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REVIEW

Genetics of coronary artery disease and myocardial infarction

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Abstract

Atherosclerotic coronary artery disease (CAD) comprises a broad spectrum of clinical entities that include asymptomatic subclinical atherosclerosis and its clinical complications, such as angina pectoris, myocardial infarction (MI) and sudden cardiac death. CAD continues to be the leading cause of death in industrialized society. The long-recognized familial clustering of CAD suggests that genetics plays a central role in its development, with the heritability of CAD and MI estimated at approximately 50% to 60%. Understanding the genetic architecture of CAD and MI has proven to be difficult and costly due to the heterogeneity of clinical CAD and the underlying multi-decade complex pathophysiological processes that involve both genetic and environmental interactions. This review describes the clinical heterogeneity of CAD and MI to clarify the disease spectrum in genetic studies, provides a brief overview of the historical understanding and estimation of the heritability of CAD and MI, recounts major gene discoveries of potential causal mutations in familial CAD and MI, summarizes CAD and MI-associated genetic variants identified using candidate gene approaches and genome-wide association studies (GWAS), and summarizes the current status of the construction and validations of genetic risk scores for lifetime risk prediction and guidance for preventive strategies. Potential protective genetic factors against the development of CAD and MI are also discussed. Finally, GWAS have identified multiple genetic factors associated with an increased risk of in-stent restenosis following stent placement for obstructive CAD. This review will also address genetic factors associated with in-stent restenosis, which may ultimately guide clinical decision-making regarding revascularization strategies for patients with CAD and MI.

Key words: Coronary artery disease; Myocardial infarction; In-stent restenosis; Genetics; Heritability; Genome-wide association study; Atherosclerosis

Core tip: This review provides the most comprehensive

summary of the genetics of coronary artery disease (CAD) and myocardial infarction (MI) research with a complete, up-to-date chromosomal map of all CAD and MI-susceptible genes. We discuss the existence and significance of protective genetic factors against atherosclerosis, CAD and MI. We also summarize the current status of constructing genetic risk scores to predict long-term risks of developing CAD and MI. In-stent restenosis is a new challenge in cardiology. The genetics of in-stent restenosis are also discussed in this article.

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INTRODUCTION

Coronary artery disease (CAD) remains the number one cause of death in industrialized society. CAD alone caused approximately 1 of every 6 deaths in the United States in 2010[1]. Atherosclerotic CAD comprises a broad spectrum of clinical entities that include asymptomatic subclinical atherosclerosis and its clinical complications, such as angina pectoris, myocardial infarction (MI) and sudden cardiac death. In the early 1930s, Carl Miller in Oslo reported the co-segregation of high plasma cholesterol, xanthoma and premature coronary heart disease, providing early clues regarding a genetic component of CAD and its association with cholesterol[2]. Family clustering of CAD and MI has subsequently been well recognized and documented. Large twin studies have estimated the heritability of CAD to be approximately 50% to 60%. Understanding the genetic basis of CAD and MI will not only provide insight regarding the pathogenesis of the disease but also a basis for the development of preventive and therapeutic strategies. Research investigating the genetic architecture of CAD has proven to be a difficult and costly task due to the heterogeneities of clinical CAD and MI and its multi-decade complex pathophysiological processes that involve both genetics and environmental factors and their interactions. Together with rapid advances in molecular technology and computational capacities in genetics and genomics, the recent decade has witnessed tremendous progress in genetic and genomic studies of CAD and MI. This article provides a comprehensive overview of the clinical heterogeneity of CAD and MI, which are often assessed in genetic studies; the heritability of CAD and MI; and achievements in gene discoveries related to CAD, MI, and in-stent restenosis. The development of a genetic risk score (GRS) based on genetic risk factors related to CAD and its initial success for predicting the life-long risk of CAD is also discussed.

HETEROGENEITY OF CAD

The heterogeneity of CAD and its clinical complications introduce significant complexity in genetic studies. Clinically, the presentation of atherosclerotic CAD ranges from completely asymptomatic (subclinical atherosclerosis), angina pectoris (typical or atypical, stable or unstable), and silent MI to acute myocardial infarction (AMI) or sudden cardiac death. The Framingham Heart Study (FHS) reported that one third of all MI are unrecognized[3]. Based on autopsy, up to 50% of sudden deaths are due to MI[4]. In addition to the broad spectrum of clinical presentations, the age of onset of clinical symptoms varies dramatically. The average age at the time of first MI is 64.9 years for men and 72.3 years for women[1]. Although AMI is not uncommon in young adults (< 40 years old), an initial diagnosis of severe atherosclerotic CAD in octogenarians is rather common and is often preceded by a long, asymptomatic disease state.

Coronary artery atherosclerosis is the most common underlying pathological process responsible for the majority of clinically significant CAD. Progressive narrowing of the arterial lumen due to negative remodeling and expansion of the atheroma causes myocardial ischemia and angina pectoris. The rupture of a vulnerable atherosclerotic plaque, local activation of thrombotic mechanisms with/without severe underlying stenosis, local thrombosis formation and arterial lumen closure are accepted as underlying mechanisms of AMI. Coronary embolization of the thrombus, spontaneous coronary dissection, myocardial bridging, an anomalous origin and course of coronary artery, and coronary spasm can cause clinical symptoms/presentations that are similar to those of AMI. In addition, some unusual clinical scenarios may cause coronary insufficiency and myocardial ischemia, such as adjacent tumor compression of coronary arteries and systemic vascularitis involving coronary artery beds. In addition, the locations of atherosclerotic stenosis along the coronary tree vary significantly. Isolated aorto-ostial stenosis (ostia of the left main or right coronary artery) and bifurcation lesions are more apparent in relation to turbulent flow and the endothelial response to the flow dynamics. Diffuse atherosclerosis is more commonly observed in patients with diabetes mellitus (DM). The heterogeneity of the location of CAD within the coronary tree may reflect a different set of genetic influences on atherogenesis. The location-dependent effects of PECAM-1 on atherogenesis in animal models have been reported[5], and the results suggest that the heterogeneity of the location of atherosclerotic CAD may represent different genetic influences of atherosclerosis under different dynamic flow conditions.

These heterogeneities of phenotypic characterization, pathological etiologies of CAD and MI and the complex molecular and cellular pathogenesis of atherosclerosis (summarized in Table 1) contribute to the difficulties associated with the identification of genes that are

Table 1 Heterogeneities in coronary artery disease/myocardial infarction

Clinical manifestation	Underlying pathology	Pathological processes of atherosclerosis
Asymptomatic stenosis	Atheroma positive remodeling	Endothelial injury
Stable or unstable angina pectoris	Atheroma negative remodeling	Lipid deposition
Silent MI	Plaque rupture/thrombosis	Oxidative stress/response
Acute MI (NSTEMI and STEMI)	Critical stenosis/thrombosis	Inflammation
Sudden cardiac death	Embolization	Cellular proliferation/apoptosis
	Spontaneous dissection	Foam cell formation
	Anomalous origin/course	Matrix deposition/degradation
	Coronary spasm	Plaque rupture/hematoma/thrombosis
	Myocardial bridging	Neovascular formation

MI: Myocardial infarction.

important for CAD and MI[6].

HERITABILITY OF ATHEROSCLEROTIC CAD

Clinical and population-based studies have demonstrated that genetic factors play important roles in CAD and MI. The phenomenon of family clustering of CAD was repeatedly reported in the 1950s and 1960s[7,8]. Slack and Evans demonstrated that a history of early onset ischemic heart disease (IHD) of first degree relatives was significantly associated with and predicted early onset IHD (< 55 in men and < 65 in women)[9]. The subsequent FHS confirmed that a family history of premature CAD, defined as the presence of a first degree relative with a diagnosis of CAD at < 55 years of age in men and < 65 years of age in women, is an independent risk factor for CAD[10]. The strength of hereditary in determining the risk of CAD increases with an increasing number of affected first-degree relatives and with a younger age of onset[11]. In a multivariate analysis, the extent of CAD based on coronary angiography was also strongly associated with the history of parental CAD independently of plasma lipids, obesity, hypertension, cigarette smoking and alcohol intake[12-15].

Twins have fascinated human communities and provided invaluable opportunities to identify the genetic component of diseases. Identical twins who concordantly develop early onset, angiographically proven CAD, in whom many of the same coronary arteries are even involved, provide highly suggestive information regarding the influence of genetics on CAD[16-20]. Early in 1967, Cederlöf *et al*[21] observed a concordance of 21.7% of CHD history in monozygotic twins, as compared with 6.1% in dizygotic twins. The Swedish Twin Registry and Danish Twins Registry are the two largest twin registries in the world. The Swedish Twin Registry has captured all of the twins born since 1886 and includes 20966 twins. A longitudinal follow-up of 36 years found that if one twin died of CAD, the relative risk of the development of fatal CAD in the second twin was 8.1 for monozygotic twins (MZ, identical twin)[22] and 3.8 for dizygotic twins (DZ, non-identical twin). The estimated heritability of CAD is 57% (50%-59%) in male twins and 38% (25%-50%) in female twins[23]. The influence of genetics is evident across the age range of 36 to 86 years. The Danish Twins Registry (8000 twin pairs) reported an increased incidence of CAD and CAD deaths in MZ twins of subjects with CAD compared with DZ twins (44% *vs* 14%), with an estimated heritability of 0.53 in males and 0.58 in females[24]. In general, CAD is widely accepted to have a heritability of 50% to 60%.

Large-scale prospective, population-based epidemiology studies, such as the Western Collaborative Group Study[25], Health Professional follow-up study[26], the Nurses' Health Study[27], the FHS[10], the British Regional Heart Study[28], the German PROCAM study[29], and the Utah Cardiovascular Genetic Research[30], confirmed the strong independent association among family, parental history of CAD and MI and the occurrence in offspring[31,32]. These genetic factors are independent of traditional risk factors (TRF) for the disease. TRFs, such as hypertension, diabetes mellitus, hypercholesterolemia, low physical activity, obesity, C-reactive protein, plasma homocysteine, and tobacco use are well known to have their own complex genetic components with individual heritability values that have been estimated in twin studies[33]. Figure 1 illustrates the contribution of genetic factors directly or through TRFs in the development and manifestation of CAD. The collective effects of genetic factors, environmental factors, and age determine the development of atherosclerotic CAD and its complications.

GENE DISCOVERY FOR CAD AND MI

Early candidate gene and linkage analyses have identified numerous causal genes and mutations that underlie rare, Mendelian monogenic CAD. Many of these genes and mutations are involved in lipid metabolism. Recently, a combination of a large pedigree of familial CAD and high-throughput genomic sequencing technology led to the discovery of a panel of new possible causal gene mutations for CAD. Table 2 summarizes the genes and mutations that are considered to be causal for atherosclerotic CAD.

Table 2 Genes and mutations identified as causal for monogenic familial coronary artery disease

Categories	Genes	Chrom	OMIM	Mutations	Ref.	GWAS[1]
Monogenic *CAD* genes	*ST6GALNAC5*	1p31.1	610134	G295A (p.*337Qext*20 stop-loss)	[52]	No
	CYP27A1	2p35	606530	G674A (p. Arg225His)	[51]	No
	MEF2A	15q26.3	608320	21-bp del in exon 11	[34,38]	No
	LRP6	12p13.2	610947	G1079A (p. Arg611Cys) T1298C (p. Asn433Ser)	[44,159]	No
Gene mutations cause high LDL	*LDL receptor*	19p13.2	606945	> 1000 variants	[55]	Yes
	PCSK9	1p32.3	603776	9 gain-of-function mutations	[63]	Yes
	ApoB-100	2p24.1	144010	C10580G (p. Arg3527Gln) C10800T (p. Arg3531Cys) rs515135	[59,60,84]	Yes
	LDLRAP1, ARH	1p36.11	603813	ARH1: 432 ins A (p. FS170stop) ARH2: G65A (p. Trp22ter)	[66]	No
Mutations cause low HDL	*ABCA1*	9q31.1	205400	Many ABCA1 LoF alleles Rs2230806 > A	[78,158]	Yes
	LCAT	16q22.1	606967	> 80 mutations Rs5923 ↑ CAD in Egyptians	[160]	Yes
Mutations cause high TG	*Apo C-II*	19q13.2	207750	ApoCII$_{St. Michael}$ p. Gln70Pro	[85,86]	No

[1]The association between the genetic variant and the risk of CAD and MI is also discovered in GWAS. GWAS: Genome-wide association studies; CAD: Coronary artery disease; MI: Myocardial infarction; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; OMIM: Online Mendelian Inheritance in Man.

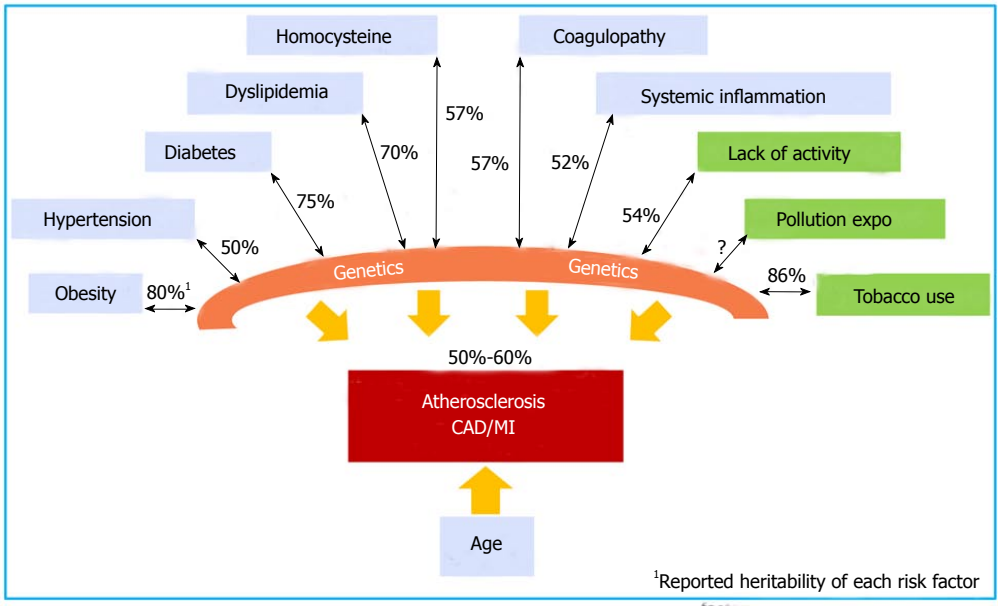

Figure 1 Atherosclerosis is a multi-decade pathological process involving complex interactions between genetics and environmental factors. The estimated heritability of CAD and MI is 50% to 60%. The heritability of each individual risk factor is indicated as a %. CAD: Coronary artery disease; MI: Myocardial infarction.

Monogenic CAD genes

Despite the clinical heterogeneity of CAD and MI as described above, the phenomenon of familial clustering of CAD and collections of large pedigrees with multiple members in multiple generations provided an opportunity to perform linkage analysis and gene discovery. In recent years, three potential causal genes and their responsible mutations for pedigrees with apparent Mendelian autosomal dominant (AD) CAD and MI have been identified (below and Table 2). The common feature of these mutations is co-segregation with the phenotype in the index kindred and a presence in unrelated cohorts. Functional analyses of these genes also supported their potential involvement in the pathogenesis of CAD and MI. However, the potential roles of these familial CAD causal genes and mutations in general population are unknown. Further validation with additional pedigrees and experimental models is warranted.

MEF2A: MEF2A is a transcription factor that belongs to the monocyte enhancer factor (MEF) family. MEF2A is expressed in blood vessels during embryogenesis as an early marker of vasculogenesis and interacts with a variety of molecules that are known to be involved in cardiovascular pathogenesis. Wang *et al*[34] performed a

genome-wide linkage analysis of a large kindred group consisting of 13 individuals and designated the first AD *CAD* gene (adCAD1) at chromosomal location 15q26 within a region containing approximately 93 genes including *MEF2A*. Considering the relevance of MEF2A in vasculogenesis, sequencing of the *MEF2A* gene revealed a 21-base pair deletion in exon 11 of the *MEF2A* gene that was present in all 10 affected living members but not in unaffected individuals[34]. This D7aa *MEF2A* mutation co-segregated with the CAD phenotype in an AD manner in the index pedigree. Interestingly, the same exon 11 deletion in *MEF2A* was reported in other CAD individuals from different ethnic backgrounds[35-37]. Missense mutations in exon 7 of *MEF2A*, resulting in a loss-of-function of MEF2A, were also found in premature CAD individuals, but not in age-, ethnicity- and BMI-matched controls lacking angiographic evidence of CAD[38,39]. Further studies have demonstrated that the deletion of 7 amino acid leads to defective trafficking of MEF2A in a dominant-negative manner. Consequently, MEF2A entry into the nucleus is blocked, which is crucial for the ability of MEF2A to regulate gene expression. It is plausible that functional abnormalities in D7aa MEF2A lead to cellular abnormalities in endothelial cells and vascular smooth muscle cells, which in turn participate in processes associated with atherogenesis. The discovery of the mutation in *MEF2A* in CAD resulted in significant hope of genetic testing for CAD. However, conflicting reports demonstrating the lack of an association between *MEF2A* gene mutations and CAD in other cohorts has raised doubts concerning a causal role of *MEF2A* in CAD[40-42]. In particular, two individuals carrying the D7aa *MEF2A* mutation did not appear to have CAD before the reported age of CAD development, while members of the same family who developed CAD carried the normal *MEF2A* gene[41]. However, potential genetic or environmental modifiers may reduce the phenotypic penetration. Polymorphisms that do not change *MEF2A* transcriptional activation are not associated with an increased risk of CAD[43], and the actual prevalence of functional *MEF2A* mutations in the general population is not yet known.

Low-density lipoprotein receptor-related protein 6: Mani *et al*[44] studied a group of extreme outlier kindred with an extraordinary prevalence of premature CAD presenting an almost uniform presence of hypertension, hypercholesterolemia, type Ⅱ DM, obesity and an absence of cigarette smoking in affected individuals. The extreme familial clustering and segregation of phenotypes (both premature CAD and risk factors) was transmitted as a highly penetrant AD trait[44]. Genome-wide linkage analysis revealed strong linkage between the familial trait and markers of chromosome 12p, which spans 750 kb and contains only six annotated genes [*ETV6, BCL2L14,* low-density lipoprotein receptor-related protein 6 (*LRP6*), *MANSC1, LOH12CR1* and *DUSP16*]. Sequencing of the candidate gene *LRP6* revealed the causal variant, a missense substitution of R611C in a conserved EGF-like domain of LRP6. LPR6 functions as a co-receptor with Frizzle proteins for Wnt ligands. Functional studies of the *LRP6*$_{R611C}$ mutation revealed a dominant-negative decrease in Wnt signaling. Although there was complete linkage of LRP6$_{R611C}$ and hypertension, high low-density lipoprotein (LDL), TG and a prevalence of DM2 in the index kindred, the frequency of *LRP6*$_{R611C}$ was very rare in general population. Recently, a polymorphism in *LRP6* intron 2 was found to be associated with the presence and severity of angiographic CAD in a Chinese case-control study[45]. A common variant of *LRP6*, rs2302685 (a non-synonymous coding sequence SNP in exon 14, T>C, p Ile1062Val), was initially found to be associated with late-onset Alzheimer's disease in Caucasians[46] followed by carotid artery atherosclerosis[47] and, very recently, CAD[48]. Ilr1062Val variant reduces Wnt/β-catenin signaling and anti-apoptotic activities in cultured cells and arterial walls. Additional missense mutations in the *LRP6* gene that correlate to its extracellular domain have been identified by the sequencing of exons and the promoter of *LRP6* in premature CAD patients and controls in different Chinese cohorts. All of these missense mutations cause loss of function (LoF) *via* reduced Wnt signaling activity and attenuated human umbilical vein endothelial cell proliferation *in vitro*[48].

CYP27A1: A large pedigree with well-defined familial traits together with massive parallel sequencing technology and bioinformatics computational and statistical tools has dramatically accelerated the pace of the discovery of disease-causing genes[49]. The identification of potential causative mutations in the *CYP27A1* gene that co-segregated with a familial AD CAD phenotype is one recent example. Inanloorahatloo *et al*[50] performed whole-exome sequencing of two affected members in a large group of AD kindred with premature CAD. An *in silico* un-biased algorithm identified two candidate variants, c. G674A (p. Arg225His) in *CYP27A1* and c. A241T (p.Ile81Phe) in NTRK2, which were further sequenced in all of the available members of the kindred. The variant c.G674A (p. Arg225His) in *CYP27A1* co-segregated with the CAD status. The *CYP27A1* gene encodes the sterol 27-hydroxylase involved in cholesterol and 25-hydroxy vitamin D3 synthesis. The amino acid p. Arg 225 that was affected by the identified variant is highly conserved in paralogous and orthologous proteins, suggesting its functional importance. This variant was not observed in 500 ethnically matched controls without a history of cardiac disease. Furthermore, an additional four disease-specific variants in the *CYP27A1* gene were discovered by sequencing the *CYP27A1* exons in 7 out of 100 unrelated CAD patients. Although disease-causing variants of the *CYP27A1* gene were considered relatively rare, potential disease-causing variants reached up to 7% in Iranian patients with CAD. To date, potential CAD-causing *CYP27A1* variants have not been reported in other populations, and the prevalence of

these variants in the general population is unknown. The mechanism underlying the possible causal role of mutant *CYP27A1* in atherosclerotic CAD remains uncharacterized. Recently, *CYP27A1*-deficient mice in an ApoE-deficient background exhibited a 10-fold reduction of aortic atherosclerosis after challenge with a high-fat diet associated with a 2-fold reduction of total plasma cholesterol, LDL, and very low-density lipoprotein (VLDL) as well as a 2-fold elevation of high-density lipoprotein (HDL). These results suggested that CYP27A1 regulates cholesterol homeostasis, and alterations of its activities may subsequently lead to atherosclerosis[51].

ST6GALNAC5: The *ST6GALNAC5* gene is the newest addition to the group of causal genes for familial CAD. InanlooRahatloo *et al*[52] studied a highly inbred Iranian pedigree of AD premature CAD. Unbiased GWAS combined with whole-exome sequencing of two affected members identified a polymorphism, G295A, in the *ST6GALNAC5* gene that resulted in a p. Val99Met mutation. Targeted sequencing of all of the available members confirmed the co-segregation between this variant and the CAD phenotype. A search of *ST6GALNAC5* mutations in other Iranians with confirmed CAD revealed a p.*337Qext*20 mutation in two unrelated patients with CAD (2 out of 160). Interestingly, one of the patients who carried this p.*337Qext*20 stop-loss mutation had one sibling with CAD and two unaffected siblings; a genetic analysis of the family again showed co-segregation of the mutation with disease status. *ST6GALNAC5* encodes sialyltransferase 7e, a member of the sialyltransferase family. Sialyltransferases add sialic acids (acetylated derivatives of neuraminic acid) to the termini of carbohydrate chains in glycoproteins and glycolipids. Elevated sialyltransferase activity in blood cells and serum sialic acid levels[53] are associated with atherosclerosis and CAD. *In vitro* functional studies of proteins encoded by these two mutated *ST6GALNAC5* genes revealed a two-fold increase in sialyltransferase 7e enzymatic activity[52]. Given that: (1) an un-biased approach was applied in the identification of the *ST6GALNAC5* mutation in a large CAD pedigree; (2) convincing evidence demonstrates the co-segregation of the *ST6GALNAC5* mutation with the familial CAD/MI phenotype; (3) additional functional mutations have been identified in the *ST6GALNAC5* gene in unrelated CAD/MI patients and families; (4) no functional variations were identified in affected family members and unrelated controls; and (5) the available evidence supports the notion that sialic acid and sialyltransferase activity are involved in the pathogenesis of atherosclerotic arterial disease, it is reasonable to conclude that gain-of-function mutations in ST6GALNAC5, such as p. Val99Met and p.*337Qext*20, are monogenic causal genes for CAD. The prevalence of functional *ST6GALNAC5* gene mutations in the general population and in patients with CAD is unknown. The mechanism by which mutant ST6GALNAC5 causes atherosclerosis and CAD and its potential role in targeted therapy or in the prevention of CAD remain unknown.

Monogenic lipid disorders

Serum lipid levels, particularly elevated LDL cholesterol and HDL, are important risk factors for the development of atherosclerotic CAD. Mutations in genes involved in lipid metabolism have been identified and demonstrated to be causal for dyslipidemia and related atherosclerotic CAD and MI with variable penetration. These genes and their mutations in association with CAD and MI have been extensively reviewed in the literature[54] and are presented in Table 2. Depending on the primary abnormality in lipid metabolism and its effects on CAD, monogenic lipid disorders can be categorized into primary elevated LDL (LDL receptor, ApoB-100, PCSK9, and LDLRAp or ARH), a primary reduction of HDL (ApoA1 in primary hypoalphalipoproteinemia, ABCA1 in Tangier disease, and the lecithin:cholesterol acyltransferase (*LCAT*) gene in Norum disease and Fish-Eye disease), and primary elevated triglycerides (TGs) (LPL, ApoC-Ⅱ in type Ib hyperlipoproteinemia and the *ABCG5/8* gene in Sitosterolemia).

Genes and variants that are the primary cause of high LDL cholesterol: Mutations in genes encoding the LDL receptor, apolipoprotein B-100 (an LDL receptor ligand) and the pro-protein convertase subtilisin kexin type 9 (PCSK9) cause AD familial hypercholesterolemia (FH). Patients who harbor homozygous [low-density lipoprotein receptor (LDLR)] mutations (1 in 1 million) display a 6- to 10-fold increase in plasma LDL-C from birth and experience CAD/MI in early childhood. The early atherosclerosis observed in children who are homozygous for FH is not associated with any other risk factors that suggest that elevated LDL alone can produce atherosclerosis in humans. Carriers of heterozygous LDLR mutations, demonstrating a frequency of 1/500 in the general population, display a 2-fold increase in low-density lipoprotein cholesterol (LDL-C) levels from birth and are at risk to suffer CAD and MI at 30s years of age. Approximately 5% patients with CAD and MI under the age of 60 years carry heterozygous LDLR mutations. A total of 1741 sequence variants (1122 unique variants) have been recorded in the British Heart Foundation LDLR database[55] (http://www.ucl.ac.uk/ldlr/Current/summary.php?select_db=LDLR&show=sum). These mutations are present in the form of exonic substitutions, small exonic rearrangements, large rearrangements, promoter variants, intronic variants, variant sin the 3' untranslated sequence, point mutations, splice site mutations, and large deletions. These mutations are equally distributed throughout the gene[56,57]. Genetic testing of all known LDLR variants is available. This test is often considered as the first step in a stepwise genetic analysis for FH followed by tests to assess the *ApoB-100* and *PCSK9* genes[58].

Apo B-100 is a unique protein component in lipoproteins originating from the liver (VLDL, IDL, and LDL). Apo B-100 is also required for the synthesis, assembly, and secretion of hepatic TG-rich lipoproteins, and it binds to heparin and various proteoglycans found in arterial walls. The most important function of Apo B-100 is to bind the LDLR *via* its LDLR-binding domain to mediate the clearance of LDL from plasma. Two mutations in the *Apo B-100* gene, C10580G (p. Arg3527Gln)[59] and C10800T (p. Arg3531Cys), result in the alteration of LDLR binding affinity. In addition, these mutations cause familial ligand-defective hypercholesterolemia (OMIM 144010) and are associated with early atherosclerotic arterial disease[60]. The frequency in the unselected general population of the Arg3527Gln and Arg3531Cys mutations is approximately 1 in 500 and 1 in 3000, respectively. Most recently, by taking advantage of whole-exome sequencing and linkage analysis of an AD hypercholesterolemia pedigree, a third mutation (p. Arg50Trp) was identified[61].

Linkage analysis of two large French ADH pedigrees resulted in the identification of two mutations in the *PCSK9* gene (1p34.1-1p32), which encodes a protein that is also known as a neural apoptosis-regulated convertase 1 (NARC-1)[62]. A total of 9 gain-of-function mutations in *PCSK9* genes in families with ADH have been reported[63]. These mutations cause a decreased number of LDLR, elevated levels of serum total and LDL cholesterol, and phenotypes of tendon xanthomas, premature CAD, MI and stroke. SNP rs11206510 (risk allele T) located in the *PCSK9* gene is also associated with an increased risk of CAD and MI in an unbiased GWAS study[64]. However, loss-of-function mutations in *PCSK9* identified by exome sequencing of individuals with extremely low LDL levels in the Atherosclerosis Risk in Communities study (ARIC) and Dallas Heart Study cohorts revealed that these mutations led to hypocholesterolemia and were protective against CAD and MI[65]. Secreted PCSK9 protein functions as an LDLR chaperone, binds to the EGF-A domain of the LDLR, decreases receptor recycling to the cell surface and promotes lysosomal degradation. Although the contribution of the PCSK9 gain-of-function mutation in ADH is rather small (< 3%), elucidation of the PCSK9 gain-of-function in ADH has shed light on the potential for the development of cholesterol-lowering agents by reducing the circulatory level of PCSK9 (PCSK9 inhibitors). These discoveries have resulted in the development of PCSK9 inhibitors as novel cholesterol-lowering agents.

Approximately 50 individuals of Mediterranean or Middle Eastern origin carry homozygous mutations in the autosomal recessive hypercholesterolemia (*ARH*) gene. *ARH* (1p34-1p35) was subsequently cloned and named LDL receptor adaptor protein 1 (LDLRAP1), which encodes a phosphotyrosine-binding domain protein and is required for LDLR internalization in hepatocytes. Two mutations, 432insA in exon 4 causing FS170Stop (*ARH1*) and the nonsense mutation G65A in exon 1 (p. trp22ter), account for most of the known cases of ARH in Sardinia[66]. The third mutant was a result of an ancient recombination between *ARH1* and *ARH2*. In addition, 4 Italian ARH individuals from the mainland carried homozygous *ARH1*. Overall, ARH mutations are rare[67].

Genes and variants that are primary causes of low HDL cholesterol: Approximately 40% of patients with CAD have a low level of high-density lipoprotein cholesterol (HDL-C; < 40 mg/dL per current guidelines or age and sex-adjusted plasma HDL-C levels below the 10th percentile). Prospective cohort studies also suggest that low HDL-C is a significant, independent risk factor for CAD. An estimated 50% to 70% of the variations in HDL-C in the human populations are due to genetic factors, and the majority remain undefined[68].

Apolipoprotein A1 (Apo AI) is the major apolipoprotein in HDL-C and is a key determinant of the levels and metabolism of HDL-C. Apo AI functions as a cofactor for LCAT, which is responsible for the formation of most cholesterol esters in the plasma. Apo AI also promotes the efflux of cholesterol from cells. *ApoAI* mutations cause AD familial hypoalphalipoproteinemia. The homozygous loss of *ApoAI* leads to a complete absence of Apo AI and HDL-C levels < 5 mg/dL with normal LDL-C and TG levels. Heterozygous LoF Apo AI carriers have HDL-C levels that are approximately 50% less than normal HDL-C levels. ApoAI gene polymorphisms are associated with decreased HDL and an increased risk of premature CAD[69]. Yamakawa-Kobayashi *et al*[70] analyzed sequence variations in the *ApoAI* gene in Japanese children with low levels of HDL (below the first percentile in the general population) and found 3 frameshift and 1 splice site mutation with possible deleterious effects. They estimated the frequency of hypoalphalipoproteinemia due to *ApoAI* mutations to be 6% in subjects with low HDL cholesterol and 0.3% in the general Japanese population[70]. The A164S variant of the *ApoAI* gene identified by sequencing of the *ApoAI* gene in 190 Copenhagen City Heart Study participants predicts an increased risk of IHD [hazard ratio (HR) 3.2, 95%CI: 1.6-6.5], MI (5.5, 95%CI: 2.6-11.7) and overall mortality (2.5, 95% CI: 1.3-4.8). Despite comparable levels of plasma lipids and lipoprotein, including HDL-C and *ApoAI*, in A164S heterozygotes, heterozygous A164S carriers exhibit a decrease in survival by more than 10 years ($P < 0.0001$) compared with non-carrier controls[71,72]. In addition, two *ApoAI* variants (*ApoAI$_{Paris}$* and *ApoAI$_{Milano}$*) were associated with a reduced risk of CAD, suggesting the occurrence of cardioprotective effects[73].

The ATP-binding cassette transporter (ABCA1) is involved in the initial phase of reverse cholesterol transport and the egress of free intracellular cholesterol and phospholipids from extrahepatic cells. Homozygous LoF *ABCA1* variants are causal factors for the rare Tangier disease, which results in extremely low HDL-C

levels, a 40% reduction of LDL-C compared with the general population, and an increased risk of early CAD[74,75]. A total of 200 LoF mutations in the *ABCA1* gene have been reported (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ABCA1, last accessed on August 6, 2014). Heterozygous carriers of the ABCA1 mutation exhibit an approximately 50% reduction in HDL-C without alterations in the levels of LDL-C and an increased risk of premature CAD[76]. The frequency of heterozygous carriers of *ABCA1* mutations is estimated as approximately 3:1000 in the general population[77]. The R219K polymorphism in the *ABCA1* gene is associated with a reduced risk of CAD, suggesting that this polymorphism provides protective effects against the disease[78].

LCAT catalyzes the esterification of free cholesterol with acyl groups derived from lecithin as an essential step in the maturation of HDL-C. Homozygous LoF in the *LCAT* gene causes rare autosomal recessive Norum disease with very low HDL (< 5th percentile), elevated TGs and decreased LDL-C. Greater than 80 genetic variants in the *LCAT* gene have been identified and reported to be associated with 29% of the individuals with low levels of HDL-C in the Netherlands[79]. However, the association between low levels of HDL-C caused by *LCAT* deficiency and an increased risk of CAD is not as certain as the associated risk of ApoAI[80]. This phenomenon may be explained by the observation that LCAT deficiency mainly causes decreased levels of ApoAII. HDL particles containing ApoAI, but not ApoAII, possess "anti-atherogenic" effects.

Genes and variants that are primary causes of elevated TGs: Plasma TGs predominantly occur in the form of intestinally synthesized chylomicrons (CMs), remnants in the postprandial state and hepatically synthesized VLDL in the fasted state. Plasma TG levels are a polygenic trait and is influenced by environmental factors and lifestyles, *i.e.*, diet, physical activities and tobacco use. Large epidemiological studies have demonstrated that plasma TG concentrations are a strong independent risk factor for CAD[81].

Lipoprotein lipase (LPL) is the rate-limiting enzyme in converting VLDL to LDL. Homozygous LoF mutations in *LDL* genes cause LPL deficiency in rare (approximately 1 in a million) AR type Ⅰ hyperlipoproteinemia characterized by marked hypertriglyceridemia with a decrease in HDL and LDL, eruptive xanthoma, hepatosplenomegaly, recurrent pancreatitis, and in some cases, premature atherosclerotic arterial disease. Greater than 100 LoF variants have been identified[82]. Sequencing data have suggested that rare LPL variants are actually common in patients with elevated TG levels[83]. Variants of LPL genes that are negatively associated with plasma levels of TG, positively associated with HDL and inversely associated with a risk of CAD have also reported, suggesting potential protective genetic variants against CAD[84]. ApoC-Ⅱ is an activator of LPL. A homozygous LoF ApoC-Ⅱ deficiency results in rare AD type Ⅰb hyperlipoproteinemia with extremely elevated TG and chylomicron levels in the plasma, causing recurrent pancreatitis and, in some cases, ApoCII$_{St.\ Michael}$ (Gln70Pro)[85] and other conditions[86], leading to premature ischemic vascular disease. The significance of these mutations in general population remains to be explored.

Sitosterolemia is characterized by hyperabsorption and the retention of dietary cholesterol and sterols, including plant and shellfish sterols, leading to high levels of plant sterols in the plasma, the development of tendon and tuberous xanthomas, accelerated atherosclerosis, and premature CAD. LoF mutations in ABCG5 (encoding sterolin-1) and ABCG8 (encoding sterolin-2) cause sitosterolemia. All of the probands identified in the sitosterolemia pedigree have homozygous mutations in either ABCG5 or ABCG8[87]. The prevalence of ABCG5 and ABCG8 heterozygous carriers and their effects on cholesterol metabolism and atherosclerotic disease in the general population remain unclear.

Genes and polymorphisms associated with CAD

Monogenic traits and their causal genetics only explain a small proportion of the genetics of CAD and MI. Prior to the completion of human genome sequencing, *CAD* and *MI* gene discovery largely employed pedigree-based linkage analysis and positional cloning with the limited availability of genomic markers. The Human Genome Project, HapMap project, and 1000 Genomes Project provided a reference of 3.2 billion nucleotide base pairs of the human genome and 3 million single nucleotide polymorphisms (SNPs) distributed throughout the genome. These SNPs serve as high-density genomic markers for the entire genome. The developments of high-density microchips containing millions of SNPs, high-throughput analytic technology, and powerful biostatistics data mining tools have permitted genome-wide association studies (GWAS). GWAS is a non-hypothesis-driven, unbiased analysis of the potential associations between traits of interest (disease, phenotypes, *etc*.) and genomic markers (SNPs) consisting of tens of thousands of cases and controls[88]. In 2007, SNPs located in 9p21 were identified as strongly associated with CAD and MI based on the results of four nearly simultaneous publications. Numerous GWAS studies have been subsequently conducted, involving tens of thousands of CAD and MI cases and controls inclusive of a large spectrum of demographic, geographic and ethnic backgrounds. The largest meta-analysis of GWAS data reported by the CARDIoGRAMplusC4D Consortium included a total of 63746 CAD cases and 130681 control subjects and confirmed/identified 46 CAD susceptibility loci[84]. Together with a 6q21 locus that was identified in the Chinese Han population by Wang *et al*[89] and an additional 3 CAD susceptibility variants identified by IBC 50K[90], which were not confirmed in the CARDIoGRAMplusC4D Consortium study, a total of 50 GWAS-identified CAD susceptibility genomic loci were

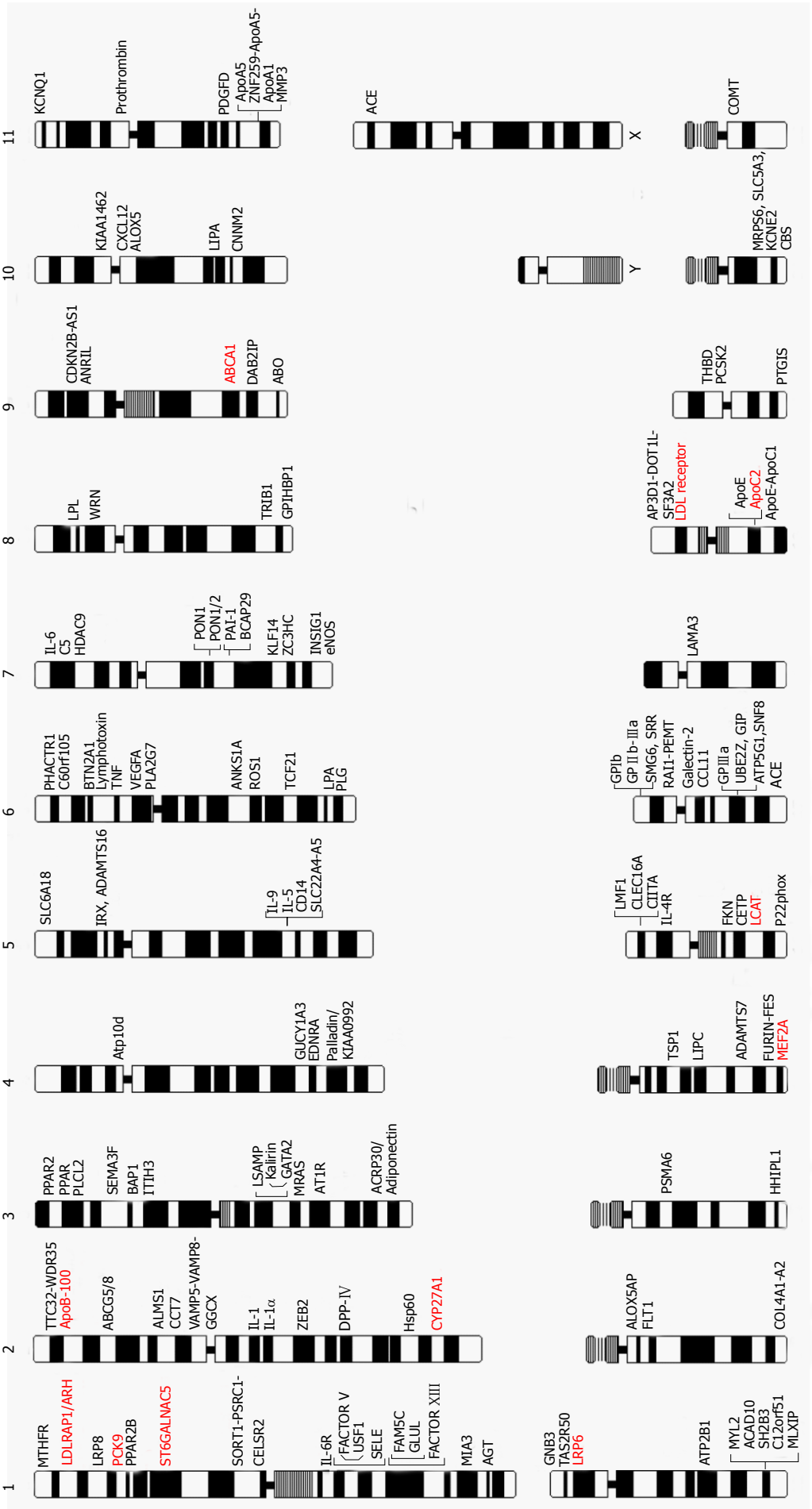

Figure 2 Chromosome map of genes reported to be causal for, susceptible to and associated with coronary artery disease and myocardial infarction in the literature. Genes in red are causal genes for monogenic familial CAD and MI. CAD: Coronary artery disease; MI: Myocardial infarction.

reported. Recent reviews have focused on GWAS discovery of CAD and MI susceptibility loci[91-95].

The journey to understand genetic/genomic architecture of CAD and MI continues with steady progress. Figure 2 provides a complete map of the genes and loci that are reported in literature to be causal for, susceptible to, or associated with the risk of CAD and MI identified by either linkage analysis and/or association analyses with

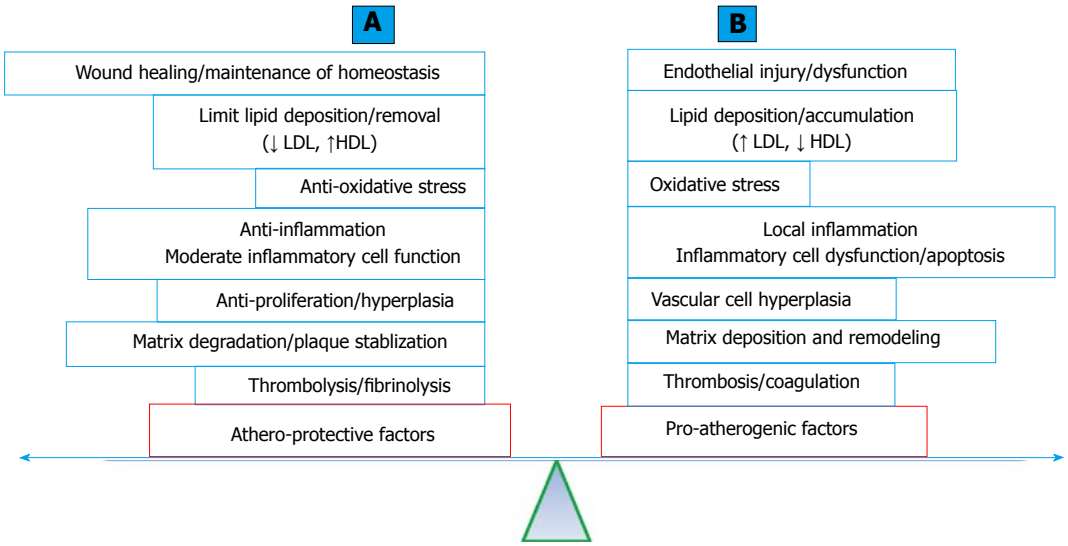

Figure 3 The balance between athero-protective (A) and pro-atherogenic (B) factors involved in the development of atherosclerosis. Obstructive atherosclerotic arterial disease results from the loss of balance between these two factors. A: A list of factors and processes that provide protective effects against atherogenesis; B: A list of factors and processes that promote atherogenesis. HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

either candidate-gene or genome-wide approaches. The genes and mutations that are potentially causal for monogenic CAD and MI families are also presented in Table 2.

Post-GWAS challenges in CAD genetics

The ultimate goals of the study of the genetics of CAD and MI are to reveal genes and their products involved in the development of CAD and MI, understand the molecular and cellular pathophysiology and subsequently establish risk stratification strategies to direct prevention and also develop effective therapeutic approaches. Despite the total of 147 genes (Figure 2) with variants that are causative for or associated with CAD and MI, these genes only explain less than 20% of the heritability of CAD and MI. Furthermore, the biological functions and pathophysiological roles of most of the gene variants and genomic loci are not fully understood. Early attempts to use genetic risk markers of CAD to predict long-term outcomes were not successful, and many challenges remain before the ultimate goal of understanding the genetics of CAD can be realized.

Searching for unexplained heritability: GWAS is based on the hypothesis of a "common disease, common variant". The sensitivity of GWAS in detecting a significant association between genetic variants and traits is limited to high frequency variants (5%)[96]. Regarding CAD and MI, most of the GWAS-identified variants individually or in combination confer relatively small increments in risk (1.1- to 1.5-fold) and explain only a small proportion of heritability. The well-recognized sources for the missing heritability of complex traits[97-99] include: (1) larger numbers of variants with a smaller effect that remains to be identified; (2) a low minor allele frequency (0.5%-5%) or rare variants (< 0.5%) with a larger frequency that are not detectable using the available arrays; (3) structural variants (*i.e.*, copy number of variants due to insertion or deletions, inversions, or translocation) are poorly captured by SNPs; (4) a low power to detect gene-gene interactions; and (5) an inability to detect gene-environment interactions using the current GWAS methodology. Strategies have been suggested and explored to overcome these pitfalls, including: (1) analyzing phenotypically well-defined cases and controls[100], increasing the numbers of participants[84], and utilizing extreme phenotype groups[54,101]; (2) developing powerful biostatistics tools to enrich the signal and detect sensitivities and to capture the additive effects of variants, gene-gene interactions and rare variants[102,103]; (3) integrating available functional information, *i.e.*, eQTLs, protein structure/function predictions, and known pathways and networks related to the traits, to prioritize GWAS signals[104] and performing integrative analyses[105]; (4) customizing fine mapping SNPs or use next-generation sequencing regions of interest to capture rare variants and structural variants; and (5) considering the epigenomic regulation of gene expression.

Many current phenotypes are subjectively measured and may represent numerous underlying biological processes. Misclassifying a phenotype can reduce power in GWAS relative to expectations based on power calculations of idealized homogeneous populations. Strong genotypic effects that are important in a small homogeneous subgroup could have a small or even negligible effect within an entire population[106].

Functional annotation of known genes and variants: Numerous genes and variants associated with CAD and MI have been explored in the last two decades. Candidate gene approaches (positional cloning, linkage analysis, and candidate-gene asso-

Table 3 Genetic variants associated with a reduced risk of coronary artery disease/myocardial infarction (protective factors against coronary artery disease/myocardial infarction)

Chr	Gene	Protective alleles	Ref.
1p13	*Rs599839 A>G Rs646776 T>C*	C/G haplotype	[161,162]
1q22	*E-selectin*	G2692A; C901T	[163]
1q31	*GLUL*	Rs10911021 T>C, TT allele	[164]
1q31	*IL-10*	G(-1082)A, GG genotype	[165]
1p34	*LRP 8*	TCCGC	[166]
2p21	*ABCG 5/8*	Rs41360247	[167]
3p25	*PPARγ2*	Pro12Ala homo	[168-170]
3p25	*PPARγ*	C161T	[171]
3q27	*Adiponectin*	Rs1501299 (G276T), TT allele	[172-174]
8q21	*FABP4*	Rs77878271	[175]
6p12.3	*PLA2G7*	R92H	[176]
6p25.3	*FXIII*	Val34Liu	[177,178]
7q21.3	*PON1/2*	Gln192Arg	[112,116]
7q32.3	*KLF14*	Rs4731702 T/T allele	[179]
7q36	*INSIG1*	Hap3 (T/G/A)	[180]
9q31.1	*ABCG1*	G1051A, r219K, KK allele	[78]
11q23.3	*APOC3*	R19X	[181]
13q34	*FVII*	R353Q; QQ allele A2 allele (without a 10 bp insertion)	[182]
16q13	*FKN*	T280M allele; Rs4329913; Rs7202364	[183,184]
16q24	*NADPH p22phox*	C242T	[185,186]
17p13.2	*GP1ba*	Thr/Th; TT haplotype	[187]
21q22.1	*MRPS6*	C699T (TT) or T1080C (CC)	[64]

ciation analysis) provide a direct link between the pathogenesis of CAD/MI and candidate genes. Variants or chromosomal loci identified by GWAS however do not associate with specific genes or pathways. Only one-third of the 45 CAD loci reported in the largest CAD and MI GWAS study contain a known functionally relevant candidate gene[84]. The 9p21 locus is the CAD locus discovered by GWAS and remains the strongest association with CAD in the human genome. However, the SNPs defining the 9p21 association with CAD are all located in intergenic locations rather than in coding or regulatory regions. Functional annotation of the 9p21 locus in association with CAD has focused on the two closest protein-coding genes, *CDKN2A* and *CDKN2B*, and an additional *CDKN2B* antisense noncoding RNA (ANRIL). The systematic functional annotation of these *CAD* and *MI* gene variants and loci will provide insight regarding the pathophysiology of the disease. These functional studies will require a combination of tissue, cell and animal model systems as well as assessments at the levels of gene expression, protein modification and metabolism[107,108].

Protective genetic factors against CAD and MI: Atherosclerosis is a multi-decade pathological process. A simplified pathologic process (Figure 3A) includes: (1) endothelial injury; (2) lipid particle deposition (fatty streak formation); (3) local cellular and inflammatory responses (early atheroma formation). The process is followed by (4) atheroma progression with the formation and expansion of the necrotic core, fibrous cap, matrix accumulation and various degrees of plaque instability; and (5) intertwined with various degrees of thrombosis formation[109]. The standard human genetic approach to CAD involves searching for genetic risk factors or susceptibility genes for the disease. Could genetic factors be protective against atherosclerosis and CAD and MI? Protective genetic factor against CAD and MI may antagonize the effects of genetic risk factors and potentially explain a portion of the missing heritability. The presence of protective genetic factors will alter the predictive value for CAD and MI using risk variants. The identification of protective genetic factors may shed light on the mechanisms of CAD and MI and further guide the development of strategies or therapy.

Clinical practice often encounters individuals of an advanced age with multiple TRF for CAD and MI without demonstrated angiographic evidence of CAD. The offspring of centenarians have a significantly reduced rate of cardiovascular complications[110]. The current understanding of the pathophysiology of atherosclerosis and CAD and MI supports the notion that the development and progression of atherosclerosis is an accumulating effect of the imbalance between athero-protective and pro-atherogenic processes (Figure 3B). Approximately 500 genes reported in the literature have been tested for their effects on atherogenic processes in atherosclerosis-prone mouse models (ApoE deficiency or LDL receptor deficiency with a high-fat diet) with transgenic (gain-of-function), knockout (LoF) or both genetic modifications. LoF mutations in approximately half of these genes accelerate atherosclerosis in a mouse model, whereas gain-of-function mutations significantly reduced atherosclerosis. It is plausible to suggest that these genes normally function as protective factors against atherosclerosis. In contrast, the remaining half of these reported genes exert pro-atherogenic effects that are normal or consistent with their gain-of-function mutations[111] (a complete list of these genes is available from the authors upon request).

Most genomic association studies have been designed to identify CAD/MI susceptibility genes or polymorphisms. The largest genetic study to assess the impact of common genomic variation on the risk of CAD reported a total of 45 CAD susceptibility loci and an additional 104 likely independent SNPs that were associated with an increased risk of CAD and MI, explaining approximately 10.6% of the heritability. No protective variants were reported[84]. Candidate gene-based association studies identified polymorphisms that are significantly associated with a reduced risk of CAD and MI (Table 3). However, the results obtained for many of these potentially protective genetic loci against CAD are conflicting.

HDL provides protection against atherosclerotic CAD partially through its anti-oxidative effects. Serum paraoxonase is responsible for most of the antioxidant

properties of HDL. Human paraoxonase is encoded by the family of *PON1, PON2* and *PON3* genes. Low serum PON1 activity is associated with an increased risk of CAD and its severity[112,113]. Many candidate gene association studies have revealed that *PON1* (Leu55Met, Gln193Arg) and *PON2* (Ser311Cys) polymorphisms are associated with the risk of CAD[114] and its angiographic severity[115]. For example, an association study in a single center of consecutive patients who underwent coronary angiography revealed a significant dose-dependent association of the *PON1* genotypes (192 Q/R) and serum PON1 (QQ192 > QR192 > RR192) as well as an inverse association with systemic indices of oxidative stress. In addition, 192 Q (QQ and QR) was associated with a decreased risk of cardiovascular and all-cause mortality[116]. The *PON1/PON2* haplotype comprising M55, Q1192 in PON1 and Cys 311 in *PON2* is associated with a significant protective effect against the risk of MI[117]. PON1-deficient mice in an $ApoE^{-/-}$ background fed a high-fat diet exhibit significantly exaggerated atherosclerosis compared with $ApoE^{-/-}$ mice carrying the wild type *PON1* gene[118,119]. Germline transgenic or transient adenoviral vector-mediated overexpression of atheroprotective PON1 (55L/192Q) in $ApoE^{-/-}$ mice revealed protective effects against atherosclerosis with $ApoE^{-/-}$ without transgenic PON1[120,121]. Multiple layers of evidence suggest that genetic polymorphisms in *PON1* and *PON2* lead to an increase in serum paraoxonase activity that may provide protective effects against CAD. However, the frequency of these variants in the general population remains to be determined.

GENETIC RISK SCORE TO PREDICT THE RISK OF CAD AND MI

Primary prevention of CAD is gauged based on the risk categories derived from the risk assessment with TRFs, such as the Framingham risk score (FRS) in the United States[122], the SCORE risk equation in Europe[123], the Reynolds risk score for women[124] and the PROCAM risk score in Germany[29]. The discovery of causal genetic factors for monogenic CAD and MI, such as monogenic lipid disorders, have made it possible to perform clinical genetic screening of family members and to provide enhanced primary prevention to carriers of causal mutations. This approach has been shown to be cost-effective[125]. The association between genetic polymorphisms and the risk of CAD and MI provides an opportunity to use genetic information and develop a GRS to improve the risk prediction of CAD and MI in the general population and subsequently guide preventive strategies. The GRS is calculated either in an un-weighted manner by adding allele numbers (0 for no risk allele, 1 for one allele and 2 for both alleles) with a weighted GRS typically by using the reported effect sizes from the reference studies as weights for the risk allele counts or with a weighted GRS mean, which is derived by dividing the sum of the weighted GRS allele counts by the number of the SNPs. The association of GRS with the risk of the CAD endpoint has been assessed. Thus, GRS is evaluated if the addition of GRS to the traditional risk scoring model improves the discrimination measured using AUC or C statistics or results in risk category net reclassification improvement (NRI). To be clinically applicable, GRS must eventually be validated in independent prospective studies. The expectations are high[126]. Research on this topic is growing. The scientific community has provided guidelines regarding the design, performance and reporting of studies investigating genetic risk prediction[127,128]. However, the outcomes have been mixed.

Early in 2004, Humphries *et al*[126] reported a non-significant improvement of the risk prediction power to *PROCAM* risk score with the addition of ApoE genotype information in the Northwick Park Heart Study Ⅱ (NPHSⅡ) cohort (ROC increased from 0.65 to 0.67, $P = 0.11$). The addition of genetic variants of *IL6* and *PPARα* did not result in any improvement of the CAD prediction power of the PROCAM score[126]. Chromosome 9p21.3 has demonstrated the strongest association with CAD in GWAS studies. The addition of the genotype of SNP rs10757274 A>G in the 9p21.3 locus did not significantly improve the predictive value of the FRS, but it improved the reclassification of coronary heart disease (CHD) risk and guided primary prevention for a high-risk population in a prospective study[129]. This conclusion was subsequently confirmed in studies using independent cohorts[130-133]. Statistical modeling revealed that larger numbers of genetic variants, higher odds ratios (OR) and the genotype frequency of individual variants can improve the discriminative accuracy of area under the receiver operating characteristic curve (AUC) using the genetic score to predict the risk of CAD and MI[134,135]. The application of 100 established variants with ORs ranging from 1.13 to 1.42 can achieve an AUC of 0.76, which is comparable to most of the currently used conventional risk scoring systems[135]. A rapid increase in the number of studies reporting the development and validation of GRS to predict the risk of CAD has been recently noted[136,137].

Morrison *et al*[138] calculated the GRS based on the number of risk alleles of 11 CAD-associated SNPs identified in the Atherosclerosis Risk in Communities Study (ARIC) cohort and combined the results with the ARIC Cardiovascular Risk Score (ACRS) to predict CAD. These researchers found that the addition of GRS to the traditional risk score significantly increased the AUC to predict the risk of CAD in blacks and suggested improved CAD risk prediction in whites[138]. In a large prospective cohort study with a median of 10.7 years of follow-up, Ripatti *et al*[139] found that individuals with a GRS in the top quintile derived from 13 multi-locus SNPs of CHD exhibited a 1.66-fold increased risk of CHD adjusting for TRF. However, the GRS did not improve the C index over the TRFs and family history or the net reclassification of risk categories. Paynter *et al*[140] prospectively studied GRS from 101 SNPs in the

large Women's Genome Health Study with a median follow-up of 12.3 years and found that the GRS did not improve the discrimination or reclassification of the ATP Ⅲ risk score. Most recently, by choosing SNPs that were repeatedly and reproducibly confirmed in multiple GWAS studies using improved statistics tools and systematic risk stratifications of TRFs, GRS added significant predictive value to improve risk predictions[141-146]. For example, Thanassoulis *et al*[142] constructed a GRS with 13 CAD risk SNPs and assessed participants in the FHS. These researchers not only confirmed the association between GRS and incident CHD and a high coronary artery calcium score (CAC) but also demonstrated that GRS modestly but significantly improved the risk reclassification for incident CHD and significantly improved the discrimination for a high CAC[142]. However, the addition of 16 newly discovered SNPs to the GRS (total of 29 SNPS) did not improve the performance of the GRS in contrast to previous *in silico* computations[135]. Tikkanen *et al*[146] derived a weight GRS using 28 SNPs associated with risk for CAD and MI in the large FINRISK study cohort with up to 19 years of follow-up for CHD. These researchers discovered a highly significant independent association between GRS and the risk of CHD. The addition of GRS to TRF with and without a family history significantly improved both the risk discrimination for all end points and the reclassification of individuals in the intermediate-risk category (clinical NRI = 27%). Similar results were validated in additional independent cohorts. Furthermore, this GRS was used as a novel risk marker in the 2-stage population screening study Emerging Risk Factors Collaboration. The addition of GRS screening of individuals with intermediate risk per TRF screening reclassified 19% of the group into the low- and 12% into the high-risk category, who thus became eligible for more aggressive primary prevention[146].

GRS derived from genetic variants associated with TRF for CAD is generally confirmed by an association with the disease, but it does not improve the discrimination of CAD and MI derived from the TRF assessment[142]. Kathiresan *et al*[147] calculated genetic scores for 5414 subjects in the Malmo Diet and Cancer Study based on the number of unfavorable alleles of nine SNPs with associations with LDL or HDL cholesterol levels. In this study, the genetic score was an independent risk factor for incident CVD over a median of 10.6 years of follow-up and modestly improved the clinical risk reclassification (Adult Treatment Panel Ⅲ, ATP Ⅲ classification) for individuals in the intermediate-risk category (26% rate of reclassification). However, this genetic score did not improve the risk discrimination[147]. Isaacs *et al*[148] derived a GRS from 95 blood lipid loci with common genetic variants with confirmed cumulative effects on subclinical atherosclerosis and clinical CAD and MI, but the score did not improve the clinical AUCs in combination with FRS. Similarly, Guella *et al*[149] analyzed a weighted GRS based on the top SNPs in 12 loci in the hemostatic gene pathway and found a 2.69-fold increased risk of early onset MI in subjects in the highest GRS quintile compared with those in the lowest quintile[149]. The predictive value of this weighted GRS has not been studied in a prospective study.

Compared with traditional risk assessment, the advantages of GRS are evident and include the following characteristics: (1) GSR is highly stable over a life time. This information permits the early identification of individuals who are at risk and the implementation of early intervention; (2) Current technology allows the simultaneous measurement of large numbers of genetic variants; (3) The presence of specific genetic risk alleles may provide information regarding targeted preventive intervention; and (4) Most of the SNPs identified by GWAS do not correlate with known TRFs. SNP-based GRS offers complementary information for risk prediction. GRS derived from CAD-associated SNPs provides significant additional predictive power that exceeds TRFs based on both AUC and NRI criteria. Genomic technology has also reduced the cost associated with genotyping a large number of SNPs. It is reasonable to predict that the GRS of CAD will eventually be a component of clinical practice. A number of questions remain to be addressed: (1) The potential difference of predictive values among candidate gene approach identified variants *vs* GWAS variants. Genetic association studies using a candidate gene approach often consist of a small sample size and cannot be replicated in different populations. The minimal criteria for a genetic variant to be included in CVD clinical risk management is recommended, including a meta-analysis based on the data from a minimum of three different independent studies that comprise at least a total of 1000 cases[126]. Potential causal variants for familial CAD and MI (Table 2) are low frequency, high impact variants. The allele frequencies in the general population remain to be determined. The appropriate techniques to incorporate these variants into the GRS remain to be addressed; (2) Protective genetic variants against CAD and MI can potentially attenuate predisposing effects of risk alleles. The number and frequency of protective genetic factors against CAD and MI in the population remain be determined. It will be interesting to evaluate how these protective variants influence the GRS calculation and its predictive power; (3) Gene-gene interactions: The synergistic effects between genetic variants have been reported in association with the risk of CAD[150,151]. Consideration of the combined effects in the GRS model may facilitate risk prediction; and (4) Gene-environmental interactions. The effects on the risk of CAD and MI by certain environmental factors depend on genetics in a "context dependency" fashion. In addition to the overall calculated GRS, information about specific genetic variants may guide personalized preventive intervention. For example, the information obtained for the *ApoEe4* allele is associated with exaggerated CAD and MI risk in tobacco smoker but not in non-smoker.

Table 4 Genetic variants associated with the risk of in-stent restenosis

Chr locations	Gene symbols	Genetic polymorphisms	Effects on risk of ISR	Pathway involved	Ref.
1p36.3	*MTHFR*	C677T	↑ ISR	Metabolism	[188]
1q32.1	*IL10*	G(-2849)A; G(-1082)A; A4259G	↑ ISR	Inflammation	[189]
1p35.1	*CX37*	C1019T	↑ ISR in men	Inflammation	[190,191]
2q14	*IL 1B*	C(-511)T	↑ ISR	Inflammation	[192]
2q14.2	*IL-1RN*	T8006C	↓ ISR	Inflammation	[154]
3p21.3	*GPx-1*	C599T (rs1050450)	↑ ISR	Thrombosis	[193]
		rs8179164 A>T	↑ ISR		[194]
3p24	*KAT2B*	rs6776870 G>C; rs2929404 T>C; rs17796904 T>C; rs4858767 G>C	↑ ISR	Epigenetic/gene expression	[194]
3q24	*AGTR1*	rs5182 T>C	↑ ISR	Vascular homeostasis	[194]
3q24	*P2RY12*	P2Y12 Haplotype H1 (5 P2Y12 ht-SNPs)	↑ ISR	Thrombosis	[195]
3q27	*Adiponectin*	T(+45)G rs2242766	↑ ISR	Inflammation	[196]
4q13	*IL-8*	A(-251)T + C(781)T	↑ ISRS	Inflammation	[197]
4q28	*FGB*	rs1044291 T>C	↑ ISR	Thrombosis	[194]
5q12	*CCNB1*	rs350099 C>T (TT); rs350104 T>C (CC); rs164390 T>G (GG); TT/CC/GG haplotype	↑ ISR ↑↑ ISR	Cell cycling	[198]
5q31.1	*CD14*	C(-260)T	↑ ISR	Inflammation	[199]
5q34	*miRNA-146a*	rs2910164>G (G/C)	↑ ISR	Inflammation	[200]
		rs2910164>G (C/C)	↓ ISR		
6p21.3	*TNFa*	T (-857)C +C(-1031)T	↑ ISR	Inflammation	[201]
6p21.3	*RAGE*	T(-374)A	↓ ISR	Inflammation	[202]
6q25.1	*aER*	PvuII (C/T) > (TT)	↑ ISR in women	Cell cycling	[203]
7q22.1	*PAI-1*	5G/5G	↑ ISR (smoker) ↓ ISR (nonsmoker)	Thrombosis	[204]
7q36.1	*eNOS*	298C/T (p. Glu298Asp)(rs1799983>T); T(-786)C	↑ ISR	Cell proliferation	[193,205,206]
11q22.3	*MMP12*	rs12808148 C>T; rs17099726 G>T	↑ ISR	Matrix deposition	[194]
11q22.2	*IL-18*	G(-137)T	↑ ISR	Inflammation	[207]
11q13.4	*UPC3*	C(-55C)T	↑ ISR	Metabolism	[208]
12p13.1	$p27^{kip1}$	(-838)AA	↓ ISR	Cell cycling	[209]
12q13.11	*VDR*	Block 2 AA haplotype	↑ ISR	Metabolism	[210]
		rs11574027 T>G; rs11574077 G>A	↑ ISR		[194]
13q12	*ALOX5AP*	rs10507391 T>A; rs17216473 G>A	↑ ISR	Lipid metabolism	[211]
		rs17222814G>A	↓ ISR		
17p13.1	*p53*	Arg72Pro	↑ ISR	Cell cycling	[212]
17q23.3	*ACE*	D allele: no 287-bp Alu repeats insertion in intron 16	↑ ISR	Cell cycling	[29-32,213-215]
19p13.2	*ICAM-1*	K469E	↑ ISR	Cell-cell interaction	[216]
21q22.3	*CD18*	C1323T	↓ ISR	Inflammation	[217]
22q13.1	*HO-1*	> 29 TG repeats in promoter	↑ ISR	Oxidative stress	[218]

ISR: In-stent restenosis.

It would be particularly important to advise smoking cessation in *ApoEe4* carriers[126]. Although, the inclusion of *ApoEe4* in the GRS calculation may overestimate the risk for non-smokers.

GENETICS OF IN-STENT RESTENOSIS

Percutaneous coronary intervention (PCI), an effective and safe alternative treatment modality for obstructive CAD, has become one of the most commonly performed therapeutic medical procedures since it was first performed by Grüntzig *et al*[152] in 1977 (http://www.ptca.org/nv/timeline.html. Last accessed on 8/30/3014). Restenosis, which is defined as a renarrowing of the treated vessel area that equals or exceeds 50% of the lumen in the adjacent normal segment, is an entity that is produced with the birth of PCI. The process often results in recurrent symptoms that require repeated intervention. Early experiences in balloon angioplasty revealed a restenosis rate of greater than 50%. The implantation of bare metal stents reduces the restenosis rate to 20% to 30%, mainly *via* the elimination of early elastic recoil and negative remodeling. The development of drug-eluting stents (*i.e.*, silorimus and paclitaxel as prototype-eluting drugs) further reduced the rate to 5% to 15%, as demonstrated in large randomized controlled trials[153]. Despite the advancement of PCI equipment and technology, late luminal loss due to in-stent restenosis (ISR) remains the "Achilles heel" for interventional cardiologists treating CAD.

ISR is a complex disease. Patient factors, such as older age, hypertension, diabetes mellitus and a history of restenosis, increase the risk of ISR[154], whereas tobacco use decreases the risk. Lesion characteristics, such as chronic total occlusion, a small vessel diameter, long lesions, the degree of calcification, ostial/bifurcation

lesions, and restenosis lesions, are associated with an increased risk of restenosis. Procedural-related factors, such as multiple stents, bare metal stents, small diameter and/or long stents, stent fracture, under-expansion, and the presence of edge dissection, increase the risk of restenosis[155]. The pathophysiology of ISR is not fully understood. Compared with balloon angioplasty, stent placement achieves greater acute gain (greater lumen caliber), prevents acute elastic recoil and plaque prolapse, and reduces negative remodeling. However, greater injury to the deeper arterial layers, de-endotheliarization and accumulating layer of platelets and fibrin on the stent surface in association with stent deployment trigger an increased inflammatory response and wound healing process, which includes leukocyte infiltration, cytokine and growth factor release, VSMC activation and proliferation, and matrix production. These processes result in neointimal hyperplasia, the main process of ISR, and neo-atheroma formation during late luminal loss[156]. It is well known that these processes, in particular the cell cycle regulation and inflammatory responses, involve a sophisticated regulatory network consisting of a multitude of proteins. The abundance, modification, and temporal and spatial expression of these proteins are controlled by genetic elements. Genetic factors are hypothesized to influence the risk of ISR for individuals undergoing PCI with stent placement.

Traditional epidemiological genetics has not yet established the heritability of ISR. Candidate gene association analyses using genetic markers, genomic polymorphisms or SNPs have revealed many important associations between genomic variants and the risk of ISR. Table 4 summarizes the published gene polymorphisms associated with the risk of ISR. These genes participate in the regulation of the cell cycle [CCNB1, p27kip1, eNOS, miRNA-146a and p53); inflammation (IL1B, IL1RN, IL8, IL18, TNFα, CD18, CD14, ICAM1 and CX37]; oxidative stress (RAGE, eNOS and HO-1); metabolism/hormonal regulation (ALOX5AP, vitamin D receptor (VDR), α-estrogen receptor (αER), methylenetetrahydrofolate reductase (MTHFR), adiponectin, UPC3, and FBG) and coagulation/thrombosis [factor V leiden, fibrinogen beta chain (FGB), GPx-1, PAI-1, and P2RY12]; epigenetic regulation of gene expression (KAT2B), matrix deposition and degradation (MMP12); and the renin-angiotensin-aldosterone system (RAAS) system (ACE and AGTR1), which is also related to the maintenance of vascular hemostasis (ICAM-1). Although most of the polymorphisms identified in these genes are associated with an increased risk of ISR, some genetic variants were found to have protective effects against ISR, such as the TT genotype in exon 11 of CD18 (ITGB2), the A allele of *ALOX5AP*, the A allele of the $p27^{kip1}$ gene, the AA genotype at position -374 of the RAGE promoter, the CC alleles in miRNA-146a, and allele 2 (C allele) in the *IL-1RN* gene. There are discrepancies in the literature reporting candidate gene-based association analyses and unbiased GWAS studies that involve significantly heterogeneous cases and controls as well as relatively small sample sizes[157]. Further validation and physiological annotation of most of these associations between polymorphisms and the risk of ISR in future studies will be essential.

Discoveries of genetic factors associated with the risk of ISR will not only provide insight regarding the molecular mechanisms underlying the pathogenesis of ISR but also facilitate the development of novel strategies or agents to prevent ISR. More importantly, a complete understanding of genetic risks for ISR will provide clinicians with prognostic information to tailor revascularization strategies, PCI with stenting or coronary artery bypass grafting (CABG). Understandably, patients, in particular younger patients with significant CAD who possess genetic risk factors for ISR, will theoretically benefit more from CABG, and patients carrying protective genetic factors for ISR may benefit from PCI stenting to avoid surgery.

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REVIEW

Population-level differences in revascularization treatment and outcomes among various United States subpopulations

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Abstract

Despite recent general improvements in health care, significant disparities persist in the cardiovascular care of women and racial/ethnic minorities. This is true even when income, education level, and site of care are taken into consideration. Possible explanations for these disparities include socioeconomic considerations, elements of discrimination and racism that affect socioeconomic status, and access to adequate medical care. Coronary revascularization has become the accepted and recommended treatment for myocardial infarction (MI) today and is one of the most common major medical interventions in the United States, with more than 1 million procedures each year. This review discusses recent data on disparities in co-morbidities and presentation symptoms, care and access to medical resources, and outcomes in revascularization as treatment for acute coronary syndrome, looking especially at women and minority populations in the United States. The data show that revascularization is used less in both female and minority patients. We summarize recent data on disparities in co-morbidities and presentation symptoms related to MI; access to care, medical resources, and treatments; and outcomes in women, blacks, and Hispanics. The picture is complicated among the last group by the many Hispanic/Latino subgroups in the United States. Some differences in outcomes are partially explained by presentation symptoms and co-morbidities and external conditions such as local hospital capacity. Of particular note is the striking differential in both presentation co-morbidities and mortality rates seen in women, compared to men, especially in women $\leqslant$ 55 years of age. Surveillance data on other groups in the United States such as American Indians/Alaska Natives and the many Asian subpopulations show disparities in risk factors and co-morbidities, but revascularization as treatment for MI in these populations has not been adequately studied. Significant research is required to understand the extent of disparities in treatment in these subpopulations.

Key words: Revascularization; Myocardial infarction; Cardiovascular; Disparities; Minorities

Core tip: Disparities persist in the care of myocardial infarction (MI) in women and racial/ethnic minorities in the United States. They arrive at the hospital later, present with more risk factors and co-morbidities, and are less likely to receive guideline treatments. Women and blacks are less likely to receive revascularization. Younger women have more in-hospital mortality, and both blacks and women have greater long-term risk for death, recurrent MI, and re-hospitalization. Disparities in risk factors and co-morbidities among Hispanics/Latinos are complicated by the many subgroups. American Indians/Alaska Natives and Asian subpopulations have been much less studied, but surveillance data indicate more risk factors and co-morbidities among these subgroups.

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INTRODUCTION

Despite recent general improvements in health care, significant disparities persist in the cardiovascular care of women and racial/ethnic minorities, even when income, education level, and site of care are taken into consideration[1]. A lack of significant improvements in cardiometabolic risk factors, including hypertension, dyslipidemia, obesity, and cardiorenal metabolic syndrome, combined with increased prevalence of diabetes among blacks, accounts for much of the observed racial differences. Eliminating racial/ethnic disparities alone could prevent an estimated 1.1 million hospitalizations a year[2].

Explanations suggested for the observed continued disparities include socioeconomic considerations and elements of discrimination and racism that affect socioeconomic status and access to adequate medical care.

Socioeconomic status

Patients with acute myocardial infarction (AMI) living in poorer regions were of advanced age and more likely to be non-white and presented with more co-morbidities and were more likely to be smokers. Lower education was associated with more mature age, nonwhite race, more co-morbidity, and lower ejection fraction[3]. In the PREMIER study, lower levels of socio economic status were associated with higher risk of mortality and re-hospitalization in patients hospitalized for AMI. Patients with lower income levels had worse initial overall symptoms and clinical presentation at admission and worse quality of care. Baseline clinical status largely explained the excess mortality but not re-hospitalization[4].

Achieving less than a high school level of education was linked with a 67% increase in one to five-year mortality in women and a 37% increase in men among nearly 16000 Medicare patients admitted for myocardial infarction (MI) from 1991 to 2001, adjusting for a number of clinical factors. Education level was associated with 1- to 5-year MI recurrence in men only[5]. Patients with high financial stress had worse physical and psychological health, worse disease-specific overall quality of life, and more angina 1 year after hospitalization than patients without such stress in a study of 2344 AMI patients discharged in 2003 and 2004. Four-year mortality rates did not differ[6].

Current cardiovascular health disparities

Cardiovascular disease (CVD) health disparities continue to exist among women and minorities. Krieger[7] proposed an "ecosocial" approach to the study of discrimination and health. She posited that inequitable race relations simultaneously benefit the group claiming racial superiority at the expense of those deemed inferior[7]. Minority patients with acute coronary syndrome (ACS) are at greater risk for the full spectrum of cardiac disease including MI, re-hospitalization, and mortality than non-minority patients[8,9]. American Indians/Alaska Natives (AI/AN) have significantly higher rates of obesity, diabetes, CVD, CHD, stroke, and stroke-related death than the general United States population. AI/AN women are particularly at risk[10].

Interventional studies designed to reduce disparities in CVD risk factors and outcomes have included hypertension, hyperlipidemia, tobacco cessation, physical inactivity, and heart failure management. These studies were few, limited by not enough patients, had short follow-up times, and showed only modest clinical gains[11]. Investigators have found "compelling evidence" of disparities in cardiac interventions between whites and blacks. These are not explained by confounding factors such as insurance coverage and disease severity[12].

Table 1 provides a current summary of racial and gender cardiovascular disparities.

REVASCULARIZATION

Revascularization is an accepted treatment for MI, with recommendations available on the use of CABG *vs* PCI, including anatomical considerations[14]. Although ST-segment elevation myocardial infarction (STEMI) incidence decreased between 2001 and 2010, PCI for STEMI increased by 33.5% among patients aged 65 to 79 years and by 22% for those ⩾ 80 years[15].

PCI has continued to be inferior to CABG for anatomical conditions such as left main disease. Despite advances in both procedures, risk for repeat revascu-

Table 1 Summary of current minority disparities related to cardiovascular disease[13]

Total CVD prevalence and total CVD mortality are higher in females than in males
Black males have higher prevalence than white males (44.4% *vs* 36.6%) and higher mortality (369.2/100000 *vs* 278.4/100000)
Black females have higher prevalence than white females (48.9% *vs* 32.4%) and higher mortality (260.5/100000 *vs* 192.2/100000)
Mexican American males have lower prevalence than white males (33.4% *vs* 36.6%)
Mexican American females have lower prevalence than white females (30.7% *vs* 32.4%)
The prevalence of having ≥ 2 risk factors is highest among blacks (48.7%), followed by AI/AN (46.7%), and lowest among Asians (25.9%). The prevalence is similar among men (37.8%) and women (36.4%)
The prevalence of having ≥ 2 risk factors is lower among college graduates (25.9%) than among those with less than a high school diploma (52.5%); a similar disparity in prevalence of risk factors is seen among those making ≥ $50000/yr (28.8%) *vs* those making < $10000/yr (52.5%)
Among older Americans (≥ 65 yr), hypertension is more prevalent in women than in men (57% *vs* 54%) and women have a significantly lower rate of hypertension control
Hypertension increased from 1988 through 2002 in both blacks and whites: From 35.8% to 41.4% in blacks (44.0% among black females) and from 24.3% to 28.1% in whites
Blacks develop hypertension earlier in life and have higher average blood pressures. As a result, blacks have a non-fatal stroke rate 1.3 times that of whites and a fatal stroke rate 1.8 times that of whites. Blacks also have a rate of death attributable to hypertension 1.5 times greater than that of whites and a 4.2-times-higher rate of end-stage kidney disease
Black and Mexican American males have higher mean LDL levels than white males (blacks, 115.9 mg/dL; Mexican Americans, 119.7 mg/dL; whites, 115.1 mg/dL); both black and Mexican American females have lower mean LDL levels than white females (blacks, 114.2 mg/dL; Mexican Americans, 115.0 mg/dL; whites 115.7 mg/dL)
Among men, non-Hispanic blacks (38%) and Mexican Americans (36%) are more likely than non-Hispanic whites (34%) to be obese. Among women, non-Hispanic blacks (54%) and Mexican Americans (45%) are more likely to be obese than non-Hispanic whites (33%)
The prevalence of physician-diagnosed diabetes mellitus in adults > 20 yr is highest in non-Hispanic blacks (12.6%) followed by Hispanics (11.8%), Asian Americans (8.4%), and non-Hispanic whites (7.1%). The prevalence of diagnosed diabetes in adult Asian Indians is more than twice as high (14%) as that in Chinese (6%) or Japanese (5%) Americans. Death rates per 100000 attributable to diabetes mellitus are 23.1 for white males, 43.6 for black males, 15.6 for white females, and 35.1 for black females
The age-adjusted prevalence of diabetes in AI/AN adults aged < 35 yr rose from 8.5% to 17.1% between 1994 and 2004; the rate was higher in females in all age groups

LDL: Low-density lipoprotein; CVD: Cardiovascular disease; AI/AN: American Indians/Alaska Natives.

larization still appears to be higher with PCI[16]. Mohr *et al*[17] found no significant differences between CABG and PCI in all-cause death or stroke, but patients with intermediate or high SYNTAX scores treated with PCI had more serious adverse cardiac and cerebrovascular events at 5-year follow-up. CABG, compared to PCI, offered significant protection from long-term mortality[18], but PCI offers advantages in accessibility. A recent expert consensus found no difference in either in-hospital or 30-d mortality with primary PCI between sites with and without on-site surgical backup[19].

Coronary revascularization has become one of the most common major medical interventions in the United States, with over 1 million procedures yearly[20]. Even so, revascularization is used less in both female and minority patients[21,22]. Local hospital capacity helps to explain the revascularization disparities between black and white AMI patients[23].

This review presents recent data on disparities in co-morbidities and presentation symptoms, care and access to medical resources, and outcomes in revascularization as treatment for ACS.

WOMEN

Co-morbidities and presentation symptoms

Among 6746 STEMI patients undergoing primary PCI, stratified by age (< 65 years, ≥ 65 years), hypertension was higher in both groups of women than in men, and younger women had a higher likelihood of being current smokers. Older women also had more diabetes than men[24]. In a cohort that included 15120 women, women were less likely than men to be taking cardioprotective medications in the first year after their diabetes diagnosis[25]. The impact of this lack of adherence to medications on cardiovascular disparities is difficult to estimate, but crude mortality in MI patients has been found to be highest in those with diabetes[26].

In the Variation in Recovery: Role of Gender on Outcomes of Young AMI Patients (VIRGO) study, younger female AMI patients (18 to 55 years) had worse pre-event health than men, including more diabetes, dyslipidemia, and obesity. They also had significantly more angina, stroke, and congestive heart failure, worse physical function, and poorer quality of life than male AMI patients in the same age group[27,28]. Similar results were found in a cohort of younger AMI patients (≤ 55 years) from the Translational Research Investigating Underlying disparities in Acute Myocardial infarction Patients' Health Status (TRIUMPH) study[29,30]. Lower resting metabolic rates in black women may contribute to the higher levels of obesity seen in black women compared to white women[31].

Between 1997 and 2009, awareness of CVD as the leading cause of death in women increased significantly, but black and Hispanic women still had significantly less awareness than white women. Only 53% of the women interviewed would call 9-1-1 if they thought they had symptoms of a heart attack[32]. Hispanic women were also less likely to know the symptoms of a heart attack and more likely to underestimate their weight[33].

Chest pain is critical in the decision to initiate dia-

gnostic testing for ACS upon presentation, yet up to 35% of AMI patients do not report chest pain, which can lead to misdiagnosis and a higher risk of death. Female ACS patients aged 55 years or younger had a higher probability of presenting without chest pain and with NSTEMI. This was not associated with markers of coronary disease severity[34,35]. However, women aged 65 and older were actually less likely to present without chest pain than similarly aged men[36]. African American women were more likely to present with stomach associated symptoms and less chest related signs than white women and had significantly greater all-cause and cardiovascular mortality[37].

Women with STEMI have increased left ventricular filling pressures during acute STEMI *vs* men, independent of age, high blood pressure, and size of the infract. This suggests that pulmonary capillary wedge pressure may mediate the effect of sex on outcomes post-STEMI[38].

Seeking better risk estimates for women, Cook *et al*[39] compared the Adult Treatment Panel Ⅲ (ATP-Ⅲ) score, the Framingham risk score and the Reynolds Risk Score CVD model. The ATP-Ⅲ overestimated the risk for coronary heart disease and the Framingham CVD model overestimated the risk for major CVD. After recalibration, the Reynolds Risk Score was better calibrated for both black and white women than either of the Framingham-based models[39]. A high-sensitivity troponin assay that incorporated diagnostic thresholds that were specific for women and men increased the ability to diagnose MI in women compared to a single-threshold contemporary assay, but this was not as effective in men. Women with MI identified by the high-sensitivity assay or by both assays had the highest risk for death or recurrent MI at 12 mo[40]. Independent predictors of obstructive CAD in women with chest pain and an abnormal stress test included body mass index (BMI) < 30 kg/m^2, a history of smoking, low high-density lipoprotein (HDL), a significant family history of early heart disease, age ⩾ 55 years, lateral abnormality on stress imaging, and exercise capacity < 5 metabolic equivalents. the risk score had a negative predictive value of 80%[41].

Care/medical resources

Disparities in care for AMI among women fall largely into three categories: The likelihood of hospitalization, the time to hospital or to guideline treatment from onset of symptoms, and the administered treatments themselves.

Between 1992 and 2010, rates of hospitalization for AMI per 10000 Medicare enrollees were significantly lower in both black and white women, persisting as hospitalization rates for AMI in general declined. Rates of PCI within 30 d of AMI continued to be significantly lower in both black and white women. Mortality differences by race declined, but remained higher in women[42].

Between 1960 and 2008, women had consistently longer prehospital delay from symptom onset (median 1.8 to 7.2 h *vs* 1.4 to 3.5 h in men). The characteristics associated with delay in females included being older, not being married, having a previous history of MI, being alone during symptom onset, and not wanting to bother anyone[43]. The time from the first appearance of symptoms-to-balloon time was also significantly longer in women than in men, largely driven by later presentation to the hospital. Women were more often treated with just medical management and were more likely to receive medications such as a diuretic or warfarin on discharge, whereas men more often received b-blockers and statins. Compared with men, women had significantly higher levels of major adverse cardiovascular events; major bleeding; death; and target vessel revascularization for ischemia in-hospital and at 30 d. In women, higher rates at these end points persisted at 3 years[44]. At hospital arrival, female STEMI patients had delays in both door-to-code and code-to-balloon times. Independent determinants of delays in door-to-balloon times included female sex, hypertension, maximum ST-elevation, office hours, and triage category[45].

Among ACS patients aged 18 to 55 years, women had significantly less income; more diabetes mellitus, hypertension, family history of CVD, and previous CVD events; and more depression and anxiety before symptom onset. Females were less likely to have a diagnosis of STEMI and more likely to have a diagnosis of unstable angina. Women were less likely to receive ECG or fibrinolytic therapy within established time benchmarks but did not differ from men in timely PCI. Females with STEMI were less likely to have reperfusion therapy than males, and females with NSTEMI were less likely to have PCI, although the proportions of male and female patients with NSTEMI who had cardiac catheterization were similar. The determinants of poorer access to care included anxiety, more risk factors, and lack of chest pain at presentation[46].

STEMI patients aged ⩽ 45 years generally had more non-traditional cardiovascular profiles and had lower in-hospital mortality, but younger women had significantly poorer quality of care, with longer delay in door-to-thrombolytic time, and higher in-hospital mortality rates than younger men[47]. In patients receiving care for CVD in Veterans Health Administration facilities in 2010 and 2011, women had higher mean LDL cholesterol levels than men but were significantly less likely to receive statin treatment according to recent cholesterol guidelines[48]. Between 2008 and 2011, patients hospitalized for non-ACS indications who had in-patient STEMI were more likely to be older and female and less likely to undergo cardiac catheterization or PCI. These patients had more than 3-fold greater in-hospital mortality[49].

At 30 d after discharge, no difference was found by race/ethnicity since inception of the Medicare Part D

prescription drug benefit in usage of statins, β-blockers or ACE inhibitors but women were less likely than men to be using β-blockers and angiotensin-converting enzyme inhibitors. At 12 mo, black and Hispanic women were the least likely to be adherent, followed by white, Asian, and other women and by black and Hispanic men[50].

Golden *et al*[51] looked at cardiovascular testing after evaluation for chest pain. This analysis focused on the physician-patient discussions and how these affect patient decisions around cardiovascular testing. The primary outcomes were sex differences in recommendations for testing. Physicians were less likely to tell women their symptoms could result from heart disease or to recommend cardiovascular testing or cardiac catheterization. No patients in this study did not follow the recommendations of their doctors[51].

Hormone replacement and combined hormone contraception

A recent analysis of data from the large Women's Health Initiative, including 13 years of follow-up, found the risk for both CHD and stroke to be higher with combined estrogen plus progesterone in all age groups, but risk for MI was slightly reduced in the 50 to 59 year age group. Among women with previous hysterectomy taking estrogen alone, women aged 50 to 59 years had slightly less risk for CHD and MI, but not older women taking estrogen alone and the risk for stroke and venous thrombosis was higher. These findings do not support the use of hormone therapy, although it might be a reasonable option to manage menopause symptoms during early menopause[52]. A more recent Cochrane review of 19 trials largely confirmed these results[53].

The potential added risk of MI associated with combined hormonal contraceptives has also been controversial. Most women who take oral contraceptives take a combined estrogen-progestin preparation and today's contraceptives have much smaller doses of estrogen than earlier versions. In a very large Danish cohort, women taking doses of 30 to 40 μg ethinyl estradiol had a roughly 2-fold risk of both thrombotic stroke and MI, but risk varied with the type of progestin. Those taking 20 μg ethinyl estradiol had approximately a 1.5-fold risk of both thrombotic stroke and MI with all types of progestin except drospirenone, which offered no excess risk[54]. In a more recent but much smaller study in Turkey that also examined MI/PCI sequelae, women taking a contraceptive containing 30 μg ethinyl estradiol combined with drospirenone had increased risk for STEMI. Following PCI, patients had increased thrombus burden, were less likely to have complete ST resolution, and were more likely to develop congestive heart failure than women not taking the contraceptive. Confounding factors in this study included the small number of oral contraceptive users and the fact that women taking contraceptives were more likely to be smokers, suggesting that oral contraceptives should not be prescribed to smokers, especially if aged more than 35 years[55]. It has been noted that taking combined hormonal contraceptives is safer than pregnancy and delivery[56].

Outcomes

Age-adjusted CVD mortality rates in the United States from 1980 through 2002 declined more in men than women (52% among men and by 49% among women). However, between 2000 and 2002, the mortality rate in women aged 35 to 54 years increased by 1.5% despite declining in this group during the earlier study years. By contrast, in both men and women aged ⩾ 55 years, declines in mortality rate accelerated between 2000 and 2002[57].

In a decade long prospective study of STEMI patients undergoing primary PCI, hypertension was more prevalent in both younger (< 65 years) and older (⩾ 65 years) women than in men of the same age. Younger women were more likely to smoke, have less obstructive CAD, and to have a family history of CVD than younger men, whereas older women had more diabetes than older men but were less likely to smoke. Overall mortality was greater in women, but younger women had more risk of mortality at 30 d and at 1 year than men of the same age, whereas older women had significantly increased risk of mortality only at 30 d, not at 1 year[24].

In-hospital mortality was higher in women for both STEMI and NSTEMI but more so for STEMI patients. However, younger women actually were the main drivers in this difference in mortality. Among NSTEMI patients, in-hospital mortality rate differences reversed among women ⩾ 70 years, who had better in-hospital survival than men of the same age[58]. Similarly, women were older than men for both STEMI and NSTEMI diagnoses and were less likely to be treated with PCI or CABG for either. Female STEMI patients had more in-hospital mortality than men of similar ages in all age categories except 80 to 89 years. Female NSTEMI patients had higher rates of in-hospital mortality than men of the same age through age 69 years, but women ⩾ 70 years had better survival than men of the same age[59].

AMI hospitalization rates did not decline in either sex in United States patients aged 30 to 54 years between 2001 and 2010, but women had more co-morbidities, longer hospital stays, and more in-hospital mortality than men across all ages. In-hospital mortality declined significantly for women but not for men[60]. In adults ⩾ 20 years of age hospitalized for AMI, younger women had a higher rate of renal disease, diabetes, systolic heart failure, and malignancies than similarly aged men. Women ⩽ 55 years experienced a significant increase in AMI rates, which did not occur in men in this age group. Women also had higher 30-d mortality rates than men, although this declined in both sexes

over the 10 years of the study. Women 20-55 had 45% higher odds of 30-d mortality than men of the same age, which persisted over the study. Only women ⩾ 75 years of age had borderline better mortality rates than men[61]. In a systematic review of between-sex AMI mortality, unadjusted mortality was higher in women at both 5 and 10 years. Sex differences in long-term mortality after AMI were largely explained by differences in age, co-morbidities, and differential treatment usage by women compared with men[62].

An analysis of outcomes by sex and long-term outcomes by sex and type of stent found that women had more in-hospital complications, including mortality, MI, bleeding, and vascular complications. At 30 mo, women had a slightly lower adjusted risk for death, but there weren't any significant sex-related differences in adjusted rates of MI, bleeding, or revascularization. Males and females benefited similarly from the use of drug-eluting stents[63]. The most significant predictors of re-hospitalization for ACSs within 1 year were CABG prior to hospitalization for the AMI, female sex, and in-hospital PCI. No difference was found in risk of ACS re-hospitalization by type of stent, but the strongest predictors of revascularization were multi-vessel disease and in hospital PCI with a bare metal stent[64].

Significant pre-PCI predictors of 30-d re-admission comprised gender, age, Medicare or other government insurance, a history of heart failure and kidney disease. Predictors after PCI included not receiving a prescription for b-blocker upon discharge, vascular complications, and prolonged length of stay[65].

At age 45, the risk of death increased more significantly in white men than black men. White men had six times the increased risk of death compared to white women, whereas black males had only twice the increased risk of fatal CHD compared to black women. The risk of mortality between sexes equalized by age 95 in both blacks and whites. Adjustments for CHD risk factors did not explain this disparity between races in gender difference in CHD mortality[66].

Sex differences in perceived stress could be a central explanation for gender based differences in post-AMI recovery. Women had significantly higher baseline stress, mostly explained by co-morbidities, state of physical and mental health, intra-family conflicts, caregiving demands, and financial hardship. Higher stress was associated with worse female recovery at 1 mo post-AMI in angina, overall quality of life, and mental health[67]. A study to distinguish the effects of gender role *vs* biological sex on quality of life after ACS found that at baseline and at 1, 6, and 12 mo, women had clinically significant lower Health Related Quality of Life scores than men. Social support and gender-related variables such as housework responsibility were statistically significant predictors of physical limitation, angina frequency, and disease perception, but biological sex predicted only physical limitation[68].

The status of CVD in women internationally

Women in other countries with health care systems vastly different from that of the United States have been found to experience similar disparities. Recent studies in Spain, China, Germany, Vietnam, and Italy found equivalent differences in presentation symptoms and co-morbidities, access to treatments, and outcomes compared to men[69-73].

Table 2 summarizes disparities in women in presentation, treatment, and outcomes of acute coronary syndrome.

BLACKS

Co-morbidities and presentation symptoms

Differences between blacks and whites at presentation for ACS often fall into three categories: Demographic factors such as income and education, risk factors and co-morbidities, and symptoms.

A constellation of cardiometabolic risk factors, including high blood pressure, high cholesterol, obesity, diabetes mellitus, and chronic kidney disease, coupled with physical inactivity, smoking, and poor eating habits, is more prevalent in blacks and contributes to CVD disparities[2].

Black patients with NSTE ACS in the CRUSADE quality improvement initiative, from 2002 to 2003, were younger; had a higher prevalence of hypertension, diabetes mellitus, congestive heart failure, kidney failure and history of stroke than whites. African American patients had a lower rate of private insurance or primary cardiology care and more likely to be uninsured. Blacks were less likely to be prescribed clopidogrel and GP IIb/IIIa inhibitors or to have diagnostic cardiac catheterization or PCI than white patients. High-risk African Americans had a lower incidence of CABG than high-risk white patients[74].

African Americans in the PREMIER study[75] were more likely than white patients to have Medicaid, no education beyond high school, household income less than $10000, and a prior incidence of heart failure. Comparing rates of hypertension, hypercholesterolemia, diabetes, obesity, and current smoking, more blacks than whites had hypertension and diabetes within each age-sex group. Black men ⩾ 55 years were more likely to smoke, but no differences were observed in the group < 55 years of age. The prevalence of multiple cardiac risk factors was significantly higher for blacks, particularly black women, with 60% of older (⩾ 55 years) and 54% of younger (< 55 years) black women having three or more risk factors[76].

Black patients with confirmed ACS upon presentation were significantly younger and had less education and lower incomes, significantly longer prehospital delays, more hypertension, higher rates of diabetes, higher BMI, and reported more current tobacco use than whites. They were more likely to experience palpitations, chest pressure, and chest pain, and to

Table 2 Summary of disparities in acute myocardial infarction co-morbidities and presentation symptoms, care and access to medical resources, and outcomes in women

Co-morbidities and presentation symptoms
More hypertension and diabetes than men[24]
More diabetes, dyslipidemia, obesity, angina, stroke, and congestive heart failure; worse physical function; and poorer quality of life than men[28]
More hypertension, diabetes, lung disease, depression, and angina; worse general health scores; poorer physical function; and worse quality of life than men[30]
Women ≤ 55 yr of age more likely than men to present without chest pain or with NSTEMI[35]
Women < 45 yr of age more likely than men to present without chest pain, but this reversed with age[36]
Risk less likely to be accurately assessed by standard models or assays[39,40]
More likely than men to be older and have hypertension, hyperlipidemia, and congestive heart failure and less likely to have previous history of MI or revascularization[44]
Women ≤ 55 yr of age more likely to have low income, more diabetes, more hypertension, more family history of CVD, more previous CVD events, and more depression and anxiety; less likely to have diagnosis of STEMI and more likely to have NSTEMI or unstable angina[46]
Higher baseline stress than men[67]
Care/medical resources
Lower rates of hospitalization for AMI and lower rates of PCI as treatment for AMI compared to men[42]
Longer pre-hospital delay from onset of symptoms compared to men[43]
Longer symptom-onset-to-balloon time than men and more likely to be treated with medical management only; less likely to receive b-blockers and statins on discharge[44]
Greater delays than men in both door-to-code and door-to-balloon times[45]
Less likely than men to receive ECG or fibrinolytic therapy within guideline times, to have reperfusion therapy with STEMI, or to have PCI with NSTEMI[46]
Longer door-to-thrombolytic time than men[47]
Less likely than men to have statin treatment for high cholesterol[48]
Women with in-hospital STEMI less likely to have cardiac catheterization or PCI than men[49]
Less likely than men to be using ACE inhibitors, angiotensin receptor blockers, and β-blockers 30 d after discharge[50]
Less likely than men to be told their symptoms could be related to heart disease or to have cardiovascular testing or cardiac catheterization recommended[51]
Less likely than men to be treated with either primary PCI or CABG[73]
Outcomes
Greater mortality than men at 30 d and at 1 yr in women < 65 yr, but only at 30 d in women ≥ 65 yr[24]
Greater in-hospital mortality than men for both STEMI and NSTEMI in women ≤ 69 yr[58]
Greater in-hospital mortality than men for STEMI in women < 80 yr, and greater in-hospital mortality than men for NSTEMI in women ≤ 69 yr[59]
More in-hospital mortality for AMI than men[60]
Higher 30-d mortality rates for AMI than men up to age 75 yr[61]
Higher post-AMI mortality rates than men at both 5 and 10 yr[62]
More in-hospital complications than men, including mortality, MI, bleeding, and vascular complications[63]
More likely than men to be re-hospitalized for ACS within 1 yr[64]
Worse recovery than men at 1 mo post-AMI in angina, overall quality of life, and mental health[67]
Clinically significant lower health-related quality of life scores than men at 1, 6, and 12 mo following ACS event[68]
Higher re-hospitalization rates and lower quality of life than men at 6 mo after AMI[69]
Greater risk of 1-yr re-hospitalization for AMI and higher 1-yr mortality than men[93]

AMI: Acute myocardial infarction; CVD: Cardiovascular disease; STEMI: ST-segment elevation myocardial infarction; CABG: Coronary arterybypass grafting; PCI: Percutaneous coronary intervention; ECG: Electrocardiogram; ACE: Angiotensin converting enzymes; ACS: Acute coronary syndromes.

report more severe symptoms than whites. A higher percentage of black patients received lidocaine, but there was no other significant treatment difference. At 1 mo follow-up, blacks reported significantly more symptoms and more clinic visits than whites. Blacks continued to have more symptoms at 6 mo, but health service usage no longer differed[77].

Care/medical resources

Disparities in clinical care for blacks with AMI fall largely into two categories: The time to hospital or to guideline treatment from onset of symptoms, and the administered treatments themselves.

Black patients undergoing PCI were more apt to be younger and female and to have more hypertension and other chronic illnesses including prior MI, history of gastrointestinal bleeding, and worse baseline hemoglobin. They were also more likely to be on Medicaid or to be uninsured. No differences were found in treatment for STEMI between whites and blacks, but more black patients than white patients had PCI for NSTEMI and more white patients had emergent CABG. No difference was seen in in-hospital mortality rates between whites and blacks. In a propensity-matched subcohort of African American and white patients, blacks were not as likely to receive prasugrel or drug-eluting stents[78].

Time from first arrival to first drug was longest for blacks, but was actually significantly longer for all minority patients than for whites. Door-to-balloon times were significantly longer for blacks, Hispanics, and Asian/Pacific Islanders than for whites. The differences remained significant when controlling for specific hospitals[79]. Cavender *et al*[80] found insignificant differences in door-to-balloon times when comparing white, black, and Hispanics and similar in-hospital mortality rates between groups. However, after controlling for the usual confounding factors, black race was

associated with less likelihood of door-to-balloon time under 90 min (a quality-of-care indicator for treatment of STEMI) compared to white race[80]. Among Medicare beneficiaries with AMI admitted to hospitals without revascularization facilities in 2006, black patients were transferred to a PCI ready hospital more slowly than whites (median 1 d for whites, 2 d for blacks), but the risk-standardized mortality rate in the revascularization hospitals did not differ between races[81].

In a study of hospitalizations for ischemic heart disease in Massachusetts in the pre- and post-health care reform periods (November 2004 to July 2006, and December 2006 to September 2008), blacks had 30% less likelihood of receiving revascularization than whites in pre-health reform Massachusetts. This disparity has persisted post-reform, as have somewhat smaller disparities in Hispanics. Asians were slightly more likely to receive revascularization than whites. Patients living in more educated communities, men, and patients with private insurance were more likely to have revascularization treatment both before and after reform. The adjusted odds of in-hospital mortality were higher in the post-reform period than in the pre-reform period, but no differences were observed in 1-year mortality by race/ethnicity, education level, or sex[82].

Blacks were the least likely to be treated with revascularization in an analysis of data from 12555 patients admitted with AMI in New York city in 1996. Whites were older and more likely to have congestive heart failure. Hispanics were more likely to survive than whites, but blacks and whites did not differ significantly in survival. Non-revascularized blacks and Hispanics were more likely to be discharged alive than non-revascularized whites[83].

In an analysis of data from AMI patients with Medicare, private insurance, as well as those were uninsured or on Medicaid, in nine states (from 2000 to 2005). Blacks and Hispanics were significantly less likely than whites to be revascularized, regardless of insurance status. After adjusting for demographics, co-morbidities, and hospital clustering, blacks were approximately 25% less likely than whites with similar insurance to be treated with revascularization and Hispanics about 5% less likely[84].

The impact of hospital and physician effects on disparities in revascularization treatment was examined in 119386 initial episode AMI patients, aged ⩾ 65 years, and all fee-for-service Medicare recipients in Florida (from 1997 to 2005). Black and Hispanic patients were younger, more likely to be female, and more likely to have diabetes mellitus than white patients. The unadjusted rates of intervention were significantly higher in whites than in either blacks or Hispanics. Black-white disparities for these procedures persisted despite adjustment for age, gender, co-morbidities, socioeconomic status, and hospital characteristics. Hispanic-white disparities held for catheterization and PTCA, but were no longer significant for CABG. Hospital fixed effects were found to not be the full reason behind disparities in cardiac treatment. Physician fixed effects accounted for some disparities in treatment and entirely explained Hispanic treatment differences[85]. Li *et al*[23] assessed data for AMI patients in Pennsylvania from 1995 to 2006 and found African Americans were significantly less likely to be treated with either CABG or PCI within 3 mo of AMI. The PCI rate disparity was more in counties with the lowest AMI hospital capacity[23].

Improvements in process-of-care quality measures were assessed using more than 2 million AMI hospitalizations in 2005 or 2010. Despite significant narrowing of the racial/ethnic gap in performance rates among United States hospitals on these quality measures, the gap in PCI rates between blacks and whites in 2010 remained three times the size of the gap in PCI rates between Hispanics and whites[86].

Outcomes

Black Medicare beneficiaries, aged ⩾ 68 years, admitted with AMI from 2000 to 2005 to non-revascularization hospitals, were significantly less likely to be transferred to a hospital with PCI facilities or to receive revascularization, and had significantly higher 1-year mortality than white patients. After adjustment, disparities between transfer and revascularization rates remained significant. Black patients had lower mortality at 30 d, but significantly higher mortality thereafter, regardless of hospital type[87].

Black patients undergoing PCI had significantly more cardiovascular co-morbidity and had a higher likelihood of presenting with an AMI. At 6 mo, patients of both races had equivalent survival. However, at 5 years, blacks had significantly higher incidence of AMI, congestive heart failure, and mortality than white patients[88]. Nonwhite patients in the TACTICS-TIMI 18 randomized trial had a higher probability of death, MI, or re-hospitalization after adjustment for medical characteristics. Rates of protocol-guided angiography and revascularization were similar in white and non-white ACS patients, but nonwhite patients were significantly less likely to take their cardiac medications, to undergo non-protocol mandated angiography, and to receive a stent if undergoing PCI. They also had less procedural success with PCI. Nonwhite patients had significantly worse prognosis than white patients after adjustment for baseline characteristics[89]. In the BARI 2D trial Black, white, and Hispanic patients with diabetes treated similarly showed similar risk for death or risk for death, MI, or stroke at 5 years and in all, better risk factor control was associated with higher 5-year survival[90].

After adjusting risk in Medicare recipients aged ⩾ 65 years who had CABG performed in 2007 and 2008, using patient characteristics, socioeconomic status, and relative hospital quality, nonwhite patients had a 34% higher risk of death following CABG. Hospitals treating the largest proportion of nonwhite patients

had the highest risk-adjusted mortality for both white and nonwhite patients and hospitals treating the smallest proportion of nonwhite patients had the lowest mortality for both white and nonwhite patients[91]. In a retrospective study of revascularization outcomes in patients with left main CAD, black race and age were the only two independent predictors of adverse cardiac outcomes at 1 year following revascularization[92].

In a national sample of more than 2 million Medicare patients hospitalized for AMI from 1999 to 2010, the incidence of an index AMI declined from 1283 per 100000 person-years in 1999 to 830 in 2010, but the percentage of nonwhite patients increased from 11.0% to 12.7%. The risk of 1-year re-hospitalization for AMI declined in both white and black patients, but the decline was larger in whites (27.7%) than in blacks (13.6%), so that at the end of the study period, the discrepancy between whites and blacks actually increased. All-cause 1-year mortality declined in both sexes and both races between 1999 and 2010, but females had consistently higher 1-year mortality rates than males[93].

In the Family Cardiac Caregiver Investigation To Evaluate Outcomes (FIT-O) study[94], blacks and Hispanics were less likely to report statin use before admission, but statin use after discharge was not significantly different. Patients with a statin prescription at discharge were significantly less likely to be dead or readmitted at 30 d, independent of demographic characteristics or co-morbidity. At 1 year, blacks and Hispanics were 23% more likely than white/Asian patients to be dead or readmitted. This was not associated with statin prescription before or after hospitalization. After adjusting for co-morbidities, the investigators found that race/ethnicity did not predict death or re-hospitalization at 1 year, but age > 65 years, having a caregiver, and lacking health insurance remained significant predictors[95].

O'Neal *et al*[96] found that median survival for black CABG patients with pre-operative β-blockers was 14 years, compared with 11 years for black patients who did not have pre-operative β-blockers. White patients who had pre-operative β-blockers had median survival of 15 years *vs* 13 years for those without pre-operative β-blockers. Despite the absolute difference in median survival, the magnitude of the drugs' effect on survival was statistically similar for black and white patients[96].

Table 3 summarizes disparities in blacks in presentation, treatment, and outcomes of acute coronary syndrome.

HISPANICS

Co-morbidities and presentation symptoms

One challenge in preventing CVD in United States Hispanics is understanding the diversity within that community as it is not at all a homozygous population.

The population's genetics, exposures and related cultural experiences have tremendous variation[97]. Some recent studies that include results for Hispanics have already been presented in the sections on women and blacks.

The Hispanic Community Health Study/Study of Latinos includes individuals from a number of Hispanic backgrounds. The overall rate of high cholesterol was 52% among men (range: 48% in Dominicans and Puerto Ricans to 55% in Central Americans) and 37% in women (range: 31% in South Americans to 41% in Puerto Ricans). About 37% of men were obese (range: 27% in South Americans to 41% in Puerto Ricans) and 43% of women were obese (highest among Puerto Ricans). Approximately 26% of men actually smoking currently (highest in Puerto Ricans) and 15% of women were current smokers (21% in Cuban women, 32% in Puerto Rican women). Puerto Ricans had the highest rates of obesity and current smoking. Central American men and Puerto Rican women had the highest hypercholesterolemia. The presence of having ⩾ 3 risk factors was highest among Puerto Ricans and those who either were United States born or had lived in the United States for 10 or more years[98].

A higher level of education was linked with a higher probability of high blood pressure and large waist measurement in both male and female Mexican Americans born in the United States and those born abroad. The odds of diabetes increased with education among United States-born Mexican American women. Foreign-born Mexican American women who had lived in the United States for 5 to 19 years had the highest risk of diabetes. The odds of having hypertension were 26% lower among Mexican-born men residing in the United States under 5 years, 39% lower for Mexican-born men in the United States 5 to 19 years compared with Mexican-born men in the United States for ⩾ 20 years. Foreign-born males who had lived in the United States for fewer than 5 years had the least probability of diabetes and a large waist circumference[99].

Data from both the National Health and Nutrition Examination Study and the earlier Hispanic Health and Nutrition Examination Study looking at first- and second-generation Mexican Americans showed that first- and second-generation men did not differ in diabetes incidence, cholesterol levels and framingham risk score (FRS). Smoking levels were lower in second-generation men; they also had lower HDL cholesterol levels, and the degree of hypertension was higher than first-generation men. Neither FRS nor diabetes rates differed between first- and second-generation women. The levels of HDL cholesterol were higher in second-generation women and the rates of smoking and total cholesterol was also lower though rates of hypertension were higher than first-generation women[100].

Hispanic patients were more likely to be younger and have diabetes, and less likely to have previous MI or prior revascularization in a comparison to white STEMI patients enrolled in the Get with the Guidelines Registry. Hispanics had a higher probability of being uninsured. Hispanic patients experienced noteworthy time delays in

Table 3 Summary of disparities in acute myocardial infarction co-morbidities and presentation symptoms, care and access to medical resources, and outcomes in blacks

Co-morbidities and presentation symptoms
More likely than whites to have dyslipidemia, hypertension, obesity, insulin resistance, hyperglycemia, diabetes, and chronic kidney disease and to be physically inactive, smoke, and have poor eating habits[2]
More likely than whites to be younger and female and to have hypertension, diabetes, congestive heart failure, renal insufficiency, and history of smoking and stroke; less likely to have private insurance or cardiology care and to be uninsured[74]
More likely than whites to have Medicaid as insurer; to have no education beyond high school; to have low income; and to have a history of congestive heart failure, hypertension, and diabetes[76]
Likely to be younger and to have less education than whites; to have more hypertension, diabetes, higher BMI, and more current tobacco use; also more likely to experience palpitations, chest pressure, and chest pain[77]
More likely than whites to be younger and female and to have more hypertension, diabetes, renal insufficiency, history of smoking, congestive heart failure, previous MI, history of gastrointestinal bleeding, and lower baseline hemoglobin; also more likely to be on Medicaid or uninsured[78]
Care/medical resources
Less likely to be treated with either PCI or CABG within 3 mo of AMI than whites[23]
Longer door-to-drug and door-to-balloon times than for whites[79]
Less likely than whites to have door-to-balloon times < 90 min[80]
Likely to be transferred to a revascularization hospital more slowly than whites[81]
Less likely than whites to receive revascularization treatment[82]
Less likely than whites or Hispanics to receive revascularization treatment[83]
Less likely to be treated with revascularization than whites regardless of insurance status[84]
Less likely than whites to receive cardiac catheterization, PTCA, or CABG[85]
Persistently lower PCI rates in blacks compared to whites[86]
Less likely than whites to be transferred to a hospital with revascularization services or to be revascularized[87]
Less likely than whites to take their cardiac medications, to undergo non–protocol mandated angiography, or to receive a stent if undergoing PCI; less procedural success with PCI[89]
Outcomes
More likely to be discharged alive when not treated with revascularization than whites not receiving revascularization[83]
Lower mortality than in whites at 30 d post-AMI but higher thereafter[87]
Higher rates of recurrent AMI, congestive heart failure, and mortality than whites at 5 yr post-PCI[88]
Higher risk of death, recurrent MI, or re-hospitalization than whites[89]
Higher risk of death than whites following CABG[91]
More likely than whites to have adverse cardiac outcomes at 1 yr post-revascularization[92]
Consistently more likely than whites to have AMI re-hospitalization at 1 yr[93]
Both with and without pre-operative β-blockers, shorter median survival times with CABG than white patients[96]

PTCA: Percutaneous transluminal coronary angioplasty; AMI: Acute myocardial infarction; BMI: Body mass index; CABG: Coronary arterybypass grafting; PCI: Percutaneous coronary intervention.

triage and subsequent related reperfusion, but the use of acute medications and primary PCI was the same in the two groups. Mean in-hospital stay was longer for Hispanics, but in-hospital mortality did not differ significantly. Hispanic patients had less evidence-based discharge care. Despite these disparities, Hispanics had clinical outcomes that did not differ significantly from those of non-Hispanic whites[101].

In STEMI patients who received PCI between 2004 and 2007, patients receiving a bare metal stent (BMS) were more likely to be Hispanic and uninsured; had higher rates of surgical or PCI revascularization, peripheral vascular disease, and diabetes; and had significantly longer hospital stays and a trend toward higher all-cause mortality. Hispanic ethnicity was not an independent predictor of BMS use[102].

Among Mexicans, Hispanics, and non-Hispanic whites presenting with NSTE ACS, Mexicans were younger; had less hypertension, hyperlipidemia, renal failure, and prior revascularization; and were more likely to smoke than Hispanic and non-Hispanic white patients. Mexicans and Hispanics had a significantly higher incidence of diabetes. Acute medication use was similar in all three groups, but Mexican patients were less likely to have revascularization. Mortality was similar in all three groups[103].

Care/medical resources

The literature on disparities in AMI treatment for Hispanics is sparse. Some pertinent data were presented in the previous section, and we here briefly reiterate specific results for Hispanics from studies already cited in earlier sections. These disparities are primarily in the time to treatment and the specific treatments Hispanics received.

Door-to-drug and door-to-balloon times were significantly longer for Hispanic patients than for white patients when receiving primary PCI for STEMI; some of this disparity was explained by the hospitals in which Hispanics were treated[79]. Cavender *et al*[80] found that median door-to-balloon time was marginally longer for Hispanics than for whites. Hispanic ethnicity was not associated with lower odds of door-to-balloon times ⩽ 90 min. There was no association between race/ethnicity and in-hospital mortality.

Hispanics were not as likely as non-Hispanic whites to get in-hospital revascularization regardless of insurance (Medicare, private insurance, uninsured/

Table 4 Summary of disparities in acute myocardial infarction co-morbidities and presentation symptoms, care and access to medical resources, and outcomes in Hispanics

Co-morbidities and presentation symptoms
More likely than non-Hispanic whites to have hypertension, diabetes, and renal failure and to lack health insurance[95]
More likely than non-Hispanic whites to be younger and to have diabetes, but less likely to have previous MI or prior revascularization[101]
More likely than non-Hispanic whites to have diabetes[103]
Care/medical resources
Longer door-to-drug and door-to-balloon times than for whites[79]
Longer door-to-drug and door-to-balloon times than for whites[79]
Less likely than whites to receive catheterization or PTCA[104]
Outcomes
Hispanic patients with diabetes somewhat less likely at 5 yr to be dead, have MI, or have stroke than white patients with diabetes[90]
More likely to be dead or re-hospitalized at 1 yr than non-Hispanic whites[95]
In-hospital mortality increases with age and is higher among Hispanic females[105]

MI: Myocardial infarction; PTCA: Percutaneous coronary angioplasty.

Medicaid) but were more likely than black patients to receive revascularization[84]. Hispanics were less likely than whites to receive catheterization or PTCA, whereas black patients were less likely than white patients to receive catheterization or CABG and were somewhat less likely to have a stress test or echocardiogram[104]. Disparities in the treatment of Hispanic patients *vs* white patients dropped significantly between 2005 and 2010, indicating more equitable care in the same hospital[86].

Outcomes

Data are very limited on clinical outcomes in Hispanic patients treated for AMI. We here briefly reiterate data from studies already cited, but with specific reference to Hispanics, and note one additional recent study of Puerto Rican patients.

Patients with first MI hospitalized in 2007 in San Juan, Puerto Rico, had an average age of 64 years. Women made up 45% of the study population, but the incidence rate per 100000 was significantly higher for men (198) than for women (134). Women were less likely to receive medications such as aspirin, recommended statins, ACE inhibitors, β-blockers, or to have interventional procedures[105].

Hispanic patients with diabetes treated intensively for cardiac risk factors had a smaller risk of death/MI/stroke at 5 years than white diabetics with similar treatment, but the differences were not significant[90]. Hispanic patients with pre-existing coronary heart disease, admitted to a cardiovascular service, were less likely to report statin use before admission and more likely to be dead or re-hospitalized at 1 year than white patients. They also had higher rates of high blood pressure and diabetes and less likely to have health insurance[97].

Table 4 summarizes disparities in Hispanics in presentation, treatment, and outcomes of acute coronary syndrome.

OTHER GROUPS

Recent data are largely lacking on CVD risk factors in general, on CVD health status, and especially on revascularization as treatment for AMI among minority groups other than blacks and Hispanics. A few studies have already been discussed that either originally included some groups but tossed those study participants from the analyses because they were too few to lead to any conclusions or simply added them to other populations (*e.g.*, whites/Asians). Here we include a brief look at some recent attempts to elucidate risk factors, treatment status, or outcomes among other minority groups.

A study of the prevalence of dyslipidemia among minority populations in the United States included Asian Americans, Mexican Americans, and blacks, compared to non-Hispanic whites. Outcome measures were elevated levels of triglyceride, low HDL and high LDL cholesterol levels. Hispanic/Latino patients from subgroups other than Mexican Americans were excluded because of small numbers. Filipino and Mexican American women had the highest prevalence of high triglyceride levels and high LDL cholesterol levels. Asian Indian and Mexican American women had the highest prevalence of low HDL cholesterol levels. Mexican American women and all Asian subgroups except Korean women had higher prevalence of high triglyceride levels than white patients, but black patients had the lowest prevalence of high triglyceride levels (18.1%). In general, the prevalence rates of all three dyslipidemia types were higher in men[106].

Among Chinese, South Korean, Asian Indians, Japanese, Filipinos, and Vietnamese-the six largest Asian subgroups in the United States-Asian Indian men and women and Filipino men had the largest proportionate mortality burden from ischemic heart disease relative to non-Hispanic whites. The mortality impact of hypertension and cerebrovascular disease, namely hemorrhagic CVAs, was more elevated in every Asian-American subgroup than in whites[107].

AI/ANs have higher rates of obesity than any other group, and they are rising at a faster rates compared with non-Hispanic whites. Metabolic syndrome was found in higher rates in AI/AN men and women,

compared with the general population. The prevalence of diabetes among AI/ANs is almost three times the prevalence of diabetes among non-Hispanic whites. The rates of self-reported heart disease, stroke, and cardiovascular mortality were higher in AI/ANs than in whites. Differences in the prevalence of hypertension and hyperlipidemia were more equivocal. English surveys may underestimate the prevalence of these risk factors[10].

Bradley *et al*[79] used time between hospital arrival and reperfusion therapy as their main outcome measure in an analysis of individuals being treated for STEMI. Black patients had the longest door-to-drug time, followed by Asians/Pacific Islanders and Hispanics; all had significantly longer door-to-drug times than white patients. Minorities on a whole had significantly longer door-to-balloon times than whites.

Despite a vastly different health care system, a study in the Netherlands found disparities among minority populations there similar to those observed in the United States Investigators looked at differences between first-generation ethnic minority groups (Antillean, Chinese, Indonesian, Moroccan, South Asian, Surinamese, and Turkish) and the ethnic Dutch population after first hospitalization for AMI or congestive heart failure. Mortality rates at 28 d and 5 years were significantly higher among the migrant groups than in the ethnic Dutch. The rate of AMI re-admission for the migrant groups was nearly a third larger than for ethnic Dutch. Mortality rates for migrants after congestive heart failure differed, with a lower 28-d mortality rate among Moroccans and Turks and a higher 5-year mortality rate among Surinamese, Chinese, and South Asians. Re-admission rates for congestive heart failure were generally higher among migrant groups than in the ethnic Dutch population[108].

CONCLUSION

As this review documents, disparities persist in risk factors, health status, and co-morbidities at presentation; in the allocation of treatments; and in outcomes in revascularization as treatment for MI and, more generally, outcomes associated with CVD between female and minority patients and the general white patient population.

Women, for example, typically have more risk factors and are likely to present with more co-morbidities such as diabetes, dyslipidemia, and obesity than men. This is exacerbated among minority female groups compared with white women. Models intended to assess and estimate risk do not estimate women's risk accurately. Women have longer pre-hospital delay, and once admitted, have more limited access to guideline treatments such as PCI than men. Younger women especially have substantially higher rates of in-hospital and early and long-term mortality than men and are more likely to be re-admitted. These differences decline with age.

Blacks similarly have more risk factors such as dyslipidemia, hypertension, and obesity, and are more likely to present with co-morbidities, especially diabetes. Black patients have poorer access to guideline treatments such as PCI or CABG and experience more long-term mortality, congestive heart failure, and re-admission for AMI.

Disparities among Hispanics are more challenging to characterize, not least because so many Hispanic/Latino groups are represented in the United States population. The range of risk factors and co-morbidities seen across these various groups makes generalizing very difficult. Furthermore, in several studies, Hispanics seemed to have access to comparable care and to have outcomes that are comparable or slightly better than those in white patients, even if their initial presentation does not seem as promising.

The data show many disparities in all three of these groups but offer little explanation for these disparities. One thing women, blacks, and Hispanics often have in common in the United States is lower incomes, which can translate into less access to health insurance and thus less continuity of care. These groups often live in poorer neighborhoods and, for a variety of reasons, may have poorer health habits than their wealthier neighbors. Residential location can mean care in less-than-optimal hospital settings. Several studies reviewed here showed that hospital quality can have a major impact on the quality of treatments and on outcomes. The logical expectation that better access to coverage would translate into better care and better outcomes does not seem to be borne out in the early results from Massachusetts, where health care reform implementation is nearly 10 years old.

Huge knowledge gaps still exist, especially in studies of treatments for AMI and outcomes in minority groups beyond blacks and Hispanics. Little is known about revascularization as treatment for AMI/ACS in AI/ANs or the several Asian subpopulations in the United States or, for that matter, in Hispanic subpopulations. With advances in revascularization techniques and particularly the promising results obtained with drug-eluting stents, new randomized trials and comparative treatment studies that oversample these groups are needed. Admittedly, these can be difficult groups to study because of small numbers, but much too little is known about how to better treat these populations and how to resolve disparities in outcomes.

We'd like to end on a positive note by pointing out that racial/ethnic gaps in treatment quality measures are narrowing and that where rigorous adherence to treatment guidelines is enforced for study purposes, benefits to patients are uniform regardless of race or ethnicity. The news does not seem to be so good for women, however, and much research is still needed to understand observed disparities in outcomes among

younger female patients.

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REVIEW

Surgical perspectives in the management of atrial fibrillation

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Abstract

Atrial fibrillation (AF) is the most common cardiac arrhythmia and a huge public health burden associated with significant morbidity and mortality. For decades an increasing number of patients have undergone surgical treatment of AF, mainly during concomitant cardiac surgery. This has sparked a drive for conducting further studies and researching this field. With the cornerstone Cox-Maze III "cut and sew" procedure being technically challenging, the focus in current literature has turned towards less invasive techniques. The introduction of ablative devices has revolutionised the surgical management of AF, moving away from the traditional surgical lesions. The hybrid procedure, a combination of catheter and surgical ablation is another promising new technique aiming to improve outcomes. Despite the increasing number of studies looking at various aspects of the surgical management of AF, the literature would benefit from more uniformly conducted randomised control trials.

Key words: Atrial fibrillation; Cardiac surgery; Surgical management; Surgical ablation; Minimally invasive

Core tip: The surgical management of atrial fibrillation (AF) is a rapidly developing field. Existing surgical

techniques are constantly evolving in order to achieve more minimally invasive procedures. Additionally, the relatively new ablative modalities are being increasingly used, either alone or in conjunction with surgical techniques; attempting overall better and less invasive results. This review looks at the current surgical techniques and ablative modalities available for managing AF, where each section is re-enforced with the current most up to date guidelines on the use of each of these modalities.

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INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and it has been associated with substantial morbidity and mortality. The lifetime risk of AF between the ages of 40-95 is 1 in 4, as demonstrated by the Framingham study[1]. In the general population AF has been shown to occur in around 1%-2%[2].

Patients suffering from AF are at risk of thromboembolic events, including stroke leading to disability or death. One out of every five strokes is secondary to AF, with those resulting from AF being more disabling and having increased clinical significance[1,3].

The first-line treatment of AF has traditionally been anti-arrhythmic drugs, whether attempting rate and or rhythm control. However, long-term anticoagulation is not without risks as it can interfere with quality of life and increase morbidity. There is still considerable variation in the pharmacological treatments of AF in clinical practice[2].

Catheter based ablation is an alternative to medical therapy, as a minimally invasive intervention which can provide relatively good results[4].

The role of surgery in the management of AF has been introduced following the ground-breaking "Maze" procedure described and developed by Cox *et al*[5] in a series of publications. The "Maze" procedure has been the cornerstone of surgical management of AF[3-8]. The Cox-Maze Ⅲ procedure is regarded as the gold-standard surgical treatment of AF having the highest success rates; greater than or equal to 90% long-term freedom from AF[4,9,10]. However, the procedure is highly invasive and technically difficult, requiring a high level of surgical expertise, thus limiting its use to a few specialist centres[9].

These limitations have fuelled further research in the field of surgical management of AF with a view to develop less invasive but equally effective alternative techniques.

This has led to the development and use of multiple ablative devices using various energy sources, as well as less surgically invasive Cox-Maze lesions allowing replacement of incisions with ablation lines[2]. There is still on-going research and development of new techniques, within this rapidly evolving field.

AF BACKGROUND AND DEFINITIONS

Electrophysiological basis of AF

In 1998, Haïssaguerre *et al*[9] made an important discovery regarding the origin of atrial ectopic beats in paroxysmal AF. At the time it was known that chronic AF was the result of re-entrant circuits, though no information was available about its origin.

The spontaneous initiation of AF was studied using intra-cardiac monitoring, angiography and fluoroscopy looking specifically at the electrical activity preceding the onset of AF. A total of 69 foci were identified as being responsible for the origin of atrial ectopic beats, in a study of 45 patients. The striking majority (94%) of the foci were found to be in the pulmonary veins with others including the right and left atria. Radiofrequency catheter ablation was subsequently utilised to ablate those foci in order to abolish spontaneous depolarisation. Sinus rhythm was achieved and maintained in 28 patients (62%) at a follow up period of 8 ± 6 mo[9]. This discovery led to the pulmonary vein isolation (PVI) approach.

The identification of abnormal depolarisation foci, helped in understanding the origin and development of AF. It became apparent that ectopic atrial depolarisation is responsible for the initiation of AF, whilst macro re-entrant circuits are responsible for its propagation (Figure 1)[10].

Definition and classification of AF

AF is defined as a supra-ventricular arrhythmia where asynchronous atrial activation occurs, leading to worsening atrial mechanical function[2,4,11].

According to the definitions given by ACC/ESC/AHA 2006, Society of thoracic surgery (STS), ESC and EACTS clinical guidelines committee, there are five types of AF. These are defined as: First diagnosis, paroxysmal, persistent, long-standing persistent and permanent AF (Table 1).

AF can also be classified as primary or secondary according to its origin. Primary AF occurs in patients with no underlying cardiac disease whilst secondary AF occurs as a result of pre-existing cardiac disease. This has implications when looking at the surgical management of AF. Whilst patients with secondary AF benefit from concomitant treatment alongside other cardiac surgical procedures, catheter ablation alone is a more suitable treatment modality for paroxysmal primary AF[12-15].

SURGICAL MANAGEMENT OF AF

Correct nomenclature for surgical procedures used in AF management

According to 2012 HRS/EHRA/ESC guidelines the term

Table 1 The five types of atrial fibrillation as classified by European Heart Rhythm Association and European Association For Cardio Thoracic Surgery

Type of AF	Duration	Definition
First diagnosis	-	First episode of AF irrespective to duration or severity
Paroxysmal	48 h	Self-terminating (usually within 48 h); may continue for up to 7 d. After 48 h it is unlikely that spontaneous conversion will occur Anticoagulation must be considered
Persistent	> 7 d	Requires termination by cardio-eversion with drugs or direct current
Long standing persistent	⩾ 1 yr	Rhythm control strategy
Permanent	-	Presence of arrhythmia is accepted and rhythm control interventions are not pursued[1]

[1]In case of rhythm control interventions, the permanent AF should be re-designated as long standing persistent AF. AF: Atrial fibrillation.

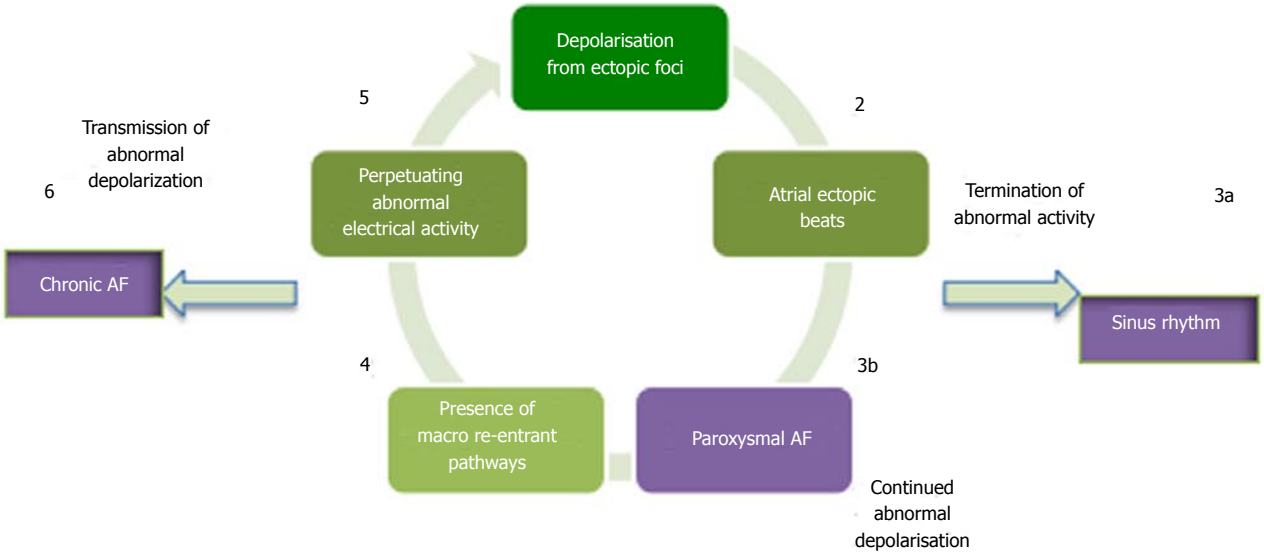

Figure 1 Electrophysiological basis of paroxysmal and chronic atrial fibrillation. AF: Atrial fibrillation.

"Maze" procedure should only be used to refer to the Cox-Maze Ⅲ lesion set. The above term should not be used for other less extensive lesion sets. Furthermore, the suggested terminology for ablative procedures is as follows: (1) full Cox-Maze lesion set; (2) left atrial appendage (LAA) lesion set; and (3) PVI[4,14].

The Cox-Maze "cut and sew" procedure

Cox-Maze Ⅰ and Ⅱ: Cox *et al*[6] first described the Cox-Maze procedure in 1991. The Cox-Maze Ⅰ (CM-Ⅰ) procedure was initially developed, consisting of a set of lesions generating an "electrical maze" through the atria. The linked atrial segments were created surgically by cutting and sewing in order to interrupt and eliminate macro re-entrant circuits[7-10]. The Maze Ⅰ procedure had several limitations including left atrial dysfunction and failure to generate sinus tachycardia on exertion. The Cox-Maze Ⅱ (CM-Ⅱ) procedure was subsequently developed aiming to overcome those limitations. In CM-Ⅱ a revised lesion set was used, in an attempt to improve conduction between the atria. However, this led to a technically more challenging procedure with the same drawbacks as CM-Ⅰ[7-10].

Cox-Maze Ⅲ: The Cox-Maze Ⅲ (CM-Ⅲ) procedure shortly followed which overcame the limitations of the previous two procedures. Atrial transport function was preserved allowing depolarisation from sinoatrial node to atrioventricular node for efficient atrial contraction. CM-Ⅲ achieved 93% freedom from AF during an 8.5 year follow up, with successful cardioeversion of all cases on one anti-arrhythmic drug. Additionally, the need for pacemaker was abolished and recurrence of AF reduced[7-10]. Equally encouraging results from the CM-Ⅲ were generated by the Cleveland Clinic and Mayo Clinic[16,17].

Despite its high success rates the procedure remained technically challenging and highly invasive. A median sternotomy and cardiopulmonary bypass (CPB) is required for CM-Ⅲ, limiting its use to patients undergoing concomitant heart surgery, as it is deemed to be too invasive to be solely performed for AF treatment.

The Cox-Maze Ⅳ: A few years later, the Cox-Maze Ⅳ (CM-Ⅳ) was developed initially described by Gaynor *et al*[18] in 2004. A different lesion set to CM-Ⅲ was used and the traditional "cut and sew" technique was replaced with the use of ablative devices. Namely, the combined use of bipolar radiofrequency ablation together with

cryoblation was used in that study. Moreover, CM-Ⅳ had the added advantage of being technically simpler than CM-Ⅲ, meaning that it could be used in a greater number of centres. The lesion set used was similar to CM-Ⅲ and it still had to be performed under CPB[18,19].

Widespread variation has been observed in the lesion sets used to treat AF. Since the publication of CM-Ⅲ the lesion sets have been altered slightly in CM-Ⅳ and are continuously being altered in the current literature. The main reason for the constant alteration of the lesion sets is that surgeons are striving to achieve minimally invasive procedures, with the best clinical outcomes possible[12].

PVI

The PVI approach was developed following the discovery of the pulmonary veins being a key area of ectopic depolarisation, resulting in atrial ectopic beats and ultimately leading to AF. The PVI approach has been used in a number of trials and was shown to produce good results in paroxysmal AF.

When first performed by Haïssaguerre *et al*[9] 62% of patients were free from AF in a median follow up of 7 mo. In studies that followed, empirical isolation of the pulmonary veins became the most common approach as it was observed that the ectopic foci contributing to AF were often variable.

In the study by Haïssaguerre *et al*[9], 94% of the ectopic foci were found in the pulmonary veins. Thus, one would expect that by ablating those foci freedom from AF would be close to 90%. However, this has not been the case in studies looking at long term (5 year) freedom from AF using PVI, with the success rate being as low as 30%-50% and the recurrence rate up to 70%[20,21].

The reduced effectiveness of PVI can be attributed to incomplete transmural lesions and pulmonary vein reconnection. Another reason for the low success rates is the fact that the pulmonary veins are not the only triggers of AF. Exclusively using PVI may not be sufficient in patients with persistent and long-standing AF, as demonstrated by studies where ablation of additional areas improved the outcomes[22-24]. However, due to large inter-study variation in ablation methodology it is difficult to assess the effectiveness of PVI alone.

Exclusion of LAA

The LAA has been associated with occurrence of stroke in patients with AF. Studies have demonstrated that around 90% of the thrombi are found in the LAA, making it the primary source of emboli. This has led to the conclusion that successful closure of the LAA would reduce the risk of such thromboembolic events[25,26]. Several studies have investigated the link between LAA exclusion and reduction in stroke in patients with AF.

Healey *et al*[27] (2005) performed an RCT of 77 patients undergoing CABG where 52 of them underwent LAA occlusion. However, occlusion was successful in just 66% of the patients. One patient had an intraoperative ischaemic stroke and another had a TIA. Twelve percent of the patients had subsequent self-reported strokes on follow-up done *via* a questionnaire.

Kanderian *et al*[28] (2008) demonstrated that 55% of 137 patients who underwent LAA closure had a successful procedure. Eleven percent of the patients that had a successful closure had a subsequent stroke or TIA compared to 15% of those with unsuccessful procedure. These results were nevertheless found to be non-significant.

García-Fernández *et al*[29] (2003) looked at 205 patients undergoing mitral valve surgery, with 58 of them having LAA ligation. Successful ligation was seen in 89.7% of patients. Absence of LAA ligation was found to be an independent predictor of thromboembolism following mitral valve surgery. Systemic emboli occurred more frequently in the group of patients that did not receive ligation.

Contrasting the above studies, Almahameed *et al*[30] (2007) found a significantly increased rate of stoke in patients with LAA occlusion, looking at 136 patients that underwent LAA ligation during mitral valve surgery.

The above studies demonstrate the heterogeneity of existing results when looking at LAA exclusion. Success rate is variable between the studies, ranging from 55% to 93%. According to the EACTS guidelines, there is insufficient evidence to prove that LAA exclusion has a benefit in terms of stroke reduction or mortality[2].

ABLATION USING ENERGY SOURCES

Various energy sources have emerged over the last decade striving to replace the traditional "cut and sew" technique by replicating the transmural lesions whilst using a less invasive approach[31]. Nevertheless, the vital pre-requisite for successful AF ablation, as demonstrated by the CM-Ⅲ lesion set, is that the lesions need to be completely transmural and contiguous bilaterally. In addition, it is fundamental that the lesions are placed in the correct pattern[10,12,18]. Therefore, an important caveat when looking at new ablation techniques is the ability to achieve complete transmurality of lesions. The ablative energy modalities available for surgical treatment of AF are compared in Table 2.

Radiofrequency ablation

Radiofrequency ablation (RFA) works by conducting an alternating electrical current through the myocardium. The energy from this electrical current gets dissipated through the myocardial tissue as heat, causing coagulative necrosis and resulting in an area of non-conducting myocardium. Complications of RFA include injury to collateral structures such as the pulmonary veins, oesophagus and coronary arteries[14,25].

Unipolar RFA

The effectiveness of unipolar RFA during concomitant cardiac surgery has been investigated by several studies.

Table 2 Comparing and contrasting the various available ablation modalitie

Ablation modality	Mode of action	Advantages	Complications	Transmural lesions	Current limitations
RFA	Controlled thermal damage and lesions caused by electrical current	Less operating time Reduced technical difficulty	Intercavity thrombus Pulmonary vein stenosis Oesophageal and coronary artery injury	Variable	Confirmation of transmurality Variation between instruments
Cryoablation	Targeted scarring by cooling tissue using high-pressure argon and helium Initial cellular destruction followed by fibrosis and full thickness disruption	Visual confirmation of transmurality Less damage to surrounding tissues and vascularity Less endocardial thrombus Electrical isolation of atria	Coronary artery and phrenic nerve injury Atrioesophageal fistula	Yes	Variable success rate
Microwave	Production of lesions by thermal injury	Minimal collateral damage Minimal scar formation Lower risk of VTE	Coronary artery damage potential	Variable	Less effective compared to other modalities Limited evidence
HIFU	Creation of localised hyperthermic lesions using a focused beam of ultrasound energy	Fast epicardial lesions Future potential advantage visualisation of thickness by ultrasound and tailor made lesions	Atrioesophageal fistula Pericardial effusion Phrenic nerve injury	Yes endocardial only	High rate of complications Limited evidence currently not recommended outside trials
Laser	Use of high energy optical beams to create thermal lesions	Well demarcated lesions Non-arrythmogenic Rapid lesions	Crater formation Perforation Tissue loss Poor visibility of scar	Yes	Limited evidence currently not recommended outside trials

RAF: Radiofrequency ablation; HIUF: High intensity focused ultrasound.

Johansson *et al*[32] (2008) looked at patients undergoing CABG in combination with unipolar RFA. Patients were followed up for 32 ± 11 mo (with intermediate follow up at 3 and 6 mo) looking for sinus rhythm. In the RFA group 62% of patients were in sinus rhythm compared to 33% in the non-RFA group. Patients who were in paroxysmal or persistent AF were more likely to remain in sinus rhythm than patients with permanent AF. The presence of sinus rhythm at 3 mo was found to be a high predictor of the patient remaining in sinus rhythm at further follow up.

Khargi *et al*[33] (2005) looked at a cohort of patients with permanent AF undergoing open-heart surgery (CABG, aortic and mitral surgery) together with unipolar RFA. Sinus rhythm was observed in 79% of patients undergoing CABG/aortic surgery and 71% of mitral surgery patients. Notably, adding RFA did not increase mortality compared to cardiac surgery alone.

Bukerma *et al*[34] (2008) looked at patients fulfilling the criteria for permanent AF who underwent concomitant cardiac surgery and unipolar RFA. Contrasting the high success rates seen at the aforementioned studies, just 52% of patients maintained sinus rhythm at 5-year follow up.

All of the above studies used 24 h outpatient holter ECG monitoring to detect the presence of sinus rhythm. An important pitfall is that asymptomatic AF can be easily missed, as the monitoring is not continuous during the follow up period. This needs to be taken into account when designing future studies[2].

Unipolar RFA combined with concomitant cardiac surgery is considered to be effective in restoring sinus rhythm. Higher degrees of success rates are seen in paroxysmal or persistent AF, young age and smaller LAD[2].

Bipolar RFA

The effectiveness of bipolar RFA during concomitant surgery has also been explored.

A meta-analysis conducted by Chiappini *et al*[35] (2004) looked at 6 non-randomised studies of patients with AF undergoing RFA as an adjunct to cardiac surgery, where 76% freedom from AF was achieved at 13.8 mo follow up with the overall survival rate being 97.1%.

Similarly, von Oppel *et al*[36] (2009) looked at patients with persistent AF undergoing concomitant cardiac surgery and bipolar RFA compared to cardiac surgery alone. Seventy-five percent of patients in the RFA group were free of AF at a 1-year follow up with more than 60% of patients having restoration of left atrial contraction.

A best evidence topic on the effectiveness of bipolar RFA during concomitant cardiac surgery conducted by Basu *et al*[37] (2012), revealed that bipolar RFA was more successful in restoring sinus rhythm for at least 1 year when performed together with cardiac surgery. In addition, a high survival rate was observed and the procedure required an average of 15 additional minutes of cross clamp time.

Bipolar RFA used in conjunction with cardiac surgery has a higher success in restoration of sinus rhythm compared to cardiac surgery alone. There is limited evidence to conclude whether bipolar RFA is more

effective than unipolar RFA. Further studies comparing the two modalities are needed[2].

Cryoablation

Cryoablation works by cryothermal energy, generated by the use of pressurised liquid nitrous oxide resulting in cooling of the surrounding tissue. Tissue injury occurs by the creation of ice crystals within the cells disrupting the cell function and electrical conductivity. Additionally, microvascular disruption ensues resulting in cell death. Complications of cryoablation include phrenic nerve injury, atrioesophageal fistulas and coronary artery injury[14,24]. Several studies have proved the efficacy of cryoablation in the treatment of AF.

The PRAGUE 12 (2012) was a randomised multi-centre trial that is considered a milestone trial for AF. It looked at 224 patients with AF undergoing valve replacement or coronary surgery. The patients were randomised in two groups with group A undergoing surgical ablation and group B having no ablation. In the ablation group, 96% had treatment with a cryoprobe. The procedure performed consisted of PVI, mitral annulus lesion, LAA lesion and a connecting lesion. At 1 year follow up 60% of patients that underwent ablation were in sinus rhythm compared to 36% in the untreated group. At 1 year follow up no clinical benefits were seen in patients who underwent AF surgery; however the study is still ongoing and results for the 5-year follow up are yet to be published[38].

Camm *et al*[39] (2011) conducted a best evidence topic looking at the effectiveness of cryoablation during concomitant cardiac surgery. Nine studies were reviewed, including RCTs and retrospective studies. Cryoablation was found to be an acceptable surgical intervention, achieving sinus rhythm in 60%-82% of patients at 12 mo follow up.

Blomström-Lundqvist *et al*[40] (2007) conducted a RCT looking at patients undergoing concomitant AF surgery and mitral valve repair. The results demonstrated that undergoing cryoablation for AF treatment significantly increased the return to sinus rhythm. 73.3% of patients in the cryoablation group were found to be in sinus rhythm compared to 42.9% of those undergoing mitral valve surgery alone. However, it is worth noting that patients in the cryoablation group had an increased rate of complications. No significant increase in the mortality or morbidity was seen in either group.

Cryoablation during concomitant cardiac surgery has been proven to achieve good rates of sinus rhythm and it is more successful in patients with paroxysmal AF as opposed to permanent AF. Increased complication rates from cryoablation were seen in just one study. Finally, a lack of 24 h monitoring meant that accurate assessment of AF resolution was difficult[2,4].

Microwave ablation

Microwave ablation works by producing a well-demarcated lesion through thermal injury. Its main advantage is that it produces good epicardial lesions and can be used in minimally invasive techniques.

MacDonald *et al*[41] (2011) conducted a best evidence topic regarding the effectiveness of microwave ablation for AF treatment during concomitant heart surgery. Eleven studies were reviewed with a large degree of heterogeneity observed between the studies. The success rate ranged between 65%-87% over a variable follow up period between 6-12 mo. The conclusion was that microwave ablation is not currently recommended due to limited evidence and unclear long-term success rates.

Lin *et al*[42] (2010) compared microwave ablation to bipolar RFA. A RCT was conducted where patients were randomised to a radiofrequency or a microwave ablation group. Patients were then followed up at 3, 6, 9 and 12 mo and then annually. With a mean follow up of 24 mo, freedom from AF in the radiofrequency group was 88.7% compared to 71.2% in the microwave ablation group (P = 0.0008). Thus bipolar radiofrequency ablation was demonstrated to be superior to microwave ablation.

Kim *et al*[43] (2010) compared cryoablation to microwave ablation in patients with mitral disease and AF. They demonstrated a 5-year freedom rate from AF of 61.3% in the microwave group compared to 79.9% in the cryoablation group. Additionally, microwave ablation was associated with more frequent AF recurrence rates.

Microwave ablation is currently considered to be less effective than other ablation modalities, based on the limited evidence[2]. Further studies are needed to investigate the effectiveness of microwave ablation as a definite treatment of AF.

High intensity focused ultrasound ablation

High intensity focused ultrasound (HIFU) is a relatively new ablative modality and works by creating a localised thermal lesion using a focused beam of ultrasound energy. HIFU has been proven to create permanent transmural lesions when applied epicardially. It has the advantage that CPB is not needed and can be performed on the beating heart. HIFU can also be delivered *via* a balloon catheter in order to facilitate circumferencial ablation of pulmonary veins[44].

Neven *et al*[45] (2011) performed PVI using HIFU and subsequently followed up the patients for 2 years. An oesophageal temperature-guided safety algorithm was used in an attempt to minimise the complications. At 2 year follow up the success rate from the procedure was comparable to that of radiofrequency ablation. However, severe complications were not prevented despite the use of a safety algorithm. Complications included atrioesophageal fistula, pericardial effusion and phrenic nerve palsy. They concluded that HIFU did not meet the safety standards required for AF treatment, mainly because phrenic nerve palsy and atrioesophageal fistula were still common severe complications. The clinical use of HIFU has currently been halted.

Davies *et al*[46] (2013) followed up 110 patients undergoing HIFU ablation for AF treatment. At a 2-year

follow up 49% of patients remained in sinus rhythm. The percentage of patients in sinus rhythm was given for each of the pre-operative AF types: 81% for paroxysmal AF, 56% for persistent AF and 18% for long standing AF. The conclusion was that HIFU is safe and effective for use in paroxysmal AF, however alternative ablation strategies should be considered for persistent and long standing AF.

Klinkenberg *et al*[47] (2009) and Schmidt *et al*[48] (2009) have also demonstrated that despite relatively high percentages of freedom from AF there is a high complication rate when using HIFU ablation. They have concluded that further research is needed to assess optimal ablation techniques.

HIFU ablation is currently not recommended outside trials due to the high rates of complications reported and its success rates being inferior to other ablative modalities[2]. Further studies are looking at the success rates of HIFU combined with cardiac surgery are needed, as the evidence available is currently limited.

Laser ablation

Laser ablation for AF treatment works by using laser energy to create localised hyperthermic lesions[14,20].

Gal *et al*[49] (2015) used an endoscopic laser balloon ablation system to perform PVI with 58% of patients remaining free from AF at follow up with no anti-arrhythmic drugs. They concluded that laser ablation has a low risk of complications and its success rate was comparable to other ablation modalities.

Šedivá *et al*[50] (2014) performed PVI using visually guided laser ablation in 194 patients. In 1 year follow up 82.3% of patients remained free from AF in the paroxysmal AF group and 75% in the persistent AF group; this percentage remained close to 75% in 3 and 4-year follow up. They concluded that visually guided laser ablation is an effective and safe modality to be used in clinical practice with good clinical outcomes and low rates of complications.

Hamman *et al*[51] (2009) used a diode pumped laser to perform a left modified or complete CM-III lesion set. The results observed were very encouraging with a 95% freedom from AF and 76% freedom from all tachyarrhythmias. However, it needs to be noted that the study was only a small one, consisting of 28 patients.

Currently, laser ablation is not approved for clinical use outside trials due to limited available evidence to support its effectiveness and safety.

CATHETER ABLATION, SURGICAL ABLATION AND HYBRID APPROACH

As well as using different devices for AF ablation, there are several methods to perform the ablation procedure. Traditionally the two options were catheter and surgical ablation.

Surgical ablation can be performed with the conventional surgical approach during concomitant cardiac operations or with less invasive approaches such as mini sternotomy, mini thoracotomy or VATS.

Catheter ablation is considered to be the least invasive approach and it is used to create endocardial lesions. In some studies catheter ablation for AF has been shown to be 80% effective, with 70% of patients not requiring any anti-arrhythmic drugs at intermediate follow up[52].

The hybrid approach consists of a combination of surgical and catheter procedures. This has emerged in an attempt to ensure good epicardial and endocardial transmurality of lesions as well as attempting to enhance the long-term success of AF ablation. In essence, this approach aims to overcome the limitations of surgical and catheter procedures alone.

The hybrid procedure can be performed as a single or a two-stage procedure with advantages to each. When performing a single procedure undergoing anaesthesia twice and having further hospital admission are avoided. In contrast, when performing a two-stage approach with an average interval of 1-3 mo, the lesions are more likely to have healed and have stable conductive properties (Figure 2)[53].

Gaita *et al*[54] (2013) looked at a hybrid approach involving surgical cryoablation and transcatheter RFA in order to perform PVI and left atrial isolation. The procedure was performed in 33 patients, 73% of which were in sinus rhythm, at a mean follow up of 10 ± 3.1 years. At the end of the follow up period 81% of patients with a complete lesion set were in sinus rhythm compared to 43% of those with an incomplete lesion set. Electrophysiological evaluation of lesion transmurality of was used, aiding in significant improvement of outcomes.

Kumar *et al*[55] (2014) used a hybrid approach consisting of bipolar radiofrequency devices epicardially and cryoballoon endocardially. This approach was found to be feasible and safe, though the results should be interpreted with caution as the study consisted of only 7 patients.

Bulava *et al*[56] (2015) combined surgical thoracoscopic RFA with catheter RFA performed 6-8 wk later in a staged hybrid method. At 12 mo follow up after a completed hybrid ablation 94% of patients were in sinus rhythm.

A systematic review by Je *et al*[57] (2015) compared the endocardial Cox-Maze procedure, epicardial surgical ablation and hybrid procedure. The results demonstrated that minimally invasive Cox-Maze procedure with CPB support was the most effective treatment for stand-alone AF with a higher success rate seen at 12 mo following the procedure.

The hybrid approach is a promising new procedure that could significantly improve outcomes for patients undergoing surgical treatment for AF. It has been shown to have a mortality rate close to 0% with long-term success rates approaching 95% similar to the cut and sew Cox maze procedure. Thus, in the mildly symptomatic lone AF population, the hybrid procedure could become the standard of care in the near future[58].

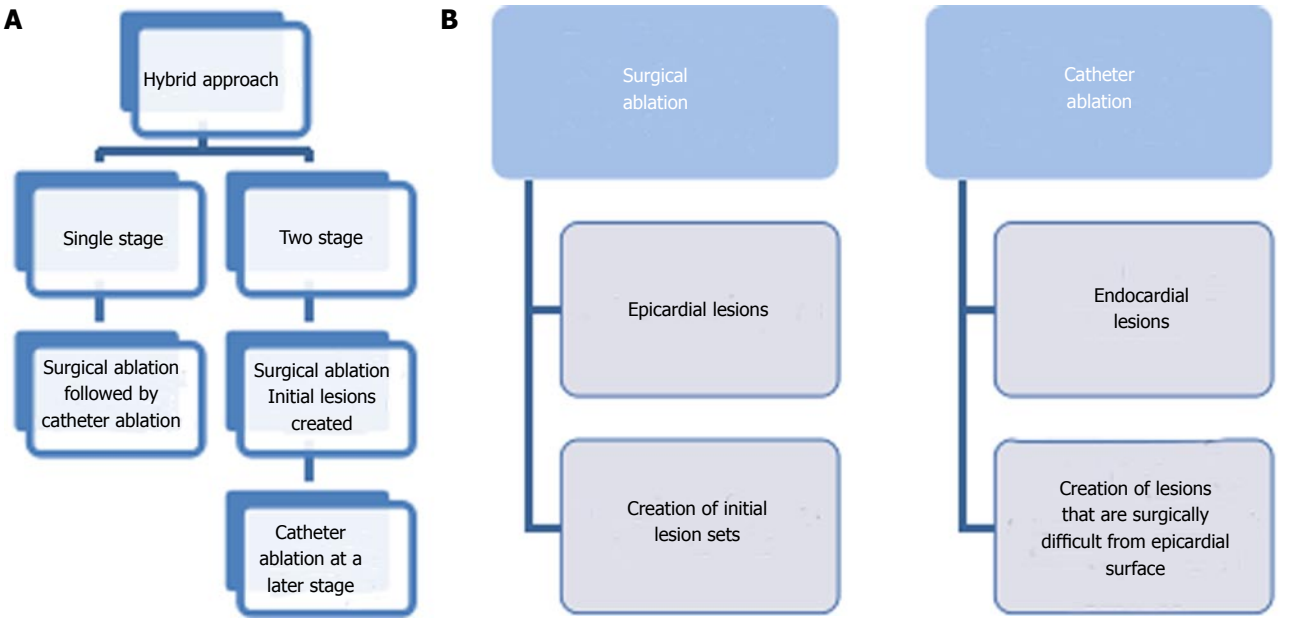

Figure 2 Schematic representation of the hybrid approach and the two stages of the hybrid approach (A and B).

Further studies would be essential in order to identify the combination of modalities that yields the most successful results when performing a hybrid procedure.

CONCOMITANT HEART SURGERY AND SURGICAL TREATMENT OF AF

Surgical treatment of AF can be performed alone as well as in conjunction to cardiac surgery. Several studies have compared the efficacy of concomitant cardiac and AF surgery to cardiac surgery alone. Mitral valve surgery is the most common procedure that has been combined with AF ablation. It is known that 30%-50% of patients undergoing mitral valve surgery present with AF, which itself leads to increased risk of stroke and reduction in survival rates[59].

Phan *et al*[59] (2014) conducted a meta-analysis on surgical ablation for AF during mitral valve surgery. The results demonstrated that the addition of AF ablation led to a significantly greater number (64.4% *vs* 17.9%, $P < 0.00001$) of patients in sinus rhythm at a follow up period of > 12 mo. There was no increase in mortality, need for pacemaker implantation, stroke or thromboembolism risk.

A RCT conducted by Gillinov *et al*[60] (2015) consisted of a group of 260 patients with persistent or long-standing persistent AF. It compared patients undergoing mitral valve replacement therapy either with or without surgical ablation. The results showed that addition of AF ablation to mitral valve surgery led to a significant increase ($P < 0.001$) in the rate of freedom from AF in 1 year. The control group had 29.4% freedom from AF when compared to 63.2% in the ablation group.

The Left atrial radiofrequency ablation during mitral valve surgery: A prospective randomized multicentre study (SAFIR)[61] study (2009) was a multi-centre double-blinded centrally randomised trial involving four university hospitals. It compared patients undergoing left atrial RFA combined with mitral valve replacement to those undergoing mitral valve replacement alone. The results were in favour of the combined ablation and mitral valve replacement. At 12 mo follow up the freedom from AF was 95.2% in the combined group *vs* 33.3% in the control ($P < 0.005$).

The Surgical Atrial Fibrillation Suppression Study (2011) was a RCT that looked at patients undergoing RFA performed in conjunction to cardiac surgery. They concluded that surgical RFA for AF during concomitant cardiac surgery significantly reduces AF burden. However, 13% of patients had asymptomatic AF episodes only identified on continuous monitoring. This was deemed to have significant implications for the definition of successful surgical AF ablation as well as the need for post-operative anti-arrhythmic and anticoagulants[62]. Potential disadvantages of this combined approach are the increased rates of permanent pacemaker insertion and increased operative time[27,59-62].

The use of energy sources for AF ablation is recommended in concomitant cardiac surgery because of good evidence supporting the efficacy of the combined procedure[2]. However, further studies are needed to investigate combination of surgical AF ablation with specific cardiac operations. This will help in identifying the combination of ablative modality and cardiac surgery yielding the best results.

Tables 3-9 summarise the studies discussed in the sections above, looking at each modality available for surgical AF ablation.

POST-OPERATIVE ANTICOAGULATION AND FOLLOW UP

Post-operative anticoagulation

A best evidence topic was conducted by Michael Gray

Table 3 Adapted from 2012 Heart Rhythm Society/European Heart Rhythm Association/European Society of Cardiology guidelines

Indications for concomitant surgical ablation of AF
Symptomatic AF refractory or intolerant to at least one Class 1 or 3 antiarrhythmic medication
Paroxysmal: Surgical ablation is reasonable for patients undergoing surgery for other indications (IIa, C)
Persistent: Surgical ablation is reasonable for patients undergoing surgery for other indications (IIa, C)
Longstanding persistent: Surgical ablation is reasonable for patients undergoing surgery for other indications (IIa, C)
Symptomatic AF prior to initiation of antiarrhythmic drug therapy with a Class 1 or 3 antiarrhythmic agent
Paroxysmal: Surgical ablation is reasonable for patients undergoing surgery for other indications (IIa, C)
Persistent: Surgical ablation is reasonable for patients undergoing surgery for other indications (IIa, C)
Longstanding persistent: Surgical ablation may be considered for patients undergoing surgery for other indications (IIb, C)
Indications for standing alone surgical ablation of AF
Symptomatic AF refractory or intolerant to at least one Class 1 or 3 antiarrhythmic medication
Paroxysmal: Stand alone surgical ablation may be considered for patients who have not failed catheter ablation but prefer a surgical approach (IIb, C)
Paroxysmal: Stand alone surgical ablation may be considered for patients who have failed one or more attempts at catheter ablation (IIb, C)
Persistent: Stand alone surgical ablation may be considered for patients who have not failed catheter ablation but prefer a surgical approach (IIb, C)
Persistent: Stand alone surgical ablation may be considered for patients who have failed one or more attempts at catheter ablation (IIb, C)
Longstanding persistent: Stand alone surgical ablation may be considered for patients who have not failed catheter ablation but prefer a surgical approach (IIb, C)
Longstanding persistent: Stand alone surgical ablation may be considered for patients who have failed one or more attempts at catheter ablation (IIb, C)
Symptomatic AF prior to initiation of antiarrhythmic drug therapy with a Class 1 or 3 antiarrhythmic agent
Paroxysmal: Stand alone surgical ablation is not recommended (III, C)
Persistent: Stand alone surgical ablation is not recommended (III, C)
Longstanding persistent: Stand alone surgical ablation is not recommended (III, C)

AF: Atrial fibrillation.

Table 4 Adapted from the surgical treatment of atrial fibrillation guidelines by the European Association for Cardio-Thoracic Surgery Clinical Guidelines Committee guidelines

Use of ablative modalities
Unipolar radiofrequency ablation
Concomitant unipolar RFA for AF treatment together with cardiac surgery is effective in restoration of sinus rhythm
Success rates vary between 54%-83% at medium term follow up (at least 12 mo)
Safe procedure - no additional risks
Success rates are higher with: paroxysmal or persistent AF, younger age, smaller LAD
Class IIa recommendation based on multiple small retrospective studies (Level C)
Bipolar radiofrequency ablation
Higher success rates in restoring sinus rhythm compared to no ablation in concomitant cardiac surgery
On average the cross clamp time is increased by 15 min
There is limited evidence to suggest superiority of bipolar over unipolar RFA
1 prospective trial has provided evidence demonstrating superiority of bipolar RFA over microwave ablation
Class I recommendation based on 3 RCTs and multiple small prospective studies (Level A)
Cryoablation
Acceptable intervention for AF treatment during concomitant surgery with acceptable sinus rhythm conversion rates between 60%-82% at 12 mo
Cryoablation is most successful in patients suffering from paroxysmal as opposed to permanent AF (suggested by 6 out of 9 studies reviewed)
Class IIa recommendation based on 1 small RCT and multiple prospective and retrospective studies (Level B)
Microwave ablation
Less effective intervention for AF treatment based on the limited evidence
Success rates in the longer term are less clear - the only RCT to date has found outcomes inferior to RFA
Class III recommendation based on 1 small RCT and multiple small prospective and retrospective studies (Level B)
HIFU
Currently not recommended as an intervention for the treatment of AF during concomitant surgery outside clinical trials due to limited evidence
Success rates seem to be inferior to those of other devices
Significant concerns have been reported
Class III recommendation based on cohort studies (Level C)
Exclusion of laa and standing alone surgical ablation
Exclusion of LAA
No proven benefit of surgical LAA exclusion in terms of stroke reduction or mortality
Ineffective LAA occlusion and potentially increased stroke risk due to poor technique was seen in many studies
Devices designed for LAA exclusion should be preferentially used rather than a cut and sew or stapling technique, if LAA is to be performed
Class IIa recommendation based on multiple cohort studies and one pilot RCT (Level B)
Stand alone surgical ablation
Surgery can be considered for symptomatic patients who are refractory or intolerant to at least 1 anti-arrhythmic medication
Considered for patients with paroxysmal, long standing and persistent AF who prefer surgery to catheter ablation or have failed catheter ablation
Results of both catheter-based and surgery-based ablation should be discussed with the patient
Class IIa recommendations based on 1 RCT and multiple cohort studies (Level B)

RFA: Radiofrequency ablation; AF: Atrial fibrillation; LAD: Left anterior descending; RCT: Randomized controlled trial; HIFU: High intensity focused ultrasound; LAA: Left atrial appendage.

Table 5 Summary of results from studies included looking at Cox-Maze procedures

Procedure	Ref.	Sample size	Mean follow-up period	Outcome	Important findings
Cox-Maze	Cox *et al*[7]	178 patients	8.5 yr	93% freedom from AF	Cox-Maze procedure developed
Cox-Maze	McCarthy *et al*[16]	100 patients	3 yr	90.4% in sinus rhythm or atrial pacing	Associated with low perioperative and late morbidity rates
Cox-Maze	Schaff *et al*[17]	221 patients	6 yr	90% in sinus rhythm	CM procedure was useful in patients requiring valvuloplasty for mitral regurgitation
Modified Cox-Maze with bipolar RFA	Gaynor *et al*[18]	40 patients	6 mo	91% in sinus rhythm	Modification of CM-III shortened and simplified the procedure with no change in short-term efficacy

RFA: Radiofrequency ablation; AF: Atrial fibrillation.

Table 6 Summary of results from studies included looking at pulmonary vein isolation and left atrial appendage

Procedure	Ref.	Sample size	Mean follow-up period	Outcome	Important findiNGS
PVI	Haïssaguerre *et al*[9]	45 patients	8 ± 6 mo	Sinus rhythm achieved in 28 patients (62%)	69 foci identified as the source of ectopic atrial beats in 45 patients
PVI	Chao *et al*[21]	88 non-paroxysmal AF patients	36.8 mo	The long-term freedom period of AF was 28.4% after a single procedure	CHADS2 score of >/3 and left atrial diameter found to be significant predictors of recurrences
LAA obliteration	Healy *et al*[27]	RCT - 77 patients with risk factors for stroke	8 wk follow-up with trans-oesophageal echocardiography	Complete occlusion achieved in 45% (5/11) of patients through the use of sutures and in 72% (24/33) using a stapler	Surgical LAA can be safely done during a routine CABG; expertise is key to its success rates
LAA excision or exclusion	Kenderian *et al*[28]	137 patients	Post-operative trans-oesophageal echocardiography	Successful LAA closure 73% with surgical excision and 23% with suture exclusion. Evidence of stroke in 11% of successful LAA closure and 15% of unsuccessful LAA closure (P = 0.61)	High proportion of surgical LAA closure. LAA excision more successful than exclusion
LAA obliteration + Mitral valve replacement	García-Fernández *et al*[29]	58 patients	69.4 mo trans-oesophageal echocardiography	46% of patients had an embolism. Risk of embolism increased by 11.6 in incomplete/absence of LAA ligation	Absence of LAA ligation and presence of left atrial thrombus identified as independent predictors for stroke
LAA exclusion during mitral valve surgery	Almahameed *et al*[30]	136 patients	3.6 ± 1.3 yr	12.3% of patients had thromboembolic events, 71% of which occurred in patients undergoing mitral valve repair	There were more thromboembolic events in patients not prescribed warfarin on discharge

PVI: Pulmonary vein isolation; LAA: Left atrial appendage.

et al[63] looking at the safety of stopping anticoagulants following successful surgery for AF.

Looking at compiled data of 10 000 patient-years follow up, they concluded that discontinuation of warfarin following AF surgery is safe. An annual thromboembolic stroke rate of 0.3%-8% in patients who were discontinued off warfarin was seen in these studies, where warfarin was stopped after AF surgery at a mean of 3.6 mo (0-8 mo) after AF surgery. It is worth noting here that care needs to be taken when interpreting those results, since the scarcity of good quality RCTs means that their conclusion was mainly based on low quality evidence such as observational studies.

Stroke risk also varied according to the procedure performed. PVI performed as an isolated procedure, as well as being the procedure most extensively evaluated, was shown to have the lowest stroke risk off warfarin (0%-0.4% per annum). In contrast, concomitant cardiac surgery such as mitral valve repair was found to considerably increase the thromboembolic stroke rate. Thus, mitral valve surgery was found to be risk factor for late thromboembolic stroke in patients undergoing concomitant AF surgery.

In summary, the best evidence topic concluded that discontinuation of warfarin at 3 mo post-operatively would be feasible in selected patients, following consi-

Table 7 Summary of results from studies included looking at radiofrequency ablation

Procedure	Ref.	Sample size	Mean follow-up period	Outcome	Important findings
Concomitant RFA	Johansson *et al*[32]	39 patients undergoing CABG	32 ± 11 mo	62% freedom from AF in ablation group compared to 33% in non-ablation group	Sinus rhythm at 3 mo was highly predictive of long-term sinus rhythm
Concomitant RFA	Khargi *et al*[33]	128 patients in permanent AF (Group 1: mitral valve surgery, group 2: aortic valve surgery or CABG)	3, 6 and 12 mo ECG and sinus rhythm confirmed with 24hrs ECG	71% post-operative sinus rhythm in group 1 *vs* 79% in group 2	Concomitant RFA in mitral valve surgery and aortic valve surgery or CABG is equally effective
Concomitant RFA	Beukema *et al*[34]	258 patients with permanent AF	43.7 ± 25.9 mo	Sustained sinus rhythm in 69% of patients at 1 yr, 56% at 3 yr, 52% at 5 yr and 57% at the latest follow up	RF modified maze procedure abolished AF in the majority of patients
Concomitant RFA	Chiappini *et al*[35]	Review of 6 studies - 451 patients in total	13.8 ± 1.9 mo	97.1% overall survival rate, 76.3% ± 5.1% overall freedom from AF	RFA is a safe and efficient procedure to cure AF in patients undergoing concomitant heart surgery
Concomitant RFA	Von Opell *et al*[36]	49 patients with AF of more than 6 mo duration	At discharge, 3 and 12 mo post procedure	Return to sinus rhythm 29% 57% and 75% (at discharge, 3 mo and 12 mo post-procedure) in the cardioblate group *vs* 20%, 43% and 29% respectively in the control group	Concomitant RFA resulted in 75% conversion rate to sinus rhythm compared to the control group (39%)
Concomitant RFA	Budera *et al*[38]	Multicentre RCT involving 224 patients with AF undergoing cardiac surgery with (n = 117) or without ablation (n = 107)	30 d	At 1 yr follow up, 60.2% of patients were in sinus rhythm in the ablation group compared to 35.5% in the control group. 1 yr mortality was 16.2% and 17.4% respectively	Concomitant ablation increases postoperative sinus rhythm with no effect on peri-operative complications
Concomitant RFA	Blomström-Lundqvist *et al*[40]	Double-blind randomized study of 69 patients undergoing mitral valve surgery with or without epicardial left atrial cryoablation	6 and 12 mo	At 6 mo follow-up, 73.3% of patients in the cryoablation group regained sinus rhythm *vs* 45.7% of patients with mitral valve surgery alone (P = 0.024). At 12 mo follow-up, the results were 73.3% *vs* 42.9% respectively (P = 0.013)	Concomitant left atrial epicardial cardioablation is significantly better in regaining sinus rhythm in patients with permanent AF compared to mitral valve surgery alone
Concomitant RFA	Chevalier *et al*[61]	Prospective, multicentre, double-blinded RCT involving 43 patients with mitral valve disease and permanent AF	12 mo	At 12 mo, sinus rhythm was maintained without any arrhythmia recurrences in 57% of patients in the RFA group *vs* 4% in the control group (undergoing mitral valve surgery only)	Left atrial RFA is an effective procedure in patients suffering with long-term AF and co-existing valvular disease
Concomitant RFA	Veasey *et al*[62]	100 patients in paroxysmal or persistent AF undergoing cardiac surgery were enrolled	6 mo	75% freedom of AF at 6 mo follow-up post concomitant RFA. The AF burden decreased from 56.2% post-operatively to 27.5% at 6 mo post-operatively. 13% of patients had asymptomatic AF episodes identified *via* continuous monitoring	Concomitant RFA successfully reduces AF burden but based on these results, the importance of post-operative antiarrhythmic medication and anticoagulation should be evaluated

RFA: Radiofrequency ablation; AF: Atrial fibrillation; ECG: Electrocardiogram; CABG: Coronary artery bypass grafting.

deration of the patient's individual risk factor profile[63].

The above is in agreement with the 2012 HRS/EHRA/ESC Guidelines, which concluded that surgical intervention for AF is not recommended solely to discontinue warfarin or other anticoagulants. Furthermore, cessation of anticoagulants in patients post-ablation is not recommended if the stroke risk is high as measured on CHAD52. However, if a patient is not high risk on CHAD52 and has been in sinus rhythm for a significant continuous period they could change their warfarin to aspirin alone[14].

The EACTS clinical guidelines committee also recommend cessation of anticoagulants at 3 mo following established AF ablation procedures; provided that the patient is in sustained sinus rhythm and that their stroke-risk profile has been considered and deemed to be low[2].

POST-OPERATIVE FOLLOW UP

The STS workforce on evidence-based surgery published a document on reporting results from AF surgery. This included reporting regular interval ECG assessments and encouraging the widespread use of implantable recording devices to assess AF[64].

The STS workforce in accordance with 2012 HRS/EHRA/ESC Guidelines, recommend that the entrance and exit block should be reported and demonstrated intra-operatively[14,63]. Additionally, the 2012 HRS/EHRA/ESC Guidelines recommend follow-up of at least one

Table 8 Summary of results from studies included looking at high intensity focused ultrasound

Procedure	Ref.	Sample size	Mean follow-up period	Outcome	Important findings
HIFU	Neven *et al*[45]	Two-year follow-up of 28 people with paroxysmal AF 9 (n = 19) and persistent AF (n = 9) undergoing	Median follow-up 738 d	Following a median follow-up of 738 d, 79% of patients were free of AF. Following a repeat procedure with radiofrequency ablation, 18% of patients maintained freedom of AF	Success rates of HIFU are comparable to radiofrequency ablation but complication rates remain higher for HIFU
HIFU	Klinkenberg *et al*[47]	15 patients with AF refractory to antiarrhythmic medication underwent HIFU for PVI	24 mo	At 6 mo 40% of patients with 1 epicardial PVI gained sinus rhythm. After 1.3 ± 0.6 yr, 27% of patients had sinus rhythm after 1 epicardial pulmonary vein isolation	Success rate was low in epicardial pulmonary vein isolation done through right-sided VATS using HIFU and was associated with substantial complications
HIFU	Schmidt *et al*[48]	22 patients with paroxysmal AF who underwent PVI using HIFU	median follow-up of 342 d	71% of patients remained free of any AF/AT recurrence without antiarrhythmic drugs after a procedure	The 12F-HIFU induces a very rapid pulmonary venous isolation in patients

HIFU: High intensity focused ultrasound; PVI: Pulmonary vein isolation; LAA: Left atrial appendage.

Table 9 Summary of results from studies included looking at the hybrid approach

Procedure	Ref.	Sample size	Mean follow-up period	Outcome	Important findings
Hybrid approach	Kuman *et al*[55]	A cohort of 7 patients with AF undergoing a hybrid procedure	Follow-up at 3, 6, 9 and 12 mo post-procedure	After a follow-up of 40 ± 3 mo, 6 out of 7 patients were in sinus rhythm	The hybrid approach is a safe and feasible technique to AF ablation
Hybrid approach	Bulava *et al*[56]	50 consecutive patients with long-standing AF who underwent the procedure	Follow-up at 3, 6, 9 and 12 mo post-procedure and thereafter after every 6 mo	94% of patients were in sinus rhythm, 12 mo after the procedure No arrhythmias were present in any patient after 12 mo	The hybrid approach is extremely effective in maintaining sinus rhythm compared to radiofrequency catheter ablation or surgical ablation alone
Hybrid approach *vs* Cox-Maze *vs* epicardial ablation	Je *et al*[57]	Systematic review of 37 studies with a total of 1877 patients	12 mo	Operative mortality for the Cox-Maze, epicardial ablation and hybrid approach were 0%, 0.5% and 0.9% At 12 mo, rates of sinus rhythm restoration for the above were 93%, 80% and 70% respectively	The Cox-Maze procedure with cardiopulmonary bypass revealed the highest success rate 12 mo post-procedure compared to the hybrid approach and epicardial approach

AF: Atrial fibrillation.

year with a minimum of 24-72 h holter monitoring, trans-telephonic monitoring, 30 d auto event triggered monitoring or outpatient telemetry[2,14].

The EACTS Guidelines recommend routine testing of entrance and exit block after AF surgery to establish creation of effective lesion sets. They also recommend following the 2012 HRS/EHRA/ESC Guidelines for reporting results of surgery and in publications[2].

CURRENT GUIDELINES IN SURGICAL MANAGEMENT OF AF

The 2012 HRS/EHRA/ESC Guidelines have summarised the indications for surgical interventions of AF. Their statements were in accordance with their 2006 guidelines and their subsequent updates. The guidelines include the indications for concomitant surgical ablation of AF (Table 3) and the use of ablative modalities in each AF category (Table 4)[14].

LIMITATIONS AND FUTURE CONSIDERATIONS

The surgical management of AF is a rapidly evolving field with multiple studies exploring the various surgical options available. The current literature includes a few RCTs as well as several prospective, retrospective and cohort studies. When looking at these studies, there are certain limitations that need to be considered.

First, there is a variable definition of "success" when reporting results. This is often vaguely described as "freedom from AF" though not being further defined. Conversely, a few studies report "success" as patients being in sinus rhythm at follow up. Standardisation on reporting the outcomes of studies would be invaluable when looking at results from different studies. This

would enable accurate comparisons in order to draw reliable conclusions. Guidelines aiding standardisation of results reporting have been published by STS[64] and following them has been recommended by EACTS and HRS/EHRA/ECAS[2,14].

Secondly, there is vast heterogeneity regarding the follow up periods used in each study. Follow up periods ranging from 1 mo to 5 years have been seen across different studies. The lack of a standardised follow up interval makes it very difficult to reliably compare results. The STS workforce has published a document regarding the reporting of results and recommended follow up periods in surgery for AF[64].

When looking at the current literature it is evident that an array of lesion sets is being used when performing AF surgery. An explanation for this can be the move towards minimally invasive surgery attempting to yield good results, whilst using less invasive techniques. Additionally, it is widely accepted that the CM-Ⅲ procedure has a limited use due to its technically challenging nature. This has led to the development of other lesion sets that are less invasive and easier to perform. It would be worthwhile establishing some common lesion sets that could be used in studies. Currently, the EACTS recommended using the terminology "Maze" procedure, "PVI" and "LAA" when describing the different lesion sets[2]. This would eliminate the bias of using slightly different lesion sets in each study and enable the results of the studies to be compared accurately.

Furthermore, there is limited data available on the comparison of different energy sources in AF ablation. Several studies have looked at the use of individual ablative modalities for AF treatment during concomitant cardiac surgery[59-62]. However, very few studies compared the ablative modalities to each other[42,43]. Future studies performing this comparison would be vital, as evidence is needed in order to identify the ablation modalities with the highest success rates and least complications.

Looking at the patient population recruited in the studies, there is a large variation between AF types in the patients included. A few studies have included a population with a single AF type such as permanent AF and looked at ablation modalities in that population[33,34,36,50]. More studies looking at patient populations with specific AF types would be useful in finding out which ablation modality works better for each AF type.

Additionally, the hybrid approach is a promising new procedure that could potentially improve success rates in the surgical management of AF. Over the recent years an increasing number of studies have been looking at this new approach[53-57]. More studies exploring the hybrid approach are needed in order to obtain reliable results as to whether the hybrid procedure should be clinically recommended for the surgical management of AF, as well as identify the best combination of modalities to be used in this procedure.

Finally, an important consideration for future studies is the big gap in literature when looking for RCTs, in order to obtain reliable results and be able to make good clinical recommendations. There is definitely a need for more, well-conducted prospective RCTs looking the various ablative modalities[65].

CONCLUSION

In conclusion, AF is a public health burden associated with substantial morbidity and mortality. Surgical management of AF is currently recommended in paroxysmal or persistent AF during concomitant heart surgery. Stand alone surgical ablation for AF can be considered with caution in patients who are intolerant or refractory to antiarrhythmic medication. Several studies have produced promising results using the new ablative modalities, which emerged over the last few years. Nevertheless, there is still a requirement for additional high quality RCTs in order to be able to make reliable evidence-based recommendations regarding the surgical management of AF.

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REVIEW

Ventricular repolarization measures for arrhythmic risk stratification

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Abstract

Ventricular repolarization is a complex electrical phenomenon which represents a crucial stage in electrical cardiac activity. It is expressed on the surface electrocardiogram by the interval between the start of the QRS complex and the end of the T wave or U wave (QT). Several physiological, pathological and iatrogenic factors can influence ventricular repolarization. It has been demonstrated that small perturbations in this process can be a potential trigger of malignant arrhythmias, therefore the analysis of ventricular repolarization represents an interesting tool to implement risk stratification of arrhythmic events in different clinical settings. The aim of this review is to critically revise the traditional methods of static analysis of ventricular repolarization as well as those for dynamic evaluation, their prognostic significance and the possible application in daily clinical practice.

Key words: Ventricular repolarization; Arrhythmias; QT interval; Cardiovascular diseases; Drugs

Core tip: The analysis of the role of ventricular repolarization perturbations as potential triggers of malignant arrhythmias has increasingly gained interest, particularly as a potential tool for the risk stratification of arrhythmic events in different clinical settings. Several measures of ventricular repolarization have been developed and tested in clinical practice. This review critically revises the traditional methods of static analysis as well as those for dynamic evaluation, their prognostic significance and the possible application in daily clinical practice.

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INTRODUCTION

The electrocardiogram (ECG) is widely used in clinical practice for the diagnosis of cardiac arrhythmias, conduction disturbance, structural changes of the myocardium, myocardial ischemia, drug effects, and electrolyte and metabolic disorders. The ECG waveforms are the expression of transmembrane action potentials (APs) of atrial and ventricular myocytes[1].

ECG recording in a normal cardiac cycle is composed of two basic processes: Depolarization and repolarization. Ventricular depolarization and activation is represented by the QRS complex, whereas ventricular repolarization (VR) is expressed as the interval from the beginning of the QRS complex to the end of the T wave (QT interval). VR is a complex electrical phenomenon which has been studied in detail[2,3].

It is a crucial step in cardiac electrical activity consisting of a recovery period with the return of the ions to their previous resting state, which corresponds with the relaxation of the myocardial muscle, thus setting the stage for the next depolarization and contraction. On the surface ECG, VR is made up of the J-wave, ST-segments, and T and U waves[1]. Over the years previous studies have emphasized the role of VR alterations in predisposing to lethal arrhythmias[4,5] and thus the analysis of VR has increasingly gained interest, particularly as a potential tool for the risk stratification of arrhythmic events[6,7], integrating other well-established parameters[8].

This review aims to critically revise the available static and dynamic methods of VR analysis and their application in clinical practice.

PATHOPHYSIOLOGICAL BACKGROUND OF VENTRICULAR REPOLARIZATION MEASURES

VR measures have been proposed to stratify arrhythmic risk due to their ability to reflect abnormalities in cardiac electrical activity predisposing to the occurrence of malignant arrhythmias. Cardiac structural and electrical alterations may cause abnormalities in APs, and in the refractory period and conduction velocities of adjacent myocardial areas, thus leading to spatial heterogeneity and temporal fluctuations in repolarization and favoring the onset of arrhythmias[9,10]. Furthermore, it has been demonstrated that autonomic nervous system (ANS) activity can interact with structural heart diseases, affecting the VR and promoting the onset of arrhythmias. Moreover, the modulation of VR by ANS is not limited to its influence on sinus node regulation and on heart rate (HR). In fact, there is a direct regulation by ANS on APs through the regulation of the activity of ion channels[11]. Furthermore, the effects of vagal and sympathetic systems should not be considered singularly. Sympathovagal interactions are fundamental in regulating heart function and conditions which alter the sympathovagal balance, facilitate cardiovascular instability and can lead to arrhythmias[8].

Finally, several drugs have been showed to affect VR and prolong the QT interval, inducing alterations which can predispose to ventricular arrhythmias[12,13]. From these considerations, the importance of the analysis of VR and the QT interval in the evaluation of arrhythmic risk, drug effects and liability of the ventricular arrhythmias is clear (Figure 1). Since the earliest demonstrations that a prolonged corrected QT interval (QTc) is associated with an increased risk of ventricular arrhythmias and sudden cardiac death (SCD), in patients with myocardial infarction[14], as well as in patients taking QT-prolonging drugs[15], interest in the assessment of QT prolongation grown. At the same time, the role of QTc prolongation as a marker for arrhythmic risk is controversial[16] and measures of QT dispersion at ECG did not always demonstrate the ability to predict the risk of arrhythmic events[17]. Thus, as an alternative to "static evaluation" of the QT interval, different measures of QT variability and dynamicity have been proposed, better representing the complex interactions between arrhythmic substrate, HR and ANS activity and offering a more complete assessment of VR and estimation of arrhythmic risk[18].

MEASUREMENT OF QT INTERVAL

The measurement of the QT interval represents the traditional approach to the analysis of VR.

Methodological aspects

The QT interval is calculated as the distance between the first deflection of the QRS and the end of the T wave on the surface ECG. Measurement of the QT interval by surface ECG can be performed either manually or automatically[11,19-22].

In the manual measurement, the end of the T wave is determined as the intersection between the tangent to the steepest down-slope of the T wave and the isoelectric line[22], but this method can be time-consuming and is liable to inter-reader variability. Recommendations concerning QT measurement have not proposed a single defined lead (lead Ⅱ or the lead with the largest T wave) or a mean QT derived from an arbitrary subset of leads. As consequence of this lack of a systematic approach, there is a variation in sensitivity and specificity for single lead measurement of the QT interval in predicting the risk of major arrhythmic events. The interlead QT variation associated with the observation that in healthy individuals the longest QT interval is most frequent in leads V2 to V5 and that the QT interval is

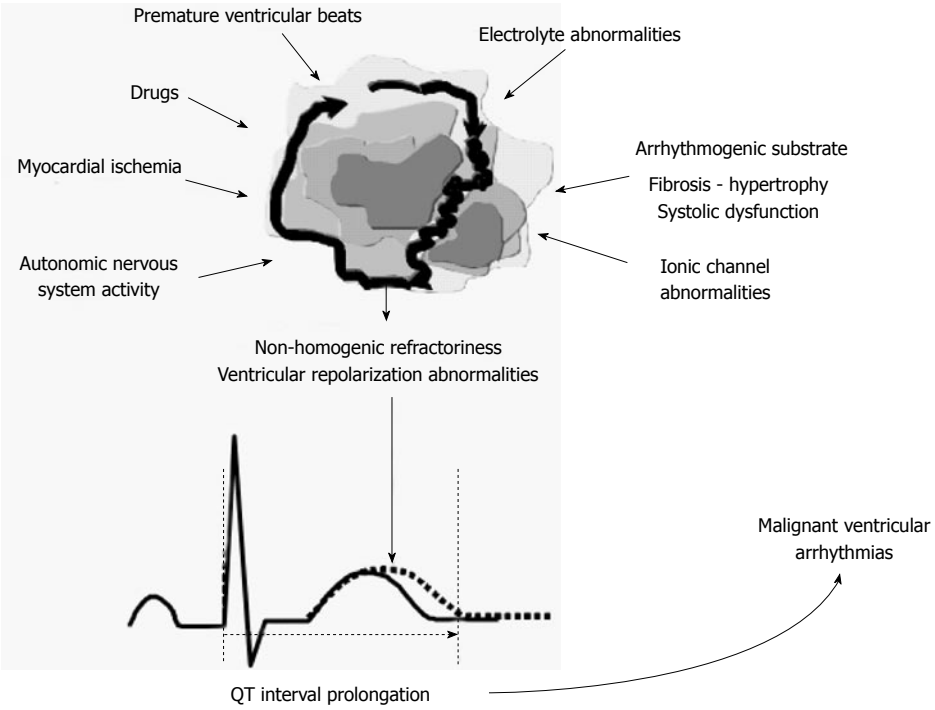

Figure 1 Structural and functional cardiac abnormalities and interaction with the other factors are able to induce non-homogenic refractoriness and abnormalities in ventricular repolarization leading to QT interval prolongation.

not closely related to T wave height makes it difficult assessing ventricular recovery by measuring a single QT interval[23-26]. The measurement of the QT dispersion addresses this challenge as subsequently explained. It is widely recognized that the typical measurement of the QT interval is subject to different interpretations due to different factors, such as autonomic tone, electrolytes imbalance, technical aspects, variations in T wave morphology, presence of U waves, and noisy baseline. The absence of agreement among experts and of a standardized measure of the QT interval contributes to interobserver variability[27,28].

The main problems regarding the automated measurement are related to the T wave morphology (flat, bifid, biphasic) and to the presence or absence of the U wave. Consequently, the measurement of the QT interval requires a lot of experience and a good interpretation of the ECG signal[29]. In addition, some of the automated QTc interval monitoring strategies are labor-intensive, and dependent on expensive technology[30] and experts disagree on the utility and efficacy of automated readings when compared with careful manual measurements[31]. The other relevant aspect in the measurement of the QT interval is its dependence on HR, the main physiological factor influencing VR. The QT interval is inversely correlated with HR prolonging at a slower HR and shortening at faster one. In order to minimise the influence of this factor on the measurement, it is essential to make a correction of the QT interval for HR (QTc). Different formulas have been developed in order to adjust QT for HR. Those most commonly used are Fridericia's formula: QTc: $QTxRR^{-1/3}$[32], and Bazett's formula: QTc: $QTxRR^{-1/2}$[33], where RR means the time interval and QT means the distance. However different opinions exist as to the best and most useful correction for HR and the evidences remain unclear and contrasting[34-36]. The normal range of QTc has been assessed by Straus *et al*[37] who established the gender-specific variability of QTc measurement. The authors subdivided the Bazett-corrected QTc interval into gender-specific groupings and further subdivided the QTc interval into normal, borderline, and prolonged categories.

Considering the lack of standardization and recommendations for the best method to measure QT, a broad of experts proposed guidelines for measuring the QT interval[31].

The expert group from the American Heart Association (AHA) and American College of Cardiology Foundation (ACCF) recommends that a QT_c over the 99th percentile should be considered abnormally prolonged. Approximate 99th percentile QT_c values for otherwise healthy postpubertal individuals are 470 ms for males and 480 ms for females[38].

Clinical implications

Marked prolongation of the QT interval is a well-established pro-arrhythmic risk factor in the general population[39] in patients with coronary artery disease[40], hypertrophic cardiomyopathy (HCM)[41], or heart failure (HF)[42] and in patients taking QT-prolonging drugs[43]. However, the clinical usefulness of QT measurement has mainly been demonstrated in both congenital[43] and acquired long QT syndrome (LQTS)[44].

Congenital LQTS

The congenital LQTS was described for the first time in 1957 and since then remarkable efforts have been made to define its pathogenesis, diagnosis and treatment[45]. Genetic studies have shown that LQTS is caused by pathogenic mutations in 15 genes encoding cardiac ion channels or membrane adaptors and thus different LQTS genotypes have been identified. Pathogenic mutations identified in the *KCNQ1* and *KCNH2* genes as well as in the sodium channel, encoded by SCN5A, are responsible for nearly 80% of all clinically diagnosed cases. All other genes together account for less than 5% of LQTS cases[46]. LQTS 1, 2 and 3 are the most common genotypes, representing 80%-90% of the total cases of inherited LQTS[47,48]. Due to limitations of space and the different purposes of this article, our focus is mainly on the diagnostic criteria of the congenital LQTS. However, details about specific triggers, clinical presentation, prevalence and genetic aspects, risk stratification and therapeutic advances are available in the literature[49-53]. As regards the diagnosis of LQTS, a scoring system, updated in 2011, and including symptoms, family history and ECG findings was proposed by Schwartz *et al*[54]. In 2013 an expert consensus, incorporating the Schwartz score, drafted the following recommendation for the diagnosis of LQTS[50]. Congenital LQTS is diagnosed when the LQTS risk score is $\geqslant$ 3.5, in the absence of a secondary cause for QT prolongation and/or in the presence of an unequivocally pathogenic mutation in one of the LQTS genes, or it can be diagnosed in the presence of a corrected QT interval for HR using Bazett's formula (QTc) $\geqslant$ 500 ms in repeated 12- lead ECG and in the absence of a secondary cause for QT prolongation. Moreover LQTS can be diagnosed in the presence of a QTc between 480 and 499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic mutation[50].

Short QT syndrome

Short-QT syndrome (SQTS) is a clinical entity originally described by Gussak *et al*[55] in 2000 as an inherited syndrome in a family with paroxysmal atrial fibrillation and constantly shortened QT intervals.

Subsequently, a high familial risk for sudden cardiac death associated with a short QT interval was demonstrated by Gaita *et al*[56] in the members of a family with a history of syncope or palpitations, as well as one case of sudden death resuscitation. A strong family history of sudden death, present in 4 generations, was detected. The family members had a very short QT interval at ECG, which never exceeded 280 and 290 ms when corrected for HR with Bazett's formula (QTc). The authors provided a definition of SQTS as characterized by familial sudden death, short refractory periods, and inducible ventricular fibrillation. The definition of SQTS became a possible diagnosis for yet unexplained SCD in patients without structural heart disease. Details of possible pathophysiological mechanisms, related genetic mutations and other possible explanations, besides the well-known channelopathy, are available in the literature[57-59].

Acquired long QT syndrome

In daily clinical practice, one of the most frequent application of QTc measurement is the monitoring of drug-induced QTc prolongation, the aim of which is to mitigate the risk of Torsade de Pointes (TdP) and other ventricular arrhythmias and prevent SCD. Several drugs have been shown to induce perturbation of VR and prolongation of QT interval, increasing the risk of ventricular arrhythmias such as TdP and SCD. There has been increased research into the mechanisms involved in drug-induced LQTS in patients in treatment with these drugs. Those generally involved in acquired LQTS are cardiac drugs such as class ⅠA and Ⅲ antiarrhythmic drugs, while the non-cardiac ones include antibiotics, antihistamines, antidepressants, antipsychotic and methadone. An updated list of drugs that can potentially cause QT prolongation is available on the Internet where they are classified into the following four categories: (1) drugs with known risk of TdP; (2) drugs with possible risk of TdP; (3) drugs with conditional risk of TdP; and (4) drugs to avoid in congenital LQTS (https://www.crediblemeds.org/new-drug-list/). Medications that cause QT prolongation generally act by blocking Ikr, a potassium-channel protein that regulates an important rapid delayed repolarizing current in phase 3 of the cardiac AP. This protein is encoded by the human ether-a-go-related gene (HERG), mutated in the LQT2 form of the congenital syndrome[60]. The blocking of Ikr causes a lenghthening of AP in cardiac myocytes and QT interval, thus increasing the risk of ventricular arrhythmias and SCD[16,61]. However many drugs blocking the Ikr current do not seem to cause TdP (*e.g.,* amiodarone). Other mechanisms involved in drug-induced LQTS have been described and concern the loss of K channels (*e.g.,* fluoxetine) and an increased inward sodium current, such as that produced by cisapride and antimony[62-65]. However, drug-induced LQTS is not easy to predict in any given individual and the same medication does not exhibit the same pro-arrhythmic effects in different individuals[16]. Indeed, some individuals taking QT prolonging drugs, may develop QTc prolongation with or without TdP, or may not experience QTc prolongation, while several authors have also demonstrated genetic predisposition to acquired LQTS[61,66]. However, only a small proportion of patients taking QT prolonging drugs experience TdP and, at the same time, TdP can develop in patients with a normal QTc interval[27]. Additional risk factors causing QT prolongation and favoring drug-induced VR alterations and TdP have been identified. They include hypokalemia, drug-drug interactions, the female gender, advancing age, genetic predisposition, hypomagnesemia, heart failure, bradycardia, and baseline QTc interval prolongation. Many risk factors are potentially modifiable and should be corrected in

those patients at risk for QT interval prolongation. It has been reported that all patients with TdP secondary to non-cardiac drugs have established risk factors. Most patients manifestating drug-induced LQTS have at least one identifiable risk factor in addition to drug exposure[13,27,67]. Female gender is the most common risk factor[68]. Age has also been detected as a risk factor for QT prolongation, particularly when combined with additional risk factors, such as female gender, personal or family history of pre-syncope or syncope, electrolyte disturbances or cardiovascular disease[69]. Impaired glucose tolerance and diabetes were observed to be associated with an increased likelihood of prolonged QTc independent of age, race, gender, education, and heart rate. In addition, persons with diabetes and multiple cardiovascular disease risk factors were more likely to have a prolonged QTc than those with Normal Glucose Tolerance and no additional risk factors, suggesting that this group may be at increased risk for cardiac arrhythmia and sudden death[70]. Hypokaliemia is a very common QT-prolonging factor and is often detected in patients with drug-induced QT prolongation and TdP and some proposals have been made as to the different mechanisms that may potentially be involved[61,71]. Other metabolic conditions correlated with QT-prolongation are hypothyroidism, hypocalcemia and hypomagnesemia as well as hepatic diseases[72,73]. The "five-year cross-sectional ECG screening Outcome in psychiatry study" revealed that patients with drug-induced LQTS had a significantly higher frequency of hypokaliemia, hepatitis C virus infection and abnormal T wave morphology. The drugs mostly involved in QT prolongation were haloperidol, clotiapine, phenothiazines and citalopram[74]. Another recognized risk factor is represented by the polytherapy and the concomitant use of medications known to prolong QT[13,75]. A prospective observation study carried out in the United States demonstrated the high prevalence of QTc prolongation in patients admitted to cardiac units, with 28% of patients presenting with QTc prolongation (defined as $\geqslant$ 470 ms in males and $\geqslant$ 480 ms in females) and 18.2% with a QTc > 500 ms at admission. The study revealed that of 251 patients admitted with QTc interval prolongation, 87 (34.7%) were subsequently administered QT interval-prolonging drugs, and 166 of the patients admitted with QTc interval > 500 ms, 70 (42.2%) were subsequently administered QT interval-prolonging drugs. Moreover additional QTc interval prolongation $\geqslant$ 60 ms occurred in 57.1% of these patients[76]. The authors also suggested that hospitalized patients are more prone to develop QTc prolongation due to the presence of other risk factors such as heart disease, electrolyte imbalance, and advanced age associated with the administration of QTc prolonging drugs. The statement from the AHA/ACCF underlined the importance of collecting the medical history and the risk factors of each single hospitalized patient in order identify those at higher risk and minimize the possibility of QTc prolongation and TdP. According to the AHA's practice standards for ECG monitoring in hospital settings, the indications for QT-interval monitoring include the following: (1) initiation of a drug known to cause TdP; (2) overdose from potentially proarrhythmic agents; (3) new-onset bradyarrhythmias; and (4) severe hypokalemia or hypomagnesemia. Due to the lack of clarity regarding the types and amounts of drugs taken in an intentional overdose situation, it is prudent to monitor QT intervals in all overdose victims[38]. To achieve a long-term reduction in the risk of drug-induced QTc prolongation and TdP in hospitalized patients, a risk score has been developed and validated. The authors suggested that, by using easily obtainable risk factors, the risk score can identify patients at highest risk for in-hospital QTc prolongation and, thus, could be incorporated into clinical decision support system to guide monitoring and treatment decisions[30].

Acquired LQTS and antipsychotic: An unclear scenario

Among the drugs known to cause QT-prolongation, antipsychotic agents are probably the most studied. Since the development and marketing of the first molecules, a close relation has been found with QTc prolongation and an increased risk of major arrhythmic events such as TdP[77,78]. Antipsychotic drugs are widely used in the treatment of schizophrenia, mood disorders and somatic symptoms.

Conventional or first generation antipsychotics have been widely recognized as being associated with an increased risk of cardiac arrhythmias, TdP and SCD[79].

Since the early 1960s, sudden unexpected deaths with antipsychotic use have been reported[77].

Among the first generation antipsychotics, the described association between thioridazine and haloperidol with TdP or SCD led to the withdrawal of thioridazine from the market by its producer in 2005 and to the use of label warnings by the Food and Drug Administration for intravenous haloperidol[80,81]. However, the rate of ventricular arrhythmia, sudden death, and unexplained or unattended death did not appear to increase with the dosage for either thioridazine or haloperidol, suggesting that low-doses of these drugs have similar cardiac safety[82]. As in the case of thioridazine and haloperidol, data on the safety profile and possible link of other first generation antipsychotics with severe arrhythmias are contradictory[83-85]. When first introduced, atypical or second generation antipsychotics were considered to be safer than first generation antipsychotics as regards the QTc prolongation and arrhythmic risk[84,86,87]. However these promising data were not univocally confirmed[88]. Among the second generation antipsychotics available, lurasidone and aripiprazole seem to have minimal effect on QTc interval[89-91]. It should be noted that patients with schizophrenia are more likely to experience SCD and QTc prolongation than individuals from the general population because of treatment-related metabolic disorders and autonomic dysfunction linked to the

underlying psychiatric illness[92,93]. Given all of these evidence, and considering the contradictory and inconclusive data available, it is easy to imagine how difficult the assessment of the individual risk in patients taking antipsychotic agents must be. In fact, drug-induced LQTS is unpredictable in any given individual. Because of all the possible medical complications associated with antipsychotics, Nachimuthu *et al*[62] and Shulman *et al*[94] highlighted the importance of a multidisciplinary approach which takes into account pre-existing heart or other diseases, personal or family history of ventricular arrhythmias or syncope, metabolic and endocrine disorders such as hypothyroidism and hypokalemia in order to reduce the risk of adverse events. Some authors recommend performing an ECG before and shortly after initiation of treatment with an antipsychotic drug in order to screen for existing or emergent prolongation of the QT interval[95]. The majority of authors recommend a baseline ECG for patients with personal or family history of cardiovascular disease or signs of arrhythmias, such as syncope, and for those patients taking another agent known to prolong QTc[96]. De Hert *et al*[97] performed a systematic review of the practice guidelines for the screening and monitoring of cardiometabolic risk in patients with schizophrenia and related psychotic disorders using the Appraisal of Guidelines for Research and Evaluation. The authors concluded that four European guidelines can be recommended for clinical use in daily clinical practice and proposed a monitoring protocol to manage cardiovascular disease risk. After a careful research of the literature, Trinkley *et al*[13] concluded that it is necessary to perform a careful and systematic monitoring of ECG and electrolytes, after the initiation of QT-interval-prolonging drugs. Where there are risk factors for QTc prolongation, patients should be trained to go to the emergency room in case of palpitations, lightheadedness, dizziness or syncope. When the QTc interval is 470-500 ms for males, or 480-500 ms for females, or the QTc interval increases by 60 ms or more from pretreatment values, dose reduction or discontinuation of the offending drug should be considered if possible, and electrolytes corrected as needed. Furthermore, if the QTc interval is $\geqslant$ 500 ms, the drug should be discontinued, and continuous ECG telemetry monitoring should be performed, or a 12-lead ECG should be repeated every 2-4 h, until the QT interval has normalized[13]. The American Psychiatric Association practice guidelines report that an absolute QTc interval > 500 ms or an increase of 60 ms from baseline requires dosage reduction or discontinuation of the agent. Serum potassium levels and an ECG should be obtained before initiating treatment with thioridazine, mesoridazine and pimozide and in the presence of cardiac risk factors, known heart disease, personal history of syncope, a family history of sudden death under 40 years age or LQTS before treatment with ziprazidone. An ECG should be performed again after a significant change in the dose of thioridazine, mesoridazine and pimozide or in the presence of cardiac risk factors, ziprasidone, or following the addition of another QT-prolonging medication[98,99]. Based on a relevant literature research, Shah *et al*[100] recently provided clinical practice guidelines for monitoring QTc intervals in patients being treated with antipsychotics. The authors reported that antipsychotics can be prescribed without pre-treatment ECG and without ECG monitoring, with the exception of patients with increased cardiac risk, antipsychotics with known risk of TdP and SCD, patients with an overdose of antipsychotic agents. In these cases ECG monitoring is recommended. If a pre-treatment ECG indicates a prolonged QTc, the ECG should be repeated and risk factors assessed and it is advisable to use lusaridone, which is the agent with minimal QTc prolongation. If QTc prolongation is assessed during antipsychotic treatment, the ECG should be repeated and monitored in case of continuous QTc prolongation in association with evaluation and correction of serum electrolytes. If a patient being treated complains of syncope or palpitations, discontinuation of the antipsychotic can be considered.

Prognostic significance in cardiovascular disease

As already outlined, several cardiovascular diseases are associated to QTc prolongation. Those known to predispose to QT-prolongation are HF, cardiac arrhythmias, bradycardia, myocardial ischemia, and HCM[40-42]. Prolonged QTc reflects cardiac repolarization prolongation and/or increased repolarization inhomogenity known to be associated with an increased risk of arrhythmias[101].

QTc prolongation has been widely observed in patients with myocardial infarction and it has been suggested as one of the earliest ECG abnormalities in transmural ischemia[102] and a prognostic marker of arrhythmic events[103].

Thus, for some time, the assessment of QTc interval has been considered an interesting tool in the evaluation of the arrhythmic risk[104,105] improving the accuracy of the personalized cardiovascular prognosis when associated to conventional risk models for cardiovascular diseases[106].

However, data available in literature concerning the role of QTc prolongation in the risk assessment are not univocal. The QTc interval did not seem a useful prognostic tool after acute myocardial infarction[107] and reduced left ventricular ejection fraction (LVEF) and frequent ventricular premature complexes were found to be the most important factors for predicting subsequent SCD after acute myocardial infarction[108].

Similarly in patients with HF, baseline QTc interval within normal limits seems to be associated with a marked reduction in mortality, suggesting its possible usefulness in identifying patients who might benefit from prophylactic treatment with antiarrhythmic drugs[109]. Prolonged QTc interval has been found to be a strong, independent predictor of adverse outcomes in patients with advanced HF with BNP levels > 400 pg/mL[110].

At the same time, prolonged QRS interval, but not prolonged QTc interval, was observed to be associated with increased long-term mortality in patients with acute decompensated HF[111].

Moreover, the role of QTc prolongation as a marker of arrhythmic risk is not widely confirmed, sometimes judged "imperfect", and nowadays its role remains controversial[112,113].

Despite the large number of studies on the evaluation of QTc in cardiovascular diseases, data about its utility are not consistent and the measurement of QTc interval in daily clinical practice is not widely carried out. Based on this background, other strategies to assess abnormalities of VR in cardiovascular diseases have been proposed.

QT DISPERSION

The "simple" measurement of QT interval cannot always permit a complete assessment of the arrhythmic risk. The analysis of the QT dispersion seems to more accurately represent the non-uniform prolongation of APs and heterogeneity of the duration of the refractory periods and the conduction velocities of adjacent myocardial areas, thus better analyzing the perturbation of VR.

Various experimental studies have highlighted the close relationship between the dispersion of the myocardial repolarization and the development of ventricular arrhythmias[114-116].

A non-invasive method to highlight the inhomogeneity of myocardial repolarization time has been proposed, *i.e.*, the measurement of the variability of the QT interval duration in the different leads of the standard 12-lead ECG[117,118].

Methodological aspects

QT dispersion (QTd) is the measurement of the variability of the QT interval on the 12-lead surface ECG, defined as the difference between the maximum QT and minimum QT calculated[119]. This phenomenon was described by Campbell *et al*[24] who demonstrated small but significant differences between the QT intervals of different leads measured by a digitizer program. The normal value of QTd is less than 50-70 ms. The standard deviation of repeat measurements is approximately 6 ms when an experienced observer measures from recordings made at 50 mm/s and 10 mV/cm. When the digitizer is used the normal rate-corrected values for QTd are between 20 ms and 50 ms with values after infarction rising to 60-100 ms and to as high as 150-200 ms in patients with LQTS[26].

There are more discrepancies about the determination of the end of the T wave, the lead group to be used, and the use of manual or automatic measurements. Surely, the greatest difficulties in the assessment of electrocardiographic VR are based essentially on the lack of universally accepted criteria for defining the end of the T wave. In particular, Kautzner *et al*[119] concluded that in the absence of more objective criteria for the separation of the T wave and U wave, measuring the QT dispersion appears to be unstable and its statistical validity is disputable.

New measurements have gradually been added to the traditional measurement of QT dispersion, among which are the following: (1) QTd in the precordial leads: The measurement is carried out only in the precordial leads, considered closer to the heart and, therefore, more reliable; (2) QTc-d in the 12 leads and in the precordial leads: The measurement is performed using the values of QTc (Bazett's formula)[120]; (3) QT "adjusted": The value of QT dispersion is adjusted to the number of leads on which the calculation is made (QTd/numbers of leads); (4) and QT "relative" and QTc "relative": Respectively, the standard deviation of the QT/QT average x 100 ratio and the standard deviation of the QTc/QTc average x 100 ratio on the 12-lead ECG.

Clinical implications and prognostic significance

The electrophysiological mechanisms through which the dispersion of ventricular repolarization may induce ventricular arrhythmias are different. Kuo *et al*[116] identified at least three: The formation of an ectopic focus; the creation of a reentry circuit facilitated by conduction from an area with long refractoriness to an area with short refractoriness; and the creation of a reentry circuit facilitated by an area with short refractoriness to an area with prolonged refractoriness. The increase in the QTd has been associated to vulnerability in the development of ventricular tachycardia (VT), particularly in patients with previous myocardial infarction[121]. A cross sectional American study compared 100 patients with coronary artery disease and a history of arrhythmia events with 70 patients with previous myocardial infarction. QTd was measured in all cases.

It was observed that QTc and QTd were consistently higher in patients with susceptibility to episodes of sustained and unsustained VT as well as in the post infarction patients[122].

The mechanism underlying the origin of VT in post infarction has been recognized to be a mechanism of reentry[122-125]. The role of increased dispersion of repolarization in the genesis of ventricular fibrillation has also been generally accepted[126] and infarct scar and reentrant circuits are known to be substrates in the pathogenesis of sustained monomorphic VT[127]. Strong evidence supports the hypothesis that dispersion of refractoriness and repolarization provides a pathophysiologic basis for reentry[128,129]. Furthermore, QTd has been shown to reflect the dispersion of recovery times and repolarization. Thus, increased QTd suggests the presence of a substrate for ventricular tachy-arrhythmias, more realistically by a reentry mechanism[117,118,121,122]. Changes in QTd evaluated in a population of patients after percuteneous coronary intervention were predictors of long-term cardiac mortality, confirming how a defective QTd recovery suggests the

persistence of repolarization inhomogeneities[130].

QT DYNAMICITY AND QT VARIABILITY

Measures of QT variability and dynamicity have been proposed as an alternative to the "static" evaluation of VR and seem to offer a more complete picture of the complexity of VR components.

Methodological aspects

Several kinds of softwares have been developed in order to dynamically analyse the QT interval and QTd from 24-h Holter recordings. Compared to the ECG static evaluation, dynamic assessment of VR allows the analysis of the relationship between the duration of the QT interval and HR changes and the effects of the ANS on both these elements. Moreover, through the measurement of the variability of VR duration and not its duration in absolute terms, this kind of analysis allows technical difficulties related to the exact definition of the T wave end to be reduced.

Dynamic behaviour of repolarization may translate in beat-to beat changes in repolarization duration and morphology[18,131].

QT-RR relationship and QT variability reflect an increased vulnerability of the myocardium and changes in autonomic HR control, which are conditions related to the increased risk of SCD[132]. The automatic assessment of VR dynamicity is based on the measure of: QT apex (QTa): The interval between the Q wave start and the T wave apex. The T-wave apex is identified by interpolation of a parabola with the peak of the T-wave.

QT end (QTe): The interval between the Q wave start and the T wave end, T-wave end is determined by the intersection of the tangent of the downslope of the T-wave with the isoelectric baseline[7]. It has been shown that the QTa is more influenced by HR changes than QTe. The interval between the apex and the end of the T wave is rate independent and is probably influenced by the ANS and by activity of M cells that seem to be the cells involved in arrhythmogenesis[133,134].

QT dynamicity is generally assessed by 24 h ECG recordings which are analyzed by a specific software able to automatically calculate, in a template of 30 s the QTa, the QTe and the correspondent RR interval. By interpolating each measure obtained the software also computes the slopes of the linear regressions between QTe and QTa and the corresponding RR interval (QTe/RR and QTa/RR). A steeper slope reflects a greater variation of QT interval for changes in RR intervals (Figure 2).

QT-RR slope, *i.e.,* the slope of the regression line between QT end and RR during a 24 h period, is considered an index of QT dynamicity related to arrhythmic events.

The automatic measure of VR by ELA system was validated by Copie *et al*[135] in a study that did not show significant differences between this kind of measurement and the manual measurement.

The QT-RR slope is highly individual. Generally the QT-RR slope is steeper in women than men and has higher values during the day than at night due to the ANS influence[136]. A steeper QTe slope indicates that the QT interval is more prolonged with longer RR intervals and shortens more with shorter RR intervals. The steeper the QTe-slope, the greater the arrhythmic risk[18]. Another parameter of QT dynamicity, QT/RR variability ratio, was proposed by Jensen *et al*[137]. This is the ratio between the standard deviation of all QT intervals and the standard deviation of all RR intervals. Whereas QT dynamicity is based on the analysis of QT/RR relationship, "QT variability" is based on the analysis of beat to beat changes in duration and the morphology of VR. HR and ANS can influence QT variability, but do not entirely explain beat to beat repolarization changes. These can depend on fluctuations in ion channel activity and number[18]. QT variability can be measured during a short-term (256 s or 30 beats) or 24-h period, distinguishing into short term and long term variability. Its measurement is not standardized, so several algorithms have been developed in order to quantify it. In 1997, Berger *et al*[9] proposed a first semiautomated algorithm able to measure temporal beat-to-beat lability of repolarization. In this instance, the operator selects the start and the end of the QT interval of one beat and the algorithm by stretching or compressing the JT segment identifies the QT interval of all other beats. Moreover, Berger *et al*[9] developed an index, called the QT variability index (QTVI), that is the log ratio between the QT interval and HR variability, both normalized by their mean values.

Most studies use this method in order to evaluate QT variability, but other algorithms have been proposed.

Burattini *et al*[138] proposed a time domain method which is able to quantify beat-to-beat variability of repolarization morphology without the need to exactly define the T wave end.

Starc *et al*[139] proposed a fully automated time-shifting algorithm that estimates the QT interval constructing separate QRS and T wave templates and shifting them in time. This algorithm has been shown to be the best method in order to measure QT variability[140]. On the basis of these considerations the assessment of QT dynamicity and/or variability allows a more complete evaluation of ventricular repolarization because they reflect the interaction between the arrhythmic substrate, the increased vulnerability of the myocardium, HR and ANS activity.

Prognostic role of QT dynamicity and variability

The increased QT variability and dynamicity probably build up repolarization heterogeneity inducing the onset of arrhythmias. Therefore a number of studies have investigated the role of an increased QT dynamicity and variability as predictor of arrhythmic events in different clinical settings. QT dynamicity was demonstrated to be able to predict major arrhythmic events in patients with both idiopathic and ischemic dilated cardiomyopathy,

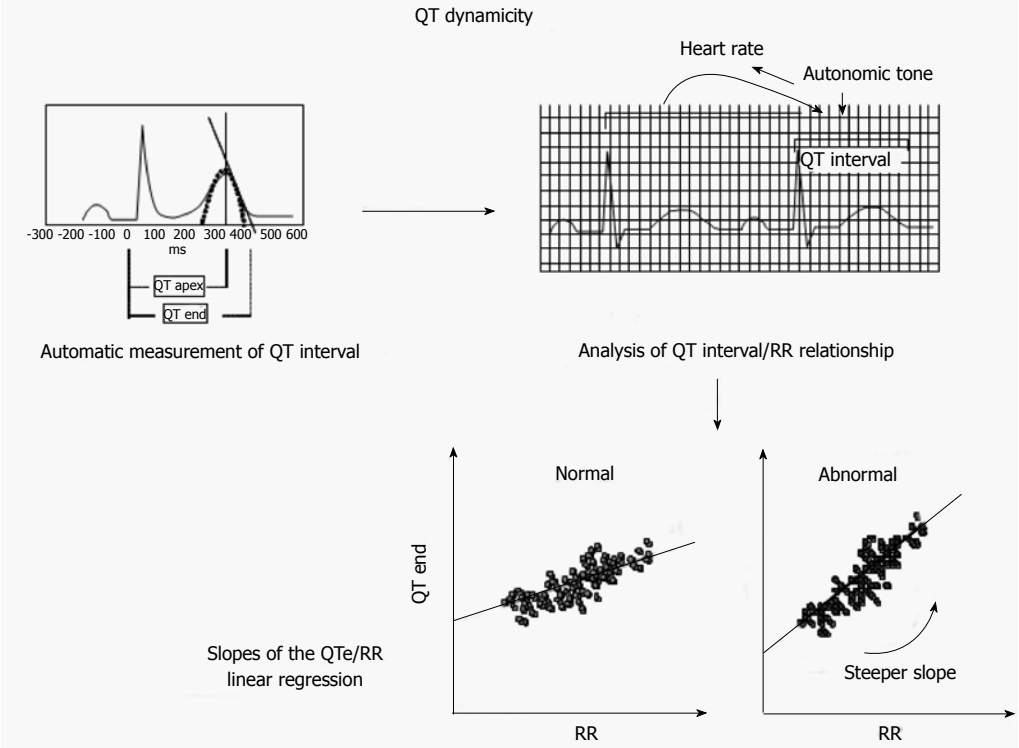

Figure 2 QT dynamicity analysis is based on automatic measurement of QT interval (QT apex and QT end) during 24 h electrocardiogram monitoring. The relationship between these measures and the corresponding RR intervals allows a slope of regression analysis to be obtained. A steeper slope reflects a wider QT variability suggesting abnormalities in ventricular repolarization.

improving the accuracy in stratifying the arrhythmic risk of these patients. Our group evaluated the role of the QT slope as a predictor of a greater risk of ventricular arrhythmias in a population of patients affected by non-ischemic dilated cardiomyopathy. At univariate analysis, QTe-slope, LVEF, non-sustained VT, and standard deviation of RR intervals (SDNN) were the variables significantly related to major arrhythmic events. At multivariate analysis only the QTe-slope, LVEF and non-sustained VT remained significantly associated with these events, independently of SDNN, a QRS duration > 120 ms or beta-blocker therapy. Combining LVEF (< 35% *vs* > 35%), non-sustained VT and QTe-slope (> 0.19 *vs* < 0.19), patients with non-sustained VT and LVEF < 35% and patients with LVEF < 35% and a steeper QTe slope presented a greater risk of arrhythmic events than patients with a higher LVEF and non-sustained VT or steeper QTe slope. Considering these variables together, the population of patients with low LVEF and presence of non-sustained VT and a QTe slope > 0.19 represented the subgroup with the highest probability of arrhythmic events[7]. Chevalier *et al*[141] showed the association between a steeper QTe slope and a greater arrhythmic risk in a population of patients with ischemic cardiomiopathy. They found that QTe slope was a more powerful independent predictor of sudden death than LVEF, HR variability and late potentials in these patients. Another parameter of QT dynamicity, QT/RR variability ratio has been shown by Jensen *et al*[137] to be related to the sudden arrhythmic death risk in a population of post-acute myocardial infarction patients. Moreover, QTe slope was shown to be important in arrhythmic risk stratification also in patients with HF, regardless of etiology, both with relatively preserved LVEF (> 35%)[142] and with reduced LVEF[143]. Recently Quinteiro *et al*[144] evaluated the role of QTe slope in arrhythmic risk stratification in a small population of patients with HCM. They found that these patients presented an impaired QT dynamicity and QT slope was helpful in order to identify high risk patients. Several studies have also demonstrated the prognostic role of "beat to beat" QT variability. Berger *et al*[9] showed that there was an increased repolarization variability in a group of patients with ischemic and non-ischemic dilated cardiomyopathy, compared with control subjects, regardless of HR. Subsequent studies confirmed the increased repolarization variability in ischemic and non-ischemic cardiomyopathy and in different clinical settings, including acute myocardial ischemia, left ventricular hypertrophy, HCM, left ventricular dysfunction and LQTS[145]. Atiga *et al*[146] demonstrated the relation between an increased QTVI and arrhythmic risk in a population of patients presenting for electrophysiologic study. The QTVI had greater values in patients with ischemic or non-ischemic heart disease than in healthy subjects, and among patients with cardiac disease those with SCD had the highest values. A QTVI $\geqslant$ 0.1 was a predictor of a higher risk of arrhythmias. Therefore the authors concluded that this index identified patients with SCD, and predicted arrhythmia-free survival. The same group also investigated the prognostic role of QT variability

in a population of patients with HCM, concluding that patients with HCM presented the increased repolarization variability and had a greater risk of SCD. The highest QTVI values were found in patients with b-MHC gene mutation, a mutation associated with a worse prognosis[147]. In a substudy of the MADIT trial, Haigney *et al*[6] have shown that, in a wide population of postinfarction patients with a low LVEF, increased QT variability was associated to the occurrence of malignant ventricular arrhythmias (VT/ventricular fibrillation). Piccirillo *et al*[148] also evaluated the role of QT variability in a population of patients with ischemic cardiomyopathy but with a LVEF between 35 % and 40% and in NHYA class Ⅰ. A QTVI greater than or equal to the 80th percentile identified a high risk of SCD, therefore this parameter might be useful to stratify the risk of sudden death in this population of patients who do not currently meet the criteria for ICD implantation for primary prevention. Tereshchenko *et al*[149] investigated the role of QT variability in a population of patients with cardiac structural disease who had undergone ICD implantation for primary or secondary prevention of SCD. This study confirmed the prognostic utility of the increased repolarization variability, able to predict the malignant arrhythmic events risk in this population of patients. Nevertheless, the same group more recently investigated the prognostic role of QT variability in a population of patients with chronic HF (NYHA class Ⅱ-Ⅲ), both with preserved and reduced LVEF and found that an increased QTVI was a predictor of cardiovascular mortality, but not of SCD, regardless of LVEF. The hypothesis of the authors was that in these patients the elevated QTVI was due to depressed HR variability, a predictor of cardiovascular mortality, and not to an increased QT beat to beat variability[150]. In a heterogeneous population of patients with mild to moderate HF, the role of QTVI in predicting increased cardiovascular mortality has been investigated. The authors observed that QTVI, as an expression of increased repolarization liability, is a marker of increased risk of cardiovascular mortality[151]. Finally, a very recent study on a population of patients with ischemic dilated cardiomyopathy, both with reduced and with preserved LVEF, confirmed the utility of QT variability in order to identify the patients with a higher risk of SCD[152]. Therefore increased QT/RR slopes and an increased QT variability can reflect a greater vulnerability of the myocardium and predispose the development of malignant arrhythmias. Thus the dynamic analysis of VR represents an interesting tool to improve the accuracy in stratifying the risk of arrhythmic events.

MICROVOLT T WAVE ALTERNANS

Definition and prognostic role

Electrical alternans is defined as a change of the amplitude of the waves of the ECG which manifests itself at alternate heartbeats. It was described for the first time by Hering[153] in 1908. Much of the interest in the alternans phenomenon has focused on alternans occurring during the repolarization phase of the cardiac APs, also known as repolarization alternans and in particular in microvolt level beat-to-beat alternation in T-wave morphology: The microvolt T wave alternans (MTWA). That has been recognized as being a strong predictor of ventricular arrhythmias, as assessed in a variety of clinical and experimental studies[154-157]. There are two main theories that explain this mechanism. The first refers to a spatial dispersion of refractoriness which results in changes in the impulse propagation and repolarization. In this case the alternation of repolarization would be secondary to the alternation in the propagation of the impulse. This would occur when the refractory period of the cell is shorter than the time between two successive activations. It would block one-way and re-entry. This hypothesis was supported by an experimental study. According to the second hypothesis MTWA would be caused by an alternation in repolarization of the APs resulting in a secondary propagation of alternans APs[158-160].

Methodological aspects

The analysis is based on the alignment of ECG cycles to the QRS complex and on the measurement of T-wave amplitude. The beat-to-beat fluctuations of the T-wave are then analyzed using fast Fourier transformation and MTWA is represented by the pronounced peak visible in the spectrum at 0.5 cycles/beat. The MTWA is considered significant when the alternans voltage exceeds a threshold (usually 1.9 microV) and if the alternans ratio K is $\geqslant$ 3. In general, we can judge as positive an alternans lasting more than 1 min, at a HR $\leqslant$ 110 beats/min[161]. Because of the strong correlation between the phenomenon of MWTA and the occurrence of ventricular re-entrant arrhythmias, its identification was proposed as a test to stratify the risk of ventricular arrhythmias[162,163].

The phenomenon is dependent on the HR. The treadmill test, a non-invasive and inexpensive test, is generally used for recognition. However, there are discordant opinions on the fact that a negative MTWA test can really identify a group of patients at extremely low risk of SCD or cardiac arrest[164-167].

Prognostic significance

Hohnloser *et al*[168] found that MWTA is predictive of major ventricular arrhythmias with a very low event rate among patients with a negative MWTA. In another meta-analysis, a positive MTWA was associated with a 2.5-fold higher risk of cardiac death and severe arrhythmias in both ischemic and non-ischemic cardiomyopathy[169]. The Alternans Before Cardioverter Defibrillator (ABCD) trial demonstrated the role of MWTA in guiding the ICD implantation for primary prevention in patients with LVEF $\leqslant$ 40%, coronary artery disease and non-sustained VT[170]. The analysis of data from five studies involving 2883 patients without ICD demonstrated that in patients with a LVEF >

35%, a positive MWTA identified those more prone to experience major arrhythmic events and SCD[171].

Despite the evidence suggesting a possible role of MWTA in predicting the arrhythmic risk, discordant data are available in the literature. Gupta *et al*[172] demonstrated that spectrally derived MTWA testing has limitations in its feasibility and is not specific enough to evaluate the arrhythmic risk and to guide strategic clinical decisions. In a population of patients with HF, Kraaier *et al*[173] observed that MTWA testing could be carried out only in a half of the population, and often resulted indeterminate. The authors concluded that MTWA treadmill testing is not suitable to stratify the risk of SCD in patients with HF.

CONCLUSION

There is much evidence that underlines the role of VR alterations in predisposing to lethal arrhythmias and the analysis of VR has become an interesting tool to refine the risk stratification of arrhythmic events. Different cardiac structural and electrical alterations may cause abnormalities in APs and in refractory period leading to perturbations of repolarization that can favor the onset of severe ventricular arrhythmias. Several measures of ventricular repolarization have been suggested with the aim of stratifying the arrhythmic risk allowing identification of those patients more prone to experiencing major arrhythmic events.

The measure of QT interval has gained growing interest since the first description of congenital LQTS and SQTS. Moreover, several factors such as drugs, advanced age, female sex, personal medical history, metabolic and electrolyte disorders can cause QTc prolongations and predispose to arrhythmic events. However, despite the vast number of studies evaluating the role of prolonged QTc as a marker of arrhythmic risk, opinions on the utility of QTc measurement in daily clinical practice are not univocal.

In order to overcome some of the limitations of QTc assessment, other strategies evaluating VR have been proposed. The analysis of the QTd seems to more accurately represent the non-uniform prolongation of APs and heterogeneity of the duration of the refractory periods and the conduction velocities of adjacent myocardial areas, allowing a better analysis of VR. The role of QTd analysis as an arrhythmic risk predictor has been well-established, but this evaluation of VR has not always been effective in identifying patients at higher risk of arrhythmic events. Moreover there are no recommended cut-off values for QTd.

Therefore, dynamic measures of VR, such as QT dynamicity and variability, reflecting the dynamic behaviour of VR, were developed as prognostic markers of arrhythmic risk. In addition, MWTA, another ECG parameter expression of alternans during VR, has been shown to predict arrhythmic events, although there are limitations in its feasibility. However there are no recommendations for the routine use of these parameters.

In conclusion, several measure of VR have been proposed but, despite the first studies concerning VR being nearly outdated, their role as a predictor of ventricular arrhythmias is not always clear and definite recommendations on their use in different clinical settings and for each single patient are still lacking and unfocused.

More attention should be paid to collecting a complete medical history of the patient and detecting the presence of well-established risk factors, cardiovascular conditions and medications known to cause QTc prolongation.

In the absence of one-size-fits all approach to the risk stratification of arrhythmic events, it is desirable to combine different measures of VR with other predictors in each specific clinical setting.

Further robust and tailored studies are required to settle the existing issues and provide useful prognostic tools for clinician.

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Cardiovascular drugs in the treatment of infantile hemangioma

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Abstract

Since the introduction of propranolol in the treatment of complicated infantile hemangiomas (IH) in 2008, other different beta-blockers, including timolol, acetabutolol, nadolol and atenolol, have been successfully used for the same purpose. Various hypotheses including vasoconstriction, inhibition of angiogenesis and the induction of apoptosis in proliferating endothelial cells have been advanced as the potential beta-blocker-induced effect on the accelerated IH involution, although the exact mechanism of action of beta-blockers remains unknown. This has generated an extraordinary interest in IH research and has led to the discovery of the role of the renin-angiotensin system (RAS) in the biology of IH, providing a plausible explanation for the beta-blocker induced effect on IH involution and the development of new potential indications for RAS drugs such as angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers in the treatment of IH. This review is focused on the current use of cardiovascular drugs in the treatment of IH.

Key words: Infantile hemangioma; Beta-blockers; Renin-angiotensin system; Angiogenesis

Core tip: This article aimed to review the different beta-blockers used in the treatment of children with infantile hemangioma, the pre-treatment cardiologic work-up required and the potential side-effects associated with beta-blockers therapy in such a young population. Other cardiovascular drugs with potential effects on infantile hemangioma including angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers are also reviewed.

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INTRODUCTION

Infantile hemangiomas (IH) are not only the most common vascular tumors in children, but also the most common soft-tissue tumors in this population, occurring in 5% to 10% of infants[1,2]. Evolution of IH is characterized by a proliferation phase, stabilization, and a progressive spontaneous involution in the first 2-10 years of age[3]. Only 10%-15% of IHs results in complications requiring treatment[4]. Beta-blockers, especially propranolol, have emerged as first-line therapy and have dramatically changed the therapeutic approach for complicated IH, leaving systemic glucocorticoids as the second-line therapy. After the first publication in 2008[5] about the efficacy of propranolol in IH, more than 500 reports in the medical literature have supported its use as first-line therapy[6-10]. The largest, randomized, placebo controlled trial involving patients with complicated IHs treated for up to 24 wk with a pediatric oral propranolol solution has been recently published[6]. Other pharmacological agents including angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin-receptor blockers (ARBs) implicated in the renin-angiotensin system (RAS) have been tested for IH[11-13] (Table 1). This article aimed to review the current indications for beta-blockers in IH, the potential effects of RAS drugs in the treatment of IH and their role as antiangiogenic agents.

BETA-BLOCKERS

Beta adrenergic receptor blockers are a class of medications which exerts their action by blocking B1 and/or B2 adrenergic receptors that exist throughout the body. B1 are primarily represented in the myocardium and kidneys while B2 receptors are the predominant beta receptor in the extracardiac vasculature, skeletal muscle and lungs. Multiple forms of beta-blockers exist including B1 selective and nonselective beta-blockade. Beta-blockers cardiac function is by partially activating the beta receptors and thus not allowing norepinephrine or epinephrine to bind to the receptor. This causes a decreased amount of G stimulatory protein activation leading to decreased intracellular cyclic adenosine monophosphate (cAMP) which then decreases phosphorylation by protein kinase A. This in the myocardium leads to decreased heart rate and contractility. In the vasculature, B2 receptors are also coupled to G stimulatory proteins which when stimulated by norepinephrine or epinephrine lead to increased cAMP and intracellular calcium loading which yields smooth muscle relaxation. B2 antagonism therefore is associated with a small degree of vasoconstriction in many vascular beds. Beta-blockers have also been found to decrease vascular endothelial growth factor (VEGF) as well as bFGF through unknown mechanisms[14]. B antagonism has also been shown to decrease the renin formation in renal cells as cAMP signaling subsequent to B receptor activation is critical for basal expression of vessel associated renin[15].

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

ACEIs function by preventing the angiotensin-converting enzyme (ACE) from cleaving angiotensin Ⅰ (AT Ⅰ) to create AT Ⅱ. Normally, renin produced in the kidney as a result of sympathetic stimulation, hypotension or decreased sodium delivery to the nephrons cleaves the protein angiotensinogen to form AT Ⅰ which is then converted by ACE to AT Ⅱ. AT Ⅱ then binds to AT I-receptors in smooth muscle and cause vasoconstriction. AT Ⅱ also causes release of norepinephrine as well as preventing the reuptake of norepinephrine in sympathetic nerves. In addition, AT Ⅱ causes an increase in the circulating aldosterone levels. By blocking these mechanisms, ACEIs therefore cause vasodilation of the vasculature with a resultant decrease in cardiac preload and afterload as well as decreasing the systemic blood pressure. ACEIs also down-regulate the sympathetic tone by preventing the release of norepinephrine in the sympathetic nerves. ACEIs additionally increase naturesis by helping to decrease aldosterone levels[11,12,16].

ANGIOTENSIN-RECEPTOR BLOCKERS

ARBs also work on the renin-angiotensin-aldosterone pathways but by a competitive antagonism of AT Ⅱ binding to the AT Ⅰ receptors. This results in the same decrease in vascular tone, sympathetic/norepinephrine release and aldosterone release. ARBs have also been shown to block transforming growth factor beta which is known to have angioproliferative properties[13,17].

BETA-BLOCKERS AND ANGIOGENESIS

Since the serendipitous discovery of the use of propranolol in the treatment of complicated IHs in 2008[5], an interest in the role of beta-blockers in hypoxia-induced angiogenesis has been raised. Other conditions, including retinopathy of prematurity (ROP) and cancer, are also characterized by the presence of hypoxia-induced angiogenesis and a potential role for beta-blockers has been advocated[18]. Most IHs do not present a premonitory mark and they become apparent 1-3 wk after birth. A blanched area, a telangiectatic patch or a bruiselike lesion may be seen as a premonitory mark in the early neonatal period in some patients. A rapid growth of IH is normally seen in the first 3-4 mo after birth, followed by a slow growth or stable phase until the age of 1 year. Spontaneous involution occurs over the next several years[2,4]. However, the growth pattern differs from each patient and lesion. Deep IHs often appear later and continue to grow for a longer time than superficial IHs. The origin of IH remains unknown, but some authors support the hypothesis that IH

Table 1 List of publications on the role of beta-blockers, angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers in infantile hemangioma

Ref.	Study type	*n*	Median age (range)	CV drugs	Conclusions
Léauté-Labrèze *et al*[6]	Prospective, clinical trial	456	103.8 ± 31.0 d	Propranolol	Propranolol was effective at a dose of 3 mg/kg per day for 6 mo
Abarzua-Araya *et al*[48]	Randomized double-blind controlled trial	23	5.2 ± 3.5 mo (2-14 mo)	Atenolol *vs* Propranolol	Atenolol appears to be as effective as propranolol
Léauté-Labrèze *et al*[10]	Randomized double-blind controlled trial	14	12.5 wk	Propranolol	Propranolol may be given very early in infants with IH, to stop IH growth and thus prevent disabling scarring
Blanchet *et al*[47]	Case series	4	2 mo (1.5-3 mo)	Acebutolol	Acebutolol seems to present advantages for use in treating subglottic hemangiomas
Bauman *et al*[43]	Randomized investigator-blind controlled trial	19	2 wk-6 mo	Propranolol *vs* Prednisolona	Both medications show similar efficacy. Propranolol should be the first line of therapy for symptomatic IH unless contraindicated or unless future studies demonstrate severe adverse effects
Chan *et al*[45]	Randomized controlled trial	41	2.5 mo (5-24 mo)	Timolol	Topical timolol maleate 0.5% gel with a maximum dose of 0.5 mg per day is a safe and effective option for small superficial IHs that have not ulcerated and are not on mucosal surfaces
Pope *et al*[46]	Cohort- blinded study	19	4.5 mo (1-92 mo)	Nadolol *vs* Propranolol	Patients with proliferative IH, treated with oral nadolol for 6 mo, experienced almost complete involution of their tumor, which was significantly different from patients treated with propranolol
Tan *et al*[11]	Open-labelled observational trial	8	12.9 wk (5-22 wk)	Captopril	The response of IH to an ACEI supports a critical role for the RAS proteins in IH
Cristou *et al*[12]	Retrospective case series	17	7.5 mo (4.5-15 mo)	Captopril	The striking improvement observed with propranolol has not been replicated with captopril. ACEI is not involved in IH involution and the mechanism of action
Itinteang *et al*[13]	Basic research-*In vitro*	6	6 mo (4-8 mo)	Ramipril Losartan	Findings suggest a key regulatory role of AT I and AT II in promoting cellular proliferation in IH, and establish a role for ACEIs and ARBs in the proliferation of IH

ACEI: angiotensin-converting enzyme inhibitors; ARBs: Angiotensin-receptor blockers; AT I: Angiotensin I; IH: Infantile hemangioma.

may actually be a response to local tissue hypoxia, a homeostatic attempt to revascularize relatively hypoxic tissue with new blood vessels[19-23]. A well-known risk factor for IH is placental insufficiency and this might be the cause of prenatal hypoxia that triggers the angiogenesis process and the development of IH[24,25]. Glucose transporter, type 1 (GLUT-1), a glucose transporter in the erythrocyte membrane, is recognized as a specific immunohistochemical marker for IH and an important sensor of hypoxia. GLUT-1 is present in IH during proliferation and involution phases and it has been recently demonstrated to be upregulated within hypoxic zones of mesenchymal tumors[26,27]. Another condition associated with hypoxia is the ROP, which is known to be related to an initial microvascular ischemic insult followed by abnormal hypoxia-induced neovascularization[28,29]. Both, IH and ROP, have a higher incidence and severity in neonates with lower gestational age and birth weight. Interestingly, both conditions occur in the early neonatal period and most of them resolve spontaneously without sequelae. VEGF is overexpressed in response to hypoxia and ischemia. Both, IH and ROP, have an overexpression of VEGF. Particularly in the retina, VEGF induces a pathological blood vessel formation at the junction between the vascularized retina and the avascular zone of the retina, also into the vitreous[30]. Anti-VEGF drugs, including bevacizumab and ranibizumab, have showed similar efficacy in the regression of ROP[31,32]. Kong *et al*[33] measured serum levels of bevacizumab and VEGF in premature infants with ROP and treated with intravitreal injection of bevacizumab and they concluded that clearance of bevacizumab from the bloodstream takes at least 2 mo in this age population. Because VEGF is crucial in the fetal organogenesis, concern about the anti-VEGF effect mediated by bevacizumab in premature infants exists. Beta-blockers have demonstrated to have a safer pharmacological profile in the pediatric population. Ristori *et al*[34] published the first demonstration that beta-blockers were protective against retinal angiogenesis in an animal model. Filippi *et al*[35] evaluated the safety and efficacy of oral propranolol administration in preterm newborns affected by an early phase of ROP and they concluded that propranolol counteracts the progression of ROP with a high incidence of adverse effects. The preterm infant with IH may not be the appropriate candidate for systemic propranolol use and instead a topical beta-blocker may be an alternative selection in this specific population. Topical ocular instillation of propranolol has

shown to be safe in animal models and on-going studies will define its role in ROP[36]. For periocular IH, timolol, another beta-antagonist, has also been reported to be effective and may be a viable alternative to systemic propranolol therapy[37].

New anti-cancer agents are being developed in response to tumor chemoresistance and severe side effects of standard chemotherapeutic agents. Several drugs, including beta-blockers, ACEIs and ARBs, were originally approved for indications other than malignancies treatment, but recent investigations support their role as cytostatic agents[38]. Adrenergic activation may play a role in carcinogenesis and tumor progression by promoting production of VEGF. Expression of beta-adrenergic receptors has been demonstrated in several tumor types, including colon cancer, hepatocellular carcinoma, lung adenocarcinoma, prostate cancer and breast cancer[39]. Recent studies have suggested that angiotensin and beta-adrenergic blockade may modulate the development and progression of cancer. Engineer *et al*[40] evaluated 262 patients with colon cancer who were exposed to ACEIs, ARBs and beta-blockers and they concluded that exposure to a combination of beta-blockers and ACEIs/ARBs is associated with decreased tumor progression, decreased hospitalizations, and increased survival in patients with advanced colorectal cancer.

BETA-BLOCKERS AND INFANTILE HEMANGIOMA

The use of beta-blockers for the treatment of IH was serendipitously discovered when a patient with a large infantile hemangioma was treated for cardiomyopathy with propranolol which prompted a change in color, softening and decrease in size of the lesion in 2008[5]. The group from France then treated 10 other patients with propranolol resulting in similar decrease in size of the IH. There have been multiple retrospective, single-institution case series reporting the benefit of propranolol in treating IH. A meta-analysis from 2013 reviewed 1162 publications and included 41 studies in the analysis comparing corticosteroids and propranolol for the treatment of cutaneous IHs[41]. Sixteen reported the outcomes from corticosteroid use in 2629 patients and 25 examined propranolol use in 795 patients with a pooled response rate of 69% for corticosteroids and 97% for propranolol (P = 0.001).

There have been three randomized controlled trials addressing propranolol use in a few different manners. The first was a small study, which randomized 40 patients to propranolol at 2 mg/kg (divided three times daily) or placebo for 6 mo[42]. Propranolol halted growth after 4 wk of use and decreased volume, color and elevation when compared to placebo. Major side effects such as hypoglycemia, hypotension and bradycardia were not reported. The next randomized trial evaluated the difference between treatment with corticosteroids and propranolol in 19 patients at 3 vascular anomalies centers[43]. Treatment occurred until toxicities developed or clinical response was achieved. The corticosteroid group had quicker decrease in size of the lesion but also had more frequent severe adverse events limiting the length of treatment. No difference in response rate to the medications of the IH was found after 4 mo of treatment though all 11 patients had discontinued the steroids due to toxicity. A third randomized trial explored the possible additive effect corticosteroids and propranolol[44]. Thirty patients were randomized to one of three groups: Propranolol (2-3 mg/kg per day), prednisolone (1-4 mg/kg per day) or combination therapy all for 3 mo. The group treated with propranolol had superior results to the prednisolone group and similar results to the combination therapy. Again, most patients treated with prednisolone stopped taking the drug early due to adverse events. The largest and most recent randomized trial examined the effect of propranolol at different doses and lengths of treatment[6]. In 456 patients, the optimal dosing was identified at 3 mg/kg per day for 6 mo with a response rate of 60% *vs* 4% for placebo. Response was defined as complete or near complete resolution of the lesion at 24 wk of treatment. After 5 wk of treatment, 88% of patients in the higher propranolol dosing group had a response to the medication. The known adverse events of hypoglycemia, hypotension, bradycardia and bronchospasm were infrequent and equivalent in both groups.

Other beta-blockers, including timolol, acetabutolol, nadolol and atenolol, have been successfully used in the treatment of IH. Topical treatment with timolol maleate gel has also been well studied with a randomized controlled trial published in 2013[45]. Forty patients with superficial hemangiomas without ulceration or mucosal involvement were randomized to topical timolol gel 0.5% (twice daily) *vs* placebo. The treated group had smaller than expected lesions and improved color at 24 wk of treatment though minimal differences were identified at earlier time points. No adverse events were discerned in the treatment group.

Since propranolol is a lipophilic nonselective beta-blocker that crosses the blood-brain barrier, sleep disturbances have been associated with its use, being less frequent with hydrophilic drugs such as atenolol and nadolol. Some investigators have highlighted the importance of the beta-adrenergic system in memory modulation and the potential long-term memory loss of children with prolonged propranolol use. A pilot, cohort study by Pope *et al*[46] compared 10 patients in the nadolol group *vs* 9 historic controls in the propranolol group, matched on age and sex. The nadolol group had a superior response at 4, 12 and 24 wk assessments, decreasing sleep disturbances and potential concerns about long-term memory loss. The difference in response may be related to the longer half-life of nadolol, which may increase compliance and steady

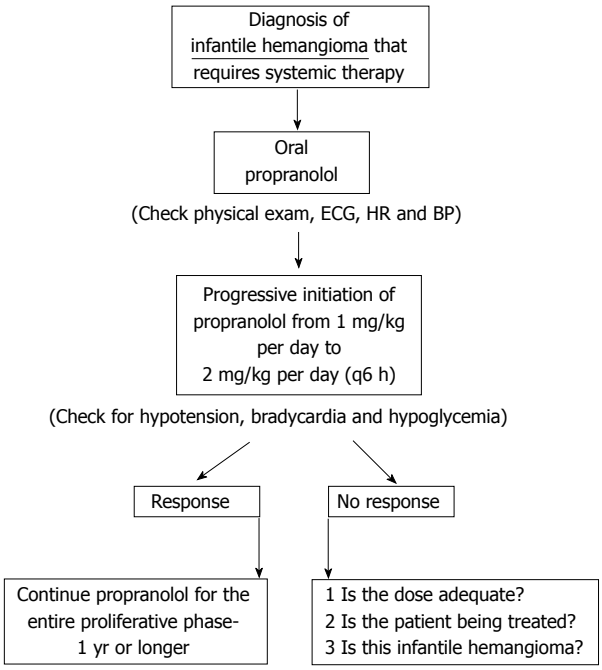

Figure 1 Therapeutic algorithm for oral propranolol treatment in infantile hemangioma. BP: Blood pressure; ECG: Electrocardiogram; HR: Heart rate.

state effect of the drug. Blanchet *et al*[47] reported good results in 3 cases of subglottic hemangioma treated with acebutolol, a cardioselective beta-blocker. Acebutolol should theoretically have less adverse effects than propranolol due to it cardioselectivity, but more studies are necessary to compare the efficacy of this agent. Atenolol is a selective, cardiac beta-blockers and may decrease possible respiratory side effects. A small study, which randomized 23 patients to treatment with atenolol or propranolol revealed equivalent response rates, 54% *vs* 60%[48]. Prospective clinical trials are required to better define the role of each beta-antagonist and the agent selection according to the patient characteristics and the type of lesion.

Cardiology evaluation prior to starting propranolol has been routinely performed; however, there is no uniform evaluation. Most centers utilize an electrocardiogram and echocardiography in infants prior to starting treatment. Although are few limitations to starting therapy with propranolol based on abnormalities found on these studies, there can be other cardiac related issues found prior to starting which may require additional treatments. One study showed 21% of patients with IH had an additional cardiac abnormality found on echocardiography[49]. Another study has shown that pretreatment electrocardiogram is of limited value for patients with an unremarkable cardiovascular history and a normal heart rate and blood pressure[50]. The duration of treatment also can vary based off of multiple factors (Figure 1). Not all infantile hemangiomas will respond to beta-blocker therapy and may not require long term treatment. Most, however, recommend at least 3 mo prior to determining that there is no effect from the beta-blocker. The long term therapy in those who respond should be at a minimum of 6 mo. If there is a significant decrease in size of the hemangioma but not complete resolution, this can be continued for 12 mo. Routine cardiac follow-up should be determined by growth that is expected to occur for that age. Infants increase their weight and thus need dose adjustments more frequently than toddler age children and therefore require more frequent evaluation. These follow-up evaluations however should consist of monitoring for symptoms secondary to the beta-blocker, dosage adjustment for weight gain, and determination of effect of treatment.

Side effects of beta-blockers include hypotension, bradycardia, hypoglycemia, seizure, nightmares, bronchoconstriction, diarrhea and somnolence[51]. The hypotension and bradycardia seen is often asymptomatic and typically associated with the first dose. Most symptomatic hypoglycemia is associated with concomitant illness or poor oral intake around the time of seizure. This is usually seen within the first few days of therapy but may occur at any time assuming the dietary intake changes. Nonselective beta-blockers are thought to prevent catecholamine induced glycogenolysis, gluconeogenesis and lipolysis which lead to hypoglycemia. Seizure is thought to be related to the hypoglycemia. Bronchoconstriction is related to the effects on the smooth muscle in the bronchi which beta agonist cause bronchorelaxation and therefore this mechanism is blocked with beta-blockers. Sleep disturbances can be very difficult to evaluate in this patient population.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS AND INFANTILE HEMANGIOMA

After the demonstration of the RAS components in the endothelium of IH, a greater interest on the role of cardiovascular drugs in the management of proliferating IH has emerged. It has been proposed that modulation of products of the RAS such as AT Ⅰ, ACE, or AT Ⅱ could represent an alternative therapeutic target. Tan *et al*[11] published an open-labelled observational clinical trial using captopril, an ACEI, in the treatment of problematic proliferating IH in 8 patients. All the lesions responded to captopril at a dosage of 1.5/kg per day with a transient mild renal impairment occurred in one patient. Treatment was discontinued at the age of 14 mo, except for one patient. In contrast, Christou *et al*[12] reported the results of 17 patients with IH who were treated with oral corticosteroid therapy and developed hypertension requiring treatment with captopril. They concluded that captopril alone did not sustain the corticosteroid-induced involution. Further clinical trials are required to

better define the role of these cardiovascular drugs.

ANGIOTENSIN-RECEPTOR BLOCKERS AND INFANTILE HEMANGIOMA

A recent study aimed to investigate the effect of the angiotensin peptides and their agonists and antagonists on cellular proliferation in proliferating IH *in vitro* samples. A significant increase in cellular proliferation in the AT Ⅰ and AT Ⅱ treated IH tissues compared with control samples was found, suggesting a potential role for ACEIs and ARBs in the proliferation phase of IH[12].

CONCLUSION

Although very few reports have been published on the role of the RAS and some cardiovascular drugs such as beta-blockers, ACEIs and ARBs in the management of IH, clinical evidence supports the use of propranolol as first-line agent for complicated lesions. More basic and clinical studies are needed to investigate the potential effectiveness of other cardiovascular drugs.

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MINIREVIEWS

Pulmonary vein stenosis: Etiology, diagnosis and management

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Abstract

Pulmonary vein stenosis (PVS) is rare condition characterized by a challenging diagnosis and unfavorable prognosis at advance stages. At present, injury from radiofrequency ablation for atrial fibrillation has become the main cause of the disease. PVS is characterized by a progressive lumen size reduction of one or more pulmonary veins that, when hemodynamically significant, may raise lobar capillary pressure leading to signs and symptoms such as shortness of breath, cough, and hemoptysis. Image techniques (transesophageal echocardiography, computed tomography, magnetic resonance and perfusion imaging) are essential to reach a final diagnosis and decide an appropriate therapy. In this regard, series from referral centers have shown that surgical and transcatheter interventions may improve prognosis. The purpose of this article is to review the etiology, assessment and management of PVS.

Key words: Pulmonary vein stenosis; Pulmonary vein stenosis etiology; Pulmonary vein stenosis causes; Pulmonary vein stenosis diagnosis; Pulmonary vein stenosis management; Pulmonary vein stenosis treatment

Core tip: Several papers in literature focus either on

the causes, diagnosis or treatment of pulmonary vein stenosis. However this is simple yet complete and updated review of all these matters that may guide physician's decision making when facing a suspected or confirmed case of this unusual disease.

Pazos-López P, García-Rodríguez C, Guitián-González A, Paredes-Galán E, Álvarez-Moure MADLG, Rodríguez-Álvarez M, Baz-Alonso JA, Teijeira-Fernández E, Calvo-Iglesias FE, Íñiguez-Romo A. Pulmonary vein stenosis: Etiology, diagnosis and management. *World J Cardiol* 2016; 8(1): 81-88 Available from: URL: http://www.wjgnet.com/1949-8462/full/v8/i1/81.htm DOI: http://dx.doi.org/10.4330/wjc.v8.i1.81

INTRODUCTION

Despite pulmonary vein stenosis (PVS) is an uncommon entity (estimated incidence about 2-3 cases per year in large centers)[1] its morbidity and mortality rates are high at advance stages[2]. The condition, linked in the past to congenital heart diseases in childhood and mediastinal processes (*i.e.,* tumors) in adults, is nowadays firstly associated to injury from radiofrequency ablation (PVA) for atrial fibrillation (AF). It is essential to consider the possibility of the disease in patients at-risk to guarantee early detection (image techniques play a key role in this regard) and treatment. The aim of this article is to review the etiology, assessment and management of PVS.

ETIOLOGY

Congenital PVS

Congenital PVS is an exceptional abnormality (0.4% of congenital heart diseases) consequence of a failed incorporation of the common right and/or left PV into the left atrium (LA) during the embryologic development of the vessel that leads to partial or complete obliteration of the PVs on one or both sides[3]. From a histological point of view its main feature is an overgrowth of connective tissue with medial hypertrophy and intimal fibrosis which results in obstruction. Even though diagnosis is usually made within the first 3 years of life, it may be delayed till adulthood in some cases[3]. Congenital PVS is frequently associated (50%) with other cardiac defects[4,5], hence imaging examination protocols applied to patients with congenital heart diseases should include a systematic evaluation of the PVs (Table 1).

ACQUIRED PVS

PVA for AF

At the present time PVA for AF has become the principal cause of PVS. Incidence derived from recent studies reaches a mean and median of 2% and 3.1%, respectively. These figures represent a significant reduction in comparison with those reported in pioneer series (mean: 6.3% and median: 5.4%, estimated from papers published between 1999 and 2004)[5]. Main factors contributing to this finding are operator experience and improvements in the procedure [changing of ablation site from the PVs antra to ostia, reduction of temperature applied to tissue, cryoablation and intracardiac echocardiography (ICE) guidance][2]. However real occurrence of PVS is probably underestimated as screening is only performed within the first 3 mo in some centers (it has been demonstrated that PVS can occur over this time period)[6] and asymptomatic patients are not always imaged.

Table 1 Causes of pulmonary vein stenosis

Congenital
Cardiac defects associated:
Total anomalous pulmonary venous return
Septal defects
Transposition of the great vessels
Acquired
Pulmonary vein ablation
Sarcoidosis
Neoplasm
Fibrosing mediastinitis
Post cardiovascular surgery

Mediastinal processes

Extrinsic compression by lymphadenopathies or granulomatous involvement may cause PVS in sarcoidosis[7,8].

Fibrosing mediastinitis, a rare complication of tuberculosis and Histoplasma capsulatum infection, characterized by uncontrolled fibrosis around the affected mediastinal lymph nodes, may lead to invasion and obstruction of the surrounding PVs[9].

Neoplasm adjacent to the PVs may cause stenosis due to compression or infiltration[10,11].

Cardiovascular surgery

Clinically significant PVS in pediatric population is most frequently seen after total anomalous pulmonary venous return repair (estimated incidence approximately equal 10%)[12,13]. Obliteration may be localized either at the level of the anastomosis of the PV into the LA or further into the center of the vessel. Isolated cases of PV injury leading to obstruction after myxoma resection[14], suture repair of a PV cannulation site[15] and lung transplantation[16] can be found in literature.

ASSESSMENT

PVS may be symptomatic when vein caliber is reduced significantly (> 50% stenosis), as a consequence of a raise in lobar wedge pressure, or lung perfusion is decreased by > 20%-25%[17-19]. Clinical manifestations, which in case of PVA normally appear 3-6 mo after the procedure, are clearly related to the number of PVs affected and include progressive exertional dyspnea, cough, chest pain (frequently following a

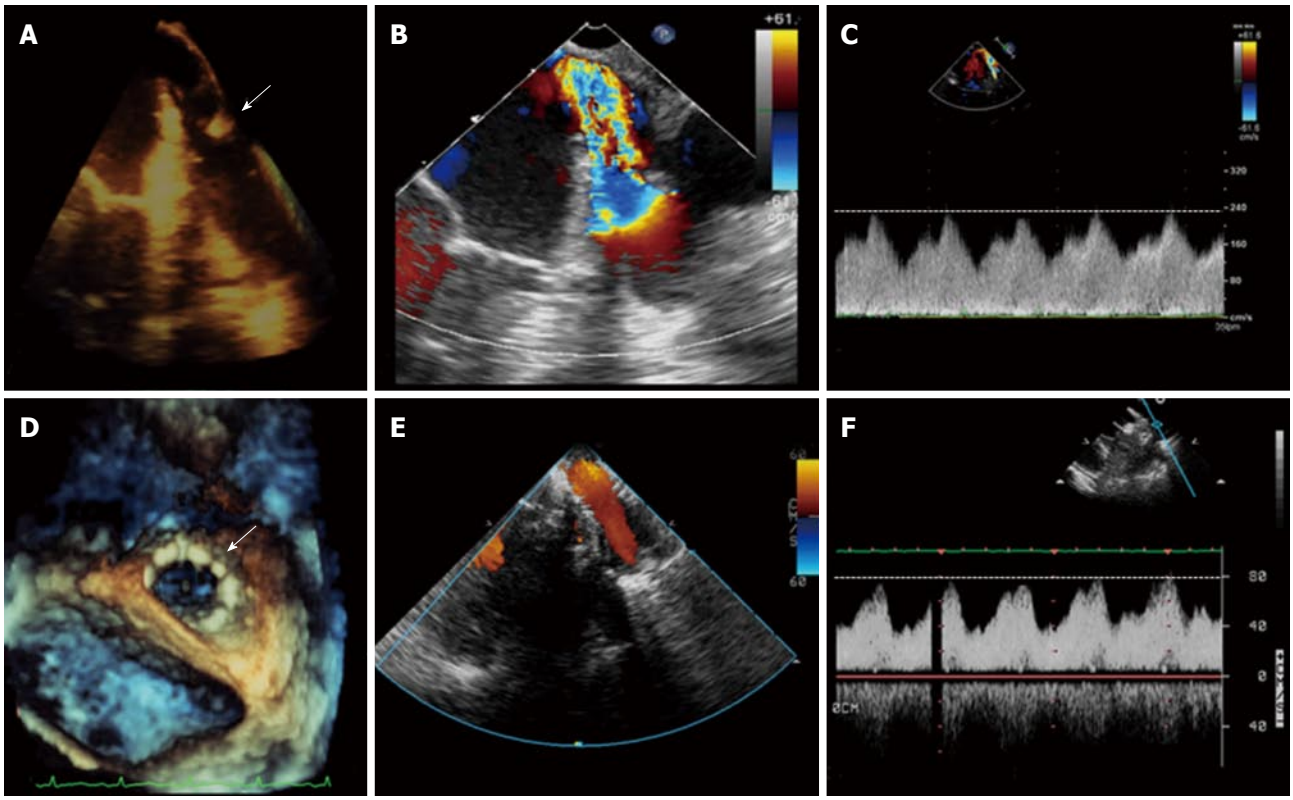

Figure 1 Transesophageal echocardiography of a patient with a recent left lung transplantation and severe congestion in the graft. A: Narrowing in the common trunk of the left PVs at the level of the sutures (arrow). Color Doppler and Continuous Wave Doppler demonstrate turbulent flow (B) and high velocity (C: peak velocity 2.4 m/s; peak gradient 23 mmHg) across the vessel which is consistent with a significant stenosis. A stent was successfully implanted at the level of the stenosis (D: "en face" 3D echo image view; arrow). Laminar flow (E) and normal velocities (F: peak velocity 0.8 m/s; Peak gradient 2.5 mmHg) were seen after the procedure.

pleuritic profile) and hemoptysis. Chest X ray may demonstrate signs of congestion (peribronchovascular and septal thickening, Kerlley B lines, alveolar edema) either diffuse or localized (mimicking other processes such as pneumonia), depending on the PVs involve[6]. Other findings can be found depending on the cause of the stenosis (*i.e.,* lung size reduction in congenital PV atresia, a thoracic mass in the case of a tumor, mediastinal calcifications in fibrosing mediatinitis or calcified mediastinal lymph nodes in sarcoidosis). As the clinical picture is nonspecific, collateral flow development may mitigate symptoms[17], and occasionally physicians do not bear in mind the possibility of the disease, the diagnosis is commonly missed or delayed. Therefore screening with available imaging modalities in patients at risk (especially those with history of PVA) who develop respiratory symptoms is warranted.

Echocardiography

Transesophageal echocardiography (TEE) is a useful tool for PV investigation. Studies have shown high diagnosis accuracy for detection of PVS after PVA (sensitivity: 82%-100%, specificity: 95%-100%) compared to other techniques [computed tomography (CT), magnetic resonance imaging (MRI) and angiography][20]. Advantages of TEE are its wide availability, avoidance of radiation exposure, low cost, and applicability to patients with ferromagnetic implanted devices (*i.e.,* pacemakers, defibrillators). There is no standard definition of PVS, nevertheless it seems that an increased maximum PV Doppler flow velocity (> 1.1 m/s) combined with color Doppler turbulence may be a reliable index[21] (Figure 1). TEE poses however noteworthy limitations including need of sedation, technical difficulties to visualize all PVs if performed by not experienced operators, non-volumetric but planar acquisition, inadequate assessment of paracardial structures, imprecise delimitation of LA-PVs junction (makes PVs ostial size a non-reliable anatomical parameter) and, even low, risk of esophageal perforation and pulmonary aspiration. 3D TEE may overcome some of these limitations.

ICE has been successfully used to guide PVA and evaluate PV ostial narrowing. The invasive nature of the technique restricts however its use to patients undergoing a redo PVA. Although diagnostic accuracy of ICE has not been investigated, an increased peak Doppler flow velocity over 1.6 m/s is consistent with PVS according to initial experiences[22].

CT

CT allows assessment of the extension of mediastinal neoplastic and non tumoral diseases infiltrating or compressing the PVs and enables the diagnosis of PVS after PVA by directly depicting vessel diameter (significant stenosis > 50%)[3,4] (Figure 2). Although the choice of CT protocol depends on daily practice in every center ECG gated scanning improves quality and allows postprocessing with 3D reconstruction software which

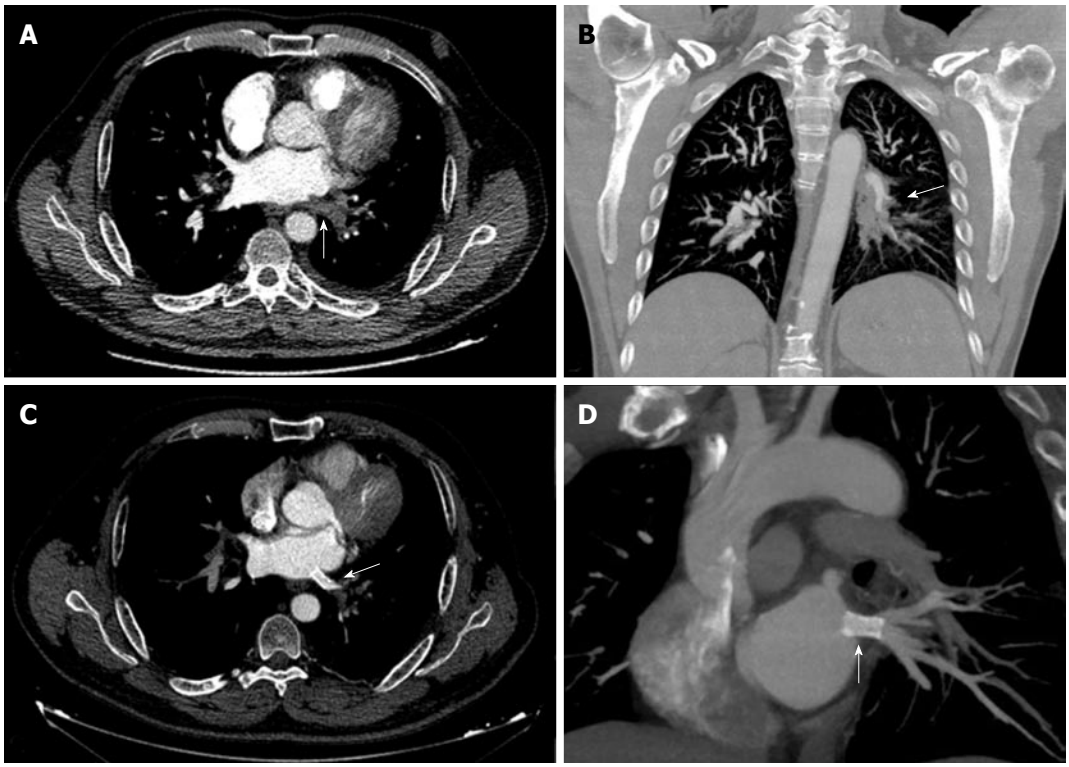

Figure 2 Computed tomography of a patient undergone radiofrequency ablation two months before and recent onset of dyspnea on exertion. A: Absent of contrast (arrow) in the left lower pulmonary vein (complete occlusion); B: Extensive infiltrate within the left lung (arrow) cause by localized edema; C and D: After stent implantation (arrows) flow was successfully restored.

permits better evaluation of the PVs ostia. The main benefits of CT are short examination time, multiplanar views and high spatial resolution, whereas disadvantages include patient exposition to ionizing radiation and need of intravenous iodine contrast agents that might impair renal function in vulnerable individuals. PVs (typically the left inferior) can be compressed between the LA and the descending aorta appearing stenotic (pseudostenosis). Differential diagnosis can be made measuring PV caliber in every phase of the cardiac cycle (fixed in cases of true PVS and variable in pseudostenosis) or imaging the patient in prone position (this maneuver eliminates LA compression and therefore pseudoestenosis) when either a multiphase scan has not been performed or findings are inconclusive[23].

MRI

MRI is diagnostic in most cases by analyzing PV anatomy (MR angiography) and flow dynamics (MR phase contrast imaging; velocity and gradients across the vessel)[24,25] (Figure 3). This modality can be also used to evaluate congenital cardiopathies and processes in the vicinity of the heart associated with a PVS (*i.e.,* neoplasm). The main advantage of MRI over CT is that it does not expose the patient to radiation. Nevertheless drawbacks are considerable: Spatial resolution is lower than CT, it is contraindicated in patients with implanted non-compatible metal devices, and it may not be possible to perform in individuals with claustrophobia, unable to cooperate, large body habitus or severe renal impairment when gadolinium contrast is needed. Additionally, scanning time is considerably long.

Perfusion imaging

Perfusion of a pulmonary lobe draining to a PV with a significant stenosis may be decreased and detected using radionuclide quantitative pulmonary flow imaging (TC99m macroaggregated albumin) (Figure 4). This test however is not valuable for an etiological diagnosis of a PVS, may be altered in other pathologies that decreased lobar perfusion (*i.e.,* pulmonary thromboembolism), is not suitable for detection of < 50% stenosis[26] and may be inaccurate if significant compensating ipsilateral PV flow is present. Moreover, even small, it implicates radiation exposure (Table 2).

Summing up, clinical manifestations and imaging test are the key elements in PVS assessment. Choice of imaging modality depends on availability, experience, and patient characteristics. Despite current guidelines do not provide a recommendation for frequency and duration of imaging screening in case of PVA most electrophysiology (EP) labs suggest a follow up test within 3-6 mo after the procedure in order to detect significant iatrogenic PVS at an early stage and avoid its sequelae.

MANAGEMENT

PVS in pediatric population

Mild and asymptomatic PVS may not need intervention;

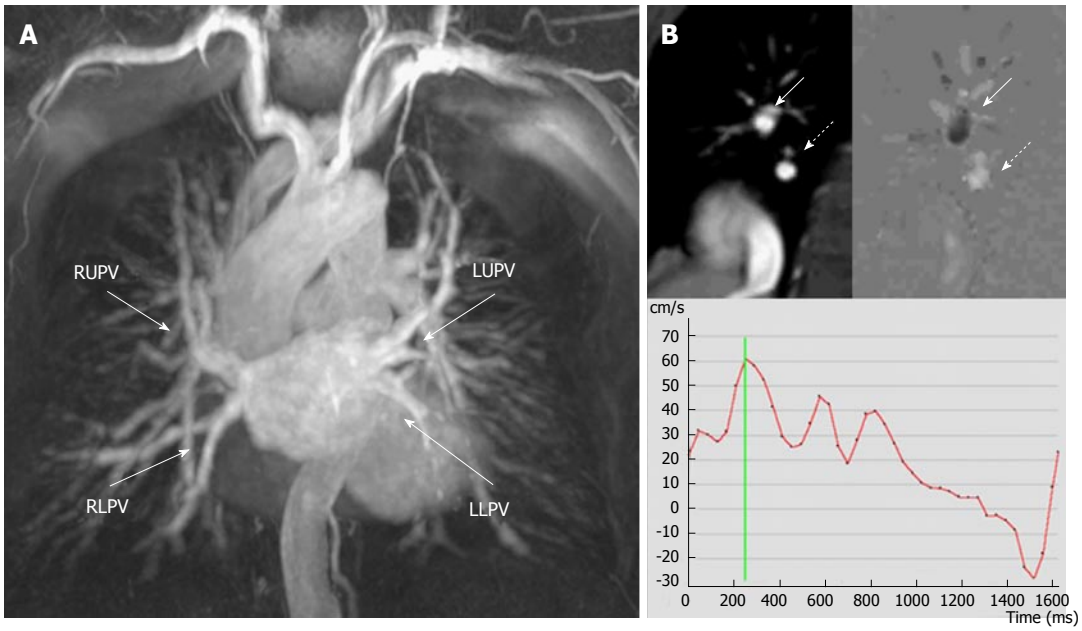

Figure 3 Magnetic resonance scan of a patient with a radiofrequency ablation procedure one month before, mild hemoptysis and fever. A: Angiography shows normal caliber of the four PVs; B: Phase contrast imaging of the right lower PV. Top left: right pulmonary artery (arrow) and right lower PV (dashed arrow). Top right: Flow map. Black or white signal depends on the direction of the flow. The PV "white flow" (dashed arrow) compares with the opposite direction of flow in the pulmonary artery seen in the same image that is "black" (arrow). Bottom: the resulting velocity-time curve demonstrates normal flow morphology and velocities in the PV. Significant PVS was excluded. RUPV: Right upper pulmonary vein; RLPV: Right lower pulmonary vein; LUPV: Left upper pulmonary vein; LLPV: Left lower pulmonary vein; PV: Pulmonary vein.

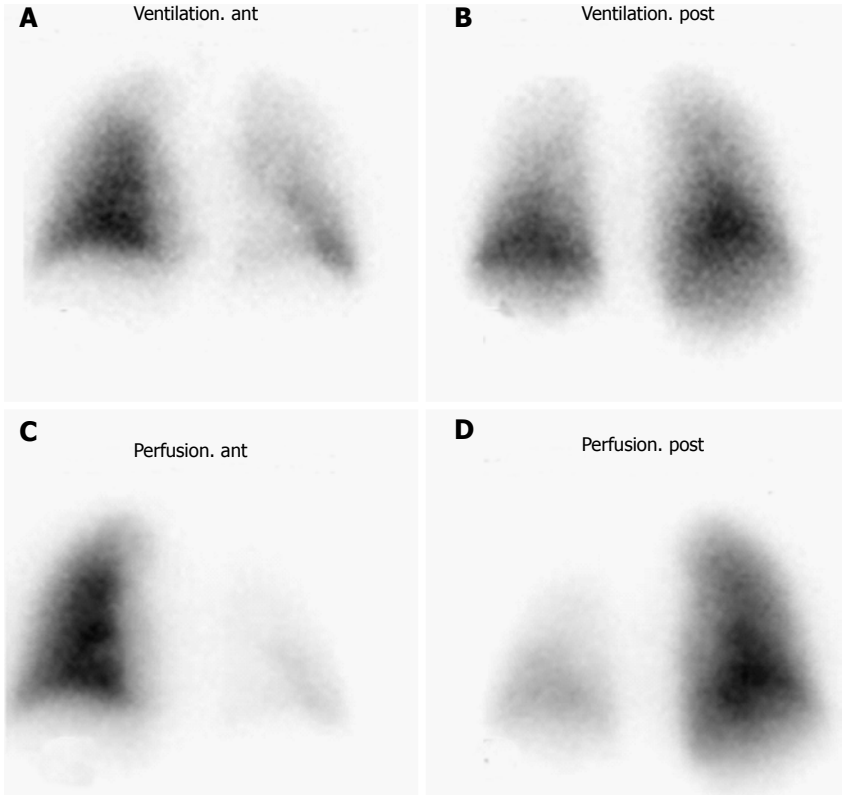

Figure 4 Radionuclide lung ventilation/perfusion scan performed three months after radiofrequency ablation in a patient with shortness of breath. A and B: Normal ventilation; C and D marked hypoperfusion within the left lung consistent with significant left PV stenosis which was demonstrated on a CT scan. PV: Pulmonary vein; CT: Computed tomography.

clinical and image surveillance is advised as the disease can evolve over time. Surgery is the preferred approach in most congenital or acquired significant symptomatic PVS. The conventional interventions

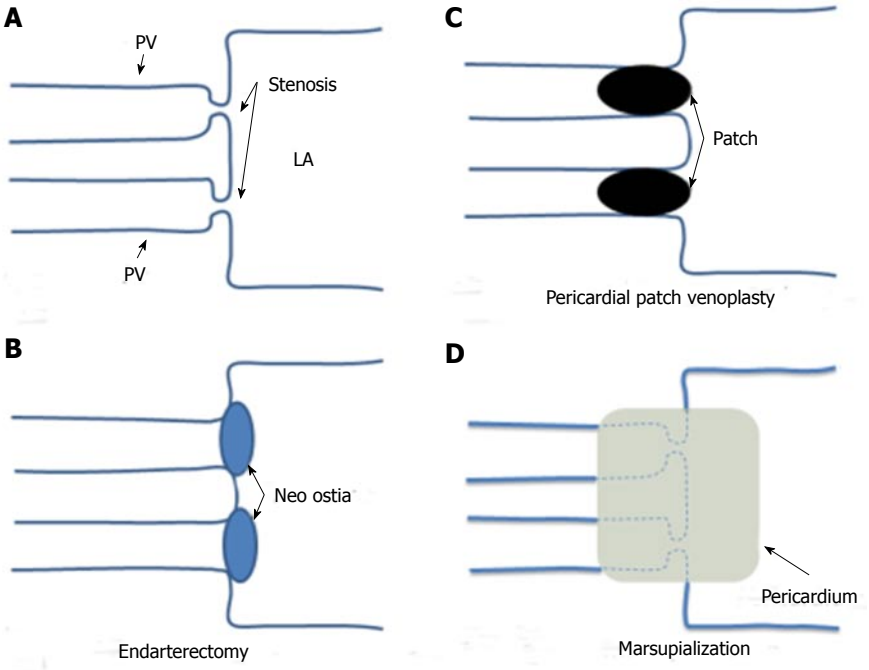

Figure 5 Surgical techniques for pulmonary veins. A: Schematic representation of a bilateral pulmonary vein stenosis at the ostia of the vessels; B: Endarterectomy; the stenotic tissue has been excised and the PVs directly anastomosed to the LA; C: Pericardial patch venoplasty; the stenotic tissue has been resected and a pericardial patch anastomosis has been used to enlarge the tightened ostia of the vessels; D: Sutureless marsupialization: the veins ostia have been incised longitudinally, excess fibrotic tissue has been excised and *in situ* pericardial flaps have been sewn directly to the left atrium so direct stiches over the cut edges of the pulmonary veins are avoid. PV: Pulmonary vein; LA: Left atrium.

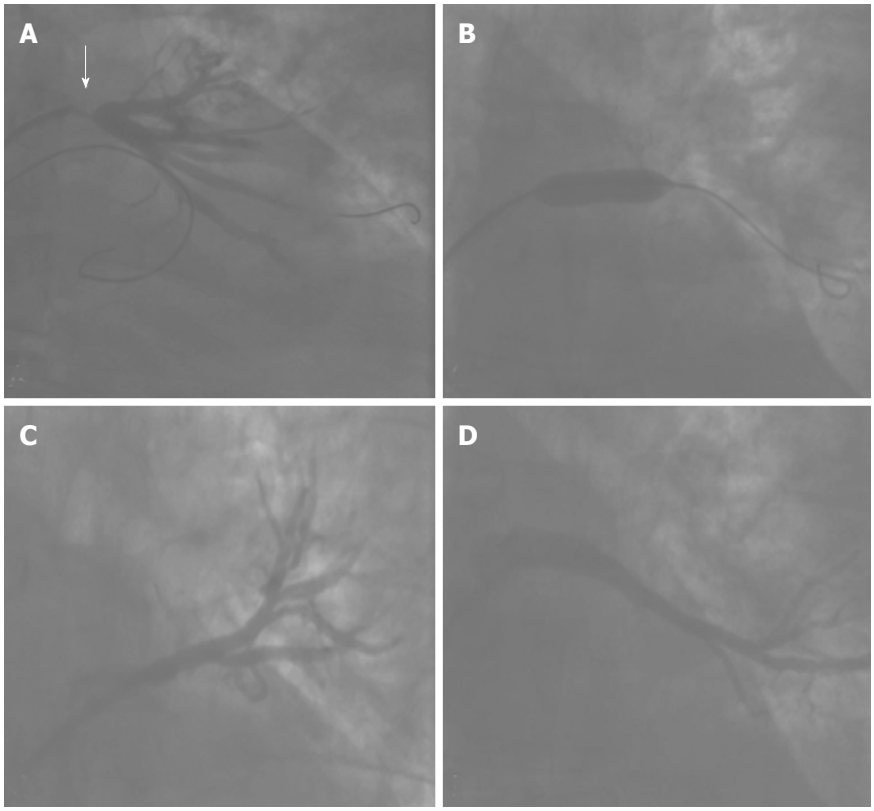

Figure 6 Stent implantation in a pulmonary vein stenosis. A: Angiography showing a critical stenosis in the ostium of the left lower pulmonary vein; B: Bare metal stent release; C and D: Final result. The stenosis was resolved. Normal flow can be seen in the main superior (C) and inferior (D) branches of the vein.

(Figure 5) include: (1) endarterectomy (excision of the stenotic ring and direct anastomosis of the PV to the LA endocardium); and (2) pericardial patch venoplasty (resection of the stenotic tissue and patch anastomosis

Table 2 Advantages of imaging modalities used for pulmonary vein stenosis evaluation

	TEE	CT	MRI	VQ
Availability	Yes	Yes	No	No
Non-invasive	No[1]	Yes	Yes	Yes
Caliber assessment	No	Yes	Yes	No
Functional assessment	Yes	No	Yes	Yes
Evaluation of surrounding tissues	No	Yes	Yes	No
Radiation avoidance	Yes	No	Yes	No

[1]TEE is generally considered a semi-invasive technique. TEE: Transesophagueal echocardiography; CT: Computed tomography; MRI: Magnetic resonance imaging; VQ: Ventilation perfusion scan.

to enlarge the tightened segment). The newer sutureless marsupialization technique (the pericardium surrounding the affected PV is directly attached to the LA so direct stiches over the cut edges of the vessel are avoided) can help to prevent deformation of the suture line and reduce tissue growth stimulus decreasing therefore restenosis risk[27]. Overall, published surgical outcomes are modest; only half of cases are free from reintervention or death at 5 years[27,28]. Pneumectomy may be mandatory in cases of severe or uncontrolled hemoptysis and lung transplantation has been performed in patients with relentless PVS progression and severe pulmonary hypertension[29]. There is limited experience with percutaneous interventions in childhood; angioplasty is technically challenging (high pressures are needed to released stenosis and in case of stent implantation prosthesis should allow future expansion to adult dimension (> 12 mm) and results are suboptimal (repeated dilatations are frequently needed as instent restenosis rate is high)[30].

PVS in adult population

Transcatheter therapy is the most common chosen approach (Figure 6). While evidence of treatment of PVS due to extrinsic compression, infiltration or cardiac surgery is restricted to cases reports in literature[16] several small studies have evaluated the efficacy of percutaneous interventions for PVS after PVA. There are discrepancies among EP labs about management of asymptomatic PVS. Despite most authors recommend clinical and imaging monitoring every 3-6 mo in patients with 50%-85% stenosis, some promote angioplasty if a single stenosis > 75%[17] and others in cases of a cumulative stenosis index (average stenosis of the PVs of one site) > 75%[18]. Main arguments for early intervention are: Inadequate recovery of lung perfusion at advance stages caused by fixed venoconstriction leading to permanent pulmonary hypertension; and fast progression to PV occlusion in some cases which may be difficult to amend. Regarding the technique itself stenting appears better than isolated balloon venoplasty in terms of vessel restenosis (60% *vs* 36% for PV over 8 mm)[5]. Mid to long term patency is directly related to vessel size with higher rates of restenosis observed in PV < 1 cm. Drug eluting stents may have a better restenosis profile than conventional bare metal stent however studies regarding their use this scenario are scarce[31].

Limited data about antithrombotic regimes are available: (1) anticoagulation with warfarin, with an international normalized ratio target of 2-3, is generally recommended for at least 12 mo in the case of stents > 1 cm and indefinitely for those smaller[19]; (2) dual antiplatelet therapy, added to anticoagulation, with aspirin plus clopidogrel is usually prescribed for a minimum of 3 mo, however optimal duration is not known; and (3) new oral anticoagulants (dabigatran, rivaroxaban, apixaban) or antiaggregants (prasugrel, ticagrelor) have not been tested.

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ORIGINAL ARTICLE

Basic Study

Bicuspid aortic valve hemodynamics does not promote remodeling in porcine aortic wall concavity

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Abstract

AIM: To investigate the role of type-I left-right bicuspid aortic valve (LR-BAV) hemodynamic stresses in the remodeling of the thoracic ascending aorta (AA) concavity, in the absence of underlying genetic or structural defects.

METHODS: Transient wall shear stress (WSS) profiles in the concavity of tricuspid aortic valve (TAV) and LR-BAV AAs were obtained computationally. Tissue specimens excised from the concavity of normal (non-dilated) porcine AAs were subjected for 48 h to those stress environments using a shear stress bioreactor. Tissue remodeling was characterized in terms of matrix metalloproteinase (MMP) expression and activity *via* immunostaining and gelatin zymography.

RESULTS: Immunostaining semi-quantification results indicated no significant difference in MMP-2 and MMP-9 expression between the tissue groups exposed to TAV and LR-BAV AA WSS (P = 0.80 and P = 0.19, respectively). Zymography densitometry revealed no difference in MMP-2 activity (total activity, active form and latent form) between the groups subjected to TAV AA and LR-BAV AA WSS (P = 0.08, P = 0.15 and P = 0.59, respectively).

CONCLUSION: The hemodynamic stress environment present in the concavity of type-I LR-BAV AA does not

cause any significant change in proteolytic enzyme expression and activity as compared to that present in the TAV AA.

Key words: Bicuspid aortic valve; Fluid shear stress; Aortopathy; Remodeling; Matrix metalloproteinases

Core tip: The bicuspid aortic valve with left-right cusp fusion (LR-BAV) generates a stress overload on the ascending aorta (AA) convexity, which promotes aortic medial degeneration and aortic dilation. While the wall concavity is generally spared from the disease, the protective role of the local hemodynamics has not been demonstrated. This study aimed at comparing matrix metalloproteinase biology in AA concavity tissue subjected to the local hemodynamic stresses generated by a tricuspid aortic valve (TAV) and a LR-BAV. The results suggest that the fluid stresses in the TAV AA and LR-BAV AA concavity result in similar MMP expressions and activities.

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INTRODUCTION

The bicuspid aortic valve (BAV) is present in 1%-2% of the general population[1-3] and is the most common cardiac anomaly. Despite its seemingly low incidence, the BAV is responsible for causing more valvular and vascular disease compared to all other congenital heart defects combined[4]. Unlike the normal tricuspid aortic valve (TAV) which consists of three leaflets, the BAV forms with only two[5-7]. While there are different BAV morphogenic phenotypes[8-10], the most common is referred to as the type-I BAV and is characterized by the presence of two cusps of unequal size and one fibrous raphe marking the site of fusion on the larger leaflet[5]. Type-I BAV patients not only have a higher susceptibility to develop valvulopathies that usually require surgical intervention and valvular replacement, they are also associated with increased risk of aortopathies such as aortic dilation, dissection and aneurysm[11-13]. In particular, type-I BAVs with fusion between the left- and right-coronary cusps (LR subtype) has emerged as the most aggressive in terms of risk for secondary aortopathy[14-16]. This subtype tends to result in asymmetric dilation patterns that localize to the convex region of the thoracic ascending aorta (AA) but spare the wall concavity[14,15,17].

Previous clinical studies have demonstrated that the degenerative remodeling of the aortic wall in type-I BAV patients is accompanied by increased expression of matrix metalloproteinase-2 (MMP-2) and MMP-9[16,18-20] in the disease-prone wall convexity relative to the wall concavity[14,15,21]. Those proteolytic enzymes degrade key extracellular matrix components such as collagen and elastin[22]. The respective expression and activity of those enzymes and their tissue inhibitors regulate the balance between extracellular matrix synthesis and resorption[23]. A perturbation of this delicate equilibrium can result in the progressive degeneration of the vascular wall and the loss of vessel wall integrity[24].

Interestingly, type-I LR-BAVs have been shown to generate perturbed hemodynamics characterized by a valvular jet skewed toward the non-coronary leaflet and increased shearing friction force [*i.e.,* wall shear stress (WSS)] on the convexity of the thoracic AA[25-31]. While those observations suggest a role for hemodynamics in the pathogenesis of BAV aortopathy[32-34] and despite the clear evidence for the existence of flow abnormalities in BAV aortic wall regions vulnerable to dilation, the causative effects of those abnormalities on the local weakening of the aortic wall have not been fully established. Underlying challenges hampering the *in vivo* assessment of this hemodynamic theory include the possible existence of genetic anomalies in the aortic wall, as well as the paucity of hemodynamic data in non-dilated BAV aortas. To circumvent those issues, *ex vivo* methodologies enabling the replication of the native BAV AA WSS environment on genetically normal and non-dilated AA tissue have been developed.

In our previous *ex vivo* study[35], we isolated the impact of TAV and LR-BAV hemodynamic stresses on the remodeling of the AA convexity. The WSS environments in the convex region of a TAV AA and a normal (non-dilated) LR-BAV AA were quantified computationally[28,35] and replicated in the laboratory using a shear stress bioreactor[36,37]. The remodeling response of porcine aortic tissue extracted from the AA convexity and exposed to those environments for 48 h was investigated *via* immunostaining, immunoblotting and zymography. Exposure of normal aortic tissue to BAV AA WSS resulted in increased MMP-2 and MMP-9 expressions and MMP-2 activity but similar fibrillin-1 content relative to the TAV AA WSS treatment. While this study demonstrated the susceptibility of the hemodynamic stresses experienced by the BAV AA convexity to focally mediate aortic medial degradation, the apparent protective effects of the LR-BAV hemodynamics on the AA concavity and the asymmetric development of dilation in the LR-BAV AA require further investigation. Therefore, the objective of the present study was to isolate *ex vivo* the impact of LR-BAV hemodynamic stresses on the remodeling of the disease-protected AA concavity, with a focus on MMP expression and activity.

MATERIALS AND METHODS

WSS characterization and in vitro generation

The temporal WSS variations experienced by the

Table 1 Wall shear stress signal characteristics in the concavity of the tricuspid aortic valve and left-right bicuspid aortic valve ascending aorta

	Maximum (Pa)	Minimum (Pa)	TSM (Pa)	OSI
TAV AA WSS	3.2	-2.75	0.77	0.49
LR-BAV AA WSS	3.3	-3.63	1.06	0.18

WSS: Wall shear stress; LR-BAV: Left-right bicuspid aortic valve; TAV: Tricuspid aortic valve; AA: Ascending aorta; OSI: Oscillatory shear index; TSM: Temporal shear magnitude.

concave region of a TAV AA and LR-BAV AA were obtained computationally using a previously published and validated fluid-structure interaction (FSI) model of a human aorta subjected to idealized TAV and LR-BAV flows[28]. Briefly, a realistic model of a human aortic arch was reconstructed based on histological slices (Visible Human Project, National Library of Medicine). 3D transient velocity profiles matching physiologic TAV and LR-BAV average flow rates were prescribed at the model inlet. The dynamic WSS profiles experienced by the TAV AA and LR-BAV AA concavity were captured in a rectangular region (dimensions: 8 mm × 15 mm) centered on the wall concavity and located 1 cm above the left-coronary leaflet (Figure 1A). The two WSS waveforms share important similarities both qualitatively and quantitatively, as indicated by their peak values and average magnitude temporal shear magnitude (TSM) over one cardiac cycle (Table 1). Importantly, as compared to the TAV, which generates a nearly perfectly sinusoidal WSS signal, the LR-BAV generates a double negative WSS peak, which tends to lower the signal oscillatory shear index (OSI).

The two WSS environments were replicated in the laboratory using our previously described and validated cone-and-plate bioreactor[35,37]. Briefly, the device consists of a cylindrical chamber filled with culture medium and containing a cone rotating above a stationary mounting plate (Figure 1B). The rotation of the cone generates a flow and thus, a WSS on the surface of the plate, whose intensity τ at a radial location r is directly proportional to the cone angular velocity ω:

$$\omega = [(h + r\alpha)/\mu r]\tau \quad (1)$$

where μ is the dynamic viscosity of the culture medium (0.95×10^{-3} kg/m per second), α is the cone half angle (0.5°), and h is the distance between the cone apex and the mounting plate (200 μm)[36]. The two angular velocity waveforms producing the TAV AA and LR-BAV AA WSS profiles obtained computationally were programmed into the servo drive (Gemini GV6k, Parker Hannafin) controlling the cone motion. The details of this protocol have been previously published[35].

Tissue harvest and preparation

The experiments were conducted on porcine aortas (6-12 mo) acquired from a local abattoir (Martin's Custom Butchering, Wakarusa, IN, United States) due to their structural similarities with human aortas and their well-characterized antibody specificities. Aortic tissue was harvested after on-site dissection of the hearts within 10 min of slaughter and was transported to the laboratory in sterile, ice-cold Dulbecco's Phosphate Buffered Saline (PBS, Sigma-Aldrich). This protocol has been previously implemented in our laboratory and has been shown to preserve endothelium integrity and cellular viability[35,36,38]. All subsequent procedures were performed in a sterile flow hood. Upon arrival to the laboratory, the aortas were cut longitudinally in order to expose the inner endothelial surface. Consistent with our previous study on the effects of BAV flow on AA convexity[35], two circular specimen (7-mm diameter) were excised from the AA concavity, 8 and 15 mm above the sinus of the left-coronary leaflet (*i.e.,* region least prone to dilation[14,15,39]). Samples were then randomized into fresh controls and experimental samples. Six samples were mounted to the circular plate using a button suturing technique, which has been shown not to affect the WSS level generated on the tissue[35]. The native orientation of the tissue relative to blood flow was maintained by aligning the longitudinal axis of the samples with the direction of cone motion (*i.e.,* tangential direction). Tissue conditioning to WSS was performed in an incubator maintaining a temperature of 37 ℃ and a CO_2 level of 5% for 48 h (duration sufficient for acute mechanosensitive remodeling processes to become evident in aortic tissue[34,35,40]). The system was continuously perfused with standard culture medium (Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 3.7 g/L sodium bicarbonate, 0.05 g/L ascorbic acid, 10% non-essential amino acid solution and 1% penicillin-streptomycin; all from Sigma-Aldrich) at a rate of two bioreactor volumes per hour. The perfusion system was flushed and replenished with fresh medium every 12 h.

Groups

Two experimental groups and one control group were considered to isolate the impact of TAV AA and LR-BAV AA hemodynamics on the acute remodeling response of the AA concavity: (1) fresh porcine tissue excised from the concavity of the ascending aortic wall (control); (2) fresh porcine tissue excised from the concavity of the ascending aortic wall and subjected to the local TAV AA WSS; and (3) fresh porcine tissue excised from the concavity of the ascending aortic wall and subjected to the local BAV AA WSS.

Biological analyses

Following WSS conditioning, the samples were harvested and washed three times with sterile PBS. The samples were then either frozen in optimal cutting medium for future immunostaining analysis flash frozen in liquid nitrogen for future gelatin zymography analysis.

Immunostaining: The OCT blocks were cut into

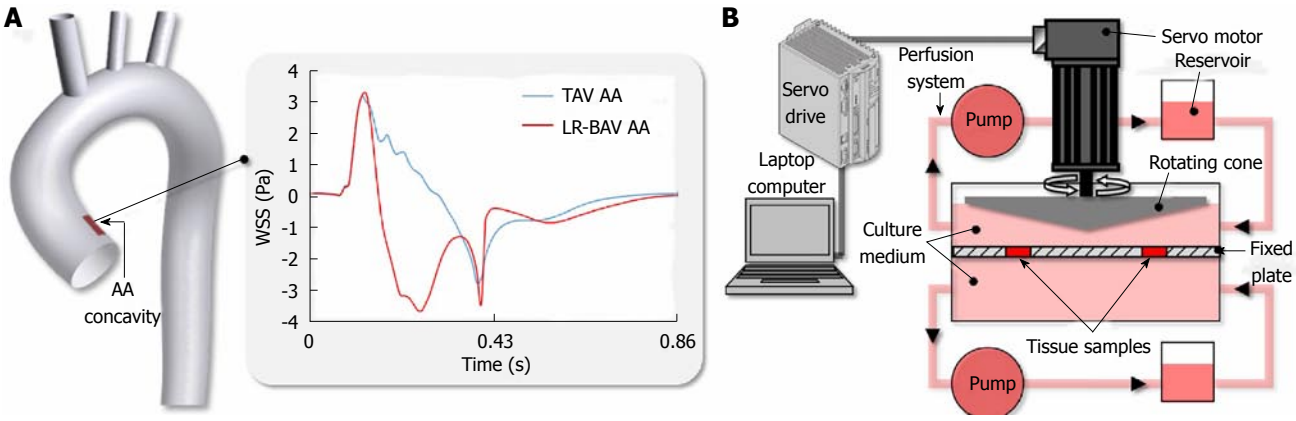

Figure 1 ***Ex vivo* methodology.** A: Temporal wall shear stress (WSS) signals captured computationally in the concavity of the tricuspid aortic valve (TAV) ascending aorta (AA) and left-right bicuspid aortic valve (LR-BAV) AA (adapted from Cao *et al*[28]); B: Shear stress bioreactor used to condition porcine AA tissue to TAV AA and BAV AA WSS.

5-μm sections using a Microm 505E cryostat (Microm International GmbH) and mounted on glass slides. The region occupied by the tissue section was circled with a fluid block pen (Immunotech) after 20 min on a heater at 37 ℃. Sections were then rinsed for 20 min in PBS. Blocking [10% Goat Serum (Sigma), 0.2% TritonX-100 (Sigma), 1% dimethyl sulfoxide (Thermo Fisher Scientific)] was performed at room temperature for 1 h. Next, MMP-2 (1:200, EMD Millipore) or MMP-9 (1:200, EMD Millipore) primary antibody was diluted in blocker and slides were incubated overnight at 4 ℃ with shaking. The following day, PBS was used to rinse the sections 3 times and secondary antibody (1:100, Santa Cruz) was incubated for 2 h at room temperature in PBS. Sections were rinsed 3 more times in PBS for 5 min each before counterstaining with 1 4′,6-Diamindino-2-phenylindole (DAPI, Sigma) and mounted with fluorescence mounting medium (Dako). Coverslipped sections were stored at 4 ℃. Fluorescence immunohistochemistry (IHC) performed on a Nikon E600 microscope was used to identify regions positively stained for MMP-2 and MMP-9 on each slide. MMP-2 and MMP-9 expression was assessed semi-quantitatively using ImageJ (National Institutes of Health, Bethesda, MD) over three image fields per sample, following our previously published methodology[38,41-43]. The overall intensity of MMP immunopositive expression was measured and normalized by the total number of cells present over each image field.

Gelatin zymography: Proteolytic activity of enzymes MMP-2 and MMP-9 was quantified using gelatin zymography. The collected supernatant protein content was quantified using a bicinchoninic acid protein assay (BCA, Pierce). Tissue lysates were loaded in equal amounts (20 μg) on a 10% zymogram gel (Bio-Rad). Gels were resolved by sample buffer (Bio-Rad) followed by 1 h incubation in developing buffer (Bio-Rad) at 37 ℃ and 5% CO_2. Stain solution (G-Biosciences) was added at room temperature, then gels were destained in deionized water at room temperature. Gels were scanned and the digital images were converted to 8-bit grayscale images before being processed in ImageJ for densitometric analysis following our previously published protocol[42,43].

Statistical analysis

Consistent with our previous study, each analysis was performed on a sample size of $N = 3$ and was quantified as mean ± SE. This sample size was shown previously to generate significant biological differences between convexity tissue specimens subjected to TAV and BAV flows[35]. Normalization to the fresh control was performed in all experimental groups. Significance was determined using ANOVA followed by a Bonferroni post-hoc test using the software SAS (SAS Institute Inc). The threshold for statistical significance was set at a P value of 0.05. Those analyses were reviewed by a biomedical statistician (Dr. Ick H Jin, Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN, United States).

RESULTS

TAV and LR-BAV hemodynamic stresses generate similar MMP expression levels in AA concavity tissue

MMP-2 and MMP-9 immunostaining results are shown in Figure 2. In fresh tissue, MMP-2 and MMP-9 expressions were moderate and consistently localized in the medial layer (Figure 2A). While MMP-2 and MMP-9 were also detected in the same region in specimens subjected to WSS, MMP-9 expression was reduced in the samples subjected to BAV AA WSS. Semi-quantification of the IHC images (Figure 2B) revealed statistically similar MMP-2 expression across the different groups (TAV AA WSS: 1.2-fold increase *vs* control, $P = 0.73$; BAV AA WSS: 1.1-fold increase *vs* controls, $P = 0.95$) and no statistical difference between the two experimental groups subjected to WSS ($P = 0.80$). While MMP-9 semi-quantification indicated a significant reduction in MMP-9 expression in tissue exposed to BAV AA WSS (0.3-fold increase *vs* control, $P = 0.01$), it revealed no statistical difference between the fresh controls and the

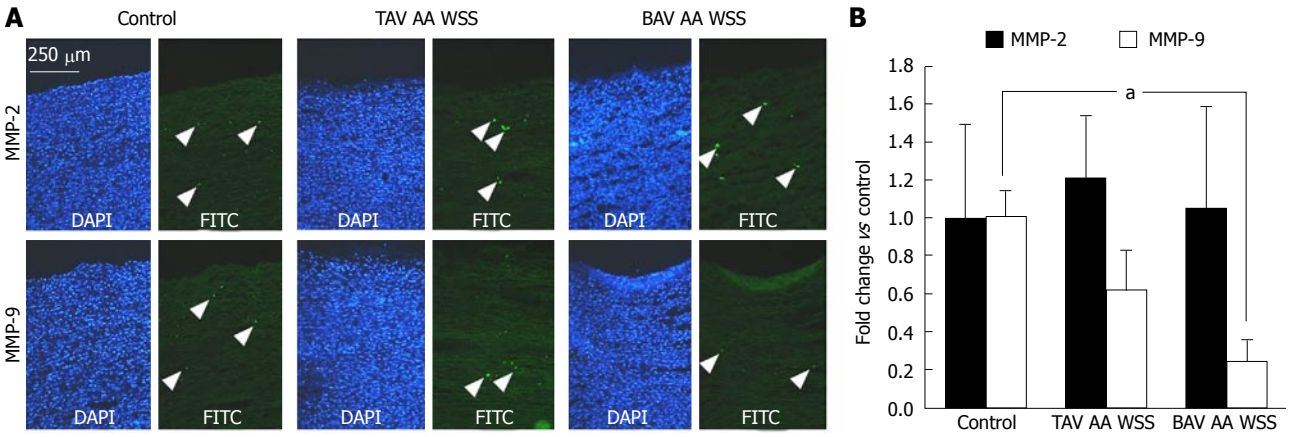

Figure 2 Matrix metalloproteinase immunohistochemistry. A: Matrix metalloproteinase (MMP)-2 and MMP-9 immunostaining with DAPI and FITC filters (blue cell nuclei, green positively stained cells); B: Semi-quantitative results ($^{a}P < 0.05$ *vs* control). DAPI: 4',6-diamidino-2-phenylindole; FITC: Fluorescein isothiocyanate.

specimens subjected to TAV AA WSS (0.6-fold increase *vs* control, $P = 0.20$) or between the two conditioned groups ($P = 0.19$).

TAV and LR-BAV hemodynamic stresses generate similar MMP activities in AA concavity tissue

MMP-2 and MMP-9 zymography results are shown in Figure 3. MMP-9 was undetectable in the zymograms. While the active form of MMP-2 was hardly detectable in the fresh controls, both latent and active MMP-2 were found in both experimental groups subjected to WSS (Figure 3A). Neither latent nor active form of MMP-9 was detected in the zymogram. Therefore, the densitometric quantification of MMP activity was only performed on MMP-2. Total MMP-2 activity was quantified as the sum of active and latent forms of MMP-2 (Figure 3B). Tissue specimens subjected to TAV AA WSS exhibited a significant increase in total MMP-2 activity relative to the fresh controls (1.5-fold increase, $P = 0.02$). While BAV AA WSS also resulted in increased total MMP-2 activity relative to the fresh controls (1.5-fold increase), the difference was not statistically significant ($P = 0.37$). No significant difference was also detected between the two experimental groups subjected to WSS ($P = 0.08$). Although the densitometric analysis performed on the latent form of MMP-2 (Figure 3C) indicated lower contents in groups subjected to TAV AA WSS (0.76-fold increase) and tissue subjected to BAV AA WSS (0.69-fold increase) relative to fresh tissue, the differences were not statistically significant ($P = 0.17$ and $P = 0.12$, respectively). No statistical difference was found between the two experimental groups subjected to WSS ($P = 0.59$). Lastly, tissue conditioning to WSS resulted in a dramatic increase in expression of active MMP-2 relative to the fresh controls, in which active MMP-2 expression was hardly detectable (TAV AA WSS: 5713-fold increase *vs* controls, $P = 0.004$; BAV AA WSS: 3877-fold increase *vs* controls, $P = 0.0008$; Figure 3D). The difference in active MMP-2 expression between the two conditioned groups remained non-significant ($P = 0.15$), but both groups exhibited a conversion from pro-(latent) to active MMP-2 after 48 h of conditioning.

DISCUSSION

We conducted an *ex vivo* study to investigate the isolated effects of BAV hemodynamic stresses on the biological remodeling of porcine AA tissue excised from the concavity of the aortic wall. The primary contribution of this study is the indication that, in the absence of any underlying congenital defect, the local hemodynamics experienced by the LR-BAV AA concavity does not trigger any acute upregulation of enzymatic protease expression or activity in the aortic medial layer.

The immunostaining and zymography analyses evidenced the absence of significant change in MMP expression and activity between the aortic wall specimens subjected to TAV AA and LR-BAV AA WSS. Those results complement our previous study on the effects of BAV flow on the biology of the AA convexity, which revealed the susceptibility of the WSS overload experienced by the convexity of the LR-BAV AA to promote aortic medial degradation *via* MMP-dependent pathways[34,35,40]. Those observations are consistent with the asymmetric presentation of aortic dilation observed in type-I LR-BAV patients and the higher vulnerability of the AA wall convexity to aortopathy[14,15,44]. The similarities in the hemodynamic stress environments present in the concavity of the TAV AA and LR-BAV AA evidenced in the present study, combined with the absence of difference in the remodeling activity of tissue exposed to those environments support a hemodynamic etiology of BAV aortopathy.

The demonstration of causality between the asymmetric BAV flow patterns and the asymmetric expression of secondary BAV complications has already been established in the context of BAV calcification, which typically affects primarily the fused leaflets exposed to WSS overload but spares the non-coronary leaflet subjected to relatively normal WSS levels[42,45]. Therefore, while the involvement of underlying genetic

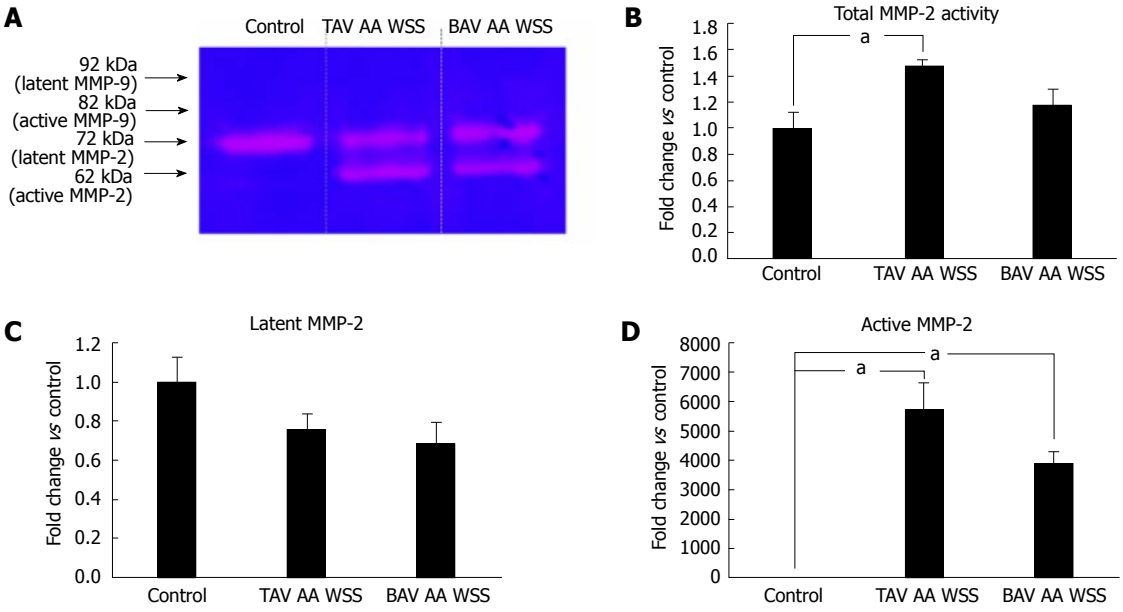

Figure 3 Matrix metalloproteinase gelatin zymography. A: Zymogram showing latent and active forms of matrix metalloproteinase (MMP)-2 and MMP-9 in fresh controls and tissue conditioned to tricuspid aortic valve (TAV) ascending aorta (AA) and bicuspid aortic valve (BAV) AA wall shear stress (WSS); B: Densitometry results for total MMP-2 enzymatic activity; C: Latent MMP-2; D: Active MMP-2 ($^aP < 0.05$ *vs* control; MMP-9 data not shown as MMP-9 was absent from the protein lysates).

abnormalities in BAV leaflets and BAV AA tissue cannot be ruled out, the present study provides one more evidence in support of the key role played by blood flow and hemodynamic stresses in BAV disease.

While no difference in protease expression and activity was detected between the tissue groups subjected to TAV AA and BAV AA WSS, some changes were measured between the fresh controls and the conditioned groups. First, tissue subjected to WSS exhibited a significant upregulation of active MMP-2 relative to fresh tissue. Although this result requires further investigation, it is important to note that, while the bioreactor was able to subject the samples to WSS, it eliminated all other forces normally present in the native environment, such as stretch and pressure. Those forces have been shown to play a critical role in the maintenance of vascular homeostasis and MMP-2 regulation[46,47]. Second, tissue exposure to LR-BAV WSS resulted in a significant MMP-9 downregulation relative to the fresh controls as suggested by immunostaining, while MMP-2 levels remained unchanged. This difference in biological response may be related to the specific mechanosensitivity of the aortic endothelium to WSS. In fact, studies conducted in our laboratory in the context of valvular tissue have demonstrated the differential sensitivity of valvular endothelial cells to WSS magnitude, directionality and frequency[41,43,48]. Following the same concept, the cells lining the aortic wall may be able to detect changes in different WSS characteristics and transduce them into different biological responses. As a result, despite the qualitative similarity between the WSS waveforms captured in the concavity of the TAV AA and LR-BAV AA, the minor differences in magnitude and directionality, as quantified by the TSM and OSI (Table 1), may be sufficient to drive a differential biological response.

MMP-9 expression was below detection level in tissue lysates and zymograms. The near absence of MMP-9 expression following exposure of aortic concavity tissue to WSS proves to be an interesting phenomenon. Animal models have indicated that MMP-2 and MMP-9 work synergistically to promote aneurysm formation[49] and that MMP-9 knockout mice are unable to develop aneurysms even under the presence of increased MMP-2 activity[50]. Therefore, the absence of MMP-9 in fresh tissue and tissue subjected to TAV AA and BAV AA WSS, which prevents the possible downstream pathological effects of MMP-2/MMP-9 synergies, may protect the aortic wall against dilation and aneurysm formation. Further *ex vivo* investigations on the combined effects of WSS, MMP-2 and MMP-9 and the modulation of the remodeling response through MMP-2/MMP-9 synergies will be necessary to test this hypothesis.

Although relationships between WSS abnormalities and biological perturbations in the aortic endothelium have been previously evidenced[51-54], more work needs to be done to isolate the impact of hemodynamic stress abnormalities on tissue remodeling. The results collected from the present study combined with those from our previous study[35] on the local effects of TAV and LR-BAV AA hemodynamic stresses on AA remodeling suggest the susceptibility of BAV hemodynamic stresses to mediate aortic medial degradation on the disease-prone wall convexity, while sparing the wall concavity. These observations suggest a critical role played by hemodynamic stresses in the development of BAV asymmetric aortopathies.

Finally, several limitations should be discussed.

The demonstration of a role for WSS in the differen-

tial remodeling state of the BAV AA convexity and concavity relied on the retrospective comparison of the present data obtained with AA concavity tissue with our previous data obtained with AA convexity tissue[35]. While ideally both sets of experiments should have been carried out using tissue specimens from the same animal, the availability of a single shear stress bioreactor prevented such mode of operation. However, the same methodology (sample size, culture techniques, biological endpoints, assays) as in the previous study was implemented to allow for the direct comparison of the results.

Second, the study only focused on the effects of WSS and neglected other important mechanical signals (stretch, pressure) normally found in the native environment. This choice is justified by two arguments. First, the objective of the study was to isolate the potential role played by WSS in the remodeling of the BAV AA concavity, in the absence of any other biochemical and mechanical signals. Second, the mechanical characterization of the TAV and BAV aortic wall provided by the FSI model revealed the absence of substantial differences in circumferential stretch and pressure between the TAV AA and BAV AA (average pressure difference: 0.4% in the convexity and 0.5% in the concavity; average stretch difference: 0% in the convexity and 0.3% in the concavity). The analysis of the WSS in both anatomies revealed more contrasted environments (TSM difference: 94% in the convexity and 38% in the concavity), which motivated and justified the investigation of their mechanobiological impact.

Third, while the study would benefit from a larger sample size, the absence of significant differences in the remodeling response of TAV AA and BAV AA concavity tissue is in agreement with the data reported in larger clinical studies that examined the asymmetric nature of aortic dilation and the spatiotemporal patterns of MMP expression in BAV AAs[14-16,55,56]. In addition, the same sample size in our previous study was able to demonstrate statistically significant biological differences between convexity specimens subjected to TAV AA and BAV AA WSS. In this context, the absence of statistical differences in the remodeling response of concavity tissue samples subjected to TAV AA and BAV AA flow is likely to be a reflection of the low impact of the concavity WSS environment on the local tissue biology rather than the consequence of a small sample size.

COMMENTS

Background

The asymmetric dilation of the ascending aortic (AA) wall downstream of the bicuspid aortic valve (BAV) is marked by aortic medial degeneration through upregulation of matrix metalloproteinase (MMP) expression and enzymatic activity. BAV AA dilation typically localizes to the AA wall convexity, which is the region impinged by the skewed BAV orifice jet and subjected to abnormally high hemodynamic wall shear stress (WSS). While previous studies have established a link between the WSS overload on the AA wall convexity and the upregulation of remodeling activity, the ability of the hemodynamic environment present in the BAV AA concavity to maintain vascular homeostasis has not been formally demonstrated.

Research frontiers

The common classification of BAV aortopathy as an inherited disorder has guided the implementation of aggressive surgical modalities including reduction aortoplasty, aortic root replacement and AA replacement. However, those procedures have been associated with a significant mortality rate when treating aortic dilation in BAV patients. Such outcome questions the suitability of those surgical approaches and justifies the need to elucidate the etiology of BAV aortopathy. The characterization of the remodelling pathways involved in the disease and the interacting mechanisms of micro-scale mechanotransduction and macro-scale hemodynamics are current hotspots in BAV and vascular research.

Innovations and breakthroughs

The authors' previous *ex vivo* characterization of the isolated effects of tricuspid aortic valve (TAV) AA and BAV AA WSS on the biology of the AA convexity revealed the ability of BAV AA hemodynamic abnormalities in the wall convexity to promote aortic medial degeneration *via* MMP-2 and MMP-9-dependent pathways. The present study is a logical extension of the authors' previous work as it investigates the contribution of the WSS environment in the seemingly disease-protected concavity of the LR-BAV AA on MMP biology. The absence of difference in the remodeling response of tissue subjected to the local WSS normally present in the TAV AA and LR-BAV AA concavity justify at least partially the asymmetric dilation pattern typically observed in LR-BAV patients and isolate, for the first time, the key role played by hemodynamic stresses in BAV aortopathogenesis.

Applications

The demonstration of a hemodynamic pathway of BAV aortic dilation may switch the clinical focus from developing aggressive surgical procedures aimed at eliminating the presumed genetically weakened AA wall to investigating new modalities aimed at normalizing BAV aorta hemodynamics or inhibiting pharmacologically the pathological remodeling cascade at an early age.

Terminology

The BAV is the most common cardiac anomaly and consists of two leaflets instead of the three present in the normal TAV. The most common LR-BAV morphotype results from the fusion between the left- and right-coronary cusps. WSS is the frictional fluid force resulting from the relative motion between the aortic wall and the surrounding blood flow. MMP-2 and MMP-9 are two proteolytic enzymes that degrade collagen, elastin and fibronectin, which are fundamental protein components of the aortic media.

Peer-review

The manuscript by Atkins *et al* proposes an *ex-vivo* model of wall shear stress applied to the lesser curvature (concavity) of porcine thoracic aorta. The *ex-vivo* model reproduces in a bioreactor the shear stress generated by a TAV and by a LR-BAV, with the aim of dissecting the role played by this single local hemodynamic factor on vascular wall remodeling of aortic concavity.

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META-ANALYSIS

Percutaneous assist devices in acute myocardial infarction with cardiogenic shock: Review, meta-analysis

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Abstract

AIM: To assess the impact of percutaneous cardiac support in cardiogenic shock (CS) complicating acute myocardial infarction (AMI), treated with percutaneous coronary intervention.

METHODS: We selected all of the studies published from January 1st, 1997 to May 15st, 2015 that compared the following percutaneous mechanical support in patients with CS due to AMI undergoing myocardial revascularization: (1) intra-aortic balloon pump (IABP) *vs* Medical therapy; (2) percutaneous left ventricular assist devices (PLVADs) *vs* IABP; (3) complete extracorporeal life support with extracorporeal membrane oxygenation (ECMO) plus IABP *vs* IABP alone; and (4) ECMO plus IABP *vs* ECMO alone, in patients with AMI and CS undergoing myocardial revascularization. We evaluated the impact of the support devices on primary and secondary endpoints. Primary endpoint was the inhospital mortality due to any cause during the same hospital stay and secondary endpoint late mortality at 6-12 mo

of follow-up.

RESULTS: One thousand two hundred and seventy-two studies met the initial screening criteria. After detailed review, only 30 were selected. There were 6 eligible randomized controlled trials and 24 eligible observational studies totaling 15799 patients. We found that the inhospital mortality was: (1) significantly higher with IABP support *vs* medical therapy (RR = +15%, *P* = 0.0002); (2) was higher, although not significantly, with PLVADs compared to IABP (RR = +14%, *P* = 0.21); and (3) significantly lower in patients treated with ECMO plus IABP *vs* IABP (RR = -44%, *P* = 0.0008) or ECMO (RR = -20%, *P* = 0.006) alone. In addition, Trial Sequential Analysis showed that in the comparison of IABP *vs* medical therapy, the sample size was adequate to demonstrate a significant increase in risk due to IABP.

CONCLUSION: Inhospital mortality was significantly higher with IABP *vs* medical therapy. PLVADs did not reduce early mortality. ECMO plus IABP significantly reduced inhospital mortality compared to IABP.

Key words: Intra-aortic balloon pump; Impella; TandemHeart; Extracorporeal membrane oxygenation; Cardiogenic shock; Meta-analysis

Core tip: Meta-analyses from observational studies represent an area of innovation in statistical science. In the present review, we identified only a small number of randomized trials, which by themselves were underpowered to assess the efficacy of the support devices on inhospital mortality. To increase the power of the analysis we included observational data, which enabled us to add 14909 additional patients to the 890 from the randomized controlled trials selected. The results of the analysis showed that: (1) intra-aortic balloon pump (IABP) used alone was associated with significant increase in inhospital mortality compared to Medical therapy; (2) percutaneous left ventricular assist devices increased, although non significantly, the mortality as compared with IABP; and (3) extracorporeal membrane oxygenation (ECMO) plus IABP had significant protective effect compared to IABP or ECMO alone.

Romeo F, Acconcia MC, Sergi D, Romeo A, Francioni S, Chiarotti F, Caretta Q. Percutaneous assist devices in acute myocardial infarction with cardiogenic shock: Review, meta-analysis. *World J Cardiol* 2016; 8(1): 98-111 Available from: URL: http://www.wjgnet.com/1949-8462/full/v8/i1/98.htm DOI: http://dx.doi.org/10.4330/wjc.v8.i1.98

INTRODUCTION

Cardiogenic shock (CS) occurs in 5% to 15% of patients with acute myocardial infarction (AMI). Despite major technical advances the inhospital mortality of these patients continues to remain unacceptably high at over 40%[1-4]. To date immediate myocardial revascularization represents the only intervention of proven benefit. Emergency percutaneous coronary intervention (PCI) is recommended if coronary anatomy is amenable and emergency surgical revascularization is recommended in case coronary anatomy is not amenable for PCI (AHA/ACC and ESC/EACTS indication: Class Ⅰ, Level B)[5-7]. In order to maintain hemodynamic stabilization before and/or after early revascularization, mechanical support with devices such as intra-aortic balloon pumping (IABP), percutaneous left ventricular assist devices (PLVADs) and complete extracorporeal life support with extracorporeal membrane oxygenation (ECMO) are often considered[8]. It is known that IABP support provides significant benefit when used in association with thrombolysis; however, it is of no benefit when used in association with PCI[4,9,10].

It is of note that current guidelines do not recommend routine use of IABP in AMI patients with CS complicating AMI (AHA/ACC and ESC/EACTS indication: Class Ⅲ, Level A), but IABP use may be considered in these patients when CS is secondary to mechanical complications (AHA/ACC indication: Class Ⅱa, Level C). Further, it is recommended that the use of LV assist devices should be restricted for short-term circulatory support (AHA/ACC and ESC/EACTS indication: Class Ⅱb, Level C)[5-7].

Because the sickest patients are often excluded from randomized controlled trials (RCTs), only few RCTs of circulatory assist devices have been conducted thus far. On the other hand, there are some data from clinical observational studies[11-15].

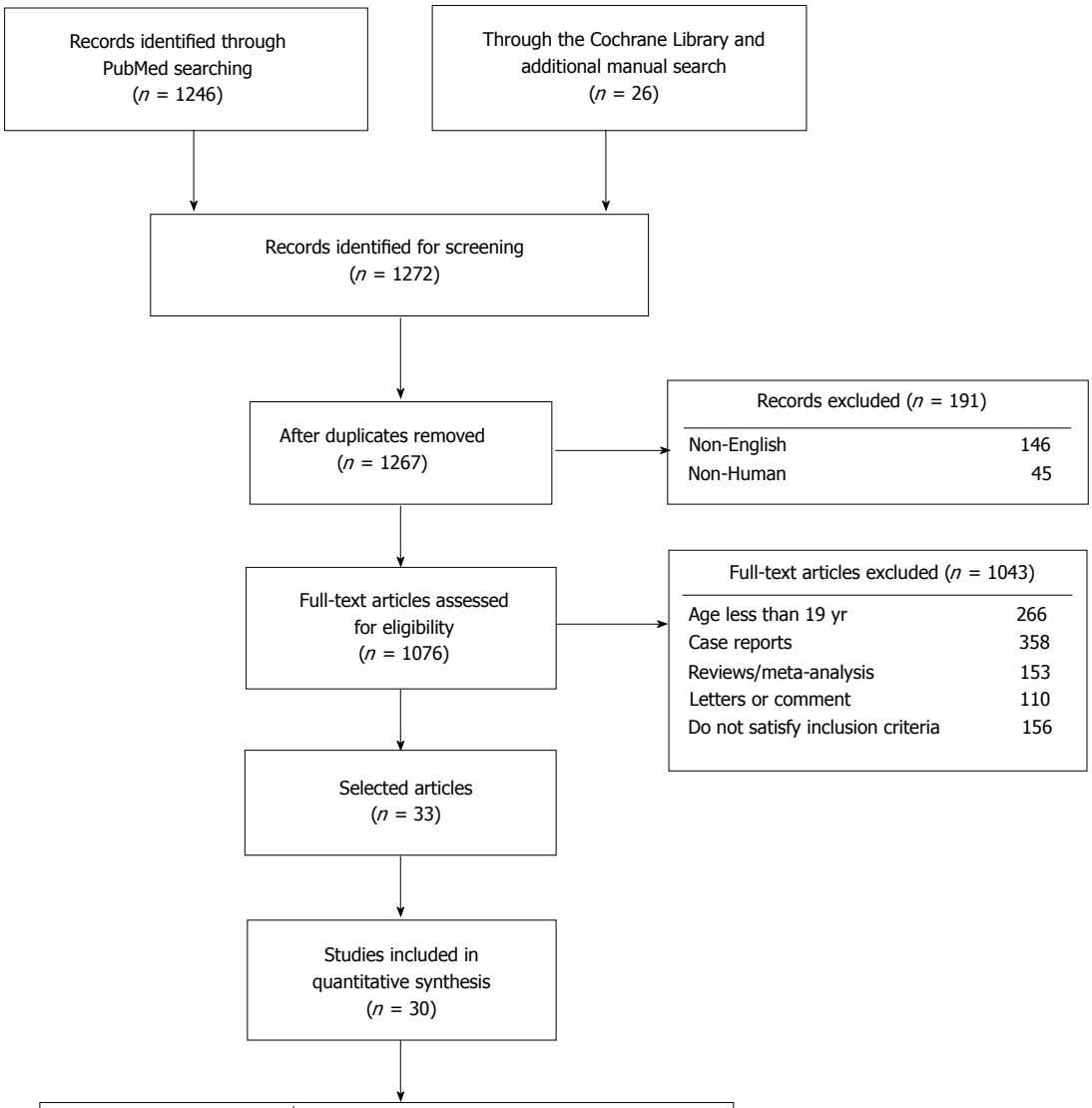
We present here a meta-analysis of available data, based on RCTs and observational studies, on the use of support devices in AMI patients with CS undergoing PCI with regard to inhospital and late mortality.

MATERIALS AND METHODS

Study definition (search and data extraction)

We performed a systematic PubMed and the Cochrane Library literature search using the terms relating to the intervention of interest "IABP" or "IABC", "Impella", "Tandemheart", "PLVADs" "ECMO" or "extracorporeal life support" or "ECLS" or "CPS" in the setting of CS in patients with AMI undergoing percutaneous coronary revascularization. We performed additional manual literature search through: (1) the reference lists of retrieved articles and published reviews; and (2) the abstracts presented at recent (last five years) International Conferences.

Two investigators independently examined the designs, patient populations and interventions used, aiming to include only studies designed to test the effect of the percutaneous support in patients with CS due to AMI and undergoing myocardial revascularization. The



Comparison	No. of studies	Inhospital mortality				Total
		Experimental group		Control group		
		Events	Total	Events	Total	
IABP *vs* medical therapy	13	2016	4360	1783	4431	8791
PLVADs *vs* IABP	6	66	118	73	153	271
ECMO + IABP *vs* IABP	3	32	88	46	76	164
ECMO + IABP *vs* ECMO	6	116	196	72	105	301
Total	28	2230	4762	1974	4765	9527
		Late mortality				
IABP *vs* medical therapy	6	1816	3491	1396	3550	7041
Overall	30	3851	7865	3167	7934	15799

Figure 1 Flow-chart of the study selection process. IABP: Intra-aortic balloon pump; PLVADs: Percutaneous left ventricular assist devices; ECMO: Extracorporeal membrane oxygenation.

search was restricted to English-language journals and excluded studies on non-human subjects as well as articles unrelated to the topic.

The study selection process is outlined in Figure 1. The exclusion criteria were data from registries or studies with lack of a control group, the absence of mortality data, the presence of different timing for the outcome or, more generally, insufficient data for risk estimation. Disagreements were resolved by asking the opinion of a third reviewer to reach consensus at each stage of the screening process. We selected all of the studies published from January 1st, 1997 to May 15st, 2015 that compared the following percutaneous mechanical support in patients with CS due to AMI undergoing myocardial revascularization: (1) IABP *vs* Medical therapy; (2) PLVADs *vs* IABP; and (3) ECMO plus IABP *vs* IABP or ECMO. CS was defined by: (1) a decrease in systolic blood pressure to $\geqslant$ 90 mmHg for more than 30 min, in the absence of hypovolemia, or requiring vasopressor support; (2) a reduction of cardiac

index to 1.8 L/min per square without support or to 2.0–2.2 L/min per square with support; and (3) elevated left ventricular filling pressures[16,17]. Moreover, profound shock was defined as systolic blood pressure less than 75 mmHg-despite receiving an intravenous inotropic agent that was associated with altered mental status and respiratory failure[18]. The acronym PLVADs included the Impella®2.5 (Abiomed, Danvers, MA, United States) and the TandemHeart (Cardiac Assist Inc., Pittsburgh, PA, United States)[14,15]. The acronym of ECMO included a modified heart-lung machine, generally consisted of a centrifugal pump, a heat exchanger and a membrane oxygenator[15,18-22].

Study outcomes

Primary and secondary endpoints: We evaluated the impact of the support devices on primary and secondary endpoints. Primary endpoint was the inhospital mortality due to any cause during the same hospital stay and secondary endpoint late mortality at 6-12 mo of follow-up.

Statistical analysis

Meta-analysis was performed separately for observational studies and RCTs comparing the following groups of patients: (1) IABP (experimental) *vs* Medical therapy (control); (2) PLVADs (experimental) *vs* IABP (control); (3) ECMO plus IABP (experimental) *vs* IABP (control); and (4) ECMO plus IABP (experimental) *vs* ECMO (control). We computed the risk ratio (RR) with 95%CI, using the Mantel-Haenszel random-effect model to take into account possible heterogeneity among the individual studies beyond that expected from chance, to point out the relative effect of the mechanical assist devices under study. We used the Forest plot to present the results graphically, to report the effect estimates for the individual studies together with the overall measure of effect. We computed the Cochran's *Q* test and I^2 statistics to quantify the homogeneity/heterogeneity among the selected studies within and between subgroups[23]. A Funnel Plot was designed as visual aid for detecting bias or systematic heterogeneity among the studies included in the meta-analysis (publication bias). A sensitivity analysis was then performed by repeating the meta-analysis after exclusion of the study(ies) falling out the 95%CI.

The meta-analysis was performed using Review Manager (RevMan) (Computer program) Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboratio, 2014[24].

We performed Trial Sequential Analysis using the program provide by "The Copenhagen Trial Unit, Center for Clinical Intervention Research CTU, Denmark; version 0.9 beta; available at www.ctu.dk/tsa" in order to assess if the studies enclosed in the meta-analysis reached the required number of participants (information size), and to construct the monitoring boundaries to detect significance and futility of the primary and secondary endpoints[25,26]. Trial Sequential Analysis was done using the effective difference in risks between the experimental (intervention risk) and control groups (basal risk) with a risk of a type I error of 5% and a power of 80%. The relative risk reductions (RRR) observed were linked to the number of patients to be treated (NNT) or to be harmed (NNH), to assess the clinical benefit or the detrimental effect corresponding to each level of RRR. All statistical tests were two-sided and α error of $\leqslant$ 0.05 was defined as statistically significant.

The statistical methods of this study were reviewed by Flavia Chiarotti, Biostatistician, Research Director from the Italian National Institute of Health.

RESULTS

One thousand two hundred and seventy-two records met the initial screening criteria. After detailed review, only 30 were selected[4,18,21,27-54]. There were 6 eligible RCTs[4,27-31] and 24 eligible observational studies[18,21,32-54] totaling 15799 patients. The main characteristics of the selected studies are reported in Table 1.

IABP vs medical therapy

In the comparison between IABP and Medical therapy, we analysed a total of 15063 patients (14273 from 12 observational studies[32-44] and 790 form 3 RCTs[4,27,31]). The data provided us by French *et al*[31] and Kunadian *et al*[44] contributed only for the analysis of the secondary outcome.

Primary endpoint: Primary endpoint was assessed in 8791 patients (8153 from 11 observational studies[32-43] and 638 from 2 RCTs[4,27]). The inhospital deaths occurred in 46.24% of patients in the experimental group and 40.24% of patients in the control. The NNH was 16 (6 more deaths every 100 patients treated with IABP). The overall analysis showed a significant risk increase (+18%, P = 0.002) in the IABP group (Figure 2). More specifically, we observed a significant risk increase in observational studies (RR = +21%, P = 0.0008) and a nonsignificant risk reduction in RCTs (RR = -3%%, P = 0.78) (Figure 2). The test for subgroup differences showed high heterogeneity among observational studies (I^2 = 63%) and between observational and RCTs (I^2 = 73.9%), providing a significantly different estimate of the IABP effect (Figure 2). In the Funnel plot, the studies by Gu *et al*[37] and by Zeymer *et al*[39] fell out of the 95%CI, thus appearing to be the potential source of bias. After the sensitivity analysis, heterogeneity decreased to a lower level among the observational (I^2 = 19%), but persisted at high levels between observational studies and RCTs (I^2 = 68.2%) (Table 2). Furthermore the overall risk in the experimental group slight decreased (RR = +15%) (Table 2). The NNH was equal to 18 (5 more deaths every 100 patients treated with IABP) (Table 3). Trial Sequential Analysis showed that the required number of participant was reached and the monitoring boundaries,

Table 1 Main characteristics of the selected studies

Ref.	Setting	Study design	Etiology of CS	Cardiac arrest	Treatment	Period	No. of pts
IABP *vs* medical therapy							
Anderson *et al*[32], 1997 (GUSTO-I)	United States, Europe	Obs.; multicenter	STEMI	No	PCI	1990-1993	37
Sanborn *et al*[33], 2000 (SHOCK Registry)	United States, Canada, Europe, New Zealand	Obs.; multicenter registry	AMI	No	PCI or CABG	1993-1997	383
Barron *et al*[34], 2001 (NRMI-2)	United States	Obs.; multicenter registry	AMI	No	PCI	1994- < 2000	2990
French *et al*[31], 2003 (SHOCK Trial 12-mo survival)	United States, Canada, Europe, New Zealand	RCT; multicenter	AMI	No	PCI or CABG	1993-1998	152
Vis *et al*[35,36], 2007 (AMC CS)	Europe	Obs.; single-center	STEMI	No	PCI	1997-2005	292
Gu *et al*[37], 2010	Asia	Obs.; single-center	STEMI	No	PCI	2003-2008	91
Prondzinsky *et al*[27], 2010 (IABP-SHOCK)	Europe	RCT; single-center	AMI	No	PCI	2003-2004	40
Stub *et al*[38], 2011	Europe	Obs.; multicenter registry	ACS	No	PCI	2004-2010	410
Zeymer *et al*[39], 2011 (Euro Heart Survey PCI)	Europe	Obs.; multicenter registry	STEMI or NSTEMI	No	PCI	2005-2008	653
Thiele *et al*[4], 2012 (IABP-SCHOCK II)	Europe	RCT; multicenter	AMI	No	PCI (95.8%), CABG (3.5%), PCI and CABG (0.7%)	2009-2012	598
Zeymer *et al*[40], 2013 (ALKK-PCI)	Europe	Obs.; multicenter registry	STEMI or NSTEMI	No	PCI	2006-2011	1913
Dziewierz *et al*[41], 2014 (EUROTRANSFER registry)	Europe	Obs.; multicenter registry	STEMI	No	PCI (49 pts), CABG (2 pts)	2005-2007	51
Kunadian *et al*[44], 2015 (BCIS registry)	Europe	Obs.; multicenter registry	ACS	No	PCI	2005-2011	6120
Kim *et al*[42], 2015 (KAMIR)	Asia	Obs.; multicenter registry	AMI	Yes	PCI	2005-2014	1214
Suzuki *et al*[43], 2015 (Tokyo CCU Network Scientific Council)	Asia	Obs.; multicenter registry	STEMI	No	PCI	2009-2011	119
PLVADs (TandemHeart, Impella® 2.5) *vs* IABP							
Thiele *et al*[29], 2005[1]	Europe	RCT; single center	AMI	No	PCI (49 pts), CABG (2 pts)	2000-2003	41
Burkoff *et al*[28], 2006[1]	United States, Europe	RCT; multicenter	AMI (70%)	No	PCI (22 pts), CABG (3 pts)	2002-2004	33
Seyfarth *et al*[30], 2008[2] (ISAR-SHOCK)	Europe	RCT; two-center	AMI	No	PCI (22 pts)	2004-2007	26
Schwartz *et al*[46], 2012[1,2]	United States	Obs.; single center	68% STEMI, 11% OHCA	Yes	PCI (63 pts), CABG (5 pts)	2008-2010	76
Shah *et al*[47], 2012[1,2]	United States	Obs.; single center	STEMI or UA/NSTEMI	No	PCI	2007-2009	17
Manzo-Silberman *et al*[45], 2013[2]	Europe	Obs.; single center registry	ACS (mainly), OHCA	Yes	PCI (54 pts)	2007-2010	78
ECMO plus IABP *vs* IABP							
Sheu *et al*[18], 2010	Asia	Obs.; single center	STEMI	No	PCI	1993-2009	71
Tsao *et al*[21], 2012	Asia	Obs.; single center	AMI	No	PCI	2004-2009	58
Perazzolo Marra *et al*[48], 2013	Europe	Obs.; single center	AMI	No	PCI	2010-2012	35
ECMO plus IABP *vs* ECMO							
Yamauchi *et al*[49], 2009	Asia	Obs.; single center	AMI	No	PCI	2000-2007	16
Chung *et al*[50], 2011	Asia	Obs.; multicenter	AMI, INCA (14 pts)	Yes	PCI (7 pts), CABG (13 pts)	2206-2009	20
Kagawa *et al*[51], 2012	Asia	Obs.; multicenter	ACS, INCA, OHCA	Yes	PCI	2004-2011	73
Aoyama *et al*[52], 2014	Asia	Obs.; single center	AMI, INCA (2 pts, OHCA 7 pts)	Yes	PCI (34 pts), CABG (4 pts)	1993-2000	38
Park *et al*[53], 2014	Asia	Obs.; single center	AMI	No	PCI (78 pts), PCI e/o CABG (10 pts), medical treatment (8 pts)	2004-2011	96
Kim *et al*[54], 2014	Asia	Obs.; multicenter	ACS	No	PCI (53 pts), CABG (5 pts)	2010-2013	58

ACS: Acute coronary syndrome; AMI: Acute myocardial infarction; CABG: Coronary artery bypass grafting; CS: Cardiogenic shock; ECMO: Extracorporeal membrane oxygenation; IABP: Intra-aortic balloon pump; INCA: In-of-hospital cardiac arrest; NSTEMI: Non-ST-elevation myocardial infarction; PCI: Percutaneous coronary intervention; PLVADs: Percutaneous left ventricular assist devices with ([1]TandemHeart, or [2]Impella® 2.5); pts: Patients; Obs.: Observational study; OHCA: Out-of-hospital cardiac arrest; RCT: Randomized controlled trial; STEMI: ST-elevation myocardial infarction; UA: Unstable angina.

Table 2 Meta-analysis before and after sensitivity analysis

Comparison/subgroup	RR							
	Before				After			
	n	I^2 (%)	Estimate (95%CI)	*P*	*n*	I^2 (%)	Estimate (95%CI)	*P*
Inhospital mortality								
IABP *vs* medical therapy								
Observational studies	11	63	1.21 (1.08, 1.36)	0.0008	9	19	1.17 (1.09, 1.26)	< 0.0001
RCTs	2	0	0.97 (0.81, 1.18)	0.78	2	0	0.97 (0.81, 1.18)	0.78
Overall effect	13	62	1.18 (1.06, 1.32)	0.002	11	24	1.15 (1.07, 1.24)	0.0002
Test for subgroup differences[1]		χ^2 = 3.83, df = 1 (P = 0.05), I^2 = 73.9%				χ^2 = 3.14, df = 1 (P = 0.08), I^2 = 68.2%		
ECMO plus IABP *vs* ECMO								
Observational studies	6	12	0.78 (0.65, 0.94)	0.008	5	0	0.80 (0.68, 0.94)	0.006
Late mortality								
IABP *vs* medical therapy								
Observational studies	3	90	0.92 (0.51, 1.67)	0.78	2	60	1.16 (0.69, 1.95)	0.57
RCTs	3	32	1.16 (0.86, 1.58)	0.34	2	0	1.56 (0.97, 2.52)	0.07
Overall effect	6	85	1.08 (0.82, 1.41)	0.60	4	0	1.38 (1.30, 1.46)	< 0.00001
Test for subgroup differences[1]		χ^2 = 0.48, df = 1 (P = 0.49), I^2 = 0%				χ^2 = 0.68, df = 1 (P = 0.41), I^2 = 0%		

[1]Between observational studies and RCTs. IABP: Intra-aortic balloon pump; RCT: Randomized controlled trial; ECMO: Extracorporeal membrane oxygenation.

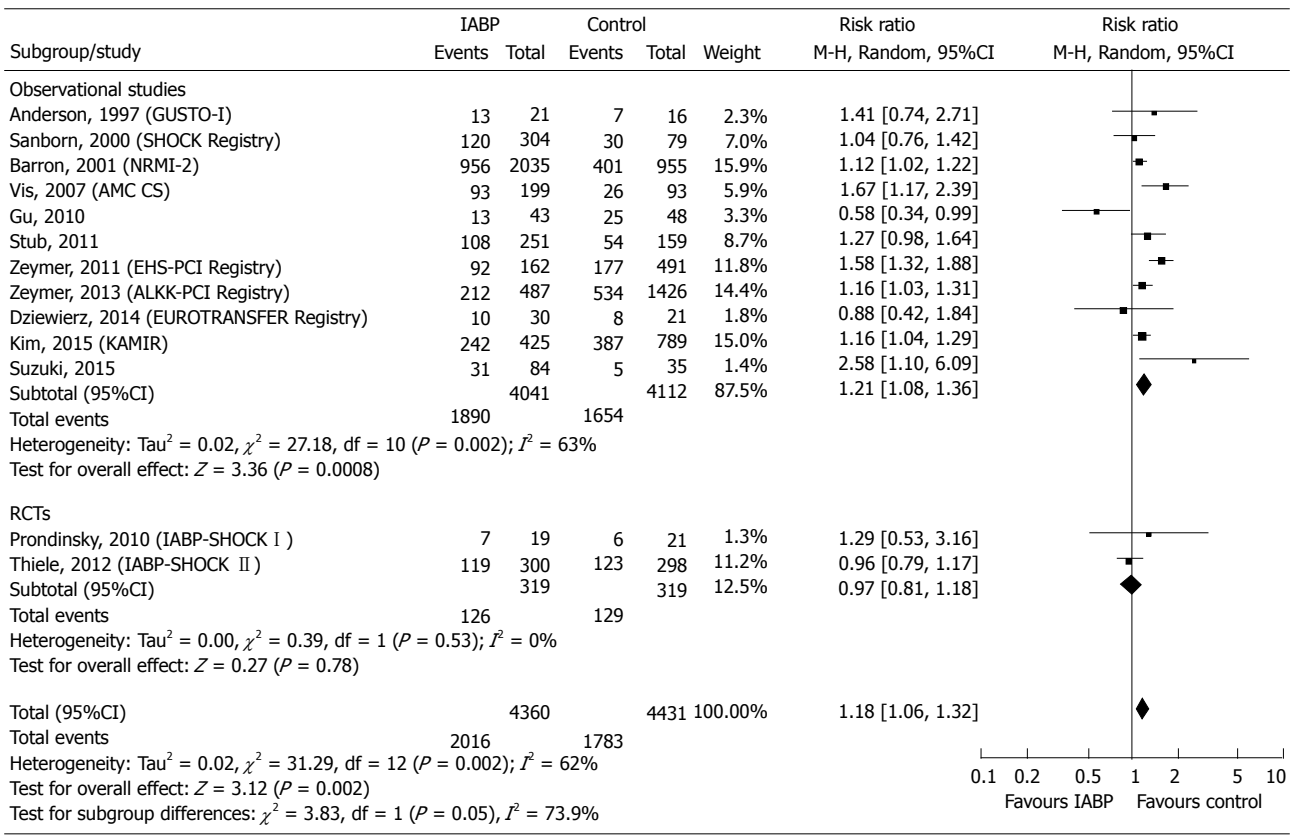


Figure 2 Meta-analysis on risk ratio of inhospital mortality between the patients with intra-aortic balloon pump *vs* medical therapy.

constructed to detect significance, were crossed by the z-curves, demonstrating a detrimental effect of IABP (Table 3, Figure 3).

Secondary endpoint: The late mortality was assessed in 7041 patients (6262 from 3 observational studies[37,41,44] and 779 from 3 RCTs[4,27,31,55,56]). Mortality rate was higher, but not significantly, in the IABP group respect to control (52.02% *vs* 39.32%). IABP reduced mortality (-8%, P = 0.78) in observational studies and increased mortality (+16%, P = 0.34) in RCTs (Figure 4). In the Funnel plot the studies by Gu *et al*[37] and by Thiele *et al*[56] fell out of the 95%CI, appearing to be the potential source of bias. When we applied the sensitivity analysis by excluding the study by Gu *et al*[37] from observational studies and the study by Thiele *et al*[56]

Table 3 Benefit - harm observed in the experimental group and result of Trial Sequential Analysis

Groups			Mortality rate (%)		RRR	Effect of experimental support				Trial Sequential Analysis	
Experimental		Control	Experimental	Control		NNT	NNH	Harm[1]	Benefit[1]	Required information size	Results
Inhospital mortality											
IABP[2]	*vs*	Medical therapy[2]	45.99	40.62	-13.22		18	5.37		2174	Conclusive
PLVADs	*vs*	IABP	55.93	47.71	-17.23		12	8.22		1161	Inconclusive
ECMO + IABP	*vs*	IABP	36.36	60.53	39.92	5			24.16	150	Conclusive
ECMO + IABP[2]	*vs*	ECMO[2]	61.29	66.67	8.06	19			5.38	Not calculable	Inconclusive
Late mortality											
IABP	*vs*	Medical therapy	52.02	39.32	-32.28		7	12.70		5984	Futility
IABP[2]	*vs*	Medical therapy[2]	52.08	37.68	-38.22		6	14.40		168	Conclusive

[1]Number of patients out of 100; [2]Comparison after sensitivity analysis.

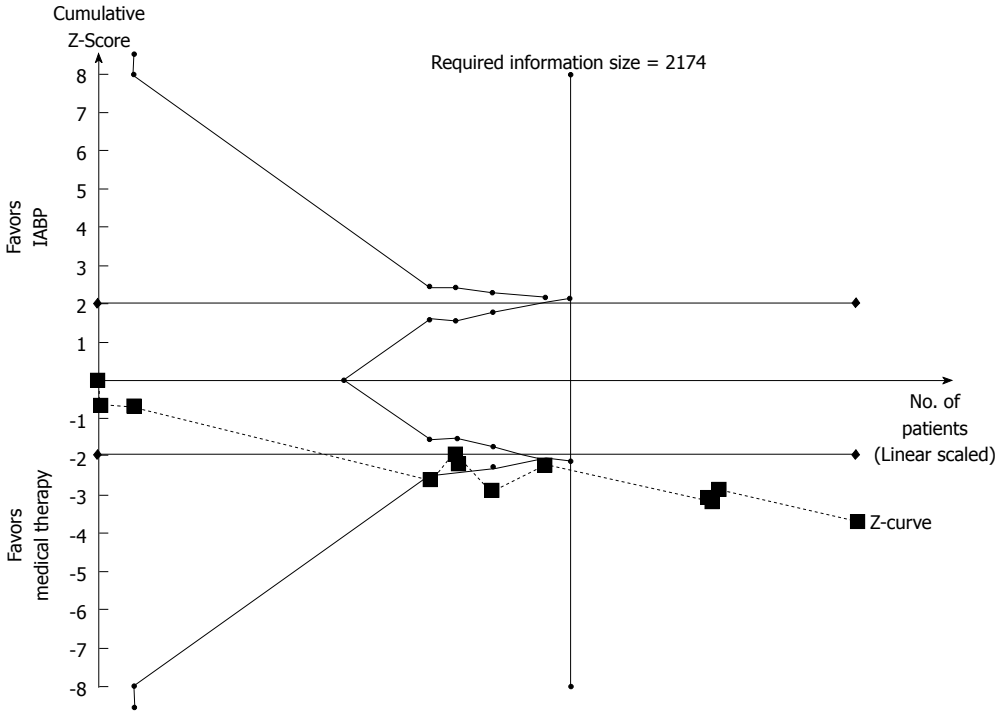


Figure 3 Intra-aortic balloon pump *vs* medical therapy: Trial Sequential Analysis on inhospital mortality. IABP: Intra-aortic balloon pump.

from RCTs, the overall I^2 decreased from 85% to 0% (Table 2). Moreover, the test for subgroup differences showed that the heterogeneity between observational and RCTs was lower (I^2 = 0%) and an overall significant detrimental effect of IABP was found (Table 2). Trial Sequential Analysis was performed: (1) by including all studies; and (2) by excluding the study by Gu *et al*[37] and that by Thiele *et al*[56] according to the sensitivity analysis (Table 3). With inclusion of all studies, there was a 32.28% mortality increase in the IABP group with about 13 more deaths every 100 treated patients. When studies by Gu *et al*[37] and Thiele *et al*[56] were excluded, IABP support resulted in a 38.22% risk increase, and Trial Sequential Analysis showed that data were sufficient to highlight the harmful effect of IABP support on the late mortality (Table 3).

PLVADs vs IABP

We compared the effect of PLVADs *vs* IABP in 271 patients; 171 from 3 observational studies[45-47] and 100 from 3 RCTs[28-30].

Primary endpoint: The overall inhospital mortality increased although not significantly, in PLVADs group compared to IABP group, both in the observational studies (+16%, P = 0.20) and the RCTs (+6%, P = 0.80) (Figure 5). The test for subgroup differences did not show significant differences between observational studies and RCTs (χ^2 = 0.13, P = 0.72, I^2 = 0%). Indeed, in the Forest plot the confidence intervals overlapped, P values of the χ^2 tests were all greater than 0.10 and the I^2 statistics were all equal to zero, showing the homogeneity among the studies within both

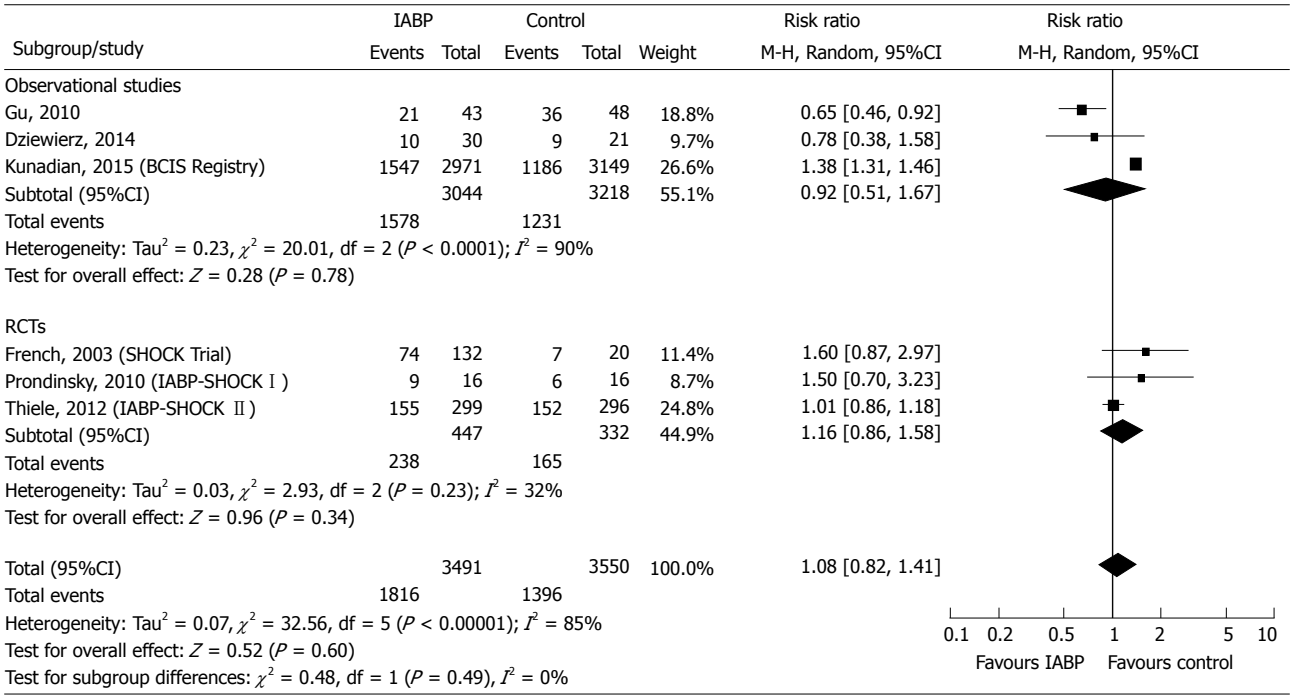


Figure 4 Meta-analysis on risk ratio of late mortality between the patients with intra-aortic balloon pump *vs* medical therapy.

observational and RCTs (Figure 5). In the Funnel plot, all studies were enclosed into 95%CI and the larger studies were plotted at the central top of the graph, demonstrating a convergence in risk estimation while increasing the sample size. RRR equaled -17.23%; when translated into clinical terms, use of PLVADs resulted 8 more deaths every 100 patients treated. For appropriate Trial Sequential Analysis, more patients would have to be included (Table 3).

ECMO plus IABP vs IABP

Primary endpoint: We compared the effect of ECMO plus IABP *vs* IABP in 164 patients from 3 observational[18,21,48]. We did not find any RCTs on the topic. In the Forest plot the χ^2 test and the I^2 statistics detected the absence of significant heterogeneity (I^2 = 7%). In the Funnel plot analysis, all studies within 95%CI were included. The inhospital mortality was higher when IABP was used alone rather than in combination with ECMO (60.53% *vs* 36.36%, respectively). ECMO plus IABP group showed a 44% RRR in mortality (Figure 6). The observed reduction was 39.92%, which means that there were 24 fewer deaths for every 100 treated patients. Trial Sequential Analysis showed that the cumulative Z-curve crossed the alpha-spending boundaries, demonstrating that a significant RRR was obtained when ECMO support was used in association with IABP (Figure 7). The required numbers of patients was reached and the meta-analysis could be considered conclusive (Table 3, Figure 7).

ECMO plus IABP vs ECMO

Primary endpoint: We compared the effect of ECMO plus IABP *vs* IABP in 301 patients from 6 observational studies[49-54]. We did not find any RCTs that analyzed this topic. We found a significantly lower inhospital mortality (RR = -22%, P = 0.008) in the group of patients treated with ECMO plus IABP compared to ECMO alone (Figure 6). In the Funnels plot analysis, only the study by Yamauchi *et al*[49] could be a potential source of bias. After the sensitivity analysis I^2 decreased to 0% while the significant effect of ECMO plus IABP *vs* ECMO remained substantially unchanged (RR = -20%, P = 0.006) (Table 2). Despite these results, Trial Sequential Analysis could not be performed because of the small number of patients included (Table 3).

DISCUSSION

All recent reviews on the use of support devices in AMI patient with CS undergoing PCI thus far show lack of a meta-analytic estimates[11-15], probably because the results were based mainly on registry data.

Meta-analyses of data from observational studies represent an area of innovation in statistical science. This analysis can be performed when the question of interest cannot be answered by a review of randomized controlled trials. Even though observational studies are prone to bias (including confounding variables), strategies to adjust for unmeasured confounding variables can be adopted[23]. In the present review, we identified only a small number of randomized trials, which by themselves were underpowered to assess the efficacy of the support devices on inhospital mortality. To increase the power of the analysis we included observational data, which enabled us to add 14909 additional patients to the 890 from the RCTs selected. Further, to avoid bias we used the Funnel plot analysis,

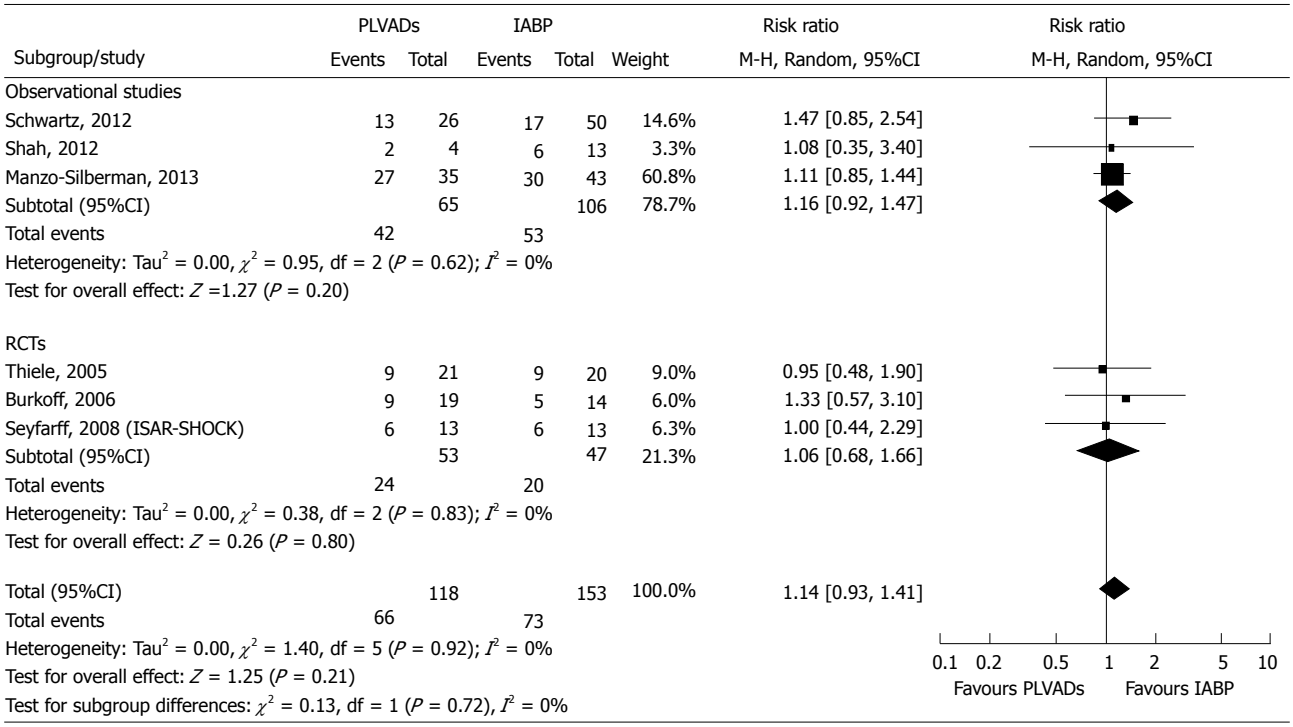


Figure 5 Meta-analysis on risk ratio of inhospital mortality between the patients with percutaneous left ventricular assist devices *vs* intra-aortic balloon pump. IABP: Intra-aortic balloon pump; PLVADs: Percutaneous left ventricular assist devices.

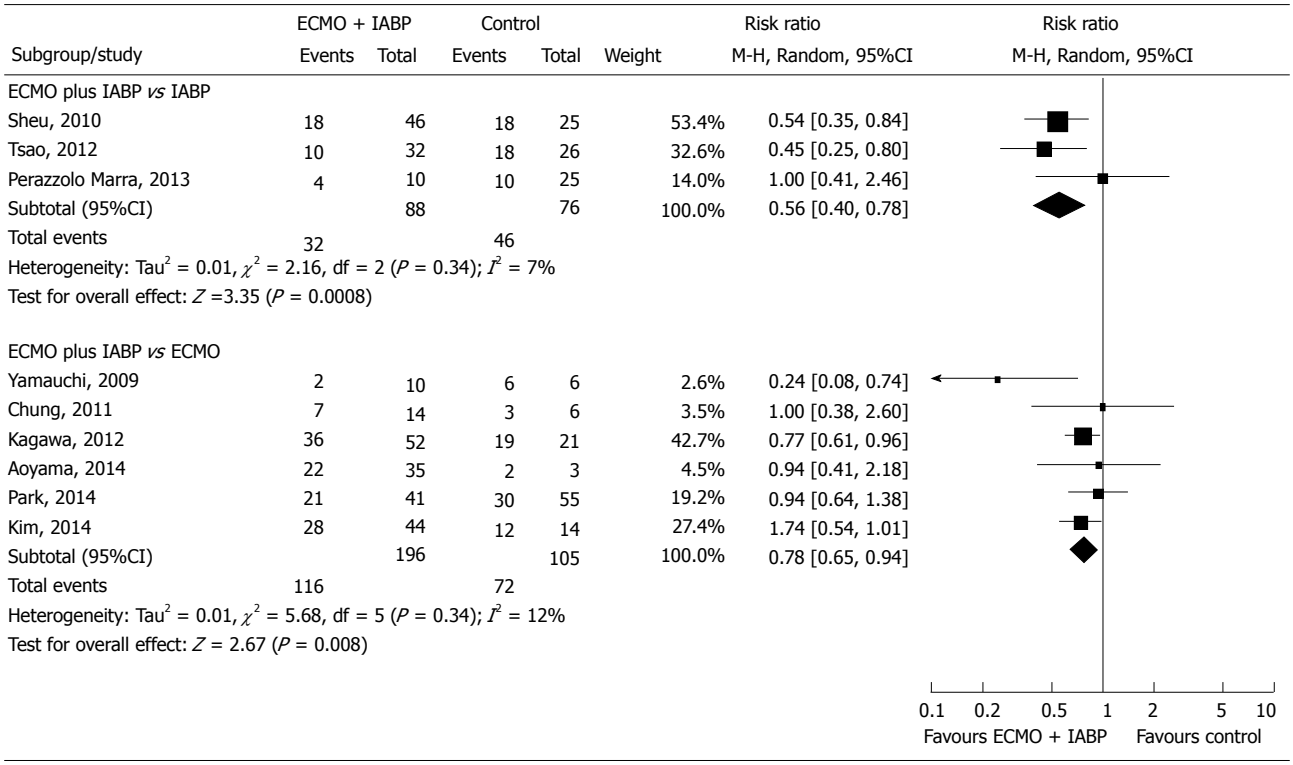


Figure 6 Meta-analysis on risk ratio of inhospital mortality between the patients with extracorporeal membrane oxygenation plus Intra-aortic balloon pump *vs* intra-aortic balloon pump or extracorporeal membrane oxygenation alone. IABP: Intra-aortic balloon pump; ECMO: Extracorporeal membrane oxygenation.

the Cochran's *Q* test and I^2 statistics to test differences between groups and subgroups. The sensitivity analysis allowed us to make comparisons not affected by excessive heterogeneity.

From the meta-analysis we can make the following conclusions

First, in the comparison between IABP *vs* Medical therapy, the analysis confirmed that IABP support

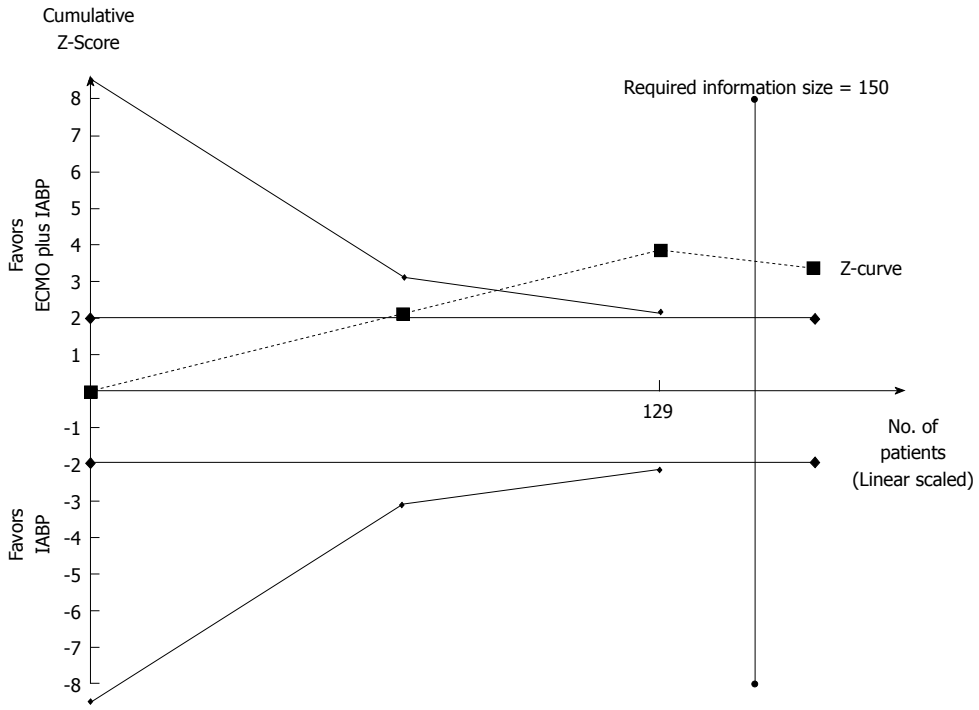


Figure 7 Extracorporeal membrane oxygenation plus Intra-aortic balloon pump support *vs* Intra-aortic balloon pump alone: Trial Sequential Analysis on inhospital mortality. IABP: Intra-aortic balloon pump; ECMO: Extracorporeal membrane oxygenation.

was associated with a significant increase inhospital mortality (Figure 2). The results of RCTs were marginal probably because of the small sample size and the results could be considered a chance occurrence (Figures 2 and 3). When we included the data from observational studies and applied the sensitivity analysis the results were affected only low heterogeneity (I^2 = 19%). Trial Sequential Analysis showed that the *Z*-curves surpassed not only the conventional boundaries but also the alpha-spending boundaries, constructed to control for type 1 error as the source of bias. Thus, the meta-analysis can be considered conclusive in terms of showing a detrimental effect of IABP (Figure 3). With regard to late mortality, we did not identify any difference in both observational studies or in RCTs. However, after sensitivity analysis a significantly higher late mortality was observed in IABP-treated patients and was confirmed by Trial Sequential Analysis, that was conclusive (Table 3).

Second, relative the comparison between IABP *vs* PLVADs, recently reported studies have failed to show a hemodynamic or survival benefit of mechanical support in AMI patients with CS and undergoing PCI. The meta-analysis by Cheng *et al*[57] dates back to 2009, performed on 3 RCTs and included 100 patients, showed that although PLVADs provided superior haemodynamic support in patients with CS compared to IABP, the use of these more powerful devices did not significantly improve early survival. Afterwards only observational studied were performed on this topic. O'Neill *et al*[58] suggested that early initiation of hemodynamic support prior to PCI with Impella 2.5 was associated with more complete revascularization and improved survival in the setting of refractory CS complicating AMI.

In our analysis, the PLVADs increased, although non significantly, the mortality as compared with IABP. The Trial Sequential Analysis showed that 1161 patients will need be analyzed in order to demonstrate its detrimental effect. Our meta-analysis was as such inconclusive and additional perspective investigations would be needed to definitive conclusion.

Third, relative to comparisons of ECMO plus IABP *vs* IABP or ECMO plus IABP *vs* ECMO, the meta-analysis showed a significant protective effect of ECMO plus IABP on inhospital mortality compared to IABP or ECMO used alone (Figure 6). Moreover, Trial Sequential Analysis showed that in the comparison ECMO plus IABP *vs* IABP the required numbers of patients was reached and the meta-analysis could be considered conclusive (Figure 7).

Potential limitation

The main limitation of this meta-analysis is the inclusion of the observational studies, since they are viewed as having less validity than RCTs, due to the absence of randomization. Indeed, we cannot exclude that CS was more severe in the IABP group compared to Medical therapy in some observational studies included in our meta-analysis. However, we repeated the analysis, including only the observational studies, between IABP *vs* control group, selected according to the same severity of shock. The results were substantially unchanged (RR = 1.11, 95%CI = 1.02 to 1.21), significantly in favour of Medical therapy. The heterogeneity was absent (I^2 = 0%). If RCTs were

added to the analysis, the heterogeneity appeared equally low (I^2 = 38%). Moreover, RCTs conducted to assess the role of haemodynamic support in patients with CS complicating AMI reported in the scientific literature are few, perhaps due to ethical issues and feasibility, involving randomization of very severely sick patients. Thus, the inclusion of well-performed observational studies may be acceptable to allow for risk estimation in such situations. Concato *et al*[59] analyzed published meta-analyses based on randomized clinical trials and observational studies that examined identical clinical topics and found that the average results of well-designed observational studies (with either a cohort or a case-control design) were markedly similar to those of the RCTs. Therefore, an integrated approach should be adopted using both experimental and observational studies, as long as well-designed and conducted. Finally, "discarding observational evidence when randomised trials are available is missing an opportunity. Conversely, abandoning plans for randomised trials in favour of quick and dirty observational designs is poor science[60]".

Another limitation was the lack of the analysis of the baseline characteristics (such as age, gender, race, *etc*.) that are recognized markers of risk. Unfortunately, these data available at baseline were not reported in the outcome.

Conclusion

The results of our meta-analysis showed that in AMI patients with CS and undergoing PCI: (1) the inhospital mortality was significantly higher with IABP support *vs* Medical therapy; (2) PLVADs increased, although non significantly, the mortality as compared with IABP; and (3) ECMO plus IABP had significant protective effect compared to IABP or ECMO alone. Trial Sequential Analysis of data on inhospital mortality in IABP *vs* control and ECMO plus IABP *vs* IABP showed that the analyses were sufficient to highlight the harmful effect of IABP and further studies would no longer be needed. Based on the results we can conclude that in CS complicating AMI: (1) routinely use of IABP and PLVADs is not recommended; and (2) the beneficial effect of the reduction inhospital mortality provided by ECMO plus IABP could be attributed to the synergistic action of the two devices in supporting the failing heart. IABP decreasing afterload and myocardial oxygen consumption, can avoid the negative effects on myocardial protection that can occur when using ECMO alone.

COMMENTS

Background

Despite major technical advances the inhospital mortality of patients with cardiogenic shock (CS) complicating AMI continues to remain high. To support the failing heart [intra-aortic balloon pump (IABP)], percutaneous left ventricular assist devices (PLVADs) and extracorporeal membrane oxygenation (ECMO) are used. Unfortunatly randomized controlled trials (RCTs) on this issue are performed in small numbers, perhaps due to ethical issues and feasibility, involving randomization of patien with CS.

Research frontiers

The question of impact of cardiac support percutaneous devices cannot be answered by a review of RCTs alone. Meta-analyses of observational studies increase the power of the analysis by adding more data to the RCTs to have more comprehensive results.

Innovations and breakthroughs

In the present study, the authors investigated the impact of IABP, PLVADs and ECMO on inhospital mortality and late survival in patients with CS complicating acute myocardial infarction (AMI) undergoing percutaneous coronary intervention (PCI). Meta-analysis of observational in addition to the RCTs enabled them to increase the power of the analysis.

Applications

The results of the meta-analysis allow us to understand the impact of percutaneous cardiac support with IABP, PLVAD and ECMO in patients with CS complicating AMI undergoing PCI.

Terminology

This systematic review and meta-analysis of observational studies and RCTs.

Peer-review

In this study, the authors collected the data from 30 published research papers (total 15799 patients) and used meta-analysis to analyze in hospital and late mortality of percutaneous mechanical support. This is an interesting study. The findings in this study have the potential to help the clinical doctor work out the guideline for reducing mortality in acute myocardial infarction patients with cardiogenic shock.

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Electrophysiologic testing guided risk stratification approach for sudden cardiac death beyond the left ventricular ejection fraction

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Abstract

Sudden cardiac death threats ischaemic and dilated cardiomyopathy patients. Anti- arrhythmic protection may be provided to these patients with implanted cardiac defibrillators (ICD), after an efficient risk stratification approach. The proposed risk stratifier of an impaired left ventricular ejection fraction has limited sensitivity meaning that a significant number of victims will remain undetectable by this risk stratification approach because they have a preserved left ventricular systolic function. Current risk stratification strategies focus on combinations of non invasive methods like T wave alternans, late potentials, heart rate turbulence, deceleration capacity and others, with invasive methods like the electrophysiologic study. In the presence of an electrically impaired substrate with formed post myocardial infarction fibrotic zones, programmed ventricular stimulation provides important prognostic information for the selection of the patients expected to benefit from an ICD implantation, while due to its high negative predictive value, patients at low risk level may also be detected. Clustering evidence from different research groups and electrophysiologic labs support an electrophysiologic testing guided risk stratification approach for sudden cardiac death.

Key words: Electrophysiologic study; Risk stratification; Sudden cardiac death; Myocardial infarction; Preserved ejection fraction

Core tip: There is a growing need for more effective risk stratification approach in order to detect those post myocardial infarction and dilated cardiomyopathy patients at high risk for sudden cardiac death (SCD) at early or even asymptomatic stage of heart failure with relatively well preserved left ventricular ejection fraction (LVEF). Although in an individual basis the SCD risk is lower among the patient population compared to the one observed among to those with a severely impaired

LVEF, epidemiologically there is a large such patient pool at risk in the community. Based on preliminary evidence these patients could be effectively and timely identified by applying a combined electrophysiologic guided approach using non-invasive electrocardiogram-related markers of risk leading to programmed ventricular stimulation testing. Using this approach, we could select those with inducible ventricular tachyarrhythmias as suitable candidates for implantable defibrillator therapy.

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TO THE EDITOR

Hilfiker *et al*[1] report on their experience with the electrophysiologic (EP) studies as risk stratifier for sudden cardiac death (SCD) surrogates among a cohort of patients with mostly organic heart disease and different levels of left ventricular ejection fraction (LVEF). They found that both the EP results as well as a reduced LVEF were significant predictors among those patients with a LVEF ⩽ 35% while the ventricular stimulation results were much more promising among those with LVEF > 35%. The latter is confirmed in our own database of post myocardial infarction (post-MI) and dilated cardiomyopathy patients who were risk stratified with EP studies for the primary prevention of SCD when presenting with a worrisome risk profile of either non sustained ventricular tachycardia or/and pre and syncope episodes despite a well maintained LVEF > 35%-40%[2-4]. This is based on a risk stratification approach aiming to define the high risk profile patient with organic heart disease beyond the reduced LVEF. Indeed there is preliminary evidence that such a mixed non-invasive[5] and invasive EP guided[4] approach may identify in a much more cost effective way not only those high risk patients with impaired LVEF but also those high risk patients with well maintained LVEF who may benefit from the prophylactic implanted cardiac defibrillators implantation timely. Such a patient population with relatively well preserved LVEF were the majority of SCD victims both in the Maastricht[6] as well as in the Oregon[7] out of hospital cardiac arrest registries. In this context we currently recruit post-MI patients at high risk for SCD despite the well maintained LVEF based on a combined non invasive and invasive EP guided approach in the ongoing prospective observational PRESERVE-EF study[8]. Is it time for a more rational EP guided risk stratification approach considering the limitations of the LVEF as a risk stratifier for SCD[9,10]?

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