>[7]</sup>, respectively, may be even harmful and were associated with increased mortality. The storage lesion and subsequent scavenging of nitric oxide (NO) through occult hemolysis after transfusion may at least in part account for these detrimental effects<sup>[15-17]</sup>. These data raise the question whether or not the mere determination of the hemoglobin levels is appropriate for risk stratification and guidance of anemia treatment in mostly elderly patients at high cardiovascular risk.

Red blood cell distribution width (RDW) is a quantitative measure of anisocytosis, the variability in size of circulating RBC. It is routinely measured in automated hematology analyzers and is reported together with hemoglobin, RBC number, and hematocrit as a component of complete blood count. RDW is typically elevated in conditions of ineffective RBC production, *e.g.*, iron or vitamin B12 deficiency, increased RBC destruction such as in hemolysis, after blood transfusion or during severe inflammation. Conceivably, RDW may represent an integrative measure of multiple pathologic processes in the elderly patient with structural heart disease, explaining its strong association with clinical short and long term outcomes<sup>[18-24]</sup>. Relevant comorbidities affecting RDW in those patients may include renal dysfunction, inflammatory stress, and nutritional deficiencies. Thus, the measurement of RDW as compared to hemoglobin may add or provide even superior information to stratification of those high risk patients with advanced aortic valve stenosis undergoing TAVI procedures.

Recent studies indicate that the detrimental effects of anemia is not only mediated by the absolute hemoglobin levels, but also by the quality of the endogenous and the substituted RBCs. Different subtypes of anemia affect the outcome after stenting in stable coronary artery disease distinctly<sup>[25]</sup>. Red cell distribution width (RDW) has emerged as a novel marker not only of the size of erythrocytes, but also as an index of quality and function of RBC<sup>[19,26]</sup>. RDW is a powerful and independent predictor of mortality in cardiac heart failure<sup>[18,20,21]</sup>. The role of RDW in anemic patients undergoing TAVI is not clear. We therefore investigated whether RDW may have the potential to act as a novel prognostic parameter for risk stratification in addition to anemia, as defined by WHO criteria.

## MATERIALS AND METHODS

### Patient selection and study design

The study population consisted of 376 patients with severe symptomatic aortic stenosis who underwent TAVI with either the Medtronic CoreValve system (Medtronic Inc, Minneapolis, MN) or the Edwards SAPIEN Valve (Edwards Lifesciences, Irvine, CA) from August 2009 to August 2013 at the Heart Center Duesseldorf. All patients were discussed in the institutional heart team and



declined for surgical aortic valve replacement due to high operative risk. All patients gave their written informed consent for TAVI and the use of clinical, procedural and follow up data for research. Study procedures were in accordance with the Declaration of Helsinki and the institutional Ethics Committee of the Heinrich-Heine University approved the study protocol. The study is registered at clinical trials (NCT01805739).

### Data collection and definitions

Collected data included patient characteristics, imaging findings, periprocedural in hospital data, laboratory results and follow up data. Blood samples for hematology and biochemistry analysis were taken from every patient before and at fixed intervals up to 72 h after TAVI including blood count and creatinine. As reported by the World Health Organisation (WHO) baseline anemia was defined as a hemoglobin (Hb) level of < 13 g/dL for men and < 12 g/dL for women. Preoperative serum creatinine values were used to calculate the baseline serum creatinine clearance using the Cockcroft and Gault equation<sup>[27]</sup>. Chronic kidney disease (CKD) was defined as a calculated serum clearance < 60 mL/min<sup>[28]</sup>. Clinical endpoints were reported according to The Valve Academic Research Consortium (VARC) consensus statement<sup>[29]</sup>. Follow up data for mortality were collected by contacting the attending physician and the civil registries. Technical appendix, statistical code, and dataset are available from the corresponding author. Participants gave informed consent for data sharing.

### TAVI procedure

TAVI procedures were performed according to current guidelines<sup>[30]</sup>. A single antibiotic shot was given shortly before TAVI procedure. All patients were referred to intensive care after the procedure. For antiplatelet therapy, patients received a combination therapy of aspirin 100 mg/d and clopidogrel 75 mg/d for three months after TAVI followed by permanent aspirin mono therapy. Patients on oral anticoagulation received clopidogrel 75 mg/d and oral anticoagulation for three months followed by oral anticoagulation.

### Statistical analysis

The statistical methods of this study were reviewed by Pablo E Verde from the Coordination Center for Clinical Trials Düsseldorf. Descriptive statistics are based on frequency tables for categorical data, means and standard deviations for continuous variables and Kaplan-Meier survival curves for time to event outcomes. Association between continuous variables are analyzed with Person's correlation coefficient and displayed graphically with scatter plots.

A recursive partitioning regression and classification was used to investigate the association between potential risk factors and outcome variables. This approach is based on the method describe by Horhorn *et al.*<sup>[31]</sup>. This technique combines an algorithm for recursive

partitioning together with a well defined theory of permutation tests. Multiple test procedures are applied to determine whether a significant association between any of the covariables and the response variable can be stated. The resulting partitioning regression analysis is graphically displayed as a classification tree. The partitioning nodes are displayed by an optimal cut-off point for continues covariables and with a classification split for categorical covariables. Each node-split is assessed with a *P*-value calculated by a permutation test. In addition, regression analysis for binary outcomes was performed using the classical logistic regression and for time to event outcomes the proportional hazard Cox's regression. In each case we report results for all covariables included in the model and with covariables selected by using a step-wise variable selection based on taking the minimum value of AIC (Akaike Information Criteria). As graphical outputs for regression analysis a forest plot is used, in this figure the odds ratio and the 95% confidence interval is displayed for each variable in the model. Data analysis was performed using the statistical software R version 3.1.0<sup>[32]</sup>, SPSS Statistics 22 (IBM®) and GraphPad (Prism®).

## RESULTS

### Baseline characteristics

Anemia was prevalent in 63.6% (*n* = 239) of our study population (Table 1). Groups with and without anemia did not differ except for chronic kidney disease (*P* = 0.001), history of myocardial infarction (*P* = 0.029), and the need for dialysis due to end-stage chronic kidney disease (*P* = 0.009).

Serum levels for baseline serum creatinine (anemia: 1.5 mg/dL ± 1.2 mg/dL vs no anemia: 1.0 mg/dL ± 0.5 mg/dL; *P* < 0.001) and C-reactive Protein (anemia: 1.4 mg/dL ± 2.0 mg/dL vs no anemia: 0.8 mg/dL ± 1.1 mg/dL; *P* < 0.001) were higher in patients with anemia whereas baseline creatinine clearance was lower in anemic patients (54.5 mL/min ± 23.6 mL/min vs 65.9 mL/min ± 22.2 mL/min; *P* < 0.001). As a marker for the variability in size of the circulating erythrocytes the RDW was higher in patients with anemia (15.4% ± 1.8% vs 14.4% ± 1.6%; *P* < 0.001).

### Procedural outcome and 30-d mortality

Clinical outcome was reported according to VARC criteria<sup>[29]</sup>. The findings are summarized in Table 2. There was no difference with regard to vascular or bleeding complications in between both groups. Overall incidence of acute kidney injury (AKI) after TAVI was higher in patients with anemia (25.1% vs 10.9%; *P* = 0.001). Further clinical endpoints as stroke (anemia: 2.9% vs no anemia: 2.2%; *P* = 0.668), myocardial infarction (anemia: 0.4% vs no anemia: 0.0%; *P* = 0.448), endocarditis (anemia: 0.0% vs no anemia: 0.0%) and need for permanent pacemaker after TAVI (anemia: 21.3% vs no anemia: 19.0%; *P* = 0.585) did not differ

**Table 1** Baseline characteristics of patients undergoing transcatheter aortic valve replacement according to the presence of baseline anemia *n* (%)

	Entire cohort ( <i>n</i> = 376)	Anemia ( <i>n</i> = 239)	No anemia ( <i>n</i> = 137)	<i>P</i> -value
Age, years ± SD	81 ± 6.1	82 ± 6.2	81 ± 5.9	0.101
Male	167 (44.4)	112 (46.9)	55 (40.1)	0.207
Weight, kg ± SD	74 ± 14.4	73 ± 14.2	75 ± 15.0	0.351
Height, cm ± SD	168 ± 8.8	168 ± 8.7	168 ± 9.1	0.685
NYHA III and IV	288 (76.6)	187 (78.6)	101 (73.7)	0.284
CAD	263 (69.9)	170 (71.1)	93 (67.9)	0.209
Previous myocardial infarction	39 (10.4)	31 (13.0)	8 (5.8)	0.029
Previous percutaneous intervention	168 (44.7)	113 (47.3)	55 (40.1)	0.181
Previous CABG	89 (23.7)	55 (23.1)	34 (24.8)	0.708
Previous valve	8 (2.1)	5 (2.1)	3 (2.2)	0.954
Previous stroke	34 (9.0)	23 (9.6)	11 (8.0)	0.604
Diabetes mellitus	93 (24.7)	59 (24.7)	34 (28.4)	0.977
Hypertension	355 (94.4)	224 (93.7)	131 (95.6)	0.441
Peripheral vascular disease	115 (30.6)	75 (31.4)	40 (29.2)	0.658
Cerebroarterial vascular disease	81 (21.5)	56 (23.4)	25 (18.2)	0.239
COPD	72 (19.1)	46 (19.2)	26 (19.0)	0.949
Atrial fibrillation	87 (23.1)	52 (21.8)	35 (25.5)	0.414
Permanent pacemaker	64 (17.0)	43 (18.1)	21 (15.3)	0.497
Chronic kidney disease	203 (54.0)	144 (60.3)	59 (43.1)	0.001
Dialysis	21 (5.6)	19 (7.9)	2 (1.5)	0.009
Aortic valve area, cm <sup>2</sup> ± SD	0.73 ± 0.2	0.71 ± 0.19	0.75 ± 0.22	0.094
Mitral regurgitation ≥ grade II	114 (30.3)	73 (32.2)	41 (31.5)	0.904
LVEF < 30%	20 (5.3)	16 (6.7)	4 (2.9)	0.253
LVEF 30%-44%	68 (18.1)	47 (19.7)	21 (15.3)	0.292
LVEF 45%-55%	49 (13.0)	29 (12.1)	20 (14.6)	0.493
LVEF > 55%	239 (63.6)	147 (61.5)	92 (67.2)	0.273
Logistic EuroSCORE, % ± SD	19.7 ± 12.9	20.5 ± 13.1	18.4 ± 12.5	0.133
Baseline hemoglobin, g/dL ± SD	11.9 ± 1.7	11.0 ± 1.1	13.6 ± 1.1	< 0.001
Baseline RDW, % ± SD	15.0 ± 1.8	15.4 ± 1.8	14.4 ± 1.6	< 0.001
Baseline serum creatinine, mg/dL ± SD	1.3 ± 1.1	1.5 ± 1.2	1.0 ± 0.5	< 0.001
Baseline GFR, mL/min ± SD	58.7 ± 23.7	54.5 ± 23.6	65.9 ± 22.2	< 0.001
Baseline CRP, mg/dL ± SD	1.2 ± 1.8	1.4 ± 2.0	0.8 ± 1.1	< 0.001
TF access	270 (71.8)	172 (72.0)	98 (71.5)	0.742
TA access	105 (27.9)	66 (27.6)	39 (28.5)	0.862
TS access	1 (0.3)	1 (0.4)	0 (0.0)	0.637

CABG: Coronary artery bypass grafting; CAD: Coronary artery disease; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; GFR: Glomerular filtration rate; LVEF: Left ventricular ejection fraction; NYHA: New York Heart Association; RDW: Red cell distribution width; TA: Transapical; TF: Transfemoral; TS: Transsubclavian.

between the groups. The incidence of a septical event was higher in patients with anemia (8.4% vs 2.2%;  $P = 0.016$ ). Overall 30-d mortality was 7.2% ( $n = 27$ ). In patients with anemia 30-d mortality was 9.2% ( $n = 22$ ) whereas 3.6% ( $n = 5$ ) of the patients without anemia died within 30 d ( $P = 0.045$ ). The partitioning regression analysis, displayed as a classification tree, showed that life-threatening bleeding ( $P < 0.001$ ) after TAVI and occurrence of AKI ( $P = 0.002$ ) were statistically relevant risk factors for 30-d mortality (Figure 1A). Stepwise multiple logistic regression analysis with all covariables and the best selected covariables (Figure 1B and C) confirmed these findings and showed that RDW was a statistically significant risk factor as well ( $P = 0.044$ ).

### Factors associated with anemia

The partitioning regression analysis using anemia as outcome parameter showed that a creatinine level > 1.1 mg/dL ( $P < 0.001$ ) and age > 83 years ( $P = 0.027$ ) were statistically relevant risk factors for anemia (Figure 2A). Stepwise multiple logistic regression analysis

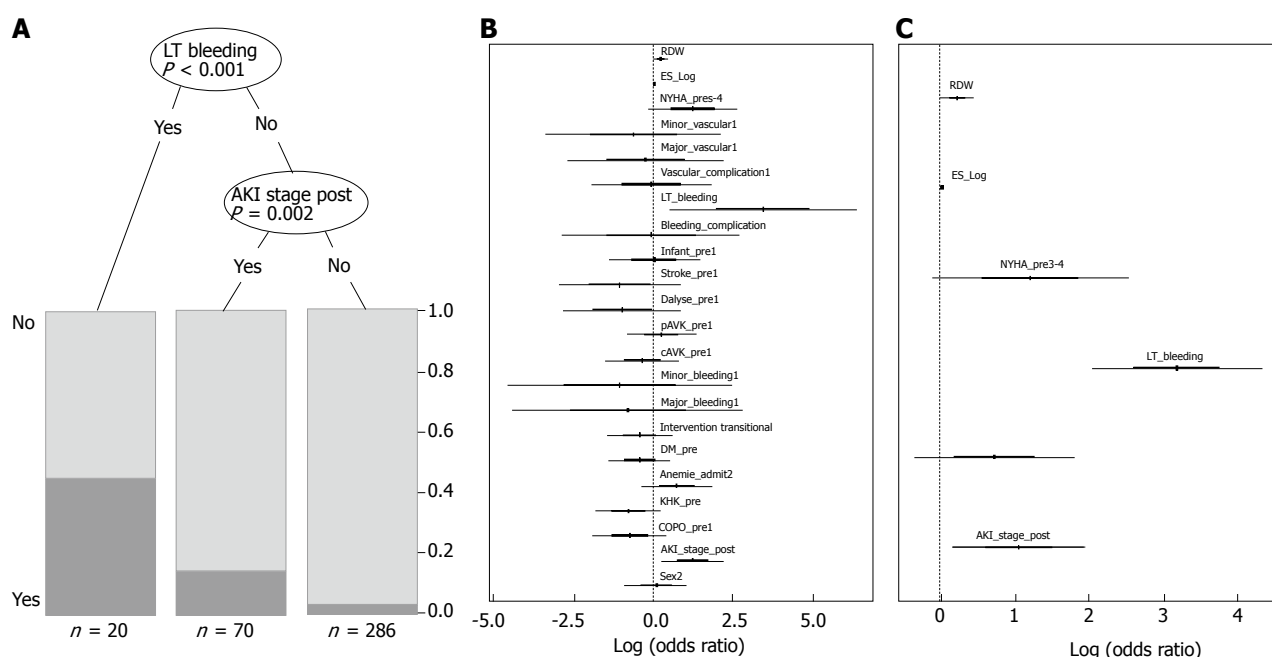
with all covariables and the best selected covariables confirmed these findings (Figure 2B and C). Mean Hb concentration in our study population was 11.9 g/dL ± 1.7 g/dL. In Figure 3A the distribution of Hb levels in our study population and marking lines for cut-off points defining anemia based on the WHO definition is shown. The distribution of RDW as a marker for the variability and function of circulating erythrocytes is shown in Figure 3B.

### Hemoglobin level and 1-year survival

One-year follow up was completed in 100% ( $n = 376$ ) of patients. The Kaplan-Meier survival curves for one-year mortality in patients with and without anemia are shown in Figure 4A. As the mean Hb concentration in our study population was 11.9 g/dL, the 1-year survival of patients grouped according to their Hb below or above this value is shown in Figure 4B. To find the best hemoglobin cut-off point to predict One-year mortality we performed a partitioning regression analysis which found a hemoglobin of 9.7 g/dL to be the optimal cut-

**Table 2** Clinical outcome of patients undergoing transcatheter aortic valve replacement according to the presence of baseline anemia *n* (%)

	Entire cohort ( <i>n</i> = 376)	Anemia ( <i>n</i> = 239)	No anemia ( <i>n</i> = 137)	<i>P</i> -value
Vascular complications				
Any vascular complications	34 (9.0)	24 (10.0)	10 (7.3)	0.372
Minor vascular complications	20 (5.3)	14 (5.9)	6 (4.4)	0.639
Major vascular complications	4 (1.1)	3 (1.3)	1 (0.7)	0.633
Bleeding complications				
Any bleeding complications	45 (12.0)	27 (11.3)	18 (13.1)	0.596
Life-threatening bleeding	20 (5.3)	12 (5.0)	8 (5.8)	0.732
Minor bleeding	21 (5.6)	12 (5.0)	9 (6.6)	0.529
Major bleeding	4 (1.1)	3 (1.3)	1 (0.7)	0.633
Percutaneous closure device failure	10 (2.7)	7 (2.9)	3 (2.2)	0.668
Acute kidney injury	75 (31.4)	60 (25.1)	15 (10.9)	0.001
Acute kidney injury stage I	44 (11.7)	33 (13.8)	11 (8.0)	0.093
Acute kidney injury stage II	1 (0.3)	1 (0.4)	0 (0.0)	0.636
Acute kidney injury stage III	30 (8.0)	26 (10.9)	4 (2.9)	0.007
Need for dialysis	22 (5.9)	18 (7.5)	4 (2.9)	0.069
Myocardial infarction	1 (0.3)	1 (0.4)	0 (0.0)	0.448
Stroke	10 (2.7)	7 (2.9)	3 (2.2)	0.668
Conversion to open surgery	8 (2.1)	6 (2.5)	2 (1.5)	0.497
Sepsis	23 (6.1)	20 (8.4)	3 (2.2)	0.016
Endocarditis	0 (0.0)	0 (0.0)	0 (0.0)	
Need for pacemaker	77 (20.5)	51 (21.3)	26 (19.0)	0.585
Length of stay > 14 d	235 (62.5)	152 (63.6)	83 (60.6)	0.561
30-d mortality, <i>n</i> (%)	27 (7.2)	22 (9.2)	5 (3.6)	0.045



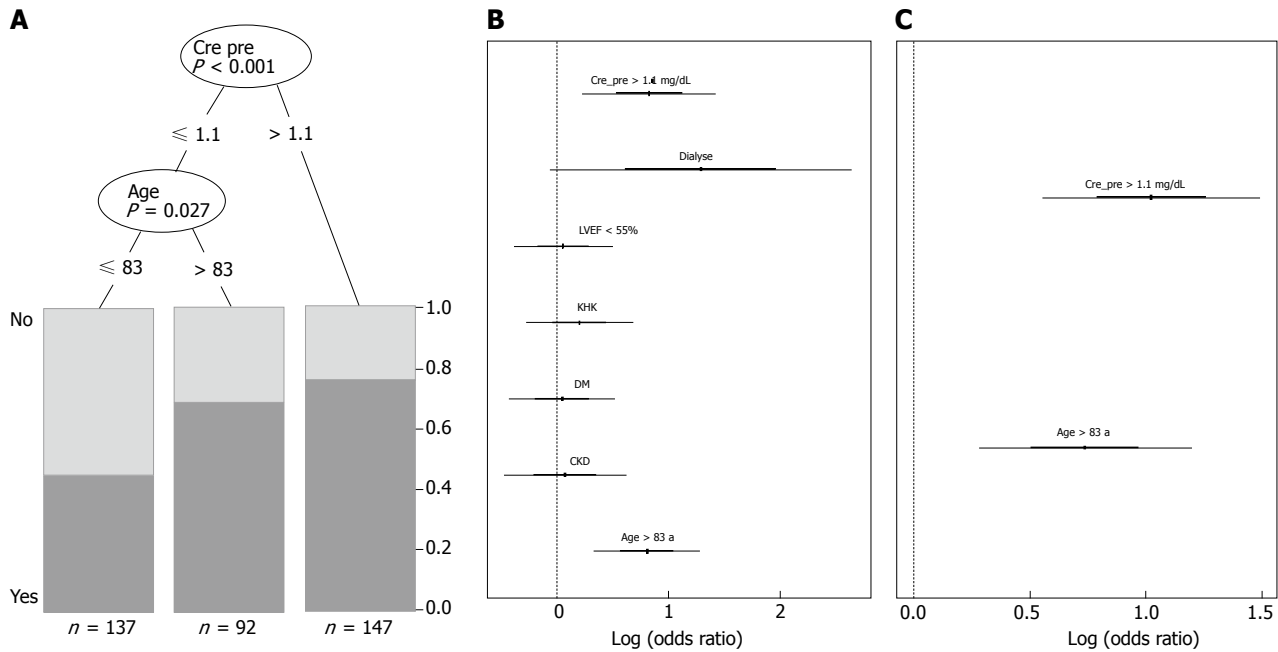
**Figure 1** Regression analysis for risk factors associated with 30-d mortality. A: Results from the classification tree with significant node-splits and distribution of patients. Life-threatening bleeding ( $P < 0.001$ ) and acute kidney injury ( $P = 0.002$ ) were found to be statistically relevant risk factors for 30-d mortality; B: Logistic regression with all covariables which were supposed to be associated with 30-d mortality. Forest plot with odds ratios and 95%CI (logarithmic scale); C: Logistic regression with the best selected covariables using AIC. Life-threatening bleeding ( $P < 0.001$ ), acute kidney injury post procedure ( $P = 0.018$ ) and RDW ( $P = 0.044$ ) were found to be statistically relevant risk factors for 30-d mortality. AIC: Akaike information criterion; AKI stage post: Acute kidney injury stage I-III post; CAD: Coronary artery disease; Cavk: Cerebroarterial vascular disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; ES log: Logistic EuroSCORE; LT bleeding: Life-threatening bleeding; NYHA: New York Heart Association; pAVK: Peripheral vascular disease; RDW: Red cell distribution width.

off point ( $P = 0.012$ ). The Kaplan-Meier survival curves of patients grouped according to their hemoglobin level above or below this cut-off point is shown in Figure 4C.

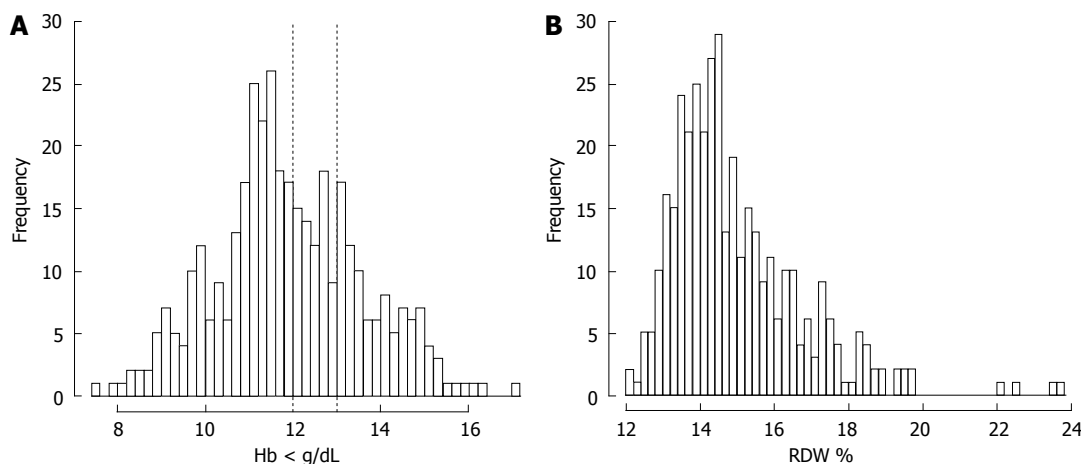
### RDW and mortality

As already described, RDW was found to be a risk

factor for 30-d mortality in our study population. The partitioning regression analysis using 30-d mortality as an outcome parameter showed a RDW cut-off point of 14% to predict 30-d mortality with the highest sensitivity and specificity (Figure 5A). In patients with RDW > 14% 30-d mortality and one-year mortality was



**Figure 2 Regression analysis for risk factors associated with anemia.** A: Classification tree with significant node-splits and distribution of patients with anemia. A creatinine  $> 1.1$  mg/dL ( $P < 0.001$ ) and age  $> 83$  ( $P = 0.027$ ) were found to be statistically relevant risk factors for anemia; B: Logistic regression with all covariables which were supposed to be associated with anemia. Forest plot with odds ratios and 95% confidence intervals (logarithmic scale); C: Logistic regression with the best selected covariables using AIC. A creatinine  $> 1.1$  mg/dL ( $P < 0.001$ ) and age  $> 83$  ( $P = 0.001$ ) were found to be statistically relevant risk factors for anemia. a: Years; AIC: Akaike information criterion; CAD: Coronary artery disease; CKD: Chronic kidney disease; Crea pre: Creatinine (mg/dL) preoperative; DM: Diabetes mellitus; LVEF: Left ventricular ejection fraction (%).



**Figure 3 Histogram of the distribution of hemoglobin and red cell distribution width levels.** A: Histogram of the distribution of hemoglobin levels. Vertical lines at 12 g/dL and 13 g/dL for population based cut-off points for women and men according to WHO definition of anemia; B: Histogram of RDW levels. Hb: Hemoglobin; RDW: Red cell distribution width.

significantly higher than in patients with a RDW  $< 14\%$  (Figure 5B).

To assess the association between hemoglobin and RDW we performed a correlation analysis (Figure 6) which revealed a significant negative correlation between hemoglobin and RDW ( $-0.36$ ; 95%CI:  $-0.45$ ,  $-0.27$ ;  $P < 0.001$ ) reflecting that an increasing severity of anemia is associated with an increased heterogeneity of red blood cell size.

### Anemia and RDW

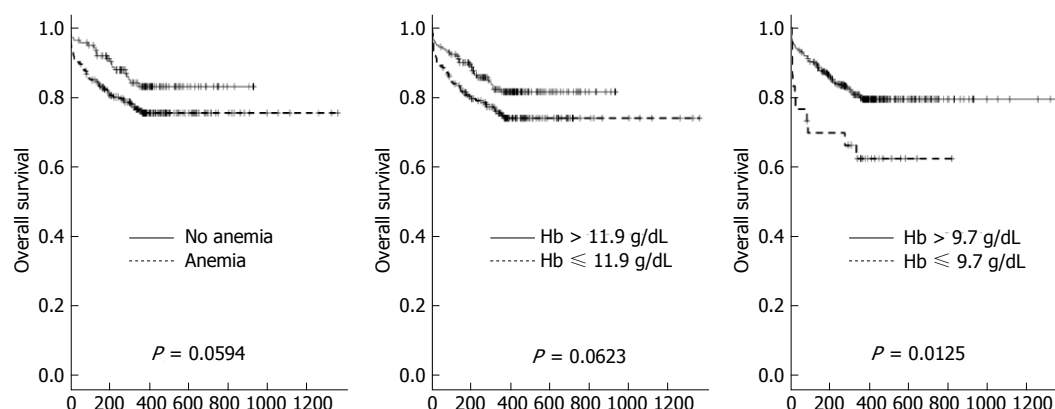
RDW has been shown to be elevated in conditions of

ineffective RBC production<sup>[19]</sup>. In our study population, anemic patients presented with a higher RDW than patients without anemia ( $P < 0.001$ ). The distribution of RDW levels in patients with and without anemia is shown in Figure 7A and B. The Kaplan-Meier survival curves of anemic patients grouped according to the presence of a RDW below or above 14% are shown in Figure 7C ( $P = 0.013$ ).

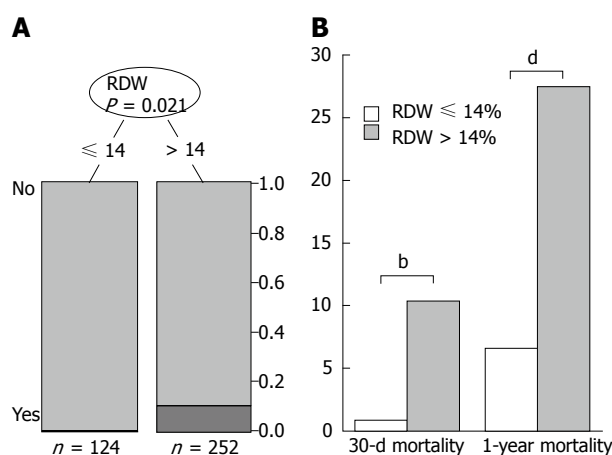
## DISCUSSION

The major findings of the present study are: (1) two

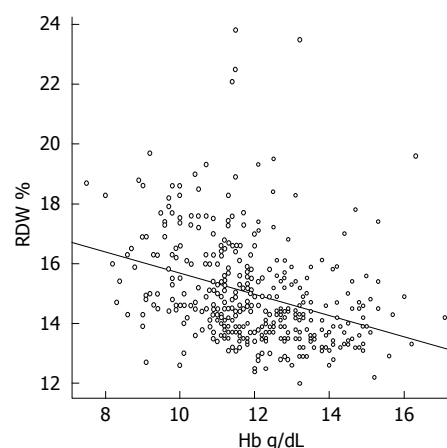




**Figure 4 Anemia and one-year mortality.** A: One-year survival curves of patients with and without anemia ( $P = 0.0594$ ); B: One-year survival curves of patients grouped according to their hemoglobin level above or below mean Hb level of 11.9 g/dL; C: One-year survival curves of patients grouped according to their hemoglobin level above or below cut-off point of 9.7 g/dL.



**Figure 5 Red cell distribution width and mortality.** A: Classification tree for 30-d mortality with significant node split at RDW 14% ( $P = 0.021$ ); B: Thirty-day ( $P < 0.01$ ) and one-year mortality ( $P < 0.001$ ) of patients grouped according to the presence of RDW  $\leq 14\%$  or  $> 14\%$ . RDW: Red cell distribution width.



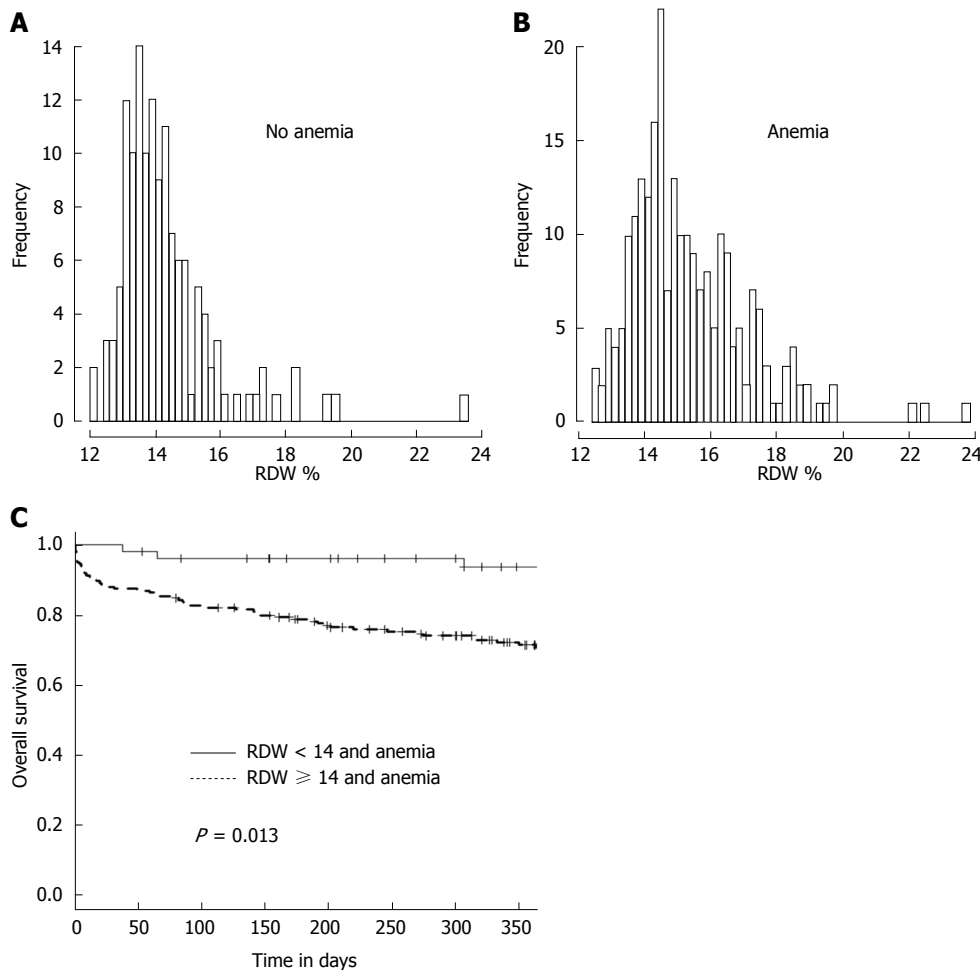
**Figure 6 Correlation of hemoglobin with red cell distribution width.** RDW and hemoglobin showed a significantly negative correlation ( $-0.36$ ; 95%CI:  $-0.45, -0.27$ ;  $P < 0.001$ ). Hb: Hemoglobin; RDW: Red cell distribution width.

thirds of TAVI patients are anemic according to the WHO definition; (2) age and level of creatinine determine independently the incidence of anemia in this population; (3) anemia affects incidence of TAVI related kidney injury and 30 d mortality according to VARC criteria for short term outcome; (4) a lower threshold of Hb (9.7 mg/dL) predicts 1 year mortality more precisely than the classical WHO definition of anemia in this patient cohort in our study; (5) absolute levels of hemoglobin are related only loosely to size, distribution and presumably function of red blood cells; and (6) a red blood cell distribution width of  $> 14\%$  is highly predictable for a reduced rate of survival in patients with aortic valve disease one year after TAVI procedure, particularly in those patients with already preexisting anemia. These findings raise the question whether or not the RDW should be integrated in the risk stratification in elderly anemic patients undergoing TAVI procedure.

#### Definition and incidence of anemia

In elderly patients with aortic valve disease the age

and the kidney function are the major predictors on the prevalence of anemia, which is similar to reports in patients with CAD and CHF<sup>[1,3]</sup>. Kidney function deteriorates with increasing age and the number of circulating RBC is critically dependent on the axis of renal stimulation of bone marrow synthesis of erythrocytes. According to the definition of the WHO, anemia was common in elderly patients with aortic valve stenosis and the mean value of hemoglobin level in the entire cohort was only 11.9 g/dL. Both the threshold levels suggested by the WHO and the mean value of Hb failed to precisely discriminate those patients at increased or reduced mortality rate in our study cohort. Only a level of  $< 9.7$  g/dL hemoglobin identified patients with a reduced survival at one year after TAVI. This finding is in line with previous reports on an increased mortality one year after TAVI with decreasing levels of hemoglobin<sup>[7]</sup>. These data imply that categorizing patients as anemic or non-anemic according to the WHO criteria might be helpful to stratify patients undergoing TAVI for their periprocedural risk and short term survival, whereas long term mortality and overall risk is better achieved with a threshold of  $< 10$  g/dL of hemoglobin.



**Figure 7** Distribution of red cell distribution width and survival of anemic patients according to their red cell distribution width. A: Distribution of RDW in patients without anemia; B: Distribution of RDW in patients with anemia; C: Survival curves of patients with anemia grouped to their RDW above or below cut off point of 14% ( $P = 0.013$ ). RDW: Red cell distribution width.

### Assessment of red blood cell function

The major task of erythrocytes is to deliver oxygen required to meet metabolic demands to tissues. Apart from the hemoglobin-dependent transport of oxygen, RBC serve many other functions. Number and distribution of RBC in the circulation are determined by their membrane and erythrocyte function<sup>[33-35]</sup>. Alterations of the redox status and the conformation of membrane regulate their shape, their distribution, passage through the microcirculation and their removal from the circulation by the reticulo-endothelial. RBC release ATP, NO, nitrite, prostanoids, chemo kinins and sulfide<sup>[36]</sup>. More recently we and others have shown that RBCs modulate their deformability, vascular tone, infarct size and thrombus formation at the endothelium through NOS/sGC signaling<sup>[37-40]</sup>. The RBC deformability and the rapid shape change are of paramount importance for the passage through the microcirculation and effective tissue perfusion. An increased RDW is associated with an impairment of RBC deformability<sup>[19]</sup>. These data may raise concerns with the view that sole measurements of hemoglobin levels reflect appropriately consequences of anemia and

their impact on outcome in cardiovascular diseases and interventions.

The distribution and width of RBC as a novel marker for adverse outcome in CHF has been described in the cohort of the CHARM trial only recently<sup>[20]</sup>. Among 36 routine laboratory values including hematocrit and hemoglobin, higher RDW showed the greatest association with morbidity and mortality. Given the association of hemoglobin with adverse outcome in CHF and CAD we evaluated the relationship of RDW and level of hemoglobin (Figure 6). We observed a moderate negative correlation as was also reported for CHF<sup>[20]</sup>. In all final multivariate models RDW was a significant predictor of short term outcome after TAVI.

### Conclusion

Age and kidney function determine the degree of anemia. The anisocytosis of red blood cells in anemic patients is emerging as an important parameter to assess short and long term mortality in patients undergoing TAVI. These findings demonstrate that RDW supplements prognostic information in addition to that derived from the WHO-

based definition of anemia.

### Study limitations

Our results have to be confirmed in larger cohorts with a longer follow up period to establish RDW as an independent and powerful prognostic marker in elderly patients with structural heart disease. In our retrospective single center cohort study we did not systematically substitute anemia with packed red blood cells and left this decision at the discretion of the interventionalists and the colleagues supervising the patients after the TAVI procedure on the ICU and the regular ward. However, we did focus on the Hb levels and RDW at the time of admittance prior to the TAVI procedure and the percentage of patients that received transfusion within the hospital was comparable in the anemic and the non-anemic group. Therefore, we believe that this did not affect outcome differences with respect to RDW (prior to TAVI) between both groups. Further, we did not investigate the treatment of anemic patients and patients with chronic kidney disease which may have been an interesting aspect.

In addition mechanistic studies focusing on RBC signaling cascades that might be altered in these elderly patients appear highly mandatory to identify potential novel therapeutic targets to improve RBC function and to determine how treatment of anemia should be guided and monitored in this elderly population with aortic valve disease.

## COMMENTS

### Background

Anemia is common in elderly patients with cardiovascular disease. An association of increased mortality with decreasing levels of hemoglobin has been shown in patients with coronary artery disease, acute myocardial infarction, cardiac heart failure and structural heart disease. Red blood cell (RBC) distribution width (RDW) is a quantitative measure of anisocytosis, the variability in size of circulating RBC. It may represent an integrative measure of multiple pathologic processes in the elderly patient with structural heart disease, explaining its strong association with clinical short and long term outcomes. Recent studies indicate that the detrimental effects of anemia are not only mediated by the absolute hemoglobin levels, but also by the quality of the endogenous and the substituted RBCs. The role of RDW in anemic patients undergoing TAVI is not clear. The authors therefore investigated whether RDW may have the potential to act as a novel prognostic parameter for risk stratification in addition to anemia, as defined by WHO criteria.

### Research frontiers

Red cell distribution width has been shown to be a novel marker not only of the size of erythrocytes, but also as an index of quality and function of RBC. It has been shown to be a powerful and independent predictor of mortality in cardiac heart failure. The results of this study contributes to evaluate the impact of prevalent anemia on outcome and to clarify the prognostic value of RDW in anemic TAVI patients.

### Innovations and breakthroughs

In this study, anemia was prevalent 63.6% of the patients and did influence 30-d mortality but did not predict longterm mortality. In contrast, a RDW > 14% showed to be highly predictable for a reduced short- and long-term survival in patients with aortic valve disease after TAVI procedure. Age and creatinine were identified as risk factors for anemia.

### Applications

This study suggests that RDW is a useful additional parameter which gives prognostic information concerning the outcome of anemic patients undergoing transcatheter aortic valve implantation.

### Terminology

Red cell distribution width (RDW): RDW is a quantitative measure of anisocytosis, the variability in size of circulating RBC. It has been shown to be a novel marker not only of the size of erythrocytes, but also as an index of quality and function of RBC.

### Peer-review

The paper is well structured, the presentation is clear and the discussion is in accordance with the results presented. The paper brings some novelty in the field.

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Observational Study

## Association of arterial stiffness with coronary flow reserve in revascularized coronary artery disease patients

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### Abstract

**AIM:** To investigate the association of arterial wave reflection with coronary flow reserve (CFR) in coronary artery disease (CAD) patients after successful revascularization.

**METHODS:** We assessed 70 patients with angiographically documented CAD who had undergone recent successful revascularization. We measured (1) reactive hyperemia index (RHI) using fingertip peripheral arterial tonometry (RH-PAT Endo-PAT); (2) carotid to femoral pulse wave velocity (PWVc-Complior); (3) augmentation index (AIx), the diastolic area (DAI%) and diastolic reflection area (DRA) of the central aortic pulse wave (Arteriograph); (4) CFR using Doppler echocardiography; and (5) blood levels of lipoprotein-phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>).

**RESULTS:** After adjustment for age, sex, blood pressure parameter, lipidemic, diabetic and smoking status, we found that coronary flow reserve was independently related to AIx ( $b = -0.38$ ,  $r = 0.009$ ), DAI ( $b = 0.36$ ,  $P = 0.014$ ), DRA ( $b = 0.39$ ,  $P = 0.005$ ) and RT ( $b = -0.29$ ,

$P = 0.026$ ). Additionally, patients with  $\text{CFR} < 2.5$  had higher PWVc ( $11.6 \pm 2.3$  vs  $10.2 \pm 1.4$  m/s,  $P = 0.019$ ), SBPc ( $139.1 \pm 17.8$  vs  $125.2 \pm 19.1$  mmHg,  $P = 0.026$ ), AIX ( $38.2\% \pm 14.8\%$  vs  $29.4\% \pm 15.1\%$ ,  $P = 0.011$ ) and lower RHI ( $1.26 \pm 0.28$  vs  $1.50 \pm 0.46$ ,  $P = 0.012$ ), DAI ( $44.3\% \pm 7.9\%$  vs  $53.9\% \pm 6.7\%$ ,  $P = 0.008$ ), DRA ( $42.2 \pm 9.6$  vs  $51.6 \pm 11.4$ ,  $P = 0.012$ ) and LpPLA2 ( $268.1 \pm 91.9$  vs  $199.5 \pm 78.4$  ng/mL,  $P = 0.002$ ) compared with those with  $\text{CFR} \geq 2.5$ . Elevated LpPLA2 was related with reduced CFR ( $r = -0.33$ ,  $P = 0.001$ ), RHI ( $r = -0.37$ ,  $P < 0.001$ ) and DRA ( $r = -0.35$ ,  $P = 0.001$ ) as well as increased PWVc ( $r = 0.34$ ,  $P = 0.012$ ) and AIX ( $r = 0.34$ ,  $P = 0.001$ ).

**CONCLUSION:** Abnormal arterial wave reflections are related with impaired coronary flow reserve despite successful revascularization in CAD patients. There is a common inflammatory link between impaired aortic wall properties, endothelial dysfunction and coronary flow impairment in CAD.

**Key words:** LpPLA2; Coronary artery disease; Arterial stiffness; Coronary flow reserve; Reactive hyperemia index

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**Core tip:** The present study is a contribution to investigate the association between the abnormalities in arterial wave reflections and coronary flow reserve. We demonstrated that augmentation of the systolic component of the central aortic pulse wave instead of diastolic is related with impaired coronary flow reserve after adjustment for several other factors potentially influencing coronary microcirculatory function. Furthermore, endothelial dysfunction as assessed by reactive hyperemia index and an inflammatory process as assessed by increased levels of lipoprotein-associated Phospholipase A<sub>2</sub> are related with increased arterial stiffness and abnormal wave reflections in coronary artery disease patients.

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## INTRODUCTION

Atherosclerosis is a complex process with many faces which include impaired coronary microcirculatory function, endothelial dysfunction, increased arterial stiffness and discrete plaque formation within epicardial coronary tree.

The measurement of peripheral vasodilator response

using fingertip peripheral arterial tonometry (PAT) provides a useful method for assessing arterial endothelial function<sup>[1-3]</sup>. Previous studies have shown an independent association of reactive hyperemia (RH-PAT) index with coronary endothelial function<sup>[3]</sup> and cardiovascular risk in patients with coronary artery disease (CAD)<sup>[4]</sup>.

Coronary flow reserve assessed by Doppler echocardiography (CFR) is a reliable, non-invasive method to identify epicardial coronary patency as well as coronary microcirculatory integrity<sup>[5-8]</sup>. The scaling values of decreasing CFR constitute a comprehensive indicator of cardiovascular risk even in the presence of critical epicardial coronary stenosis<sup>[6]</sup>.

Pulse wave velocity (PWV)<sup>[9]</sup> a valid marker of arterial stiffness, is independently related with the impairment of coronary microcirculation as assessed by CFR in patients with CAD<sup>[10,11]</sup>. Increased arterial stiffness causes an early arrival of wave reflection in systole instead of diastole and thus reduces coronary perfusion. Augmentation index (AIX), aortic diastolic reflection area (DRA) and index (DAI), derived by pulse wave analysis, are non-invasive markers of wave reflections<sup>[9,11-13]</sup>. However, the association between the abnormalities in wave reflections and coronary flow reserve in CAD patients after successful revascularization has not been fully investigated.

Lipoprotein-associated Phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) is an inflammatory biomarker related with endothelial dysfunction, carotid atherosclerosis, impaired coronary flow reserve and increased arterial stiffness in CAD patients<sup>[14]</sup>. However its association with abnormal wave reflections has not been clarified.

In the present study we hypothesized that abnormal arterial wave reflections may determine coronary flow reserve. Thus, we examined the association of abnormal wave reflections, as assessed by AIX, DRA and DAI with coronary flow reserve using Doppler echocardiography after successful revascularization in CAD patients. Finally we examined the association of wave reflection with endothelial dysfunction as assessed by RHI and with inflammatory process assessed by circulating levels of LpPLA<sub>2</sub>.

## MATERIALS AND METHODS

### Study population

We enrolled 70 patients (84.3% men, mean age  $60.2 \pm 9.8$  years) with (1) exercise- and/or stress-related angina (2) evidence of reversible ischemia during stress echocardiography or thallium scintigraphy (3) stenosis of  $\geq 50\%$  in the left main coronary artery and or  $\geq 70\%$  in one or several of the major coronary arteries before inclusion in the study as defined in the ESC guidelines<sup>[15]</sup> (Table 1). All the patients had undergone successful revascularization (PCI,  $n = 64$  or CABG,  $n = 6$ ) into their LAD within a year before inclusion in the study. PCI was considered successful when there was remained reduction in the caliber of the stenotic artery to  $< 20\%$

**Table 1** Clinical, biochemical and vascular markers of the study population

Variables	Values (n = 70)
Clinical	
Age (yr)	60.2 ± 9.8
Gender (males), n (%)	59 (84.3)
Hypertension, n (%)	38 (54.2)
DM, n (%)	23 (32.8)
Dyslipidemia, n (%)	57 (81.4)
Smoking, n (%)	43 (61.5)
FH of CAD, n (%)	25 (35.7)
SBP (mmHg)	128 ± 18
DBP (mmHg)	77 ± 10
Medications	
ASA n (%)	70 (100)
Nitrates n (%)	38 (54.3)
ACEIs/ ARBs n (%)	59 (84.2)
CCBs n (%)	12 (17.1)
Statins n (%)	65 (92.8)
β-blockers n (%)	60 (85.5)
Biochemical	
Chol (mg/dL)	198.8 ± 40.8
TG (mg/dL)	148.2 ± 79.9
HDL (mg/dL)	40.9 ± 11.4
LDL (mg/dL)	134.5 ± 35.9
Glu (mg/dL)	106.5 ± 32.5
CRP (mg/L)	2.44 ± 1.66
Lp-PLA <sub>2</sub> (ng/mL)	231.9 ± 90.9
Vascular markers	
CFR	2.65 ± 0.94
RHI-PAT	1.37 ± 0.43
PWVc (m/s)	10.32 ± 2.39
AIx (%)	35.8 ± 15.4
SAI (%)	50.6 ± 8.7
DAI (%)	49.4 ± 8.7
DRA	45.4 ± 12.6
RT (ms)	115.1 ± 22.5
SBPc (mm Hg)	133.2 ± 19.6
DBPc (mmHg)	83.3 ± 12.4

FH: Family history; CAD: Coronary artery disease; DM: Diabetes mellitus; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ASA: Acetylsalicylic acid; ACEIs: Angiotensin converting enzyme inhibitors; ARBs: Angiotensin receptors blockers; CCBs: Calcium channel blockers; Chol: Total cholesterol; TG: Triglycerides; LDL: Low density; HDL: High density lipoprotein; FPG: Fasting plasma glucose; CRP: C-reactive protein; Lp-PLA<sub>2</sub>: Lipoprotein-phospholipase A<sub>2</sub> patients with multivessel coronary artery disease before revascularisation; CFR: Coronary flow reserve; PWVc: Pulse wave velocity as measured with complior apparatus; AIx: Augmentation index; SAI: Systolic area index; DAI: Diastolic area index; DRA: Diastolic reflection area; RT: Return time; SBPc: Central systolic blood pressure.

with a final TIMI flow grade 3 without side branch loss, flow-limiting dissection, or angiographic thrombus (as visually assessed by angiography<sup>[16]</sup>). All participants attended our preventive medicine laboratory. Using valid questionnaire, we recorded pharmaceutical regimens and other cardiovascular risk factors (smoking, hypertension, diabetes mellitus, dyslipidemia, family history of CAD).

Exclusion criteria were: The presence of acute infection, malignancy, chronic heart failure (class NYHA III and IV), chronic obstructive pulmonary disease, recent major surgery, and severe chronic auto-immune diseases, liver and renal impairment. We also excluded patients with recent (within 6 mo) acute cardiovascular events.

Blood sampling for measurement of Lp-PLA<sub>2</sub> was performed on the morning before we performed echocardiography and vascular tests in all patients.

The study protocol was approved by the Local Ethics Committee, conducted in compliance with the Declaration of Helsinki and written informed consent was obtained from all patients before study entrance.

### Peripheral arterial tonometry

Measurement of peripheral vasodilator response with fingertip peripheral arterial tonometry (PAT) technology (EndoPAT; Itamar Medical Ltd, Caesarea, Israel) is increasingly being used as an alternative measure of endothelium-dependent dilation in response to reactive hyperemia<sup>[3]</sup>. The EndoPAT device records digital pulse wave amplitude (PWA) using fingertip plethysmography and consists of two finger-mounted probes, which include a system of inflatable latex air-cushions within a rigid external case. A blood pressure cuff is placed on one upper arm (study arm), while the contralateral arm serves as a control (control arm)<sup>[2]</sup>. PWA is measured continuously during three phases: A quiet baseline period, 5-min forearm occlusion (with inflation of the arterial pressure cuff to supra-systemic pressure), and reactive hyperemia following cuff release.

The reactive hyperaemia index (RHI) is calculated as follows: The ratio of the average amplitude of the PAT signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the PAT signal of a 3.5 min time period before cuff inflation (baseline)<sup>[3]</sup>. The result is further divided by the same ratio from the control arm, which allows the device to account for potential effects of systemic changes in vascular tone during testing. The final ratio is then multiplied by a proprietary baseline correction factor.

The reactive hyperemia index (RHI) measures nitric-oxide dependent changes in vascular tone<sup>[17]</sup>. An RHI < 1.35 has been related with impaired coronary endothelial function<sup>[3]</sup>. All studies were stored digitally and were analyzed by personnel blinded to clinical and laboratory data, using a computerized station.

### Pulse waveform analysis

Assessment of arterial wave reflections was performed non-invasively with the commercially available Arterio-graph apparatus (TensioMed Budapest Hungary, Ltd) by analysis of the oscillometric pressure curves registered on the upper arm with a single pressure cuff. The principle of the oscillometric method is based on plethysmography and registers oscillometric pulsatile pressure changes in the brachial artery<sup>[18]</sup>. An upper arm cuff was applied to the patient and after a first simple BP measurement, the cuff was over-inflated with 35–40 mmHg beyond the systolic BP. During systole, the blood volume having been ejected into the aorta generates pulse wave (early systolic peak, P1). This pulse wave runs down and reflects from the bifurcation of aorta, creating a second wave (late systolic peak, P2). Both early and late systolic peak were



obtained and recorded on the computer as pulse waves. The software of Arteriograph decomposes the early, late systolic and diastolic waves and also determines the onset and peaks of the waves, measuring noninvasively and other hemodynamic parameters as central systolic and diastolic blood pressure (SBPc, DBPc mmHg), augmentation index (Aix%), return time (RT in sec.) of the wave reflection, systolic area index (SAI%), diastolic area index (DAI %) and diastolic reflection area (DRA)<sup>[18]</sup>.

The Aix is defined as the ratio of the difference between the second ( $P_2$ , appearing because of the reflection of the first pulse wave) and first systolic peaks ( $P_1$  induced by the heart systole) to pulse pressure (PP), and it is expressed as a percentage of the ratio  $[Aix = 100 \times (P_2 - P_1) / PP]$ . DRA is derived by duration of the diastole and the area between the expected (theoretical) diastolic pressure curve without reflection and the truly measured diastolic curve with reflection and reflects the quality of the coronary arterial diastolic filling. SAI and DAI are the areas of systolic and diastolic portions under the pulse wave curve of a complete cardiac cycle, respectively. Thus, the higher the DAI and DRA are, the better the coronary perfusion is. Furthermore, RT is the time of the pulse wave travelling from the aortic root to the bifurcation and back, so this value is smaller as the aortic wall is stiffer<sup>[18]</sup>.

All studies were stored digitally and were analyzed by personnel blinded to clinical and laboratory data, using a computerized station.

### Echocardiography

Studies were conducted using a Vivid 7 (GE Medical Systems, Horten Norway) phased array ultrasound system using second harmonic imaging. Dr Ignatios Ikonomidis, counting more than 5500 CFR echo studies the last 10 years, has performed the echocardiographic examinations and the CFR measurements for this study<sup>[5,8,14]</sup>. All studies were stored digitally and were analyzed by two observers blinded to clinical and laboratory data, using a computerized station (Echopac GE, Horten Norway). All patients had adequate quality of images for analysis.

### Coronary flow reserve

We assessed transthoracic Doppler Echocardiographic-derived coronary flow reserve by obtaining the color-guided pulse-wave Doppler signals. In the long axis apical projections using a 7 MHz transducer, we recorded the maximal velocity and velocity-time integral in the distal LAD at baseline and during hyperaemic conditions after the intravenous administration of adenosine (0.14 mg/kg per minute)<sup>[5-8]</sup> for 3 min. Measurements of three cardiac cycles were averaged. CFR was calculated as the ratio of hyperemic to resting maximal diastolic velocity. The feasibility of the method was greater than 98% for all indices in our study cohort (initially 71 patients were recruited, but one patient was excluded due to unfeasible CFR study).

The mean CFR value of our cohort ( $< 2.5$ ) was used for subgroup analysis after previously published cutoff values for impaired CFR in CAD patients<sup>[6,19]</sup>.

### PWV measurement

The carotid-femoral PWV (PWVc) was assessed by measuring the pulse transit time and the distance travelled between the two recording sites. For pulse wave recording we used a validated noninvasive device (Complior SP<sup>®</sup>, Alam Medical, France) with capability of online wave recording. A simultaneous recording was performed by two pressure-sensitive transducers of two different pulse waves based over the right common carotid artery and the right femoral artery, respectively. Measurement of the distance between the transducers over the body surface allowed obtaining PWVc. Measurements were performed by a single observer, blinded to clinical and laboratory data, and the whole procedure has been internally validated in our laboratory<sup>[8,20]</sup>.

### Lp-PLA<sub>2</sub> levels

Serum levels of Lp-PLA<sub>2</sub> were measured in our biochemistry laboratory with a commercially available enzyme-linked immunoassay (ELISA) (PLAC test, diaDexus, Inc, San Francisco, CA) with minimum detection limit of 0.34 ng/mL<sup>[14]</sup>. The inter- and intra assay variations were  $< 5\%$  and  $8\%$ . An Lp-PLA<sub>2</sub> concentration of 235 ng/mL has been suggested to use as a clinical decision threshold<sup>[21]</sup>. Analyses were performed by personnel blinded to clinical and laboratory data.

### Statistical analysis

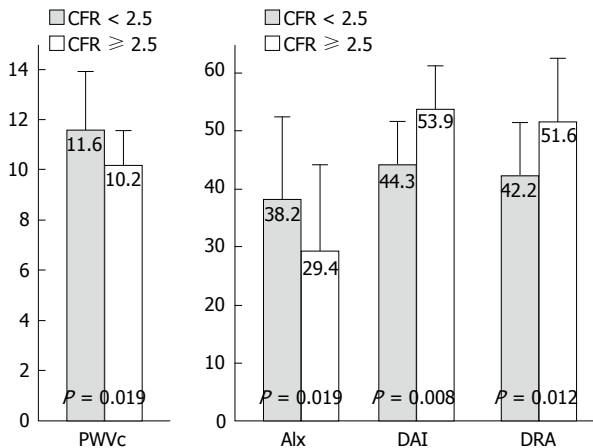
All variables are expressed as mean  $\pm$  SD. Statistical analysis was performed using SPSS 21.0 statistical software package (SPSS Inc, Illinois, United States). Categorical data were analysed using the standard chi-square test. Variables were tested by the Kolmogorov-Smirnov test to assess the normality of distribution. Parameters without normal distribution were transformed into ranks for further analysis. Patients were categorised into equal subgroups, according to the median value of CFR in our study cohort. Mean values of continuous variables were compared between groups using unpaired Student's *t*-test or the Mann-Whitney *U*-test, where applicable.

Simple linear regression was used to investigate relations between variables. Multiple linear relations were checked by multiple linear regression analysis using forward or backward procedure. Associations are presented by means of standardized regression coefficient (*b*). All covariates included in the final models were tested for interactions. Tolerance values for each covariate was  $> 0.5$  in the multivariate models.

## RESULTS

### Study population characteristics

Clinical and biochemical characteristics of our study



**Figure 1** Graphic representation of the differences in pulse wave velocity (m/s), augmentation index (%), diastolic area (%) and diastolic reflection area (%) between patients with reduced coronary flow reserve (< 2.5) and patients with preserved coronary flow reserve (≥ 2.5). CFR: Coronary flow reserve; PWV: Pulse wave velocity; Aix: Augmentation index; DRA: Diastolic reflection area; DAI: Diastolic area.

population are presented in Table 1. The mean values of the vascular parameters and the pharmaceutical regimen of the study cohort are shown in Table 1.

#### Determinants of coronary flow reserve.

In univariate analysis, a decreasing CFR was related with increasing PWVc ( $r = -0.38$ ,  $P = 0.015$ ), SBPc ( $r = -0.34$ ,  $P = 0.022$ ), Aix ( $r = -0.50$ ,  $P = 0.003$ ), SAI ( $r = -0.49$ ,  $P = 0.006$ ) as well as decreasing RT ( $r = 0.45$ ,  $P = 0.009$ ), DAI ( $r = 0.49$ ,  $P = 0.006$ ) DRA ( $r = 0.55$ ,  $P < 0.001$ ) and RHI ( $r = 0.47$ ,  $P = 0.002$ ). Furthermore, RHI was related to Aix ( $r = 0.48$ ,  $P < 0.001$ ), RT ( $r = -0.29$ ,  $P = 0.024$ ) and SBPc ( $r = 0.40$ ,  $P = 0.001$ ).

In multivariate analysis, after adjustment of age, sex, blood pressure parameter, lipidemic, diabetic and smoking status, we found that coronary flow reserve was independently related to Aix ( $b = -0.38$ ,  $r = 0.009$ ), DAI ( $b = 0.36$ ,  $P = 0.014$ ), DRA ( $b = 0.39$ ,  $P = 0.005$ ) and RT ( $b = -0.29$ ,  $P = 0.026$ ).

#### Patients with high vs patients with low coronary flow reserve

Patients were categorised in high and low CFR according to the median value of CFR. Patients with CFR < 2.5 had similar clinical characteristics with those with CFR ≥ 2.5 with the exception of higher cholesterol level, (Table 2,  $P < 0.05$ ). However, patients with CFR < 2.5 had higher PWVc, SBPc, Aix, SAI and lower RT, DAI and DRA compared with those with CFR ≥ 2.5 after adjustment for cholesterol levels (Table 2,  $P < 0.05$  and Figure 1).

Furthermore, these patients with CFR < 2.5 had higher LpPLA<sub>2</sub> compared with those with CFR ≥ 2.5 (Table 2,  $P = 0.002$ ).

#### Relation of vascular markers with Lp-PLA<sub>2</sub>

Elevated LpPLA<sub>2</sub> was related with reduced CFR ( $r =$

$-0.331$ ,  $P = 0.001$ ), RHI ( $r = -0.371$ ,  $P < 0.001$ ) and DRA ( $r = -0.35$ ,  $P = 0.001$ ) as well as increased PWVc ( $r = 0.34$ ,  $P = 0.012$ ) and Aix ( $r = 0.34$ ,  $P = 0.001$ ).

## DISCUSSION

In the present study, we found a close association between arterial wave reflection markers, as assessed by Aix, DRA and DAI, and decreasing CFR in CAD patients after successful revascularization. Furthermore, we demonstrated that diastolic component of central aortic pulse wave as expressed with DRA and DAI is an independent determinant of impaired coronary flow reserve after adjustment for several other factors potentially influencing coronary microcirculatory function. Finally we have shown that endothelial dysfunction as assessed by RHI and the inflammatory process as assessed by LpPLA<sub>2</sub> are associated with abnormal wave reflection and increased arterial stiffness.

#### Association between aortic stiffness and coronary flow reserve

Coronary flow reserve (CFR) represents the capacity of the coronary circulation to dilate following an increase in myocardial metabolic demands and can be expressed by the difference between the hyperemic flow and the resting flow curve. Impaired CFR constitutes a marker of coronary microcirculatory dysfunction and reflects the impairment of the epicardial coronary artery flow in the presence of significant coronary stenosis<sup>[6]</sup>, as well as coronary microcirculatory dysfunction<sup>[11,14]</sup>. CFR entails strong prognostic significance in stable patients with known or suspected ischemic heart disease, independently of other risk factors<sup>[22-26]</sup>. Thus, the scaling values of decreasing CFR constitute a comprehensive indicator of cardiovascular risk even in the presence of critical epicardial coronary stenosis<sup>[6]</sup>.

The association of increased PWV with the presence and prognosis of angiographic CAD has been extensively demonstrated<sup>[11,27,28]</sup>. Experimental studies have shown that low aortic compliance is associated with a reduction in coronary blood flow<sup>[29]</sup>, particularly subendocardial flow<sup>[30,31]</sup>. In a human study, Leung *et al*<sup>[32]</sup> have shown that a compliant aorta, as measured by PWV, is associated with a greater improvement in hyperemic coronary blood flow from successful PCI than a stiff aorta and this relationship persisted for PWV even after accounting for stenosis severity. Furthermore, exercise-induced rise in coronary blood flow, related to ischemic threshold, could be determined by aortic stiffness. This is supported by the findings of Kingwell *et al*<sup>[33]</sup> who found indexes of arterial stiffness were stronger independent predictors of the exercise-induced ischemic threshold than maximum coronary stenosis assessed angiographically.

In the present study, we confirm the above mentioned close relation of PWV with CFR. PWV is a marker of aortic stiffness, whereas Aix, which is largely determined by wave reflections, represents much more the vasomotor

**Table 2** Clinical and biochemical parameters of the study population divided by the median value of coronary flow reserve

	CFR < 2.5 (n = 34)	CFR ≥ 2.5 (n = 36)	P
<b>Clinical</b>			
Age (yr)	62.1 ± 9.2	58.4 ± 10.5	0.265
Males, n (%)	29 (85.2)	30 (83.3)	0.869
Hypertension, n (%)	20 (58.8)	18 (50)	0.368
Diabetes, n (%)	13 (38.2)	10 (27.7)	0.631
Dyslipidemia, n (%)	28 (82.3)	29 (80.5)	0.307
Smoking, n (%)	23 (67.6)	20 (55.5)	0.449
FH of CAD	14 (41.1)	11 (30.5)	0.334
SBP (mmHg)	130.9 ± 20.3	120.4 ± 14.8	0.011
DBP (mmHg)	77.4 ± 10.4	74.8 ± 8.8	0.058
<b>Medications</b>			
ASA, n (%)	33 (97)	34 (94.4)	0.942
Nitrates, n (%)	23 (67.6)	27 (75)	0.131
ACEIs/ARBs, n (%)	33 (97)	34 (94.4)	0.956
CCBs, n (%)	5 (14.7)	7 (19.4)	0.597
Statins, n (%)	32 (94.1)	33 (91.6)	0.547
β-blockers, n (%)	29 (85.2)	31 (86.1)	0.765
<b>Biochemical</b>			
Chol (mg/dL)	206.7 ± 44.1	190.6 ± 38.9	0.078
TG (mg/dL)	147.0 ± 57.1	143.9 ± 69.7	0.824
HDL (mg/dL)	39.6 ± 8.6	40.9 ± 12.8	0.567
LDL (mg/dL)	141.6 ± 37.8	126.6 ± 32.9	0.055
Glu (mg/dL)	100.9 ± 2.2	112.4 ± 39.9	0.126
CRP (mg/L)	2.5 ± 1.8	2.4 ± 1.5	0.279
Lp-PLA <sub>2</sub> (ng/mL)	268.1 ± 91.9	199.5 ± 78.4	0.002
<b>Vascular markers</b>			
RHI-PAT	1.26 ± 0.28	1.50 ± 0.46	0.012
PWVc (m/s)	11.6 ± 2.3	10.2 ± 1.4	0.019
AIx (%)	38.2 ± 14.8	29.4 ± 15.1	0.011
SAI (%)	55.7 ± 7.9	46.1 ± 6.7	0.008
DAI (%)	44.3 ± 7.9	53.9 ± 6.7	0.008
DRA	42.2 ± 9.6	51.6 ± 11.4	0.012
RT (ms)	106.1 ± 20.8	123.0 ± 22.1	0.015
SBPc (mm Hg)	139.1 ± 17.8	125.2 ± 19.1	0.026
DBPc (mmHg)	84.7 ± 12.1	80.0 ± 11.0	0.118

FH: Family history; CAD: Coronary artery disease; DM: Diabetes mellitus; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; ASA: Acetylsalicylic acid; ACEIs: Angiotensin converting enzyme inhibitors; ARBs: Angiotensin receptors blockers; CCBs: Calcium channel blockers; Chol: Total cholesterol; TG: Triglycerides; LDL: Low density; HDL: High density lipoprotein; FPG: Fasting plasma glucose; CRP: C-reactive protein; Lp-PLA<sub>2</sub>: Lipoprotein-phospholipase A<sub>2</sub> patients with multivessel coronary artery disease before revascularisation; CFR: Coronary flow reserve; PWVc: Pulse wave velocity as measured with complior apparatus; AIx: Augmentation index; SAI: Systolic area index; DAI: Diastolic area index; DRA: Diastolic reflection area; RT: Return time; SBPc: Central systolic blood pressure; DBPc: Central diastolic blood pressure.

tone in the small medium-sized muscular vessels downstream in the circulation<sup>[9,12]</sup>. In our study, we demonstrated for the first time that AIx is related to CFR, indicating that not only stiffness of the large elastic arteries impairs CFR, but stiffening of the smaller muscular arteries contributes as well. However, the net effect of increased systemic arterial stiffness on coronary vasodilatory reserve is thought to be mediated by reduced coronary perfusion during diastole.

Increased arterial stiffness increases the velocity of both forward and reflected pulse wave<sup>[9]</sup>. This increase in velocity of wave of pulse causes arrival of reflected

waves at the aorta during systole and not during diastole as it occurs under conditions of normal aortic elastic properties. The early arrival of the reflected waves (1) augments the systolic aortic pressure and thus increase of LV afterload, wall stress and cardiac workload leading to increased myocardial oxygen demands; (2) reduces the diastolic aortic pressure resulting in reduced myocardial perfusion<sup>[9,34]</sup>. Thus, arterial stiffness causes a mismatch between myocardial oxygen demands and myocardial perfusion resulting in reduction of coronary flow reserve after hyperemia<sup>[10,19,22]</sup>. Additionally, stiffening of the large arteries, results in reduction of their capacity to function as an elastic reservoir resulting in a greater peripheral runoff of stroke volume during systole<sup>[13,29,31]</sup>. Together with the reduced elastic recoil, the diastolic blood pressure and hence coronary blood flow is decreased.

Indeed, in our study, we found that DAI and DRA, two markers that reflect the contribution of reflected waves to perfusion of the coronary circulation, were closely associated with CFR, even after adjustment for other factors influencing CFR. This finding supports the above mentioned pathophysiological mechanism.

#### **Role of endothelial dysfunction for the relationship between coronary flow reserve and arterial stiffness**

Besides the above mentioned arterio-coronary coupling that may explain the lower coronary flow reserve associated with a stiff arterial tree, arterial stiffness may be a marker of a more generalized vascular disease process which among others, includes endothelial dysfunction. Previous studies have shown that large artery stiffness itself is influenced by endothelial function via basal release of nitric oxide<sup>[35]</sup> as well as that aortic stiffness is associated with brachial artery endothelial dysfunction<sup>[36]</sup>. On the other side, adenosine-induced CFR is also thought to be at least partly endothelium dependent<sup>[8]</sup>. Thus, endothelial function through NO production is an important determinant of coronary flow response to physiological or pharmacological stimuli<sup>[10,19]</sup>.

Reactive hyperaemia peripheral arterial tonometry (RHI-PAT) is a method to assess peripheral microvascular endothelial function and is linked to coronary microvascular endothelial dysfunction<sup>[3]</sup>, as this parameter is predominantly determined by the bioavailability of NO<sup>[16]</sup>. Both impaired CFR and reduced RHI-PAT have proven prognostic value in CAD patients<sup>[4,6,7]</sup>. In the present study we document an independent association of peripheral endothelial dysfunction, assessed by RHI-PAT, with coronary endothelial dysfunction, assessed by CFR after successful revascularization in patients with CAD. It is possible that coronary endothelial dysfunction may coexist with aortic stiffness and may contribute to abnormal coronary microcirculatory response to hyperemia, as well as impaired aortic wall properties. Furthermore, the association of RHI-PAT with AIx and RT indicates that peripheral endothelial dysfunction contribute to impaired aortic wall properties, as well as that determines at least partly, stiffening of both large

elastic arteries and smaller muscular arteries.

### Role of vascular inflammation

On the other hand increased PWV is associated with enhanced vascular inflammation and injury<sup>[20,27]</sup>. Indeed, in our study we measured LpPLA<sub>2</sub> as a marker of vascular inflammation and we found that patients with high LpPLA<sub>2</sub> levels had higher PWVc, AIX, and reduced DRA, DAI, CFR and RHI. These findings indicate a common effect of LpPLA<sub>2</sub> in all vascular territories, indicating a generalized vascular disease process which causes reduced CFR directly and/or indirectly through arterial stiffness and impaired endothelial function as we mentioned above.

### Study limitations

Our results establish a close relation between increasing PWVc, AIX, DAI, DRA, RHI-PAT and CFR in CAD patients. However, this study was not designed to verify whether this relation is causative or secondary to endothelial dysfunction and interstitial fibrosis within aortic and coronary wall in CAD patients. It is possible that the generalized vascular damage was the link between PWVc, AIX and CFR in our study.

In summary, in the present study, we demonstrated that augmentation of the systolic component of the central aortic pulse wave, as expressed by augmentation index and reduced diastolic component of central aortic pulse wave as expressed by diastolic reflection area and index are related with impaired coronary flow reserve after adjustment for several other factors potentially influencing coronary microcirculatory function. Furthermore, endothelial dysfunction as assessed by RHI and an inflammatory process as assessed by increased levels of Lp-PLA<sub>2</sub> are related with increased arterial stiffness and abnormal wave reflections in CAD patients. These findings underscore the need to assess arterial wall properties in CAD patients to better stratify the risk of future events after successful revascularization.

## COMMENTS

### Background

Atherosclerosis is a complex process with many faces which include impaired coronary microcirculatory function, endothelial dysfunction, increased arterial stiffness and discrete plaque formation within epicardial coronary tree.

### Research frontiers

Pulse wave velocity (PWV) a valid marker of arterial stiffness, is independently related with the impairment of coronary microcirculation as assessed by coronary flow reserve in patients with coronary artery disease (CAD).

### Innovations and breakthroughs

The authors demonstrated that augmentation of the systolic component of the central aortic pulse wave, as expressed by augmentation index and reduced diastolic component of central aortic pulse wave as expressed by diastolic reflection area and index are related with impaired coronary flow reserve after adjustment for several other factors potentially influencing coronary microcirculatory function.

### Applications

These findings underscore the need to assess arterial wall properties in

CAD patients to better stratify the risk of future events after successful revascularization.

### Terminology

The authors measured (1) reactive hyperemia index (RHI) using fingertip peripheral arterial tonometry (RH-PAT Endo-PAT); (2) carotid to femoral pulse wave velocity (PWVc-Complior); (3) augmentation index (AIX), the diastolic area (DAI%) and diastolic reflection area (DRA) of the central aortic pulse wave (Arteriograph); (4) CFR using Doppler echocardiography and 5) blood levels of Lipoprotein-phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>).

### Peer-review

The authors studied a group of 70 patients with CAD by means of coronary flow reserve and several indexes related to arteriosclerosis (peripheral arterial tonometry, pulse waveform analysis, carotid to femoral pulse wave velocity) and to inflammation (Lp-PLA<sub>2</sub>). As expected these indexes were impaired in patients with lower coronary flow reserve.

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## Biodegradable polymer stents vs second generation drug eluting stents: A meta-analysis and systematic review of randomized controlled trials

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**Author contributions:** Pandya B and Gaddam S were involved in acquisition of data, analysis and interpretation of data, drafting the manuscript; Raza M, Asti D and Nalluri N interpreted data; Vazzana T, Kandov R and Lafferty J critically revised the manuscript, provided their expertise and approved the manuscript.

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### Abstract

**AIM:** To evaluate the premise, that biodegradable polymer drug eluting stents (BD-DES) could improve clinical outcomes compared to second generation permanent polymer drug eluting stents (PP-DES), we pooled the data from all the available randomized control trials (RCT) comparing the clinical performance of both these stents.

**METHODS:** A systematic literature search of PubMed, Cochrane, Google scholar databases, EMBASE, MEDLINE and SCOPUS was performed during time period of January 2001 to April 2015 for RCT and comparing safety and efficacy of BD-DES vs second generation PP-DES. The primary outcomes of interest were definite stent thrombosis, target lesion revascularization, myocardial infarction, cardiac deaths and total deaths during the study period.

**RESULTS:** A total of 11 RCT's with a total of 12644 patients were included in the meta-analysis, with 6598 patients in BD-DES vs 6046 patients in second generation PP-DES. The mean follow up period was 16 mo. Pooled analysis showed non-inferiority of BD-DES, comparing events of stent thrombosis (OR = 1.42, 95%CI: 0.79-2.52,  $P = 0.24$ ), target lesion revascularization (OR = 0.99, 95%CI: 0.84-1.17,  $P = 0.92$ ), myocardial infarction (OR = 1.06, 95%CI: 0.86-1.29,  $P = 0.92$ ), cardiac deaths (OR = 1.07, 95%CI: 0.82-1.41,  $P = 0.94$ ) and total deaths (OR = 0.96, 95%CI: 0.80-1.17,  $P = 0.71$ ).

**CONCLUSION:** BD-DES, when compared to second generation PP-DES, showed no significant advantage

and the outcomes were comparable between both the groups.

**Key words:** Stent design; Drug eluting stent; Zotarolimus eluting stent; Cobalt-chromium stent; Biodegradable drug eluting stent

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**Core tip:** No direct comparison has been done so far with biodegradable polymers in drug eluting stent compared to permanent alloy in second-generation drug eluting stent. We explored the efficacy of these two stents via meta-analysis of randomized control trials in terms of definite stent thrombosis, target lesion revascularization, myocardial infarction, cardiac deaths and total deaths.

Pandya B, Gaddam S, Raza M, Asti D, Nalluri N, Vazzana T, Kandov R, Lafferty J. Biodegradable polymer stents vs second generation drug eluting stents: A meta-analysis and systematic review of randomized controlled trials. *World J Cardiol* 2016; 8(2): 240-246 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v8/i2/240.htm> DOI: <http://dx.doi.org/10.4330/wjc.v8.i2.240>

## INTRODUCTION

It's been more than two decades since the introduction of coronary stents and during this period the stent designs have been modified to improve patient safety. Bare metal stents (BMS) were trailed by first generation permanent polymer drug eluting stents (PP-DES) (Paclitaxel and Sirolimus) then followed by second generation PP-DES (Everolimus and Zotarolimus) and now biodegradable polymer DES (BD-DES) are envisaging potentially improved patient outcomes.

Stent designing is the crux of interventional cardiology research and the changes have been dynamic. Initial BMS used a simple expandable metal alloy frame work, while PP-DES use an anti-proliferative drug coating on the metal platform, glued by a binding durable polymer to hold and elute the drug over time. Beyond any uncertainty, PP-DES are superior to BMS in decreasing restenosis, however PP-DES require longer duration of dual-antiplatelet therapy to avert the risk of stent thrombosis<sup>[1]</sup>. It is now understood, the metal alloy and the permanent polymer are among the culprits for prolonged inflammation leading to very late stent thrombosis and late restenosis (termed late catch-up phenomena) and henceforth the unremitting search for safer stents<sup>[2]</sup>. The second generation PP-DES introduced few years ago, have superior metal frame work (cobalt-chromium and platinum-chromium) with thinner metal struts, enhanced biocompatible binding polymer and these stents have proven improved patient outcomes compared to its predecessors<sup>[3]</sup>. Nevertheless, the potential need for dual-antiplatelet therapy beyond one year is still an

apprehension among cardiologists and patients with second generation PP-DES. The BD-DES, unlike second generation PP-DES, will elute the anti-proliferative drug and the biodegradable polymer subsequently dissolves leaving behind a bare metal stent<sup>[4]</sup>. BD-DES is introduced with an anticipation to decrease the stent thrombosis events (especially very late events) and evading the need for prolonged dual-antiplatelet therapy.

Several randomized control trials and registries have been published in last few years, with most trials comparing first generation PP-DES to BD-DES. As anticipated, long term follow up data has shown superiority of BD-DES in decreasing very late stent thrombosis events when compared with Sirolimus (first generation) PP-DES<sup>[5]</sup>. However, there are only fewer studies comparing second generation PP-DES to BD-DES. Since second generation PP-DES is current standard of care in United States, it is of immense importance to study if the newer BD-DES offer any better outcomes. We performed a meta-analysis and systematic review of randomized control trials comparing efficacy and safety of BD-DES to second generation PP-DES (Everolimus and Zotarolimus).

## MATERIALS AND METHODS

### Literature search

Two independent investigators systematically searched PubMed, Cochrane and Google scholar database from January 2001 to April 2015. We used following keywords: "biodegradable stent", "biodegradable polymer", "biodegradable polymer drug eluting", and "biodegradable stent coronary". Reference lists from selected studies were manually searched for potentially relevant studies. Whenever available, the most recent follow up data on a study was included. The PRISMA statement was used as guidance for selection of studies to be included in the meta-analysis and is depicted in Figure 1. Randomized control trials comparing BD-DES vs second generation DES with a primary end point of definite stent thrombosis, target lesion revascularization, myocardial infarction, cardiac deaths and total death were included in the study. We found 11 trials comparing BD-DES to second generation (Everolimus or Zotarolimus) PP-DES. In ISAR-TEST 4 trial, both first generation Sirolimus and second generation Everolimus DES were used, but in our meta-analysis we only used data pertinent to second generation Everolimus DES. Given the low incidence of stent thrombosis and other outcomes, a meta-analysis was performed to prove treatment differences between these two stents.

### Study selection

Two authors screened all relevant literature by their abstract and title found by electronic search. Only trials published in English were taken into consideration. Inclusion criteria were (1) randomized control trials; (2) comparing biodegradable polymers to second generation drug eluting stents; and (3) reporting outcomes as



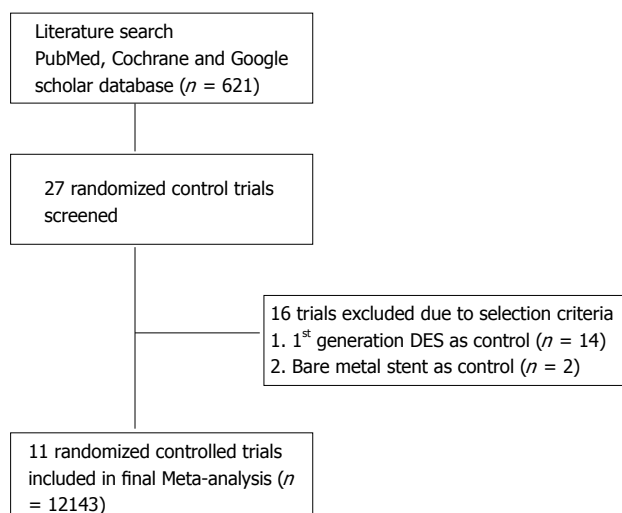


Figure 1 Study selection. DES: drug eluting stents.

a target lesion revascularization [target lesion revascularization (TLR)], definite stent thrombosis (DST), myocardial infarction (MI), cardiac deaths and total deaths. We excluded studies with first generation drug eluting stents and bare metal stents as controls and also studies performed on select population, like complex lesions or on bifurcating lesions. Randomized control trials comparing BD-DES to second generation PP-DES were only included in this study.

#### Data extraction

Data from all 11 trials were extracted by same two authors in regards to first author, year of publication, total No. of patients and No. of patients in each group (Table 1). Authors also extracted mean age group of patients, patients with DM and HTN, follow up duration and duration of use of dual antiplatelet therapy (Table 2).

#### Outcome measures

Clinical end points compared were definite stent thrombosis (DST), target lesion revascularization (TLR), myocardial infarction (MI), cardiac deaths and total deaths during the study period.

#### Statistical analysis

The results for each trial were obtained on an intention-to-treat analysis. The dichotomous and continuous endpoints from individual trials were analyzed using the odds ratio (OR) and the standard difference in mean (SDM) respectively as a parameter of efficacy with its 95%CI. We assessed heterogeneity with  $I^2$  that describes the percentage of total variation across trials due to heterogeneity rather than chance.  $I^2$  can be calculated as  $I^2 = 100\% \times (Qv - df)/Q$ , where  $Q$  is Cochran's heterogeneity statistics and  $df$  the degrees of freedom. Negative values of  $I^2$  are put equal to 0, so  $I^2$  lies between 0% (no heterogeneity) and 100% (maximal heterogeneity). The continuous outcomes were analyzed using the standard difference in mean.

Binary outcomes from individual studies were combined and the summary estimators of treatment effect were calculated using fixed-effect method. Weighting of trial data in the models was based on the inverse variance weight computed as the inverse of the squared standard error value of the effect size. A  $P$  value of  $\leq 0.05$  was regarded as significant. All analyses were performed using Review Manager (RevMan) Version 5.3 for Windows Oxford, England.

## RESULTS

### Study selection

A total of 11 RCT's with a total of 12644 patients were included in the meta-analysis, with 6598 patients in BD-DES vs 6046 patients in second generation PP-DES. The mean follow up period was 16 mo. Table 1 shows the main characteristics of the included studies. Table 2 shows the main characteristics of the BD-DES patients included in the studies. The final summary of clinical end points is depicted in Table 3.

### DST

The forest plot for summary effect is shown in Figure 2. There were a total of 34 (0.6%) stent thrombosis events in BD-DES group and 24 (0.46%) stent thrombosis in PP-DES group. The forest plot is shown in the Figure 2, with pooled OR of 1.42 (95%CI: 0.79-2.52),  $P = 0.24$ , and  $I^2$  for heterogeneity 0%.

### TRL

The forest plot for summary effect is shown in Figure 3. There were a total of 294 (4.77%) TLR in BD-DES group vs 350 (6.05%) TLR in PP-DES group. The forest plot is shown in the figure, with pooled OR of 0.99 (95%CI: 0.84-1.17),  $P = 0.92$ , and  $I^2$  for heterogeneity 0%.

### MI

The forest plot for summary effect is shown in Figure 4. There were a total of 202 (3.27%) myocardial infarctions in BD-DES group vs 238 (4.11%) myocardial infarctions in PP-DES group. The forest plot is shown in the Figure 4, with pooled OR of 1.06 (95%CI: 0.86-1.29),  $P = 0.59$ , and  $I^2$  for heterogeneity 0%.

### Cardiac deaths

The forest plot for summary effect is shown in Figure 4. There were a total of 108 (1.78%) cardiac deaths in BD-DES group vs 124 (2.18%) cardiac deaths in PP-DES group. The forest plot is shown in the figure, with pooled OR of 1.07 (95%CI: 0.82-1.41,  $P = 0.60$ ), and  $I^2$  for heterogeneity 0%.

### Total deaths

The forest plot for summary effect is shown in Figure 5. There were a total of 229 (3.65%) deaths in BD-DES group vs 236 (4.01%) deaths in PP-DES group. The

Table 1 Summary of included trials

Ref.	Trial acronym	Yr	BD-DES type	PP-DES type	Total patients	BD-DES patients	PP-DES patients
Natsuaki <i>et al</i> <sup>[8]</sup>	NEXT	2013	Biolimus	Everolimus	3235	1617	1618
Smits <i>et al</i> <sup>[9]</sup>	COMPARE 2	2013	Biolimus	Everolimus	2707	1795	912
Gao <i>et al</i> <sup>[10]</sup>	TARGET 1	2013	Sirolimus	Everolimus	458	227	231
Byrne <i>et al</i> <sup>[11]</sup>	ISAR-TEST 4	2011	Sirolimus	Everolimus	2603	652	1304
Xu <i>et al</i> <sup>[12]</sup>		2011	Sirolimus	Zotarolimus	324	168	156
Separham <i>et al</i> <sup>[13]</sup>		2011	Biolimus	Everolimus	200	100	100
Meredith <i>et al</i> <sup>[14]</sup>	EVOLVE	2012	Biolimus	Everolimus	192	98	94
Pilgrim <i>et al</i> <sup>[15]</sup>	BIOSCIENCE	2014	Sirolimus	Everolimus	2119	1063	1056
Serruys <i>et al</i> <sup>[7]</sup>	ABSORB 2	2014	Everolimus	Everolimus	501	335	166
Lee <i>et al</i> <sup>[16]</sup>		2014	Biolimus	Everolimus	500	245	255
Windecker <i>et al</i> <sup>[17]</sup>	BIOFLOW 2	2014	Sirolimus	Everolimus	452	298	154

BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.

Table 2 Main characteristics of biodegradable polymer drug eluting stents patients in the study

Ref.	Mean age	Male %	Diabetes %	Inclusion criteria	Exclusion criteria	DAPT mo	Follow up mo
Natsuaki <i>et al</i> <sup>[8]</sup>	69	77	46	SA <sup>1</sup> /ACS <sup>2</sup>	Major surgery in 30 d, cardiogenic shock	3	12
Smits <i>et al</i> <sup>[9]</sup>	63	74	22	SA/ACS	Major surgery in 30 d, cardiogenic shock	12	12
Gao <i>et al</i> <sup>[10]</sup>	59	69	14	SA/UA <sup>3</sup>	AMI <sup>4</sup> < 1 wk, CT <sup>5</sup> , LM <sup>6</sup> bifurcation, ISR <sup>7</sup>	12	12
Byrne <i>et al</i> <sup>[11]</sup>	67	75	29	SA/ACS	LM. shock, malignancy, life expectancy < 1 yr	6	36
Xu <i>et al</i> <sup>[12]</sup>	57	70	26	SA/UA	AMI < 1 wk, LM, CTO	6	24
Separham <i>et al</i> <sup>[13]</sup>	61	66	28	SA/ACS	Allergy to aspirin, plavix, heparin, stainless steel, everolimus, biolimus or contrast and pregnancy	12	12
Meredith <i>et al</i> <sup>[14]</sup>	62	80	22	Symp CAD <sup>8</sup> , Silent Ischemia	AMI, LM CAD, ISR, thrombus in target vessel	6	6
Pilgrim <i>et al</i> <sup>[15]</sup>	66	77	24	Stable CAD/ACS	Pregnancy, intolerance to aspirin, plavix, planned surgery in 6 mo	12	12
Serruys <i>et al</i> <sup>[7]</sup>	61	76	24	Evidence of myocardial Ischemia	AMI, unstable arrhythmias, LVEF <sup>9</sup> < 30	NA	12
Lee <i>et al</i> <sup>[16]</sup>	63	68	32	SA/UA/NSTEMI <sup>10</sup>	STEMI <sup>11</sup> , cardiogenic shock, allergy to aspirin/plavix/heparin/stainless steel/biolimus/everolimus, HD pts, LM CAD	≥ 12	12
Windecker <i>et al</i> <sup>[17]</sup>	63	78	28	SA/UA/Clinical evidence of myocardial Ischemia	MI within 72 h, LM CAD, triple vessel CAD, LVEF < 30%	≥ 6	9

<sup>1</sup>Stable angina; <sup>2</sup>Acute coronary syndrome; <sup>3</sup>Unstable angina; <sup>4</sup>Acute myocardial infarction; <sup>5</sup>Complete total occlusion; <sup>6</sup>Left main; <sup>7</sup>In stent restenosis; <sup>8</sup>Coronary artery disease Left ventricular ejection fraction; <sup>9</sup>Left ventricular ejection fraction; <sup>10</sup>Non ST elevation myocardial infarction; <sup>11</sup>ST elevation myocardial infarction; BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents; NA: Not available.

Table 3 Summary of clinical end points

Events	BD-DES (n = 4459)	PP-DES (n = 4221)	ODD S RATIO (95%CI)	P-value
Definite stent thrombosis	34	24	1.42 (0.79-2.52)	0.24
Target lesion revascularization	294	350	0.99 (0.84-1.17)	0.92
Myocardial infarction	202	238	1.06 (0.86-1.29)	0.59
Cardiac deaths	108	124	1.07 (0.82-1.41)	0.6
Total deaths	229	236	0.96 (0.80-1.17)	0.71

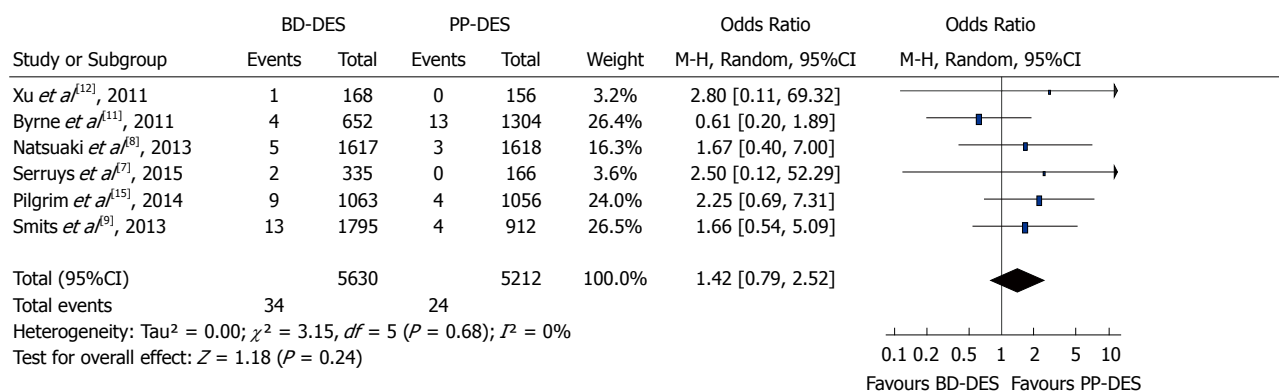
PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.

forest plot is shown in the figure, with pooled OR of 0.96 (95%CI: 0.80-1.17,  $P = 0.71$ ), and  $I^2$  for heterogeneity 0%.

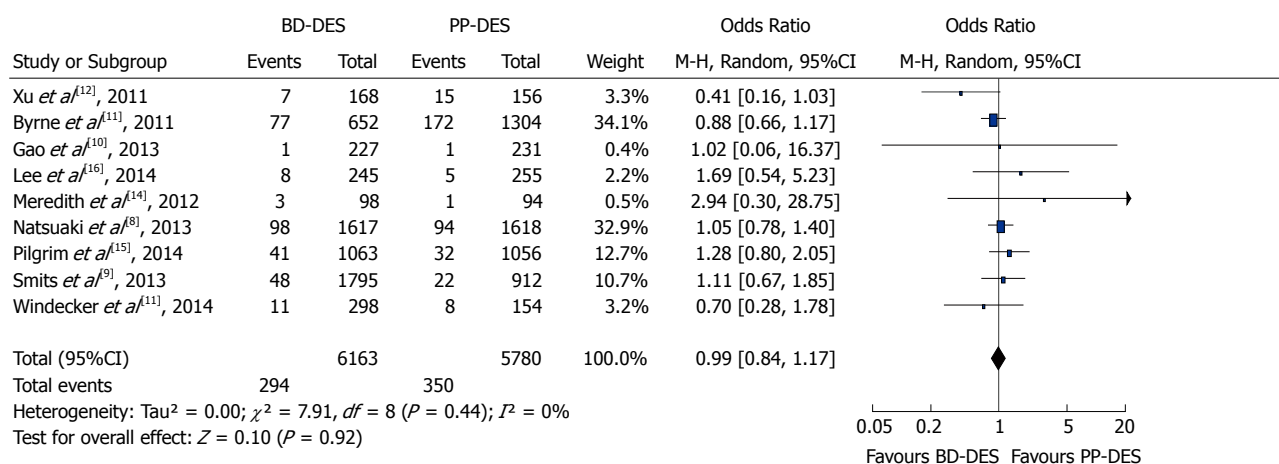
## DISCUSSION

From our study, at a mean follow up of 16 mo, BD-

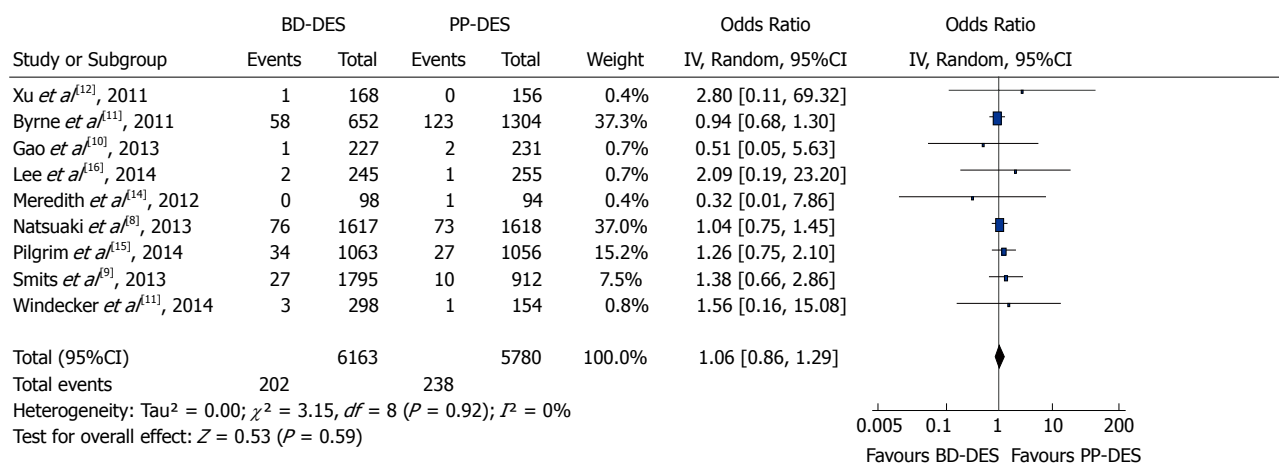
DES use did not significantly decrease mortality (OR = 0.96,  $P = 0.71$ ) or myocardial infarction events (OR = 1.06,  $P = 0.59$ ). Rates of stent thrombosis (OR = 1.42,  $P = 0.24$ ) and target lesion revascularization (OR = 0.99,  $P = 0.92$ ) were comparable between both the stents. In this study the results for BD-DES, against contrary belief, failed to show any significant



**Figure 2 Definite stent thrombosis.** BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.



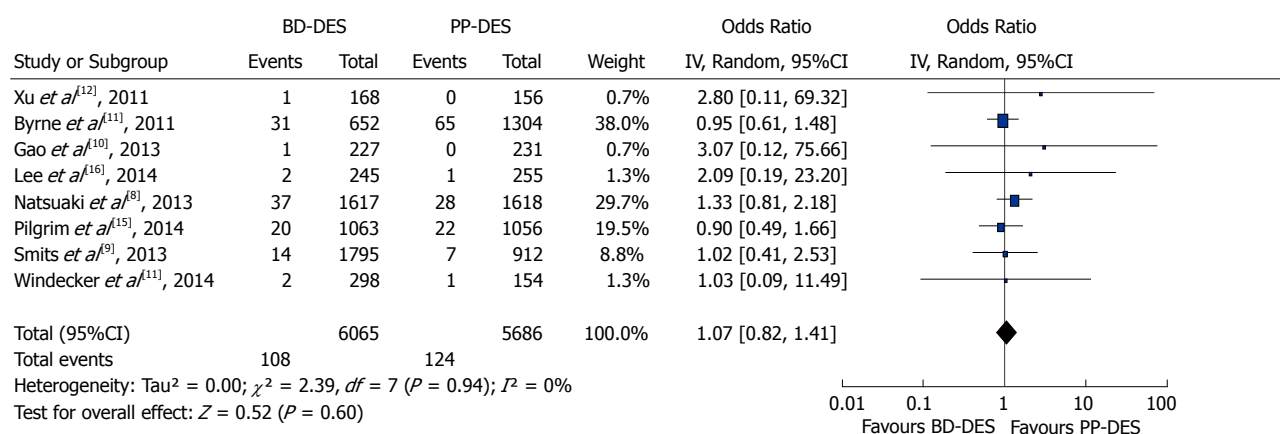
**Figure 3 Target lesion revascularization.** BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.



**Figure 4 Myocardial infarction.** BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.

decrease in stent thrombosis. Looking at individual study results, except for ISAR-TEST 4, all trials showed non-significant increase in odds of stent thrombosis compared to second generation PP-DES. However it should be remembered, BD-DES are anticipated to have decreased stent thrombosis events at long term follow up, especially after the biodegradable polymer

dissolves and leaves behind a bare metal stent. It is important to wait for long term follow up data on these trials, to observe if the very late stent thrombosis rates are lower, as seen in long term follow up data of ISAR-TEST 4. It is also crucial and remains to be observed, if the stent thrombosis events would be lower even after discontinuing dual anti-platelet therapy and the when



**Figure 5 Cardiac deaths.** BMS: Bare metal stents; PP-DES: Permanent polymer drug eluting stents; BD-DES: Biodegradable polymer drug eluting stents.

event rates are adjusted for dual-antiplatelet therapy use between both the groups. Henceforth, it is too early to come to any firm conclusions in regards to superiority of BD-DES to currently used second generation PP-DES. A recent network meta-analysis comparing BD-DES, first and second generation PP-DES and bare metal stents, concluded BD-DES are not superior to second generation BD-DES<sup>[6]</sup>. In our study, we systematically reviewed all the studies and believe long term follow up of the trials are needed before we can make any such firm conclusions.

A pooled analyses comparing ISAR-TEST3, ISAR-TEST-4 and LEADERS trial showed decreased risk of stent thrombosis with BD-DES at 4 year follow up<sup>[5]</sup>. It is likely because those trials used first generation Sirolimus DES, and the first generation stents are known to have increased late restenosis and stent thrombosis events. However, the second generation DES, use different metal alloy framework with thin struts and the binding polymer is biocompatible and hence the results of such studies cannot be extrapolated to second generation PP-DES. The time for dual anti-platelet therapy with the biodegradable stent is shorter. In fact, in some trials (Table 2), the time for DAPT is reduced to 3-6 mo, while for the drug-eluted stents can be longer. This can be an advantage in any case, since patients on DAPT may have an increased risk of bleeding, especially if unplanned surgery is needed or in case oral anticoagulation is needed for concomitant disease (atrial fibrillation or deep venous thrombosis).

Interventional cardiologists have always been welcoming to newer technology and novel stent designs. The early enthusiasm of most stents, introduced in the past, could not meet the expectations during long-term follow up. With new BD-DES being studied across the globe, we need to analyze the data more closely before drawing conclusions on their superiority to currently used second generation PP-DES.

### Limitation

The major limitation of this study is the wide variation of follow-up period. In particular, the ISAR-TEST 4 study

had the longest mean follow-up period (36 mo), and the odds ratio was completely opposite to all other included studies as pointed out. The results may change with long-term follow-up. Second, Serruys *et al*<sup>[7]</sup> included a study investigating a bioresorbable scaffold into the analysis because you aimed to compare BD-DES and PP-DES. Other minor issues are described below. BD-DES used in the RCT was of various types (Biolimus and Sirolimus) and the results should be interpreted with caution in generalizing our results to all types of BD-DES. The patient population in all these studies did vary to some degree (as described in Table 2). Also, the lesions treated and characteristics of stents used- like length and diameter along with lesion complexity could have affected the outcomes.

BD-DES when compared to second generation PP-DES, showed no significant advantage and the outcomes were comparable between both the groups. Long term follow up data is needed, to demonstrate any decrease in very late stent thrombosis events with BD-DES compared to second generation PP-DES.

## COMMENTS

### Background

Biodegradable polymer stent are currently used in Europe for PCI. Despite that there is no clear-cut evidence in literature comparing the efficacy of these two types of stent.

### Research frontiers

Now a day every effort is made to find the new design of stents, which will minimize the need for longer duration of dual antiplatelet therapy, which can be responsible for their notorious side effect in some situations.

### Innovation and breakthrough

In present study, the authors compared the efficacy of novel biodegradable drug eluting stent with the standard of care second-generation drug eluting stents in the form of meta-analysis of current randomized control trials.

### Application

The present results allow authors to think the role of biodegradable drug eluting stent in stent thrombosis, interests them in further investigating the long term outcomes in form of late stent thrombosis and duration of dual antiplatelet therapy.



## Peer-review

The present meta-analysis provides more insight into clinical practice in regards to usage of different stent designs.

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