

# World Journal of *Cardiology*

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and lungs. Pulmonary hypertension (PH) is increasingly recognised as a severe complication of HHT. PH may be categorised into two distinct types in patients with HHT. Post-capillary PH most often results from a high pulmonary blood flow that accompanies the high cardiac output state associated with liver arteriovenous malformations. Less frequently, the HHT-related gene mutations in ENG or ACVRL1 appear to predispose patients with HHT to develop pre-capillary pulmonary arterial hypertension. Differentiation between both forms of PH by right heart catheterisation is essential, since both entities are associated with severe morbidity and mortality with different treatment options. Therefore all HHT patients should be referred to an HHT centre.

**Key words:** Hereditary haemorrhagic telangiectasia; High cardiac output; Pulmonary arterial hypertension; ENG; ACVRL1; Pulmonary hypertension

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**Core tip:** Pulmonary hypertension (PH) is increasingly recognised as a severe complication of hereditary haemorrhagic telangiectasia (HHT), but the true prevalence of PH in HHT is not known. Post-capillary PH most often results from the high cardiac output associated with hepatic arteriovenous malformations. More rarely the HHT gene mutations (ACVRL1 or ENG) result in pre-capillary pulmonary arterial hypertension (PAH). Differentiation between post-capillary PH and pre-capillary PAH can be done by right heart catheterisation, and is of importance since both entities are associated with severe morbidity and mortality and have different options for treatments.

### Abstract

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disorder characterised by vascular malformations in predominantly the brain, liver

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## HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Hereditary haemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant inherited disorder with late onset penetrance (nearly 97% at the age of 60 years) characterised by vascular malformations with an estimated prevalence of 1:5000 individuals<sup>[1,2]</sup>. The abnormal vascular structures in HHT range from small telangiectasia of the skin and mucosal membranes to arteriovenous malformations (AVMs) in predominantly the brain, liver and lungs<sup>[3,4]</sup>.

### Genetics and pathogenesis

HHT consist of two main subtypes, HHT type 1 and HHT type 2, which results from mutations in the ENG gene on chromosome 9, encoding the protein endoglin and from mutations in the activin receptor-like kinase (ACVRL1) gene on chromosome 12, encoding the protein ALK-1 respectively<sup>[5,6]</sup>. A third disease-causing mutation has been found in the SMAD4 gene, causing a combination of the juvenile polyposis syndrome and HHT<sup>[7]</sup>. Most HHT families have a unique mutation and many types of mutations have been described.

The exact pathogenesis of HHT is still unclear. However, hypoxia or local hemodynamic changes could act as a possible trigger promoting tissue inflammation or endothelial cell injury<sup>[8,9]</sup>. Both endoglin, ALK-1 and SMAD4 proteins are endothelial receptors of the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily. All three proteins cooperate in the TGF- $\beta$ /ALK-1 signalling pathway, which is involved in angiogenesis. In HHT, most vessels are normal, but the mutations in ACVRL1 and ENG result in abnormal angiogenetic responses and lead to the formation of abnormal arteriovenous connections, ranging from small telangiectases that bleed easily, to large arteriovenous malformations, that can occur in every organ, but especially in the lungs, liver and brain<sup>[10,11]</sup>.

### Diagnosis

The clinical diagnosis can be based on the four Curaçao criteria<sup>[1]</sup>, which consist of: (1) Spontaneous, recurrent epistaxis; (2) Multiple telangiectasia at characteristic sites (lips, oral cavity, fingers, nose); (3) Visceral lesions (gastrointestinal telangiectasia, pulmonary, hepatic, cerebral or spinal AVMs); and (4) A first degree relative with HHT.

Three criteria suffice for a definitive diagnosis of HHT, two criteria are considered as "possible" HHT, and one or no criterion makes the diagnosis "unlikely". The positive predictive value for a definite clinical diagnosis and the negative predictive value for an unlikely diagnosis are excellent (100% and 97.7% respectively), when compared with DNA testing<sup>[12]</sup>. However, HHT has an age dependent penetrance and the clinical presentation varies among patients<sup>[1]</sup>. Therefore genetic

testing has emerged as an important tool to help make the diagnosis in paediatric patients and younger adults with a "possible" clinical diagnosis<sup>[12]</sup>.

## PULMONARY HYPERTENSION

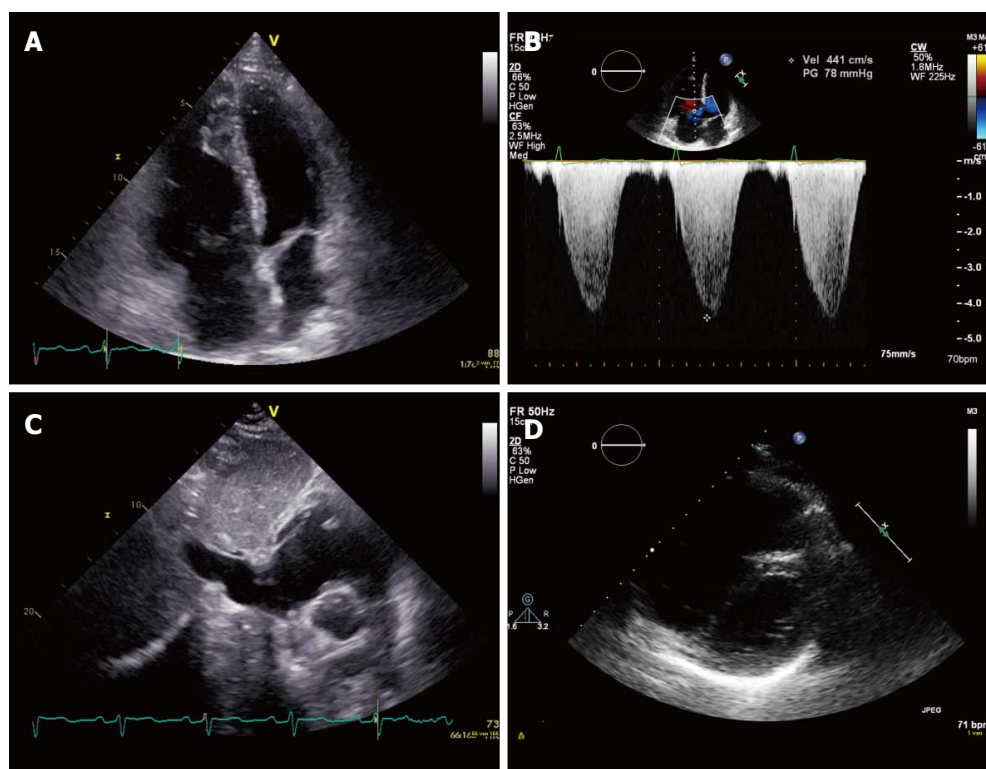
Pulmonary hypertension (PH) is a haemodynamic and pathophysiological condition defined as an increase in mean pulmonary arterial pressure (mPAP) of equal to or more than 25 mmHg as assessed by right heart catheterisation (RHC)<sup>[13]</sup>. PH is a progressive disease of many origins, affecting more than 100 million people world wide<sup>[14]</sup>. The elevated pressure in the pulmonary circulation can lead to various symptoms including limited exercise capacity and dyspnoea on exertion. The chronic elevated pressure may ultimately result in right-sided heart failure and premature death<sup>[13]</sup>.

Depending on the origin, PH can be divided into two main groups; pre- and post capillary PH. Patients with pre-capillary PH are characterised by a mPAP  $\geq$  25 mmHg, pulmonary artery wedge pressure (PAWP)  $\leq$  15 mmHg, and elevated pulmonary vascular resistance (PVR) ( $>$  3 Wood units)<sup>[15]</sup>. Pre-capillary PH can be further divided in different clinical groups, based on pathophysiological mechanisms, clinical presentation and therapeutic options (Table 1)<sup>[13]</sup>.

Transthoracic echocardiography is the cornerstone for screening in all patients suspected of PH. Typically, a dilatation of the right ventricle with septal flattening (also called D-sign) and an increase in right ventricular systolic pressure (RVSP) (sum of right ventricle-right atrium pressure gradient and estimated pressure in the right atrium based on the dimension and collapse of the inferior caval vein) (Figure 1)<sup>[13,16]</sup>.

### PH and hereditary haemorrhagic telangiectasia

PH is increasingly recognised as an important complication of HHT. HHT associated PH can occur by several mechanisms. Most often, post-capillary PH may develop as a consequence of a hyperkinetic state resulting in heart failure associated with high cardiac output (CO) due to hepatic arteriovenous malformations (HAVMs) (Figure 2)<sup>[17]</sup>, while less frequently, precapillary PH can be related to pulmonary arterial hypertension (PAH) characterised by remodeling of small pulmonary arteries with broadly similar histologic lesions than observed in idiopathic PAH<sup>[17]</sup>. The HHT-related gene mutations (ENG or ACVRL1) appear to predispose for the development of PAH<sup>[18-22]</sup>. Various studies found a high estimated prevalence of PH in HHT when screening with echocardiography<sup>[23,24]</sup>. An elevated RVSP on echocardiography was found in 9 (20.5%) out of 44 HHT patients (22 ACVRL1, 3 ENG, 19 unknown mutation), in 7 out of these 9 subjects an ACVRL1 gene was found<sup>[23]</sup>. Sopeña *et al.*<sup>[24]</sup> found a high estimated prevalence of PH (31%) in 29 hospitalised patients with HHT with a mean estimated RVSP of  $73 \pm 17.0$  mmHg measured with echocardiography. HAVMs were



**Figure 1** Characteristic echocardiogram of a patient with pulmonary hypertension. A: Apical 4-chamber view showing dilatation of the right ventricle; B: Apical 4-chamber view with Doppler signal (continuous wave) showing an increased right ventricular- right atrial pressure gradient (4.4 m/s); C: Subcostal view showing dilatation of the inferior caval vein corresponding with an increased pressure in the right atrium; D: Parasternal short axis view showing flattening of the interventricular septum (D-sign) and dilatation of the right ventricle.



**Figure 2** Hepatic arteriovenous malformations. Computed tomography with contrast in arterial fase showing extensive filling of the hepatic veins (arrows), and diffuse hepatic arteriovenous malformations (asterix).

documented in 67% of these patients. However, large observational studies including consecutive HHT patients are lacking.

Since the treatment strategies differ between post-capillary high-output PH and pre-capillary PAH, it is important to differentiate between these two different entities. RHC is the gold standard for making the diagnosis of both high-output PH and PAH<sup>[13,17,25]</sup>.

In PAH, the mPAP is usually higher with an increase in PVR and transpulmonary gradient due to arteriopathy. Most often a normal or decreased CO and PAWP is

seen. In high-output PH on the other hand, there is only a moderate increase in mPAP, with a normal PVR, elevated PAWP and most importantly, an increased CO (Table 2)<sup>[13,17]</sup>.

### High output PH

High-output heart failure is the most common initial presentation of HAVMs in HHT. Liver involvement is present in 32%-78% of the HHT patients and is predominantly seen in HHT type 2<sup>[1,26-28]</sup>. The presence of symptoms is directly associated with significant morbidity and mortality and therefore, screening for liver AVMs with Doppler ultrasound is warranted in all patients who are symptomatic or have abnormal liver enzymes<sup>[1,25]</sup>. In the majority of cases, only small telangiectasia are seen, which do not lead to symptoms. However, large HAVMs exist in typically three different and often concurrent types of intrahepatic shunting; from the hepatic arteries to hepatic veins, from the hepatic arteries to portal veins, and from the portal veins to hepatic veins<sup>[17,25]</sup>. These hepatic shunts can lead to high-output cardiac failure, portal hypertension, biliary ischaemia or encephalopathy with a wide range of symptoms<sup>[25]</sup>. Overall, symptoms due to HAVMs occur in 8% of HHT patients and predominantly in females<sup>[29]</sup>. Symptoms of high-output cardiac failure usually develop in females between 50 and 70 years of age and are characterised by dyspnoea on exertion,

**Table 1** Updated classification of pulmonary hypertension

Pulmonary arterial hypertension
Idiopathic PAH
Hereditary PAH
BMPR2
ALK1, endoglin (with or without hereditary haemorrhagic telangiectasia), SMAD9, CAV1, KCNK3
Unknown
Drug and toxin induced
Associated with:
Connective tissue diseases
HIV infection
Portal hypertension
Congenital heart diseases
Schistosomiasis
Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis
Persistent pulmonary hypertension of the newborn
Pulmonary hypertension due to left heart disease
Left ventricular systolic dysfunction
Left ventricular diastolic dysfunction
Valvular disease
Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
Pulmonary hypertension due to lung diseases and/or hypoxia
Chronic obstructive pulmonary disease
Interstitial lung disease
Other pulmonary diseases with mixed restrictive and obstructive pattern
Sleep-disordered breathing
Alveolar hypoventilation disorders
Chronic exposure to high altitude
Developmental abnormalities
Chronic thromboembolic pulmonary hypertension
PH with unclear and/or multifactorial mechanisms
Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

PH: Pulmonary hypertension; BMPR2: Bone morphogenetic protein receptor, type 2; CAV1: Caveolin-1; HIV: Human immunodeficiency virus. Adapted from Simonneau *et al*<sup>[46]</sup>, with permission of the publisher.

fatigue, orthopnoea, ascites and/or oedema<sup>[17,29]</sup>.

**Pathophysiology of high output PH:** Exercise testing in healthy persons revealed that an increase in CO leads to an elevation in pulmonary artery pressure (increase in mPAP up to 0.5 to 3.0 mmHg/L per minute)<sup>[30]</sup>.

In patients with HAVMs, shunting of blood from the hepatic arteries and/or portal veins to the hepatic veins results in a hyperdynamic state, in which the CO can be elevated two-to-three fold<sup>[31]</sup>. Besides this cause of high cardiac output, severe epistaxis or gastrointestinal bleeding in patients with HHT may lead to anaemia with a compensatory increase in CO as well.

In HHT, a multifactorial cascade will eventually lead to high-output cardiac failure. At first, the increase in CO will be compensated by dilatation of the pulmonary arteries and thereby pulmonary pressure will still be

**Table 2** Haemodynamics in pulmonary hypertension associated with hereditary haemorrhagic telangiectasia

	High output PH	PAH
mPAP (mmHg)	+	++
PAWP (mmHg)	=/+	=
PVR (Wood units)	=	++
CO (L/min)	++	-

PH: Pulmonary hypertension; PAH: Pulmonary arterial hypertension; mPAP: Mean pulmonary artery pressure; PAWP: Pulmonary artery wedge pressure; PVR: Pulmonary vascular resistance; CO: Cardiac output. +: Increase; =: Normal; -: Decrease. Adapted from Faughnan *et al*<sup>[17]</sup>, with permission of the publisher.

maintained. An increase in left atrial (LA) pressure will predispose patients for atrial fibrillation (due to enlargement of the LA) and diastolic dysfunction of the left ventricle. Increased LA pressure and impaired pulmonary vasodilatation will eventually result in PH. The combination of volume and pressure overload leads to right ventricular (RV) dilatation, decreased systolic function of the RV and subsequent right heart failure. Severe bleeding (e.g., epistaxis or gastrointestinal bleeding) may trigger the cascade because of the subsequent increase in CO<sup>[17,29,31]</sup>.

**Treatment of high output PH:** The first-line treatment of PH associated with a high-output state consists of intensive medical treatment including salt restriction and diuretics, correction of anaemia, antihypertensive and antiarrhythmic agents and digoxin if necessary<sup>[9]</sup>. In patients refractory to medical-therapy, liver transplantation is the best option, with a 5-year survival of 83% in a series of 40 patients<sup>[29]</sup>. However, a high post-operative morbidity is seen<sup>[25,32]</sup>.

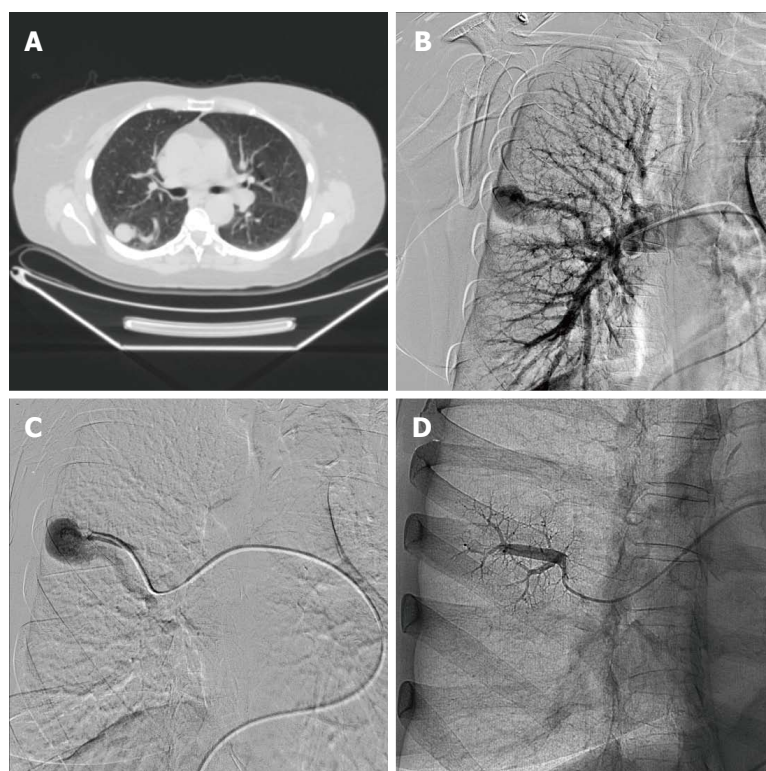
Recently, Dupuis-Girod *et al*<sup>[33]</sup> treated 25 patients with severe HAVMs and a high CO [median cardiac index (CI) 5.1 L/min per square meters (range 4.1-6.2 L/min per square meters)] with bevacizumab, a vascular endothelial growth factor inhibitor. This treatment resulted in a significant decrease in CO [median CI at 6 mo 4.1 L/min per square meters (range 3.0-5.1 L/min per square meters)], normalisation of the pulmonary pressure in 5 out of 8 patients with PH at baseline and clinical improvement of dyspnoea<sup>[33]</sup>. Other invasive treatments such as surgical hepatic artery ligation or transcatheter therapeutic embolisation of the hepatic artery are associated with a high morbidity and mortality and therefore not recommended<sup>[1,17]</sup>.

## PAH

PAH is a clinical condition characterised by the presence of pre-capillary PH due to arteriopathy with media hypertrophy and intima proliferation. It is increasingly recognised as a severe complication of HHT.

There have been a few case series that describe the association between PAH and HHT, however these series all included patients with PH in which HHT





**Figure 3 Pulmonary arteriovenous malformations.** A: Computed tomography of the chest with large pulmonary arteriovenous malformation (PAVM) in the lower lobe of the right lung; B: Pulmonary angiogram of the PAVM in the same patient; C: Selective pulmonary angiogram of the PAVM in the apex of the lower lobe of the right lung; D: Repeat angiogram after transcatheter embolisation of the PAVM with a vascular plug.

symptoms were also present<sup>[18-22]</sup>.

**Pathophysiology and genetics of PAH:** In 2001, it was demonstrated for the first time that different mutations in *ACVRL1* predispose patients for the development of PAH<sup>[18]</sup>. This was confirmed in a few case series describing the presence of PAH in patients with an *ACVRL1* mutation and clinical features of HHT<sup>[19,21,22]</sup>. Trembath *et al.*<sup>[18]</sup> described that mutations in *ACVRL1* may lead to both occlusion of the pulmonary arteries together with vascular dilatation, manifested as AVMs in HHT. Although less frequently, *ENG* mutations have also been identified in patients with both HHT and PAH, suggesting a less potent association between endoglin and PAH<sup>[18,19]</sup>. Mutations in the bone morphogenic protein receptor type 2 gene, which is another gene encoding the endothelial surface protein components of the TGF- $\beta$  receptor that is detected in approximately 70% of the patients with hereditary PAH, were not found in HHT associated PAH<sup>[34]</sup>.

**Prognosis:** The clinical outcomes of patients with PAH caused by an *ACVRL1* mutation have been analysed in 32 patients and compared to other PAH patients without this mutation. PAH caused by an *ACVRL1* mutation was found in significantly younger patients (mean age  $21.8 \pm 16.7$  years) and had a significantly shorter survival, despite similar therapy<sup>[34]</sup>. No data exist about the prognosis of patients with PAH and *ENG* mutations. The overall prognosis of PAH in general ranges from 6 mo to several years based on the underlying disease<sup>[13]</sup>.

It is noteworthy that *ACVRL1* mutation carriers may develop severe PAH without any clinical evidence of

HHT because of the early development of PAH in these patients and the late-onset penetrance of *ACVRL1* mutation for HHT manifestations<sup>[34]</sup>.

**Treatment of PAH in HHT:** No systematic evidence exists for treatment of HHT patients with PAH. It seems rational to treat patients according to the guidelines for PAH, with PAH-specific medication and supporting therapy (diuretics, oxygen, and digoxin)<sup>[13]</sup>.

Today there are three different groups of PAH specific medication; endothelin receptor antagonists (ERA), phosphodiesterase inhibitors (PDE5I) and prostacyclins. There are two case-reports that describe successful treatment of PAH in HHT patients with the ERA bosentan. After treatment, improvement of symptoms, exercise capacity and laboratory findings and a decrease in mPAP were found<sup>[35,36]</sup>.

There are no reports describing the treatment with other PAH specific medication in patients with PAH and HHT. Since there was no response to acute vasodilator challenge in 32 patients with HHT and PAH, there is probably no role for the use of calcium channel blockers in this population<sup>[17,34]</sup>. And due to an increase in bleeding complications regular treatment with oral anticoagulation is not advised<sup>[1]</sup>. However, based on recent literature, treatment with anticoagulation could be considered on a case by case basis<sup>[37]</sup>.

#### **Pulmonary arteriovenous malformations in PH:**

The coexistence of PH and pulmonary arteriovenous malformations (PAVMs) has specific clinical and therapeutic implications. PAVMs are low-resistance, high-flow abnormal vascular structures that bypass the

normal capillary filter and thereby result in permanent pulmonary right-to-left shunting (Figure 3A-C)<sup>[38-40]</sup>. Paradoxical embolisation through these PAVMs can lead to severe neurological complications, such as a stroke or brain abscess<sup>[1,40]</sup>. Contrast echocardiography is the screening test of choice (sensitivity up to 98.6%), with a direct relationship between shunt grade and prevalence of cerebral manifestations in patients screened for HHT<sup>[40-42]</sup>. To avoid neurologic and bleeding complications, PAVMs can be treated with transcatheter embolisation with coils or plugs (Figure 3D)<sup>[1,43]</sup>. It may be expected that closing this low resistance system will result in a rise in mPAP. Measuring the pulmonary pressure before and after embolisation of PAVMs in 43 patients, Shovlin *et al.*<sup>[44]</sup> found no significant increase in mPAP after embolisation, even in patients with pre-existing mild to moderate PH.

A possible explanation is a decrease in CO after embolisation which has a greater effect on the PVR than occlusion of the PAVMs. This fall in CO immediately after PAVM closure was recently described in 29 HHT patients by Vorselaars *et al.*<sup>[45]</sup>. Furthermore, PAVM-related hypoxemia can induce vasoconstriction with a concomitant increase in PVR. Both studies described an increase in saturation after embolisation which may result in a decrease in pulmonary vasoconstriction and thereby PVR<sup>[44,45]</sup>. One case report described a fatal rupture of a PAVM in a patient with severe PAH. Although patients with severe PH were excluded from the above studies, it would be prudent to consider that the higher the mPAP and PVR at baseline and the larger the PAVM, the greater likelihood of worsening PH after embolisation<sup>[44]</sup>.

### Further research and recommendations

Although a number of studies described patients with PH and HHT, no data are available about the exact prevalence of PH in the overall HHT population. Most studies used a small sample size of highly selected patients and data from RHC are lacking. Therefore we recommend to perform a systematic screening to reveal the true prevalence of both forms of PH with their different aetiologies in a HHT population.

Because of the non-specific symptoms and potentially fatal prognosis, all HHT patients should be referred to an HHT centre of excellence.

## CONCLUSION

PH is increasingly recognised as a severe complication of HHT, but the true prevalence of PH in HHT is still unknown. PH in HHT is mostly post-capillary in origin and results from high cardiac output due to HAVMs and anaemia. Rarely ACRVL-1 or ENG mutations results in pre-capillary PAH. Differentiation between both forms of PH in HHT by RHC is essential, since both entities are associated with severe morbidity and mortality with different specific treatment options. Therefore all

HHT patients should be referred to an HHT centre.

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## Insights into cardio-oncology: Polypharmacology of quinazoline-based $\alpha_1$ -adrenoceptor antagonists

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signs of ischemia.

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**Core tip:** New uses of cardiovascular drugs with proven experience and without high cost have been emerging, including to have anticancer abilities by targeting human ether-a-go-go-related gene K(+) channels, epidermal growth factor receptors, vascular endothelial growth factor receptors, as well as to overcome cancer multidrug resistance. Quinazoline-based  $\alpha_1$ -adrenoceptor antagonists (doxazosin, prazosin, and terazosin) exhibit anticancer abilities and emerging findings indicate that these drugs may have a significant role in uncontrolled hypertensive cancer patients without signs of ischemia.

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### Abstract

New uses of cardiovascular drugs with proven experience are emerging, including for treating cancer. Quinazoline is a compound made up of two fused six member simple aromatic rings, benzene and pyrimidine rings, with several biological effects. Cardiologists first used quinazoline-based  $\alpha_1$ -adrenoceptor antagonists prazosin, doxazosin, and terazosin; currently available data support their use as safe, well tolerated, and effective add-on therapy in uncontrolled hypertension with additional favourable metabolic effects. Recent findings highlight the anticancer effects of quinazoline-based  $\alpha_1$ -adrenoceptor antagonists, indicating that they may have a significant role in uncontrolled hypertensive cancer patients without

### INTRODUCTION

Despite the tremendous efforts, the medicine field has not yet come to absolute conclusions in oncology and the emerging scenario of the onco-cardiovascular patients is emerging<sup>[1]</sup>. New targeted anticancer therapies have not proven to be free from cardiovascular side effects while old anticancer therapies have shown delayed serious consequences in long-term cancer survivors<sup>[1,2]</sup>. Moreover, the heavy burden of concomitant problems and diseases requires changes in setting to prevent serious diseases such as infective endocarditis or in

Doxazosin	Prazosin	Terazosin
Quinazoline-based	Quinazoline-based	Quinazoline-based
$\alpha_1$ -adrenoceptor antagonist	$\alpha_1$ -adrenoceptor antagonist	$\alpha_1$ -adrenoceptor antagonist
Antihypertensive effect	Antihypertensive effect	Antihypertensive effect
HERG ligand	HERG ligand	HERG ligand
EGFR inhibition	EGFR inhibition	
Anti-angiogenic activity	Anti-angiogenic activity	
Cancer cell growth inhibition		Cancer cell growth inhibition
Apoptosis induction	Apoptosis induction	Apoptosis induction
Anoikis induction		Anoikis induction
	Cell autophagy induction	Cell autophagy induction
Role in MDR	Role in MDR	Weaker or no effect in MDR
Akt inhibition	Cdk 1 inactivation	G <sub>1</sub> phase cell cycle arrest
Androgen receptor downregulation	DNA damage stress induction	p27KIP1 up-regulation
Bax expression upregulation	G <sub>2</sub> checkpoint arrest	Proteasome activity downregulation
Caspase-3 activity increase	Mitochondria-mediated apoptosis induction	Ubiquitinated protein accumulation
EphA2 agonism	p53-mediated mechanism	
FGFR-2 antagonism		
Focal adhesion kinase reduction		
HIF-1 $\alpha$ inhibition		
MAPK activation decrease		
mTOR inhibition		
p27 downregulation prevention		
PDK1 inhibition		
PKB/Akt activation inhibition		
PI3K inhibition		
Rho kinase- II activation decrease		
Soluble guanylate cyclase $\alpha$ decrease		
TGF- $\beta$ and I $\kappa$ B activation		
Tubulin-polymerization-enhancing activity		

**Figure 1 Structure and polypharmacology of quinazoline-based  $\alpha_1$ -adrenoceptor antagonists doxazosin, prazosin and terazosin in cardio-oncology.** MDR: Multidrug resistance; EGFR: Epidermal growth factor receptor; FGFR-2: Fibroblast growth factor receptor-2; HIF-1 $\alpha$ : Hypoxia-inducible factor 1 $\alpha$ ; mTOR: Mammalian target of rapamycin; PDK1: 3-phosphoinositide-dependent protein kinase 1; TGF: Transforming growth factor.

perioperative oncosurgery<sup>[3-12]</sup>. The progress in cancer biology and treatment has led to a new frontier: the cardio-oncology<sup>[1-27]</sup>. New uses of cardiovascular drugs with proven experience have been emerging<sup>[1,4,5-9,27-32]</sup>, including to have anticancer abilities by targeting human ether-a-go-go-related gene K(+) (HERG) channels<sup>[5]</sup>, epidermal growth factor (EGF) receptors<sup>[9]</sup>, vascular endothelial growth factor (VEGF) receptors, as well as to overcome cancer multidrug resistance (MDR)<sup>[4,26,29,33,34]</sup>. These old cardiovascular drugs do not have high cost, however, there was a lack of noninferiority randomized, controlled trials<sup>[33]</sup>, comparing them with new anticancer therapies.

Quinazoline is a compound made up of two fused six member simple aromatic rings, benzene and pyrimidine rings<sup>[35]</sup>. The search for quinazoline-based substances as cardiovascular agents begun after pharmacological identification of quinazoline compounds having a glycine amide or  $\beta$ -alanine amide residue in the 3<sup>rd</sup> position that display a hypotensive activity. Other quinazoline derivatives have also demonstrated significant anticancer activities<sup>[26,35-39]</sup> and new molecules

have been synthesized as gefitinib, erlotinib, afatinib, and lapatinib<sup>[26,36]</sup>. Cardiologists first used quinazoline-based  $\alpha_1$ -adrenoceptor antagonists, including prazosin, doxazosin, and terazosin<sup>[26]</sup> (Figure 1). Currently available data have supported the use of these antagonists as safe, well tolerated, and effective add-on therapy in uncontrolled hypertension with additional favorable metabolic effects<sup>[37]</sup> and without association with an increased risk of heart failure<sup>[26,37-39]</sup>. New data suggest that adverse cardiac outcome of doxazosin is only among patients with moderate-to-severe ischemia on myocardial perfusion imaging<sup>[26,40]</sup>. Furthermore, it has been reported that the  $\beta$ -plus  $\alpha_1$ -blocker pretreatment (propranolol + prazosin) has led to better severity reduction of postresuscitation myocardial tissue injury and myocardial dysfunction with better neurologic function and prolonged duration of survival than propranolol treatment alone<sup>[41]</sup>. This latter finding will require certainly further evaluation.

Research has suggested several anticancer mechanisms of doxazosin, including upregulation of Bax expression, transforming growth factor (TGF)- $\beta$  and I $\kappa$ B activation<sup>[42]</sup>, focal adhesion kinase reduction<sup>[43]</sup>,



inhibition of protein kinase B/Akt activation<sup>[44]</sup>, and death receptor mediated apoptosis induction<sup>[45,46]</sup>. Doxazosin is known to be a HERG ligand, EGFR inhibitor<sup>[47]</sup>, VEGF-mediated angiogenic response antagonist<sup>[48]</sup>, and fibroblast growth factor receptor-2 antagonist<sup>[48,49]</sup>. Several signalling pathways are also inhibited from doxazosin VEGF antagonism including PI3K, Akt, 3-phosphoinositide-dependent protein kinase 1, mammalian target of rapamycin, and hypoxia-inducible factor 1 $\alpha$ <sup>[49]</sup>. In addition, doxazosin is also an agonist of receptor tyrosine kinase triggering ephrin type-A receptor 2 internalization which, in turn, suppresses haptotactic and chemotactic migration of prostate cancer, breast cancer, and glioma cells<sup>[26,50]</sup>. Notably, a tubulin polymerization-enhancing activity of doxazosin has been found<sup>[51]</sup>. A doxazosin derivative, DZ-50, impairs tumour growth and metastasis *via* anoikis<sup>[52]</sup>; similarly, doxazosin induces changes in morphology consistent with anoikis in both benign and cancerous prostatic cells and increased caspase-3 activity<sup>[43]</sup>. Moreover, doxazosin significantly decreases benign prostatic hyperplasia-induced mitogen-activated protein kinase kinase and Rho kinase-II activation and decreases expression of soluble guanylate cyclase<sup>[53]</sup> also leading to prostate cancer cell growth inhibition<sup>[54]</sup>. Doxazosin also downregulates expression of androgen receptor<sup>[54]</sup>, prevents p27 downregulation<sup>[55]</sup> and may partly reverse P-glycoprotein/MDR1-mediated cancer multidrug resistance (CMDR) and the transport of anticancer drugs<sup>[56]</sup>.

Terazosin, another quinazoline-based antihypertensive  $\alpha_1$ -adrenoceptor antagonist<sup>[57]</sup>, is also an HERG ligand<sup>[58]</sup>, a cancer cell growth inhibitor<sup>[59]</sup>, and an apoptosis and anoikis inductor<sup>[58,60]</sup>. Terazosin induces cell death which is associated with G<sub>1</sub> phase cell cycle arrest, upregulation of cyclin-dependent kinase inhibitor 1B (p27KIP1)<sup>[60]</sup>, accumulation of ubiquitinated proteins and downregulation of proteasome activity<sup>[46]</sup>. Terazosin seems to have weaker or no effects regarding CMDR<sup>[55]</sup>.

Prazosin, another quinazoline-based and antihypertensive  $\alpha_1$ -adrenoceptor antagonist<sup>[60]</sup>, is also an HERG ligand<sup>[58]</sup> and EGFR inhibitor<sup>[61]</sup>. Prazosin induces autophagic cell death *via* a p53-mediated mechanism<sup>[62]</sup> and cell apoptosis through the induction of DNA damage stress, leading to cyclin-dependent kinase 1 inactivation and G<sub>2</sub> checkpoint arrest triggering mitochondria-mediated apoptosis induction<sup>[62]</sup>. In addition, prazosin exhibits an anti-angiogenic activity<sup>[63]</sup> and its role in MDR modulation has also been suggested<sup>[55,64]</sup>. These emerging findings indicate that the quinazoline-based antihypertensive  $\alpha_1$ -adrenoceptor antagonists may have a significant role in uncontrolled hypertensive cancer patients without signs of ischemia<sup>[26,29,38,40]</sup>.

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## Recent advances in the diagnosis and treatment of acute myocardial infarction

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### Abstract

The Third Universal Definition of Myocardial Infarction (MI) requires cardiac myocyte necrosis with an increase and/or a decrease in a patient's plasma of cardiac troponin (cTn) with at least one cTn measurement greater than the 99<sup>th</sup> percentile of the upper normal reference limit during: (1) symptoms of myocardial

ischemia; (2) new significant electrocardiogram (ECG) ST-segment/T-wave changes or left bundle branch block; (3) the development of pathological ECG Q waves; (4) new loss of viable myocardium or regional wall motion abnormality identified by an imaging procedure; or (5) identification of intracoronary thrombus by angiography or autopsy. Myocardial infarction, when diagnosed, is now classified into five types. Detection of a rise and a fall of troponin are essential to the diagnosis of acute MI. However, high sensitivity troponin assays can increase the sensitivity but decrease the specificity of MI diagnosis. The ECG remains a cornerstone in the diagnosis of MI and should be frequently repeated, especially if the initial ECG is not diagnostic of MI.

There have been significant advances in adjunctive pharmacotherapy, procedural techniques and stent technology in the treatment of patients with MIs. The routine use of antiplatelet agents such as clopidogrel, prasugrel or ticagrelor, in addition to aspirin, reduces patient morbidity and mortality. Percutaneous coronary intervention (PCI) in a timely manner is the primary treatment of patients with acute ST segment elevation MI. Drug eluting coronary stents are safe and beneficial with primary coronary intervention. Treatment with direct thrombin inhibitors during PCI is non-inferior to unfractionated heparin and glycoprotein II b/IIIa receptor antagonists and is associated with a significant reduction in bleeding. The intra-coronary use of a glycoprotein II b/IIIa antagonist can reduce infarct size. Pre- and post-conditioning techniques can provide additional cardioprotection. However, the incidence and mortality due to MI continues to be high despite all these recent advances. The initial ten year experience with autologous human bone marrow mononuclear cells (BMCs) in patients with MI showed modest but significant increases in left ventricular (LV) ejection fraction, decreases in LV end-systolic volume and reductions in MI size. These studies established that the intramyocardial or intracoronary administration of stem cells is safe. However, many of these studies consisted of small numbers of patients who were not randomized to BMCs or placebo. The recent LateTime, Time, and Swiss Multicenter Trials in patients

with MI did not demonstrate significant improvement in patient LV ejection fraction with BMCs in comparison with placebo. Possible explanations include the early use of PCI in these patients, heterogeneous BMC populations which died prematurely from patients with chronic ischemic disease, red blood cell contamination which decreases BMC renewal, and heparin which decreases BMC migration. In contrast, cardiac stem cells from the right atrial appendage and ventricular septum and apex in the SCPIO and CADUCEUS Trials appear to reduce patient MI size and increase viable myocardium. Additional clinical studies with cardiac stem cells are in progress.

**Key words:** Myocardial necrosis; Type 1-5 myocardial infarctions; Troponin assays; Percutaneous coronary intervention; Fibrinolytic therapy; Thienopyridines; Cardioprotection; Bone marrow stem cells; Cardiac stem cells

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**Core tip:** The Third Universal Definition of myocardial infarction (MI) combines clinical symptoms, cardiac biomarkers and electrocardiogram (ECG) changes. Small amounts of myocardial necrosis may occur with heart failure, renal failure, myocarditis, arrhythmias, pulmonary embolism or uneventful percutaneous or surgical coronary revascularization and should be termed myocardial injury. High sensitivity troponin assays increase the sensitivity but decrease the specificity of MI diagnosis. The ECG remains a cornerstone of MI diagnosis. Primary percutaneous coronary intervention in a timely manner is the primary treatment of patients with acute ST segment elevation MI. Antiplatelet agents (clopidogrel, prasugrel or ticagrelor), in addition to aspirin, reduce patient MI morbidity and mortality. The recent LateTime, Time, and Swiss Multicenter Trials of bone marrow stem cells in MI treatment did not demonstrate significant improvement in patient LV ejection fraction in comparison with placebo. In contrast, cardiac stem cells from the right atrial appendage or ventricular septum/apex in the SCPIO and CADUCEUS Trials reduced patient MI size and increased viable myocardium. Studies with cardiac stem cells are continuing.

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## DEFINITION OF MYOCARDIAL INFARCTION

The Third Universal Definition of myocardial infarction (MI) expert consensus document was published in October 2012 by the global Myocardial Infarction Task

Force<sup>[1]</sup>. The definition of MI requires cardiac myocyte necrosis with an increase and/or a decrease in plasma of cardiac troponin (cTn). At least one cTn measurement should be greater than the 99<sup>th</sup> percentile normal reference limit during: (1) symptoms of myocardial ischemia; (2) new (or presumably new) significant ECG ST-segment/T-wave changes or left bundle branch block; (3) the development of pathological electrocardiographic (ECG) Q waves; (4) new loss of viable myocardium or regional wall motion abnormality identified by an imaging procedure; or (5) identification of intracoronary thrombus by angiography or autopsy.

Cardiac troponin (I or T) has high myocardial tissue specificity as well as high clinical sensitivity because cTn T and I are essential contractile components of myocardial cells and are expressed almost exclusively in the myocardium. Release of cardiac troponin from the myocardium can result from normal turnover of myocardial cells, myocyte apoptosis, myocyte release of troponin degradation products, increased myocyte wall permeability and bleb formation, or myocyte necrosis<sup>[1]</sup>.

Myocardial necrosis due to myocardial ischemia is defined as myocardial infarction<sup>[2]</sup>. Detection of a rise and a fall of troponin, expressed in ng/L or pg/mL, is essential to the diagnosis of acute MI<sup>[3,4]</sup>. Blood samples for the measurement of cTn should be drawn during the initial patient assessment and repeated 3-6 h later. Subsequent additional blood samples are required if further ischemic episodes occur, or when the timing of the initial symptoms onset is unclear<sup>[5]</sup>. The demonstration of a rise and fall in troponin measurements is extremely important in the differentiation of acute from chronic elevations in cTn concentrations that can be associated with structural heart disease such as patients with left ventricular hypertrophy (LVH), renal failure and heart failure (Table 1)<sup>[6]</sup>.

The ECG remains a cornerstone in the diagnosis of MI and should be acquired and interpreted within 10 min after patient presentation<sup>[7]</sup>. Since ECG changes of MI can be transient, ECGs should be acquired at 15-30 min intervals, especially if the initial ECG is equivocal. Wide spread and profound ST-T changes are associated with greater degrees of myocardial ischemia. The extent and severity of coronary stenosis, collateral coronary circulation and prior myocardial necrosis impact on the ECG manifestations of myocardial ischemia<sup>[8]</sup>. Prior ECGs, when available, should be compared with current tracings. Mimickers of ECG changes of MI such as acute pericarditis, LVH, left bundle branch block (LBBB), Brugada syndrome, stress cardiomyopathy, and early repolarization patterns should be considered in the differential diagnosis<sup>[9]</sup>.

Electrocardiographic ST-T wave criteria for the diagnosis of acute myocardial ischemia is listed in Table 2. The J point is used to determine the magnitude of the ST-segment shift. "Contiguous leads" refers to lead groups such as anterior leads (V1-V6), inferior leads



**Table 1 Causes of troponin elevation**

System	Causes of troponin elevation
Cardiovascular	Acute aortic dissection Arrhythmia Medical ICU patients Hypotension Heart failure Apical ballooning syndrome Cardiac inflammation Endocarditis, myocarditis, pericarditis Hypertension Infiltrative disease Amyloidosis, sarcoidosis, hemochromatosis, scleroderma Left ventricular hypertrophy
Myocardial injury	Blunt chest trauma Cardiac surgeries Cardiac procedures Ablation, cardioversion, percutaneous intervention Chemotherapy Hypersensitivity drug reactions Envenomation
Respiratory	Acute PE ARDS
Infectious/immune	Sepsis/SIRS Viral illness Thrombotic thrombocytopenic purpura
Gastrointestinal	Severe GI bleeding
Nervous system	Acute stroke Ischemic stroke Hemorrhagic stroke Head trauma
Renal	Chronic kidney disease
Endocrine	Diabetes Hypothyroidism
Musculoskeletal	Rhabdomyolysis
Integumentary	Extensive skin burns
Inherited	Neurofibromatosis Duchenne muscular dystrophy Klippel-Feil syndrome
Others	Endurance exercise Environmental exposure Carbon monoxide, hydrogen sulfide

GI: Gastrointestinal; ICU: Intensive care unit; PE: Pulmonary embolus; ARDS: Acute respiratory distress syndrome; SIRS: Systemic inflammatory response syndrome.

(II, III, aVF) or lateral/apical leads (I, aVL).

Supplemental leads such as V3R and V4R, in the third and fourth right intercostal spaces, indicate the electrical activity in the free wall of the right ventricle and V7-V9 indicate the electrical activity in the inferobasal left ventricular wall. In patients with inferior and right ventricular infarction, ST segments are often elevated  $\geq 0.05$  mV in V3R and V4R. In addition, ST elevation of  $\geq 0.05$  mV ST in leads V7-V9 (V7 at the left posterior axillary line, V8 at the left mid-scapular line, and V9 at the left paraspinal border), supports the diagnosis of inferobasal MI due to left circumflex coronary artery occlusion. ST depression in leads V1-V3 also may be suggestive of inferobasal myocardial ischemia (posterior infarction), especially when the terminal T wave is positive<sup>[10-12]</sup>.

**Table 2 Electrocardiogram manifestations of acute myocardial ischemia (in absence of left ventricular hypertrophy and left bundle branch block)**

ST elevation  
New ST elevation at the J point in two contiguous leads with the cut-points:  
 $\geq 0.1$  mV in all leads other than leads V2-V3 where the following cut points apply:  $\geq 0.2$  mV in men  $\geq 40$  yr;  $\geq 0.25$  mV in men  $< 40$  yr, or  $\geq 0.15$  mV in women  
ST depression and T wave changes  
New horizontal or down-sloping ST depression  $\geq 0.05$  mV in two contiguous leads and/or T inversion  $\geq 0.1$  mV in two contiguous leads with prominent R wave or R/S ratio  $> 1$

ST segment elevation of  $> 0.5$  mV is observed in lead aVR in acute left main coronary artery (LMCA) obstruction and proximal left anterior descending coronary artery (LAD) obstruction proximal to the first major septal branch. The ST elevation in aVR is more pronounced than in V1 in patients with acute LMCA occlusion. This pattern occurred in 88% of the patients with acute occlusion of LMCA group in one study<sup>[10,13]</sup>. Types of MI, five types of MI are based on pathological, clinical and prognostic differences (Table 3).

## DIFFERENTIATING BETWEEN SPONTANEOUS TYPE 1 AND ISCHEMIC IMBALANCE TYPE 2 MYOCARDIAL INFARCTION

Differentiation between type 2 and type 1 MI is challenging and needs careful clinical assessment. It is very important that the differentiation be made whether the myocardial injury is likely to be due to plaque rupture (type 1 MI), or whether it is due to an imbalance in myocardial oxygen supply or demand (type 2 MI), because the management of these two conditions is very different. While, the treatment of type 1 MI primarily includes antithrombotic therapy and/or revascularization, as clinically appropriate, the management of type 2 MI is more varied because several different mechanisms may be responsible for pathogenesis ischemic imbalance. In critically ill patients or in patients with major (non-cardiac) surgery, biomarker elevation may be caused by the direct toxic effects of endogenous or exogenous high circulating catecholamines, coronary vasospasm and/or endothelial dysfunction or fixed coronary atherosclerosis and demand-supply mismatch (Figure 1). For example, a post-operative patient with hypotension and troponin elevation due to hypovolemia or acute blood loss, requires treatment with intravascular volume replacement, including blood transfusion. In certain instances, troponin elevation due to ischemic demand may unmask severe coronary artery disease (CAD) by increasing myocardial oxygen demand in the presence of fixed coronary stenosis. Consequently once the



**Table 3 Third universal classification of myocardial infarction****Type 1: Spontaneous MI**

Spontaneous MI due to atherosclerotic plaque rupture, ulceration, fissuring, erosion, or dissection with resulting intraluminal thrombus in one or more of the coronary arteries leading to decreased myocardial blood flow or distal platelet emboli with ensuing myocyte necrosis. The patient may have underlying severe CAD, non-obstructive coronary disease or no CAD

**Type 2: MI secondary to an ischemic imbalance**

Myocardial injury with necrosis occurs due to conditions other than CAD that contribute to an imbalance between myocardial oxygen supply and/or demand such as coronary endothelial dysfunction, coronary artery spasm, coronary embolism, tachycardia-bradycardia arrhythmias, anemia, respiratory failure, hypotension, and hypertension

**Type 3: MI resulting in death when biomarker values are unavailable**

Cardiac death with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes or new LBBB, but death occurs before blood samples can be obtained, before cardiac troponins biomarkers rise, or when cardiac biomarkers were not collected

**Type 4A: MI related to percutaneous coronary intervention**

MI associated with PCI is defined by elevation of cTn values greater than five times the 99<sup>th</sup> percentile upper normal reference limit (URL) in patients with normal baseline values (< 99<sup>th</sup> percentile URL) or a rise of cTn values by > 20% if the baseline troponins are elevated and are stable or falling. In addition one of the following criterion are required: (1) symptoms suggestive of myocardial ischemia; (2) new ischemic ECG changes or new LBBB; (3) angiographic loss of patency of a major coronary artery or a side branch or persistent slow- or no coronary flow or coronary embolization; or (4) demonstration with imaging of a new loss of viable myocardium or new regional wall motion abnormality

**Type 4B: MI related to stent thrombosis**

MI associated with stent thrombosis detected by coronary angiography or autopsy in the presence of myocardial ischemia with a rise and/or fall of troponin biomarkers. One troponin measurement should be above the 99<sup>th</sup> percentile UR

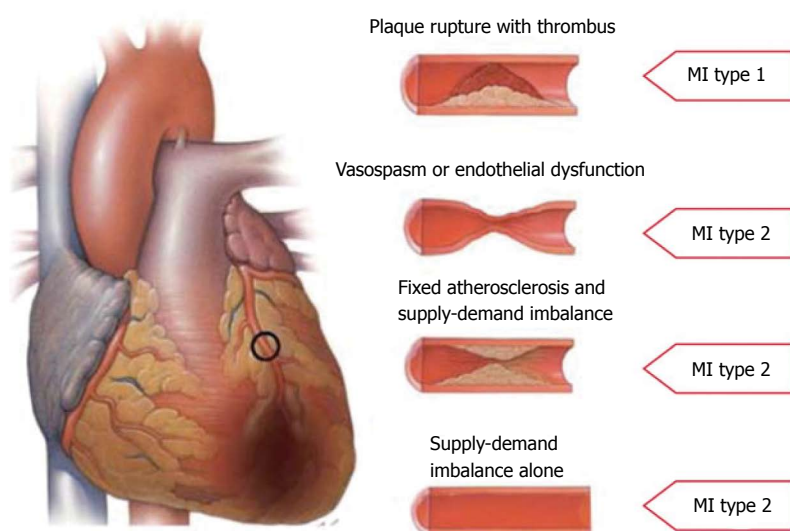
**Type 4C: MI related to restenosis**

MI associated with restenosis defined as  $\geq 50\%$  stenosis or a complex lesion demonstrated at coronary angiography after (1) initial successful stent deployment; or (2) dilatation of a coronary artery stenosis with balloon angioplasty. These coronary angiographic changes should be associated with an increase and/or decrease of cTn values > 99<sup>th</sup> percentile URL and no other significant obstructive CAD

**Type 5: MI related to coronary artery bypass grafting**

MI associated with CABG is defined by elevation of cardiac troponins greater than ten times the 99<sup>th</sup> percentile URL in patients with normal baseline cTn values (< 99<sup>th</sup> percentile URL). In addition, one of the following should be present: (1) new pathological Q waves or new LBBB; or (2) angiographic documented new graft or new native coronary artery occlusion; or (3) new loss of viable myocardium or new regional wall motion abnormality as shown by an imaging modality

Adapted from Thygesen *et al*<sup>[14]</sup>. MI: Myocardial infarction; CAD: Coronary artery disease; PCI: Percutaneous coronary intervention; cTn: Cardiac troponin; CABG: Coronary artery bypass grafting; LBBB: Left bundle branch block.

**Figure 1 Type 1 and type 2 myocardial infarctions.**

patient recovers from the acute illness, a stress test for inducible ischemia or coronary angiography can be helpful.

## MYOCARDIAL INFARCTION DUE TO RE-VASCULARIZATION PROCEDURES

The 2007 universal MI definition required the presence of cardiac biomarkers greater than three times the

99<sup>th</sup> percentile of the upper normal range limit (URL) without requirements for associated ischemic changes or complications from angiographic procedures. This resulted in approximately 15% of patients undergoing PCI being diagnosed with an AMI<sup>[15,16]</sup>. In the 2012 definition of MI, there is a more strict definition of type 4a MI<sup>[1]</sup>. Percutaneous coronary intervention related MI is defined by cTn elevation greater than *five times* 99<sup>th</sup> percentile within 48 h after the procedure with: (1) symptoms suggestive of myocardial ischemia; or

(2) new ischemic ECG changes; or (3) angiographic findings consistent with a procedural complication with loss of a major artery or side coronary artery branch, decreased coronary flow, or coronary embolization; or (4) demonstration of new loss of viable myocardium or new regional wall motion abnormality. The occurrence of procedure-related myocardial cell injury with necrosis can be detected by measurements of cardiac troponin before the procedure, 3-6 h after the procedure and, optionally, re-measurement 12 h thereafter. An increasing cTn can only be interpreted as a procedure-related myocardial injury if the pre-procedural cTn value is  $\leq 99^{\text{th}}$  percentile URL or if the troponin measurements are stable or falling. If the pre-procedural troponin is increased but is either stable or falling, an increase in cTn levels of  $> 20\%$  is used to characterize a PCI-related MI.

The relationship between troponin increases after revascularization and mortality is controversial. The evidence for the association between biomarkers and mortality has evolved over the last 15 years. Studies have suggested a stronger association with the post-PCI MB fraction of creatine kinase (CK-MB) and subsequent cardiovascular events than with cTn elevation<sup>[15,17]</sup>. The level of CK-MB measurements varied from three to ten times the URL in these studies. When analyzed in categories of incrementally increasing biomarker elevations, most contemporary PCI studies have reported associations between peri-procedural myonecrosis and mortality only for very large patient infarctions<sup>[17]</sup>. Only pre-procedure cTn elevations are correlated with subsequent mortality<sup>[18,19]</sup>. Consequently, in patients with baseline troponin elevation prior to PCI, the diagnostic accuracy of using the definition of post-PCI MI is limited.

With the application of the 2007 universal definition of post CABG MI (type 5), 42% to 82% of cardiac surgical patients had cardiac biomarker elevation greater than five times the URL<sup>[20]</sup>, but only 4% to 7% had electrocardiographic evidence required for post-CABG MI<sup>[21]</sup>. Elevation of cardiac biomarker values after CABG can occur due to myocardial trauma, with dissection of the coronary arteries, manipulation of the heart, inadequate cardiac protection, reperfusion injury, or graft failure. Any increase in cardiac biomarker values  $> 99^{\text{th}}$  percentile URL is defined as myocardial injury. The new criteria for type 5 MI in patients with CABG requires an increase in biomarkers  $> 10 \times 99^{\text{th}}$  percentile URL from a normal baseline during the first 48 h after surgery, plus new electrocardiographic Q waves or new LBBB, angiographic documentation of new graft or new native coronary artery occlusion, or imaging evidence of new regional wall motion abnormality or new loss of viable myocardium. The 2012 global MI task force emphasized that the threshold for diagnosing MI is more robust for on-pump CABG. The existing criteria for the universal definition of myocardial infarction should be used for diagnosing MI in patients who are more than 48 h after cardiac

surgery<sup>[1]</sup>.

The Society for Cardiovascular Angiography and Interventions has published an expert consensus document that defines clinically relevant myocardial infarction after revascularization (Table 4)<sup>[14]</sup>.

## REINFARCTION/RECURRENT MI

The term "reinfarction" is used for an acute MI that occurs within 28 d of a MI. If the cTn concentration is elevated, but stable or decreasing at the time of suspected reinfarction, the diagnosis of reinfarction requires a 20% or greater increase in the cTn measurement. If the initial cTn concentration is normal at the time of suspected reinfarction, the criteria for new acute MI apply<sup>[1,22]</sup>.

## TROPONIN ELEVATION IN HEART FAILURE

Based on the type of assay used, a range of elevated cTn values, indicative of myocardial injury with necrosis, may be seen in patients with a heart failure (HF) syndrome<sup>[23]</sup>. In stable heart failure patients, the median concentration of hs-cTnT is 12 ng/L, which is very close to the 99<sup>th</sup> percentile URL of 14 ng/L for this assay<sup>[24]</sup>. Hence, using hs-cTn assays, cTn concentrations may be measured in nearly all patients with HF. Many HF patients exceed the 99<sup>th</sup> percentile URL, especially those patients with severe decompensated HF syndrome<sup>[25]</sup>. While type 1 MI is an important cause of acutely decompensated heart failure, other mechanism(s) leading to troponin elevation in HF syndromes such as supply-demand inequity (type 2 MI) should be considered. Non-coronary triggers, such as anemia, cellular necrosis, apoptosis, or autophagy in the context of wall stress may cause troponin release in HF, as can the toxic effects of circulating neurohormones, toxins, inflammation, and infiltrative processes. Nonetheless, in patients with HF, troponin elevation independent of its mechanism, is strongly predictive of an adverse outcome and should not be ignored<sup>[25]</sup>.

## HIGH SENSITIVITY TROPONIN ASSAYS

Highly sensitive assays for cTnT and cTnI are available and are widely used in many parts of the world, although they are not generally used at the present time in the United States<sup>[26]</sup>. Two criteria should be met for high sensitivity troponin assays (hs-Tn). First, the coefficient of variation at the 99<sup>th</sup> percentile value should be  $\leq 10\%$ . Second, the assay should be able to measure cTn concentrations below the 99<sup>th</sup> percentile in  $\geq 95\%$  of normal individuals<sup>[27]</sup>. Compared with standard cTn assays, the hs-cTn assays have improved sensitivity and discrimination for MI, particularly in the first 3 to 6 h after symptom onset<sup>[28]</sup>. These advantages are somewhat offset by a decrease in specificity for MI<sup>[28-30]</sup> and concerns regarding the broad application of these tests, especially in populations with

**Table 4 Proposed definition of clinically relevant myocardial infarction after both percutaneous coronary intervention and coronary artery bypass grafting procedures**

In patients with normal baseline CK-MB	The peak CK-MB measured within 48 h of the procedure rises to $\geq 10 \times$ the local laboratory ULN, or to $\geq 5 \times$ ULN with new pathologic Q-waves in $\geq 2$ contiguous leads or new persistent LBBB, OR in the absence of CK-MB measurements and a normal baseline cTn, a cTn (I or T) level measured within 48 h of the PCI rises to $\geq 70 \times$ the local laboratory ULN, or $\geq 35 \times$ ULN with new pathologic Q-waves in $\geq 2$ contiguous leads or new persistent LBBB
In patients with elevated baseline CK-MB (or cTn) in whom the biomarker levels are stable or falling	The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above from the most recent pre-procedure level
In patients with elevated CK-MB (or cTn) in whom the biomarker levels have not been shown to be stable or falling	The CK-MB (or cTn) rises by an absolute increment equal to those levels recommended above plus new ST-segment elevation or depression plus signs consistent with a clinically relevant MI, such as new onset or worsening heart failure or sustained hypotension

ULN: Upper limit of normal; MI: Myocardial infarction; cTn: Cardiac troponin.

a low MI prevalence.

There is controversy regarding the metrics that should be used with hs-cTn assays for the diagnosis of AMI. In this regard, attempts have been made to define in these assays the optimal value for relative change or deltas in hs-cTn concentrations. Higher deltas increase specificity while lower ones improve sensitivity. The potential for analytical interferences with hs-cTn assays is greater than with conventional assays. Examples include reductions in hs-cTnT concentrations due to hemolysis and autoantibodies or increases due to heterophilic antibodies<sup>[31]</sup>. Studies suggest that an absolute increase of hs-cTnT values, *i.e.*,  $> 7$  ng/L over 2 h, is superior to relative percentage changes from the baseline in the diagnosis of MI<sup>[32-34]</sup>.

According to the recent guideline for the management of patients with acute coronary syndromes, blood samples for high-sensitivity cardiac troponin measurements should be obtained at presentation and 3 h after admission<sup>[35]</sup>. Measurements of hs-cTn should be repeated 6 h after admission in patients in whom the 3 h values are unchanged but in whom the clinical suspicion of MI is still high<sup>[36]</sup>.

Distinguishing between type 1 and type 2 MI is challenging with high sensitivity troponin measurements. As troponin assay sensitivity increases, the frequency of possible type 2 MI increases and the distinction from type 1 MI becomes more complicated. Moreover, the diagnostic accuracy of a baseline measurement of hs-cTn for presence of AMI in patients with renal insufficiency is poor<sup>[37]</sup>. Nevertheless, elevated hs-cTns have important prognostic implications and patients require additional evaluations because a high cTnT level is associated with all-cause and cardiovascular mortality and with incident heart failure in 3 population based studies<sup>[30]</sup>.

## TREATMENT OF ACUTE MYOCARDIAL INFARCTION

The incidence of ST segment myocardial infarction (STEMI) has gradually declined over the past decade. However it still accounts for 25%-40% of all acute coronary syndrome related hospitalizations in the United States<sup>[37]</sup>. Moreover, the incidence of acute

myocardial infarction is increasing in the developing countries<sup>[38]</sup>. Heart disease is expected to be the leading cause of death in the developing world by the year 2020. With changing dietary and personal habits, the prevalence of smoking, hypertension, diabetes, obesity and metabolic syndrome are increasing in areas of the world with large populations such as India<sup>[39]</sup>, China<sup>[40]</sup> and South America<sup>[41]</sup>. Advances made in the area of medical therapy and coronary interventions have resulted in a significant decrease in the mortality rates. Current in-hospital and one year mortality are in the order of 5%-6% and 7%-18% respectively<sup>[42,43]</sup>. During the course of last three decades, there have been significant advances in our understanding of the pathophysiology and treatment of STEMI. In addition to these scientific advances, substantial progress has been made in the areas of public awareness and guideline driven clinical practice<sup>[44]</sup>. This has led to a gradual decline in STEMI related mortality and improved patient related outcomes. However, there continues to be significant difference in the 30-d mortality rates based on the geographic region<sup>[45]</sup>, age<sup>[46,47]</sup>, gender<sup>[48]</sup> and race<sup>[49]</sup>. In addition, individuals with diabetes and chronic renal insufficiency continue to have high rates of mortality<sup>[50-52]</sup>. In the recent INFUSE-AMI (Intracoronary Abciximab and Aspiration Thrombectomy During Primary PCI for Anterior STEMI) trial<sup>[53]</sup>, diabetics compared to non-diabetics, had higher incidence of stent thrombosis at 30 d (4.3% vs 0.8%,  $P = 0.03$ ) and higher rates of major cardiovascular and cerebrovascular events at 1 year (16.5% vs 8.0%,  $P = 0.04$ ). It has been shown that patients with end-stage renal disease frequently do not receive guideline based therapies. In one registry, it has been shown that only 45% of eligible patients on dialysis received coronary reperfusion therapy, and only 70% of patients received aspirin on admission for coronary syndromes. In-hospital mortality rate from myocardial infarction is 21.3% in those on dialysis and 11.7% in those with renal disease but not on dialysis<sup>[54]</sup>.

Approximately 7% of the eligible patients with myocardial infarctions do not receive reperfusion therapy<sup>[55]</sup>. There is evidence suggesting that reperfusion therapy offers benefit in the elderly. However, age is

the strongest predictor associated with an individual not receiving reperfusion therapy<sup>[56]</sup>. Programs that focus on patient education, systematic organization of STEMI programs and standardization of clinical practice result in improved care of all groups of patients and minimize disparities<sup>[57,58]</sup>.

One of the most important components of STEMI management is getting the patients in a time efficient manner to a hospital that is capable of administering reperfusion therapies such as fibrinolytic therapy and primary percutaneous coronary intervention. Although approximately 98% of the United States population is within the reach of 911 based emergency medical service systems, patients with STEMI do not routinely utilize the system<sup>[59]</sup>. System based delays have been shown to increase STEMI related morbidity and mortality<sup>[60-63]</sup>. Hence increased community awareness and preparedness is important. In addition, regional STEMI centers with organized protocols, system based time-to-treatment goals and quality improvement programs must be established. Such efforts minimize delays and lower morbidity and mortality in STEMI patients<sup>[64,65]</sup>. In a study by Sørensen *et al*<sup>[66]</sup>, where 759 consecutive STEMI patients were divided into a group with pre-hospital diagnosis and direct referral to a primary PCI center vs a group without pre-hospital diagnosis. Pre-hospital diagnosis and direct referral resulted in shorter system delay (92 min vs 153 min,  $P < 0.001$ ).

## CORONARY REPERFUSION STRATEGIES

Fibrinolytic therapy (FT) and Primary Percutaneous Coronary Intervention (P-PCI) are the two currently available modalities of reperfusion therapies. Both of these options are extensively studied. P-PCI, when performed in a timely manner at a high patient volume center is superior to FT. However, P-PCI is not universally available<sup>[67]</sup>. Delays in door-to-balloon times (D2B) are associated with increased mortality<sup>[68]</sup>. Adherence to D2B goal of  $< 90$  min lowers mortality<sup>[69,70]</sup>. Although P-PCI is superior to FT, emphasis should be placed on timely administration of some form of reperfusion therapy rather than the mode of treatment<sup>[71]</sup>.

For patients who present to a P-PCI capable hospital, the door to balloon time should not exceed 90 min. When patients present to a hospital that is not capable of P-PCI, factors such as time of onset of symptoms, risk of bleeding, presence of acute heart failure or shock, risk of mechanical complications, time-to-transfer to a P-PCI capable hospital should be taken into consideration. In patients who present within less than 1-2 h of onset of symptoms, immediate FT may be advantageous even if the transfer times are short<sup>[72]</sup>.

## ROLE OF PRE-HOSPITAL FIBRINOLYTIC THERAPY

Multiple trials have shown the safety and efficacy of

pre-hospital FT<sup>[73-76]</sup>. This approach reduces the time to treatment by approximately 60 min and decreases mortality by 17%<sup>[77]</sup>. Similar findings were also seen in the pooled analysis of two other trials<sup>[78]</sup>. The Swedish and the French (USIC) registries showed that pre-hospital FT can be administered safely and results in reduces mortality<sup>[79,80]</sup>. At this time, pre-hospital use of FT is not commonly used in the United States but is used frequently in Western Europe and England.

## STEMI PATIENTS WITH OUT-OF-HOSPITAL CARDIAC ARREST

Approximately 70% of CAD related deaths present as cardiac arrest prior to presenting to a hospital<sup>[81]</sup>. Less than a quarter of patients presenting with sudden cardiac arrest have ventricular tachycardia or ventricular fibrillation that can be electrically converted to normal sinus rhythm<sup>[82]</sup>. Of the 60% patients who are resuscitated by emergency response teams, the median survival rate to hospital discharge is 7.9%<sup>[83]</sup>. In patients with STEMI who present with sudden cardiac arrest, timely defibrillation and hypothermia have been shown to increase survival. For every minute delay in defibrillation, there is 7% to 10% drop in survival<sup>[83,84]</sup>. Increasing access to and use of defibrillators in public places has resulted in an increase in the number of patients that are neurologically intact after sudden cardiac arrest<sup>[84-86]</sup>. In patients with out-of hospital cardiac arrest, hypothermia with temperatures between 32 °C to 34 °C increases survival. In a study of 77 patients<sup>[87]</sup>, hypothermia (with the core body temperature reduced to 33 degrees C within 2 h after the return of spontaneous circulation and maintained at that temperature for 12 h) compared to normal temperature increased the survival rates 26% to 49%  $P = 0.0046$ . In another study, survival was shown to be improved with hypothermia<sup>[88]</sup>. In patients with out-of hospital cardiac arrest in the setting of STEMI, hypothermia should be initiated as soon as possible.

## FIBRINOLYTIC THERAPY

When P-PCI is not available, FT is an alternative. It reduced mortality and morbidity when carefully administered within 12 h of symptom onset<sup>[89-94]</sup>. The usefulness of FT in patients presenting greater than 12 h from the onset of symptoms is not well established<sup>[95-98]</sup>. Fibrin specific agents such as tenecteplase, reteplase and alteplase are preferred. Tenecteplase is the most fibrin specific. None of the fibrin specific agents are antigenic. Patency rates of the infarct related artery with fibrin specific agents are approximately 85%<sup>[99-103]</sup>. Streptokinase is a non-fibrin-specific agent and can cause antigenic reactions. Infarct related artery patency rate with streptokinase is 60%-70%<sup>[104]</sup>. When the delay from first medical contact to primary PCI is  $> 120$  min, FT is indicated if the time of onset of symptoms is  $< 12$  h.



## ADJUNCTIVE PHARMACOTHERAPY WITH FIBRINOLYTIC THERAPY

The role of Aspirin and Clopidogrel with fibrinolytic therapy is well established<sup>[105-107]</sup>. Aspirin and Clopidogrel should be given prior to the administration of fibrinolytic agent. Dual antiplatelet therapy should be continued for at least one year<sup>[107]</sup>. The data on using newer antiplatelet agents like Prasugrel and Ticagrelor as an adjunct to thrombolytic therapy for fibrinolysis is not yet well established.

In addition to antiplatelet therapy, the use of adjunctive anticoagulants is supported when fibrinolytic agents are used for STEMI<sup>[108]</sup>. Unfractionated heparin, Enoxaparin and Fondaparinux can be used. However, low molecular weight heparins (LMWH) should be avoided in patients with impaired renal function (Creatinine Clearance < 30 mL/min)<sup>[109]</sup>.

## FAILED FIBRINOLYTIC THERAPY

Ongoing chest pain, lack of > 50% ST segment resolution and the absence of reperfusion arrhythmias at 60-90 min after the administration of fibrinolytics is considered failure of treatment. These parameters predict TIMI flow < 3 in the infarct artery<sup>[110]</sup>. In patients who don't respond to (FT), "rescue" PCI has been shown to be beneficial. In the Rapid Early Action for Coronary Treatment Trial<sup>[111]</sup>. The primary components endpoint of death, reinfarction, stroke, or severe HF at 6 mo, was lower among patients randomized to rescue PCI compared to conservative care or repeat fibrinolysis (event-free survival rate: 84.6% vs 70.1% vs 68.7%,  $P = 0.004$ ). This was due to reduction in reinfarction. There was no significant survival benefit. Minor bleeding was significantly higher among patients randomized to rescue PCI. However, there were no differences in major bleeding among the conservative therapy, repeat fibrinolysis or, rescue PCI groups. Similar findings of improved event free survival were reported in the Middlesbrough Early Revascularization to Limit Infarction trail. However, higher rates of stroke and periprocedural bleeding were associated with rescue PCI<sup>[112,113]</sup>. In patients with ongoing symptoms, lack of signs reperfusion, significant hypotension, severe CHF, cardiogenic shock, ECG evidence of large area of myocardium at risk, the benefit of early PCI justifies the risk of bleeding. Conservative treatment might be reasonable in a patient with improving symptoms and a limited inferior infarction despite the persistence of ST elevation.

## PATIENTS PRESENTING WITH CARDIOGENIC SHOCK

In the SHOCK trial<sup>[114]</sup>, 302 patient with STEMI with shock were randomized to medical stabilization ( $n = 150$ ) group, which included thrombolysis (63%

of patients), intra-aortic balloon counterpulsation (86%), and subsequent revascularization (25%), or to an early revascularization group ( $n = 152$ ). The primary endpoint of survival at 1 year was 46.7% for patients in the early revascularization group compared with 33.6% in the initial medical stabilization group (absolute difference in survival, 13.2%;  $P < 0.03$ ). In a prespecified subgroup analyses, only age (< 75 years vs  $\geq 75$  years) interacted significantly ( $P < 0.03$ ) with treatment. The benefit was seen only in patients younger than 75 years (51.6% survival in early revascularization group vs 33.3% in initial medical stabilization group). The benefit of early revascularization was apparent across a wide time window, extending up to 54 h after MI and 18 h after shock onset. Based on this data, STEMI patients who present with acute cardiogenic shock should undergo emergency cardiac catheterization and revascularization. This is especially true for patients younger than 75 years.

## ROUTINE EARLY ANGIOGRAPHY AFTER SUCCESSFUL FIBRINOLYTIC THERAPY

In the Grup de Análisis de la Cardiopatía Isquémica Aguda trial<sup>[115]</sup>, 500 patients with STEMI that were treated with recombinant tissue plasminogen activator were randomly assigned to angiography and coronary intervention if indicated within 24 h of thrombolysis, or to an ischemia-guided conservative approach. The primary endpoint of combined rate of death, reinfarction, or revascularization at 12 mo occurred in 9% of the angiography and intervention group compared to 21% in the conservative group ( $P = 0.0008$ ). There was a trend towards reduced rates of death or reinfarction (7% vs 12%,  $P = 0.07$ ). There were no differences in major bleeding or vascular complications.

In the Trial of Routine Angioplasty and Stenting after Fibrinolysis to Enhance Reperfusion in Acute Myocardial Infarction<sup>[116]</sup>, 1059 high-risk patients who had a STEMI received FT at centers not capable of performing P-PCI were randomized to either standard treatment (including rescue PCI, if required, or delayed angiography) or immediate transfer to another hospital and PCI within 6 h after fibrinolysis. At 30 d, the primary composite endpoint of death, reinfarction, recurrent ischemia, new or worsening congestive heart failure, or cardiogenic shock occurred in 11.0% of PCI and in 17.2% of the patients assigned to standard treatment ( $P = 0.004$ ). There was no evidence of increased major bleeding with the early invasive strategy.

In the Norwegian Study on District Treatment of ST-Elevation Myocardial Infarction trial<sup>[117]</sup> 266 patients with acute STEMI living in rural areas, where the transfer time to P-PCI are greater than 90 min, were initially treated with the combination of tenecteplase, aspirin, enoxaparin, and clopidogrel and were randomized to immediate transfer for P-PCI or to standard man-



agement in the local hospitals with early transfer, only if indicated for rescue or clinical deterioration. The primary outcome of composite of death, reinfarction, stroke, or new ischemia at 12 mo occurred in 21% vs 27% in the early invasive group and the conservative treatment group respectively ( $P = 0.19$ ). Although this study failed to demonstrate a statistically significant difference between the 2 treatment groups in the incidence of the primary composite endpoint, the incidence of death, recurrent MI, or stroke was significantly lower in the immediate-transfer group. The risk reduction was similar to that reported for high-risk patients in the Trial of Routine Angioplasty and Stenting after Fibrinolysis to Enhance Reperfusion in Acute Myocardial Infarction.

In a meta-analysis by Borgia *et al.*<sup>[118]</sup> that included 2961 patients from 7 trials, early PCI after successful fibrinolysis reduced the rate of re-infarction ( $P = 0.003$ ), the combined endpoint death/re-infarction ( $P = 0.004$ ) and recurrent ischemia ( $P < 0.001$ ) at 30-d. There was no evidence of an increase in patient major bleeding or stroke.

In the recent Strategic Reperfusion Early After Myocardial Infarction trial<sup>[119]</sup>, 1892 patients with STEMI who presented within 3 h of symptom onset who were unable to undergo primary PCI within 1 h, were randomly assigned to undergo either primary PCI or fibrinolytic therapy with bolus tenecteplase (half dose in patients  $\geq 75$  years of age), clopidogrel, and enoxaparin before transport to a P-PCI capable hospital. Emergency coronary angiography was performed only if fibrinolysis failed, which occurred in 36.3% of the patients; otherwise, angiography was performed 6 to 24 h after randomization. The primary composite end point of death, shock, congestive heart failure, or reinfarction within 30 d occurred in 12.4% of the patients in the fibrinolysis group and in 14.3% in the primary PCI group ( $P = 0.21$ ). In patients who did not undergo primary PCI within one hour of medical contact, pre-hospital fibrinolysis with coronary angiography with a median time = 17 h resulted in effective reperfusion. The incidence of intracranial bleeding was higher with FT when compared to PCI (1.0% vs 0.2%  $P = 0.04$ ).

Based on these studies, in STEMI patient who are treated successfully with FT, cardiac catheterization can be considered as part of a routine pharmacoinvasive or ischemia-guided approach > 24 h after administration of FT. Very early cardiac catheterization and PCI within 2-3 h after the administration of (FT) increases the risk of bleeding. Very early (< 2-3 h) invasive approach should be utilized for patients who require rescue PCI.

## FACILITATED PCI

Fibrinolytic agents use as adjunct to primary PCI has been studied. This approach is called facilitated PCI. Full dose or half dose of a fibrinolytic agent is administered with or without glycoprotein II b/III a (GP II b/III a) inhibitor prior to planned PCI. This approach is based

on the assumption that pre PCI pharmacotherapy will facilitate higher and faster rates of reperfusion.

The Assessment of the Safety and Efficacy of a New Treatment Strategy for Acute Myocardial Infarction trial<sup>[120]</sup> was stopped prematurely because of an increased mortality associated with facilitated PCI. In the Facilitated Intervention with Enhanced Reperfusion Speed to Stop Events trial<sup>[121]</sup>, patients were randomized to primary PCI or facilitated PCI with abciximab or facilitated PCI with half-dose reteplase and full-dose abciximab. Although the rates of death, heart failure, and ischemic outcome at 90 d for all three groups were similar, there was increased rate of major bleeding with the facilitated strategies. Because of these findings, facilitated PCI is currently not advised.

## PRIMARY PCI

Timely reperfusion with primary PCI (P-PCI) by experienced operators at an experienced center is superior to FT. Compared to FT, P-PCI results in higher rates of infarct related artery patency, higher rates of TIMI 3 flow and lower rates of complications such as recurrent ischemia, reinfarction, emergency repeat revascularization procedures, intracranial hemorrhage (ICH), and death. However, P-PCI is associated with increased rates of access site bleeding complications<sup>[122]</sup>. In addition, PCI can result in the "no reflow" phenomenon where the myocardial perfusion is inadequate despite restoration of TIMI 3 epicardial flow in the infarct related artery. No reflow phenomenon is due to a combination of endothelial injury, edema, atheroembolization, vasospasm, and myocyte reperfusion injury and inflammation<sup>[123]</sup>. Occurrence of no reflow is associated with increased mortality<sup>[123]</sup>. Multiple treatment strategies that included the use of GP II b/III a antagonists, nitroprusside, verapamil, adenosine, nicorandil, pexelizumab have not shown promising results<sup>[123]</sup>.

The benefits of P-PCI over FT are time sensitive. Door to balloon (D2B) times greater than 90-120 min can eliminate the benefits of P-PCI over FT<sup>[124]</sup>. Over the past ten years, there has been a significant reduction in the median D2B times. Although approximately 80% of the United States population lives within one hour from a P-PCI capable hospital, the majority of patients in the rural areas do not have access to such facilities. A significant increase in the number of PCI capable hospitals from 2001 to 2006 result in minimal increase in the overall patient access to such facilities<sup>[125,126]</sup>. One of the strategies to make P-PCI more accessible is to allow hospitals without onsite cardiac surgery facilities to perform PCI procedures. The Cardiovascular Patient Outcomes Research Team trial<sup>[127]</sup> showed that primary PCI can be performed safely and rapidly at hospitals without cardiac surgery back-up. Other strategies include bypassing the non PCI hospital and transferring the patients to a primary PCI capable hospital where the care and transfer protocols are standardized. These strategies have been shown to extend P-PCI to more

patients and result in better patient outcomes<sup>[127-129]</sup>.

## DELAYED PRESENTATION

In patients presenting more than 12 h after the onset of symptoms, cardiac catheterization and PCI should be considered in the setting of ongoing chest pain, cardiogenic shock, acute severe heart failure, or spontaneous or provoked myocardial ischemia.

In the Occluded Artery Trial<sup>[130]</sup>, 2166 patients with occluded infarct related arteries who presented 3-28 d after myocardial infarction and had ejection fractions less than 50% were randomized to PCI vs conservative medical therapy. The 4-year cumulative primary event rate was 17.2% in the PCI group and 15.6% in the medical therapy group ( $P = 0.20$ ). However, patients with high risk features such as New York Heart Association class III-IV, rest angina, high risk stress test, left main or three vessel diseases were excluded from the trial. The trial showed that routine PCI did not reduce the occurrence of death, reinfarction, or heart failure, and there was a trend toward excess reinfarction during 4 years of follow-up in stable patients with occlusion of the infarct-related artery 3 to 28 d after myocardial infarction. Based on this data, in patients who present more than 12 h after their symptom onset and are clinically stable, routine cardiac catheterization and PCI are not advised.

## PCI OF THE NON-INFARCT RELATED ARTERY

In patients presenting with STEMI, multivessel coronary artery disease is frequently seen and is associated with poor outcomes<sup>[131]</sup>. PCI of a non-infarct related artery prior to discharge from the hospital, at a time that is separate from the index STEMI related PCI, is indicated if there is evidence of spontaneous myocardial ischemia. However, this practice is largely based on non-randomized cohort studies<sup>[132-134]</sup>. The role of fractional flow reserve (FFR) at the time of STEMI, to evaluate the functional significance of a non-infarct related artery is not well established. In a small study by Ntalianis *et al.*<sup>[135]</sup>, FFR was useful in evaluating the functional significance of a non-culprit coronary lesion.

In the recent Preventive Angioplasty in Acute Myocardial Infarction trial<sup>[136]</sup>, 465 patients with acute STEMI who were undergoing primary PCI were randomly assigned to either preventive PCI defined as immediate PCI of any lesion with > 50% stenosis or no preventive PCI. The trial was stopped early by the data safety monitoring board. In an intention to treat analysis, the primary composite endpoint of death from cardiac causes, nonfatal myocardial infarction, or refractory angina occurred in 9% of the preventive PCI arm and 23% of the non-preventive PCI arm, respectively ( $P = 0.001$ ). It should be noted that the trial excluded patients with concomitant disease in the left anterior

descending and left circumflex arteries, patients with > 50% stenosis of the left main artery, patients with prior CABG and patient with a non-culprit artery with a chronic total occlusion.

At this time, PCI of a non-infarct related artery should be performed prior to hospital discharge if the patient has evidence of spontaneous or provokable myocardial ischemia.

## PCI TECHNIQUE BASED STRATEGIES

During the past decade, we have seen significant advances in the field of interventional cardiology as it relates to the management of acute myocardial infarction. Some of the frequently debated issues include access site (radial vs femoral), routine use of aspiration thrombectomy, and bare-metal vs drug eluting stents.

### Access site

Transradial PCI had gained widespread acceptance and is now used routinely for elective angioplasty. Major advantages with transradial approach include reductions in bleeding complications and length of hospitalizations and improved quality of life. Given these advantages, transradial PCI during STEMI has been extensively studied. Multiple randomized trials and a large meta-analysis showed that transradial primary PCI is associated with significant reduction in access site complications. In the Radial vs femoral access for coronary angiography and intervention in patients with acute coronary syndromes trial<sup>[137]</sup> 7021 patients with STEMI were randomly assigned to radial vs femoral access sites. The primary endpoint of death, myocardial infarction, stroke, or non-CABG-related major bleeding at 30 d occurred in 3.7% and 4.0% of the radial access and femoral access patients respectively ( $P = 0.5$ ). In a pre-specified subgroup analysis, non-CABG-related major bleeding at 30 d occurred in 24 patients in the radial group compared with 33 patients in the femoral group ( $P = 0.23$ ). At 30 d, 42 of 3507 patients in the radial group had large hematomas compared with 106 of 3514 in the femoral group ( $P < 0.0001$ ). In the Radial vs Femoral Randomized Investigation in ST Elevation Acute Coronary Syndrome<sup>[138,139]</sup> trial, 1001 STEMI patients undergoing primary/rescue percutaneous coronary intervention were randomized to the radial or femoral approach. The primary endpoint of cardiac death, stroke, myocardial infarction, target lesion revascularization, and bleeding at 30 d occurred in 13.6% in the radial artery group and 21.0% in the femoral artery group ( $P = 0.003$ ). Radial access was associated with significantly lower rates of cardiac mortality (5.2% vs 9.2%,  $P = 0.020$ ), bleeding (7.8% vs 12.2%,  $P = 0.026$ ), and shorter hospital stay. In the STEMI-RADIAL trial<sup>[103]</sup> 2959 patients undergoing primary PCI within 12 h of onset of symptoms were randomized to radial vs femoral approach. The primary

endpoint of access site complications and bleeding occurred in 7.2% of the femoral vs 1.4% of the radial group (80% relative risk reduction,  $P = 0.001$ ). Radial and femoral approaches are both safe and effective for PCI. Lower rates of local vascular complications may be a reason to use the radial access approach. There is some concern about longer D2B times and increased radiation exposure with radial artery access. This is mostly limited to low volume centers and operators<sup>[140,141]</sup>. Data from the randomized control trials suggests that D2B times and the cumulative radiation dose are minimally increased with radial artery catheterization. The impact of the radial artery approach on patient mortality remains unclear at this time as the reported studies are underpowered to evaluate this end-point.

### Adjunctive thrombectomy

A vast majority of patients with STEMI have large thrombus burden. It seems intuitive that thrombectomy may improve epicardial coronary flow, prevent distal embolization, reduce microvascular obstruction and the no-reflow phenomenon. However, trials that have used mechanical thrombectomy have been largely negative without any improvement in myocardial blush grade, final infarct size and overall left ventricular ejection fraction<sup>[142-144]</sup>. Recently there has been renewed interest in aspiration thrombectomy. In the Thrombus Aspiration during Percutaneous coronary intervention in Acute myocardial infarction Study<sup>[145]</sup>, 1071 patients were randomly assigned to the thrombus-aspiration group or the conventional-PCI group before undergoing coronary angiography. The primary end point of myocardial blush grade of 0 or 1 occurred in 17.1% of the patients in the thrombus-aspiration group and in 26.3% in the conventional-PCI group ( $P < 0.001$ ). At one year follow up, cardiac death occurred in 3.6% of the patients in the thrombus aspiration group and 6.7% in the conventional PCI group ( $P = 0.020$ ). In the Impact of Thrombectomy With Export Catheter in Infarct-Related Artery During Primary Percutaneous Coronary Intervention trial<sup>[146]</sup>, 155 STEMI patients were randomly assigned to standard percutaneous coronary intervention PCI ( $n = 87$ ) or aspiration thrombectomy guided PCI ( $n = 88$ ). The primary end points of myocardial blush grade  $\geq 2$  and the rate of 90-min ST-segment resolution  $> 70\%$  occurred more frequently in the thrombectomy guided PCI group (88% vs 60%,  $P = 0.001$ ; and 64% vs 39%,  $P = 0.001$ ). In a meta-analysis conducted by Bavry *et al*<sup>[147]</sup>, total of 30 studies with 6415 patients were included, a weighted mean follow-up of 5.0 mo showed that the mortality was 3.2% for the adjunctive thrombectomy group vs 3.7% for conventional PCI. In an by Kumbhani *et al*<sup>[148]</sup> data from clinical trials that randomized AMI patients to aspiration (18 trials,  $n = 3936$ ) or mechanical thrombectomy (7 trials,  $n = 1598$ ) before PCI compared with conventional PCI alone was analyzed. It showed that at a weighted mean clinical

follow-up period of 6 mo major adverse cardiac events (RR = 0.76; 95%CI: 0.63-0.92;  $P = 0.006$ ) and all-cause mortality (RR = 0.71; 95%CI: 0.51-0.99;  $P = 0.049$ ) were significantly reduced with aspiration thrombectomy. ST-segment resolution at 60 min (RR = 1.31; 95%CI: 1.16-1.48;  $P < 0.0001$ ) and Thrombolysis In Myocardial Infarction blush grade 3 post-procedure (RR = 1.37; 95%CI: 1.19-1.59;  $P < 0.0001$ ) were both improved with aspiration thrombectomy.

In the recently published TASTE<sup>[149]</sup> (The Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia) trial, aspiration thrombectomy did not show reduction in 30 d mortality. The event rate was 2.8% in the aspiration arm vs 3.0% in the routine PCI arm ( $P = 0.63$ ). This study has some significant limitations. The treating physician was aware of the group assignment. Event adjudication and review of coronary angiograms was not done in a blinded manner. Despite these limitations, the TASTE trial does suggest that routine use of aspiration thrombectomy, may not be beneficial in reducing mortality. Larger studies are needed to see if aspiration thrombectomy offers mortality benefit. The ongoing TOTAL A Trial of Routine Aspiration Thrombectomy With Percutaneous Coronary Intervention (PCI) vs PCI Alone in Patients With ST-Segment Elevation Myocardial Infarction (STEMI) Undergoing Primary PCI trial may answer some of the questions.

## TYPE OF STENTS IN THE SETTING OF PRIMARY PCI

It is now a routine practice to use coronary stents during primary PCI. Compared to balloon angioplasty, primary PCI with bare metal stents (BMS) has been shown to reduce the rates of reinfarction and target vessel revascularization. However, this does not translate into a reduction in mortality<sup>[150]</sup>. Drug eluting stents (DES) are currently being used for both elective and primary PCI. DES when compared with BMS significantly reduces restenosis rates and the need for reintervention but does not definitively reduce rates of death<sup>[151]</sup>. First generation DES such as Taxus and Cypher, when compared to BMS, can increase the risk of very late stent thrombosis<sup>[152]</sup>. Newer generation DES such as Xience, Promus and Endeavour, when compared to BMS do not increase the risk of acute or late stent thrombosis. Cobalt-chromium based everolimus eluting stents have the lowest reported rates of stent thrombosis<sup>[153]</sup>. In the Xience or Vision Stents for Management of Angina in the Elderly trial<sup>[154]</sup>, second generation, everolimus eluting DES were safely used in the elderly without increasing the risk of bleeding. Patients who are taking oral anticoagulation and present with a STEMI pose a significant challenge. Triple therapy significantly increases the risk of bleeding. In the What is the

Optimal antiplatelet and anticoagulant therapy in patients with oral anticoagulation and coronary stenting trial<sup>[155]</sup>, the group receiving warfarin plus clopidogrel had lower bleeding complications compared with the group receiving warfarin, clopidogrel and aspirin. Although the rate of stent thrombosis was not increased, this trial was not powered to evaluate the risk of stent thrombosis.

Given the advantages of marked reduction in the rates of restenosis, target vessel and target lesion revascularization and very low rates of late stent thrombosis, second generation DES should be the preferred choice during primary PCI. However, that decision should be made on a case to case basis. Factors such as bleeding risk, other indications for systemic oral anticoagulants, socioeconomic status, compliance, need for surgical procedures during the following one year should be considered. If these factors are a concern, DES implantation should be avoided. There still remain gaps in our understanding of routine use of DES in the elderly and patients who are on oral anticoagulants. Further research is need in these areas.

## ADJUNCTIVE PHARMACOTHERAPY BASED STRATEGIES

In recent years, there has been extensive research done in the area of adjunctive pharmaco-therapy. As a result, we now have multiple antithrombotic and antiplatelet agents that have been shown to reduce major adverse cardiac events in the setting of STEMI.

Unfractionated heparin (UFH) is time tested and the most familiar of all the agents. It is used frequently. When titrated to appropriate activated clotting times of 250-300 s, it is an acceptable strategy. Low molecular weight heparins (LMWN) such as Enoxaparin and Fondaparinux are not well studied in the setting of STEMI. In the STEMI Treated With Primary Angioplasty and Intravenous Lovenox or Unfractionated Heparin trial<sup>[156]</sup> 901 patients were randomized to treatment with enoxaparin ( $n = 450$ ) or unfractionated heparin ( $n = 460$ ). The composite primary endpoint of 30-d incidence of death, complication of myocardial infarction, procedure failure, or major bleeding occurred in 126 (28%) patients after anticoagulation with enoxaparin vs 155 (34%) patients on unfractionated heparin ( $P = 0.06$ ). Data from this trail suggests that enoxaparin can be safely and effectively used in patients with STEMI. In the OASIS-6 trial<sup>[157]</sup> death or reinfarction at 30 d was significantly reduced from 11.2% in the control group to 9.7% patients in the fondaparinux group ( $P = 0.008$ ). However, fondaparinux was associated with higher rates of catheter thrombosis. At this time, fondaparinux in not used as an anticoagulant in the setting of primary PCI.

The role of Bivalirudin in the setting of STEMI treated with primary PCI was tested in the Harmonizing Outcomes with Revascularization and Stents in Acute

Myocardial Infarction trial<sup>[158]</sup>. Three thousand six hundred and two patients with ST-segment elevation myocardial infarction presenting within 12 h after the onset of symptoms and were undergoing primary PCI, were randomly assigned to treatment with heparin plus a glycoprotein II b/IIIa inhibitor or to treatment with bivalirudin alone. Primary end points of major bleeding and combined adverse clinical events, including death, reinfarction, target-vessel revascularization for ischemia, and stroke within 30 d occurred in 22% of the heparin plus glycoprotein II b/IIIa inhibitor group vs 9.2% in the bivalirudin group ( $P = 0.005$ ). The risk of acute stent thrombosis within 24 h in the bivalirudin group increased, but no significant increase was present by 30 d. This was most likely secondary to a combination of adenosine diphosphate-induced platelet activation before maximal thienopyridine blockade of the platelet P<sub>2</sub>Y<sub>12</sub> receptor or by residual thrombin activity after the discontinuation of bivalirudin. Based on the results from the HORIZONS-AMI trail, it is a reasonable approach to use bivalurudin in patients with STEMI who are undergoing primary PCI. This approach may provide long term survival benefit by lowering the rate of bleeding complications.

## ADJUNCTIVE ANTIPLATELET THERAPY

### Aspirin

An initial single dose of 325 mg of Aspirin should be administered as early as possible. This should be followed by a maintenance dose of 81 mg once daily. Higher doses of Aspirin for maintenance therapy have shown to increase the risk of bleeding. In the Committee members of the Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events-Seventh Organization to Assess Strategies in Ischemic Syndromes trial<sup>[159]</sup> 25086 patients with an acute coronary syndrome who underwent cardiac catheterizatoin were randomized to either double-dose clopidogrel (a 600-mg loading dose on day 1, followed by 150 mg daily for 6 d and 75 mg daily thereafter) or standard-dose clopidogrel (a 300-mg loading dose and 75 mg daily thereafter) and either higher-dose aspirin (300 to 325 mg daily) or lower-dose aspirin (75 to 100 mg daily). The primary outcome of cardiovascular death, myocardial infarction, or stroke at 30 d was not different between higher-dose and lower-dose aspirin (4.2% vs 4.4%,  $P = 0.61$ ) or major bleeding (2.3% vs 2.3%,  $P = 0.90$ ).

### Clopidogrel

The importance of at least 12 mo of dual antiplatelet therapy with aspirin and clopidogrel in the setting of ACS with and without PCI has been well established based on the data from the Clopidogrel in Unstable Angina to Prevent Recurrent Event (CURE)<sup>[160]</sup> and PCI-CURE<sup>[161]</sup> trials. A 600 mg loading dose of clopidogrel offers rapid platelet inhibition compared to 300 mg dose<sup>[162]</sup>. In the CURRENT-OASIS 7 trail<sup>[159]</sup> the



primary outcome of cardiovascular death, myocardial infarction, or stroke at 30 d occurred in 4.2% of the double-dose clopidogrel group vs 4.4% in the standard-dose clopidogrel ( $P = 0.30$ ). Major bleeding occurred in 2.5% of the double-dose group and in 2.0% in the standard-dose group patients ( $P = 0.01$ ). Rates of stent thrombosis was lower in the double dose group (1.6% vs 2.3%  $P = 0.001$ ). Incidence of major bleeding was 2.5% in the double dose group vs 2.1% in the standard dose group ( $P = 0.001$ ). Clopidogrel 600 mg loading dose followed by 75 mg once daily for at least one year should be considered for all patients with acute coronary syndromes.

One common clinical concern with the use of clopidogrel is the variable therapeutic response. This is secondary to multiple factors such as diabetes, obesity, polymorphisms in enteric ABCB 1 and hepatic cytochrome P450 (CYP450) enzymes (CYP2C19\*2) and drug interaction that interferes with the metabolism of clopidogrel. Nearly 30% of patients have a reduced functional allele of CYP2C19\*2. This has been shown to be associated with decreased levels of the active metabolite of clopidogrel, suboptimal platelet inhibition and increased rates of major adverse cardiac events and stent thrombosis<sup>[162-165]</sup>. Based on this data, the United States Food and Drug Administration made changes to clopidogrel's prescribing information noting the potential impact of CYP2C19 genotype on clopidogrel's bioavailability and clinical response. However, in a study by Mega *et al*<sup>[166]</sup> homozygotes and heterozygotes for loss of functional allele had similar rates of primary efficacy outcomes. At this time routine testing for CYP2C19\*2 polymorphisms is not indicated. Further studies are needed to fully understand the clinical risk associated with these polymorphisms and to develop effective treatment strategies.

Proton-pump inhibitors, such as omeprazole, have been shown to interfere with clopidogrel metabolism resulting in decreased antiplatelet effect<sup>[167]</sup>. However, this does not lead to worse clinical outcomes<sup>[168]</sup>. At this time there is no strong evidence to avoid concomitant use of PPIs, when clinically indicated, in patients receiving clopidogrel.

### Prasugrel

Prasugrel is a thienopyridine class of drug that competitively antagonizes the P2Y<sub>12</sub> receptor. Similar to Clopidogrel, it is also a pro drug that requires biologic conversion to an active metabolites. In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel trial<sup>[169]</sup> 13608 patients with moderate-to-high-risk acute coronary syndromes treated with early invasive approach were randomly assigned to prasugrel, with a 60-mg loading dose and a 10-mg daily maintenance dose, or clopidogrel, with a 300-mg loading dose and a 75-mg daily maintenance dose, for 6 to 15 mo. The primary efficacy end-point of death from cardiovascular causes, nonfatal myocardial

infarction, or nonfatal stroke occurred in 12.1% of patients receiving clopidogrel and 9.9% of patients receiving prasugrel ( $P < 0.001$ ). There was significant reductions in the rates of myocardial infarction (9.7% for clopidogrel vs 7.4% for prasugrel;  $P < 0.001$ ), urgent target-vessel revascularization (3.7% vs 2.5%;  $P < 0.001$ ), and stent thrombosis (2.4% vs 1.1%;  $P < 0.001$ ). Major bleeding was increased with prasugrel 2.4% in comparison with 1.8% of patients with clopidogrel ( $P = 0.03$ ). The prasugrel group had higher rates of life-threatening bleeding (1.4% vs 0.9%;  $P = 0.01$ ), including nonfatal bleeding ( $P = 0.23$ ) and fatal bleeding (0.4% vs 0.1%;  $P = 0.002$ ). While prasugrel significantly reduced the rates of ischemic events and stent thrombosis, it increased the risk of major bleeding and did not reduce mortality. The benefits of prasugrel must be carefully weighed against the increased risk of bleeding. Prasugrel may be a preferred agent in younger, high risk acute coronary syndrome patients with large area of myocardium at risk and low bleeding risk. Prasugrel should not be used in patients with history of prior stroke, transient ischemic attacks, age greater than or equal 75, or body weight less than 60 kg. A lower dose of prasugrel 5 mg once daily has been suggested in patients who are at higher risk for bleeding. However, prasugrel 5 mg/d has not been prospectively studied.

### Ticagrelor

Ticagrelor is a cyclopentyl triazolo pyrimidine that acts on the platelet P<sub>2</sub>Y<sub>12</sub> receptor as an antagonist. It does not require conversion to active metabolite and is a reversible agent. In The Study of Platelet Inhibition and Patient Outcomes trail<sup>[170]</sup> 18624 patients with acute coronary syndromes, were randomized to ticagrelor (180-mg loading dose, followed by 90 mg twice daily) or clopidogrel (300-to-600-mg loading dose, followed by 75 mg daily). Thirty-five percent of the patients had STEMI. Overall, at 12 mo, the composite end-point of death from vascular causes, myocardial infarction, or stroke occurred in 9.8% of patients receiving ticagrelor vs 11.7% of patients receiving clopidogrel ( $P < 0.001$ ). The rate of death from any cause was also reduced with ticagrelor (4.5% vs 5.9%,  $P < 0.001$ ). In addition, there were reductions in the rates of myocardial infarction (5.8% in the ticagrelor group vs 6.9% in the clopidogrel group,  $P = 0.005$ ) and death from vascular causes (4.0% vs 5.1%,  $P = 0.001$ ). There was no difference in the frequency of stroke alone (1.5% vs 1.3%,  $P = 0.22$ ) or the rates of major bleeding (11.6% and 11.2%,  $P = 0.43$ ). However, ticagrelor was associated with a higher rate of major non CABG related bleeding (4.5% vs 3.8%,  $P = 0.03$ ), including more instances of fatal intracranial bleeding.

In a pre-specified subgroup analysis of the Study of Platelet Inhibition and Patient Outcomes trail, the net benefit of ticagrelor was smaller in the North American cohort. This was attributed to chance alone or alternatively to the frequent use of higher dose of aspirin

for maintenance therapy. Based on this observation, the dose of aspirin when used in combination with ticagrelor for maintenance therapy should not exceed 100 mg a day.

When considering adding a second drug to aspirin for dual antiplatelet therapy (DAPT), the decision should be individualized. The anti-ischemic benefits should be carefully weighed against patient comorbidities, risk of bleeding, need for long term treatment with an oral anticoagulant, cost, compliance, and the possibility of surgical procedures during the following year.

## DURATION OF ANTIPLATELET THERAPY

Current guidelines<sup>[171]</sup> support uninterrupted use of dual antiplatelet therapy for at least one year in post ACS patients regardless of invasive or conservative treatment or the type of stent (BMS vs DES). Recently, there has been significant data supporting the discontinuation of dual antiplatelet therapy 3 to 6 mo after a PCI in the setting of acute coronary syndrome.

In the Efficacy of Xience/Promus vs Cypher in Reducing Late Loss after Stenting trial<sup>[172]</sup> 1443 patients undergoing implantation of drug-eluting stents were randomized to receive 6- or 12-mo DAPT. The primary end point of target vessel failure at 12 mo was 4.8% in the 6-mo DAPT group and 4.3% in the 12-mo DAPT group ( $P = 0.001$  for non-inferiority). This study was underpowered for evaluation of death and MI.

In the Prolonging dual antiplatelet treatment after grading stent-induced intimal hyperplasia trial<sup>[173]</sup> 2013 patients were randomly assigned to receive bare-metal, zotarolimus-eluting, paclitaxel-eluting, or everolimus-eluting stent implantation. At 30 d, patients in each stent group were randomly allocated to receive up to 6 or 24 mo of clopidogrel therapy in addition to aspirin. The primary composite endpoint of death from any cause, myocardial infarction, or cerebrovascular accident at 24 mo was similar.

In the Real Safety and Efficacy of a 3-mo dual antiplatelet Therapy following E-ZES Implantation trial<sup>[174]</sup>, 2117 patients with coronary artery stenosis were randomized to 2 groups according to DAPT duration and stent type: 3-mo DAPT following zotarolimus-eluting stent (E-ZES) implantation vs 12-mo DAPT following the other (sirolimus, everolimus DES implantation). The primary composite endpoint of cardiovascular death, myocardial infarction, stent thrombosis, target vessel revascularization, or bleeding at 1 year occurred in 4.7% patients assigned to E-ZES + 3-mo DAPT compared with 4.7% patients assigned to the standard therapy ( $P = 0.001$  for noninferiority).

In the recently published Optimized Duration of Clopidogrel Therapy Following Treatment With the Endeavor Zotarolimus - Eluting Stent in the Real World Clinical Practice trial<sup>[175]</sup>, 3119 patients undergoing PCI using zotarolimus DES were randomly assigned to 3 mo vs 12 mo of dual antiplatelet therapy. The primary composite end point of all-cause death, myocardial

infarction, stroke, or major bleeding occurred in 6.0% vs 5.8%, respectively  $P = 0.002$  for noninferiority.

Although the data from these trials is reassuring and supports the discontinuation of dual anti-platelet therapy at the end of 6 mo, it is important to note that these trial included patients with stable coronary disease and low risk acute coronary syndrome. Caution should be used in extrapolating this data to patients with STEMI. At this time, dual anti-platelet therapy should be continued for at least one year without interruption when tolerated in patients with ACS.

## Role of glycoprotein IIb/IIIa receptor antagonists

Role of glycoprotein II b/III a (GP II b/III a) receptor antagonists in the setting of STEMI was extensively studied prior to routine use of dual antiplatelet therapy. Addition of a GP II b/III a receptor antagonist to combination of DAPT plus unfractionated heparin or bivalirudin failed to show benefit<sup>[176-178]</sup>. However, in a meta-analysis by De Luca<sup>[179]</sup> where 722 patients with STEMI from seven randomized trials were included, early administration of abciximab compared to late/peri-procedural administration was associated with reduction in mortality (20% vs 24.6%  $P = 0.02$ ), improvement in pre-procedural (TIMI) 3 flow (21.6% vs 10.1%,  $P < 0.0001$ ), post-procedural TIMI 3 flow (90% vs 84.8%,  $P = 0.04$ ), post-procedural myocardial blush grade (52.0% vs 43.2%,  $P = 0.03$ ), ST-segment resolution (58.4% vs 43.5%,  $P < 0.0001$ ) and distal embolization (10.1% vs 16.2%,  $P = 0.02$ ). There was no difference in the rates of major bleeding complications between early and late abciximab administration (3.3% vs 2.3%,  $P = 0.4$ ). Adjunctive use of GP II b/III a inhibitors can be considered at the time P-PCI if there is evidence of large thrombus or inadequate response to a P<sub>2</sub>Y<sub>12</sub> antagonist<sup>[179,180]</sup>. Based on the data from the HORIZONS-AMI<sup>[158]</sup> and CICERO<sup>[181]</sup> trials, a GP II b/III a receptor antagonist can be used as adjunct to bivalirudin in the presence of large thrombus or for "bail-out use" for procedure related dissection. Similar findings were also confirmed in a recent meta-analysis by Shimada *et al*<sup>[182]</sup>. In a recent MI trial<sup>[183]</sup>, intra-coronary infusion of abciximab reduced infarct size at 30 d. This approach should be considered on an individual patient basis<sup>[182,183]</sup>.

## ROLE OF CARDIOPROTECTION IN STEMI

Despite significant improvements in every area of STEMI management, adverse event rates continue to be high. Although, reperfusion therapy and the adjunctive pharmacotherapy help reestablish coronary flow, restoration of coronary blood flow can cause further injury to cardiac myocytes. This type of injury is called lethal reperfusion injury. In animal models, close to 50% of the final infarct size is due to lethal reperfusion injury<sup>[184]</sup>. This injury results from oxidative stress<sup>[185,186]</sup>, calcium overload<sup>[187,188]</sup>, inflammation<sup>[189]</sup> and rapid restoration of pH<sup>[189]</sup>. Understanding these

mechanisms at a cellular level has led to renewed interest in designing treatment strategies that target pathways that mediate lethal reperfusion injury. These strategies mediate their cardioprotective effect by multiple signaling pathways such as reperfusion injury salvage kinase (RISK) group of protective kinases. The cardioprotective signaling pathways inhibit the mitochondrial permeability transition pore and multiple other molecules<sup>[190]</sup>.

## PRECONDITIONING

Repeated, brief episodes of coronary occlusion with myocardial ischemia alternating with coronary reperfusion before a prolonged episode of ischemia, is a powerful way to limit infarct size. This is known as ischemic pre-conditioning<sup>[191]</sup>. However due to the fact that the brief episodes of ischemia need to be applied prior to an ischemic event, this approach has limited value in the setting of STEMI.

## POST CONDITIONING

Applying the principles of preconditioning after the ischemic event has been shown to be beneficial in animal models<sup>[192,193]</sup>. In a small randomized control trial, Staat *et al.*<sup>[194]</sup> showed that post-conditioning by 4 cycles of 1-min coronary angioplasty balloon inflations followed by 1 min of balloon deflation within 1 min of coronary reflow after deployment of a coronary stent reduced infarct size and improved myocardial blush grades. Similar findings have also been noted in other small studies that used different balloon inflation and deflation protocols<sup>[195]</sup>. A significant limitation of catheter/balloon based post-conditioning is that it is limited to cardiac catheterization laboratories at the time of P-PCI.

### Post conditioning by cyclosporine

Cyclosporine has been shown to be cardioprotective by inhibiting the mitochondrial permeability transition pore<sup>[196]</sup>. In a small randomized study of 58 patients, single bolus of 2.5 milligrams of intravenous cyclosporine, compared to placebo reduced infarct size by 40% as quantified by the degree of plasma creatine-kinase elevation<sup>[197]</sup>. The cardioprotective effect of cyclosporine appears to be promising.

## REMOTE ISCHEMIC CONDITIONING

Transient, repeated episodes of ischemia when applied to an organ distant from the heart have been shown to reduce infarct size<sup>[198]</sup>. This is called remote ischemic conditioning. One proposed mechanism is the release of a chemical by the distant organ that promotes cardiac conditioning. Another possibility is afferent neural pathway stimulation. In a study by Bøtker *et al.*<sup>[199]</sup>, 333 patients with a suspected first STEMI were

randomly assigned in a 1:1 ratio to receive P-PCI with or without remote conditioning that consisted of intermittent arm ischemia through four cycles of 5-min inflation and 5-min deflation of a blood-pressure cuff. The patients received remote conditioning during transport to hospital, and P-PCI in hospital. The primary endpoint of myocardial salvage index at 30 d, measured by myocardial perfusion imaging, was significantly improved in the preconditioning group (0.75 in the remote conditioning group vs 0.55 in the control group,  $P = 0.0333$ ). Given the ease of use and potential universal applicability of this approach, large-scale trials are underway to study this treatment.

## ROLE OF ADENOSINE

Adenosine, by mediating its effects *via* A<sub>1</sub> and A<sub>3</sub> receptors appears to play a key role in promoting a cardioprotective state. Although the mechanisms are complex, inhibition of the formation of mitochondrial permeability transition pores appears to be a primary mechanism<sup>[200]</sup>. Intravenous infusion of adenosine in patients with STEMI was tested in the Acute Myocardial Infarction Study of Adenosine I trial<sup>[201]</sup>. Although there was 33% relative reduction in the infarct size, this was mostly limited to individuals with large anterior wall MI. Based on this study, the Acute Myocardial Infarction Study of Adenosine II trial<sup>[202]</sup> randomized 2118 patients to 3-h intravenous infusion of low-dose adenosine (50 µg/kg per minute), high-dose adenosine (70 µg/kg per minute), or placebo before PCI or within 15 min of the initiation of fibrinolysis. There was no difference in the composite endpoint of death, new-onset congestive heart failure, or rehospitalization for congestive heart failure within 6 mo. However, subsequent post-hoc and subgroup analyses showed that there was significant reduction in the infarct size in those who received the high dose and those who received adenosine within 3 h of onset of symptoms<sup>[203]</sup>. Although the routine use of adenosine is currently not supported, early administration of high dose adenosine may reduce infarct size in patients with anterior wall STEMI with large areas of myocardium at risk.

## ROLE OF BETA BLOCKERS

Most of the data on the role of routine use of beta blockers in STEMI either predates or involves thrombolytic therapy. There is very limited data on the cardioprotective benefits of beta blockers in the setting of PPCI. In the recently published Effect of Metoprolol in Cardioprotection during an Acute Myocardial Infarction trial<sup>[204]</sup>, 270 patients with STEMI (Killip Class 2 or less) presenting within 6 h of onset of symptoms were randomized to receive intravenous metoprolol or no metoprolol. The primary endpoint of infarct size by magnetic resonance imaging was smaller after intravenous metoprolol compared with control (25.6

$\pm 15.3$  gm vs  $32.0 \pm 22.2$  gm,  $P = 0.012$ ). This trial illustrates that a commonly used inexpensive medication may play a significant role in cardioprotection in the setting of reperfusion by P-PCI for STEMI. Larger clinical trials that are powered to analyze hard clinical endpoints are needed to definitively understand the role of intravenous beta blockers in the setting of P-PCI.

The ideal duration of treatment with beta blockers after a STEMI is not well established. At the present time most patients are treated indefinitely with beta blockers after a STEMI. This is mostly based on evidence from a large meta-analysis that included 50000 patients and showed a 23% reduction in mortality at a mean follow up of 1.4 years<sup>[205]</sup>. Lower rates of reperfusion, suboptimal utilization of medical therapy, and short duration of follow up limit this data.

In a recent meta-analysis by Bangalore *et al.*<sup>[206]</sup>, that included 21000 patients with mean follow up of 44 mo, the primary composite outcome of cardiovascular death, nonfatal MI, or nonfatal stroke in post myocardial infarction patients were not significantly different the group that was treated with  $\beta$ -blockers compared with those who were not. (16.93% vs 18.60% respectively,  $P = 0.14$ ). At the present time, the data on duration of therapy with beta blockers after an MI is inconclusive. In patients with preserved left ventricular ejection fraction and without any evidence of arrhythmias and ischemia, beta blockers can most likely be stopped after one year.

## ROLE OF ATRIAL NATRIURETIC PEPTIDES

The cardioprotective effects of atrial natriuretic peptides (ANP) was tested in the Human Atrial Natriuretic Peptide and Nicorandil as Adjuncts to Reperfusion Treatment trial<sup>[207]</sup>. Following reperfusion by either PCI or fibrinolytic therapy, 569 patients with STEMI were randomized to receive a continuous infusion of ANP or placebo for 3 d. Compared to placebo, there was 14.7% reduction in infarct size by area under the curve for total creatine kinase. ANP infusion was also associated improved ejection fraction at 6 to 12 mo compared with controls (44.7% vs 42.5%). Over a median follow-up time of 2.7 years, cardiac death and rates of hospitalization were also reduced in the ANP group. Further large-scale studies are needed to fully understand the cardioprotective role of ANP.

## ROLE OF HYPOTHERMIA

In animal models, moderate hypothermia (28 °C-32 °C) offers cardioprotection by altering signaling pathways<sup>[208]</sup>. The Cooling as an Adjunctive Therapy to Percutaneous Intervention in Patients With Acute Myocardial Infarction trial<sup>[209]</sup> failed to show overall statistically significant reduction in infarct size. However, in patients with large anterior STEMI who were cooled

to temperatures of less than 35 °C prior to reperfusion there was a reduction in the infarct size (9.3% in treated patients vs 18.2% in controls;  $P = 0.05$ ).

The Rapid Cooling by Cold Saline and Endovascular Cooling before Reperfusion in patients with ST-elevation Myocardial Infarction trial<sup>[210]</sup> randomized 20 patients with STEMI undergoing P-PCI to rapid hypothermia by an endovascular catheter or standard therapy. Temperatures < 35 °C were obtained in all hypothermia patients prior to reperfusion. The primary end point of infarct size by cardiac MRI was reduced by 38% in the hypothermia group.

However, in the CHILL-MI<sup>[211]</sup> trial, hypothermia resulted in only a trend towards reduction in infarct size in patients who presented within 4 h of symptoms onset and were cooled to 33 °C large scale randomized trials are needed to determine if hypothermia during or immediately prior to P-PCI will result in significant reductions in infarct size and clinical endpoints.

## STEM CELLS IN THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

Because atherosclerotic coronary vascular disease is the major cause of death in the United States and has recently become a major cause of death throughout the world, stem cell therapy is being investigated in patients with acute myocardial infarction (AMI) to limit myocardial damage and possibly regenerate myocardium<sup>[212-214]</sup>. Two populations of stem cells have been examined in patients with myocardial infarctions and/or ischemic cardiomyopathies: adult bone marrow mononuclear cells and cardiac stem cells. Adult bone marrow mononuclear cells from bone marrow aspirates contain approximately 0.5%-3% hematopoietic and mesenchymal "progenitor" cells that secrete growth factors and cytokines that can limit myocardial inflammation and infarct size. These cells have limited ability to replicate, do not trans-differentiate into myocytes, but can chemoattract endogenous patient stem cells for repair of cardiac injury. Cardiac stem cells are specific undifferentiated progenitor cells found in the right atrial appendage and the ventricular apices of the heart that have paracrine effects, can chemoattract patient stem cells, and may transdifferentiate into myocytes for cardiac repair.

The initial ten year experience with autologous human bone marrow mononuclear cells (BMCs) in the treatment of patients with AMIs showed significant approximately 2%-3% (range 1.9%-5.4%) increases in left ventricular ejection fraction (LVEF), decreases in left ventricular end-systolic volume of -4.8 mL (range -1.4 to -8.2 mL) and reductions in myocardial infarction size of approximately 5.5% (-1.9% to -9.1%)<sup>[213,214]</sup>. These studies established that the direct intramyocardial or intracoronary administration of bone marrow mononuclear cells was safe and that significant side effects did not occur with administration of these



cells<sup>[213-214]</sup>, see Tables 5 and 6<sup>[215-237]</sup>. However many of the initial clinical bone marrow cell studies consisted of small numbers of patients and not all the studies randomized patients to treatment with either bone marrow mononuclear cells or placebo (Tables 5 and 6)<sup>[215-237]</sup>.

Since the initial treatment of patients with AMIs with BMCs, major questions have persisted about the use of these cells: what is the optimal cell for AMI treatment; when is the optimal time to inject cells for treatment; what is the viability of the stem cells prior to injection into patients; what is the best parameter to monitor cardiac patients after stem cell treatment. The LateTIME, the TIME, and the Swiss Myocardial Infarction Trials were multi-center trials that addressed the questions whether adult BMCs limit myocardial damage in comparison with patients treated with placebo and what is the optimal time of cell administration after AMIs.

## LATETIME TRIAL

The LateTIME trial was a randomized double blind, placebo-controlled trial designed to determine if delivery of adult BMCs two to 3 wk after primarily anterior wall myocardial infarction would be safe and effective in limiting infarct size and improving left ventricular (LV) function<sup>[238]</sup>. All patients in this study were successfully treated initially with primary percutaneous coronary intervention (PCI) within a median time of 4 h after the onset of chest pain. Bone marrow mononuclear cells were isolated from bone marrow aspirates in each center with a closed, automated system (Sepax, Biosafe). The Infarct volume and LV global and regional LV function were measured by magnetic resonance imaging (MRI) with gadolinium prior to intracoronary injection and 6 mo after injection. The LVEF prior to intracoronary infusion of cells or placebo in 87 AMI patients averaged 48.7% in the bone marrow cell (BMC) group and 45.3% in the placebo group.  $150 \times 10^6$  autologous BMCs or placebo were infused in 87 patients. The changes between baseline and 6 mo in the BMC group for infarct volume, LVEF, wall motion in the infarct zone, and wall motion in the border zone of the infarction were not statistically different from the placebo group<sup>[238]</sup>.

## TIME TRIAL

The TIME Trial was a double-blind, placebo controlled trial that investigated the intracoronary administration of autologous BMCs or placebo in 120 patients three or seven days after primarily anterior AMI<sup>[239]</sup>. The TIME Trial was based on the REPAIR AMI trial that reported that delivery of BMCs to patients 5 to 7 d after AMI resulted in a 5.1% absolute increase in LVEF<sup>[239,240]</sup>.

All patients had successful coronary reperfusion with coronary angioplasty within a median time of 3-4

h of the onset of ischemic symptoms. The mean LVEF in these patients was  $\leq 45\%$  by echocardiography. The mean time from PCI to bone marrow aspiration and cell processing was 3.3 d in the day 3 group and 7.4 d in the day 7 group. Bone marrow mononuclear cells were isolated in each center with a closed, automated system (Sepax, Biosafe) and the cells or placebo were infused into the coronary arteries within 12 h of aspiration and cell processing. The BMC contained 2.3% CD34 and 1.1% CD34 plus CD131 hematopoietic cells. All patients had baseline cardiac MRIs with gadolinium at day 3 or at day 7 after AMI and the MRIs were repeated at 6 mo after the AMI.

Forty-three patients received BMCs on day 3 and 36 patients received bone marrow cells on day 7 after AMI. Each patient received approximately  $147 \times 10^6$  BMCs within 12 h of aspiration and cell processing. Forty-one patients received a placebo. The median time from bone marrow aspiration to infusion directly into the infarct related coronary artery was 8.3 h. In addition, all patients received heparin during the procedure as well as aspirin and clopidogrel.

The differences between the BMC treatment and the placebo treatment in the 3 d group and in the 7 d group were not significant. When both BMC groups were combined ( $n = 75$ ) to include patients with MRI measurements at baseline and 6 mo and compared with the combined placebo group ( $n = 37$ ), LVEF in the BMC group increased from 45.2% at baseline to 48.3% at 6 mo while in the combined placebo group the LVEF increased from 44.5% to 47.8% ( $P = \text{NS}$ ). Moreover, there was no significant difference between the changes in regional wall motion in the infarct zone and the border zone between BMC and placebo groups. Infarct volumes uniformly decreased in both groups but the differences between groups were not statistically significant. No difference was observed in global or regional function in patients stratified by myocardial ischemic time.

## SWISS MULTICENTER INTRACORONARY STEM CELL STUDY IN ACUTE MYOCARDIAL INFARCTION TRIAL

The Swiss Study<sup>[241]</sup> randomized patients with AMIs with LVEF  $< 45\%$  as measured by ventriculography or echocardiography, who had been successfully treated with PCI of the infarct related artery within a median of 5 h of onset of chest pain, to either the intracoronary administration of 140-160 million autologous BMCs at a median of 6 d after AMI (early group,  $n = 58$ ) or at a median of 24 d after AMI (late group,  $n = 49$ ) or to a placebo group ( $n = 60$ ). Ninety-two percent of the patients had anterior wall infarctions. Bone marrow aspirates were performed only in patients assigned to the BMC treatment. Each 10 mL aspirate was treated with 1000 IU Heparin to prevent clot formation. The

**Table 5 Bone marrow and circulating progenitor cells in coronary artery disease patients**

Ref.	n	Randomize	Time post PCI and/or MI	Cell dose	Injection route	Baseline LVEF	LVEF change	Duration	Other findings
Assmus <i>et al</i> <sup>[215]</sup>	92	Yes	2348-2470 d	22 ± 10 <sup>6</sup> CPC 205 ± 110 × 10 <sup>6</sup> BMC	IC	CPC 39% ± 10% BMC: 41% ± 11%	CPC -0.4% BMC 2.90%	3 mo	Pts with previous MI; ↑ LVEF in BMC but not CPC
Bartunek <i>et al</i> <sup>[216]</sup>	35	Cohort	10 d	12.6 ± 2.2 × 10 <sup>6</sup>	IC	45% ± 2.5%	7%	4 mo	↑ LV regional function, perfusion; restenosis ↑
Chen <i>et al</i> <sup>[217]</sup>	69	Yes	18.4 ± 0.5 d	8-10 × 10 <sup>9</sup>	IC	49% ± 9%	18%	6 mo	↑ LVEF by ventriculogram
Erbs <i>et al</i> <sup>[218]</sup>	26	Yes	225 ± 87 d	69 ± 14 × 10 <sup>6</sup>	IC	51.7% ± 3.7%	7.20%	3 mo	↑ perfusion; ↓ ESV Pts with chronic CAD occlusion Rxed with CPC; ↓ EF by MRI; infarct size 16%
Ge <i>et al</i> <sup>[219]</sup>	20	Yes	1 d	39 ± 22 × 10 <sup>6</sup>	IC	53.8% ± 9.2%	4.80%	6 mo	↑ Perfusion by SPECT
Hendriks <i>et al</i> <sup>[220]</sup>	20	Yes	217 ± 162 d	60 ± 31 × 10 <sup>6</sup> IM	IM	42.9% ± 10.3%	5%	4 mo	CABG in Pts with previous CAD ↑ Regional but not global LV function; 6/9 with induced ventricular tachycardia
Janssens <i>et al</i> <sup>[221]</sup>	67	Yes	1 d	172 × 10 <sup>6</sup>	IC	48.5 ± 7.2	3.30%	4 mo	↓ Infarct size
Kang <i>et al</i> <sup>[222]</sup>	96	Yes	< 14 d AMI; > 14 d OMI	1-2 × 10 <sup>9</sup>	IC	52.0 ± 9.9	5.10% AMI	6 mo	G-CSF for 3 d; ↓ ESV and infarct size in AMI; = EF, ESV and infarct size in OMI
Katritsis <i>et al</i> <sup>[223]</sup>	22	Cohort	224 ± 464 d	2-4 × 10 <sup>6</sup>	IC	39.7% ± 9.3%	1.60%	4 mo	↑ Regional but not global LV function
Lunde <i>et al</i> <sup>[224,225]</sup>	100	Yes	6 ± 1.3 d	68 × 10 <sup>6</sup> (median) 54-130 × 10 <sup>6</sup>	IC	41.3 ± 11.0	=	6-12 mo	↑ LVEF in treated and controls; = EDV and infarct size
Meyer <i>et al</i> <sup>[226]</sup>	60	Yes	4.8 ± 1.3	24.6 ± 9.4 × 10 <sup>8</sup>	IC	50 ± 10	5.90%	18 ± 6 mo	↑ LVEF by MRI significant at 6 but not 18 mo
Mocini <i>et al</i> <sup>[227]</sup>	36	Cohort	AMI < 6 mo	292 ± 232 × 10 <sup>6</sup> IM	IM	46% ± 6%	5%	3-12 mo	CABG in all; troponin increased
Perin <i>et al</i> <sup>[228]</sup>	20	Cohort	ICM	25.5 ± 6.3 × 10 <sup>6</sup>	IM Trans-Endo-cardial	30% ± 6%	5.10%	12 mo	LVEF = Controls; ↑ LV perfusion
Ruan <i>et al</i> <sup>[229]</sup>	20	Yes	Approximately 1 d	NR	IC	53.5% ± 5.8%	5.80%	6 mo	↑ Exercise ↑ LV segmental contraction
Schächinger <i>et al</i> <sup>[230,231]</sup>	204	Yes	3-8 d	2.4 × 10 <sup>8</sup>	IC	48.3% ± 9.2%	6%-7%	4-12 mo	↑ EF when Rx > 4 d post MI and when EF ↑ ≤ 48.9; LV perfusion
Strauer <i>et al</i> <sup>[232]</sup>	20	Cohort	5-9 d	2.8 ± 2.2 × 10 <sup>7</sup> IM	IC	57% ± 8%	5%	3 mo	↑ Regional but not global LVEF; ↓ ESV and ↓ Infarct size
Li <i>et al</i> <sup>[234]</sup>	70	Yes	7 ± 5 d	7.3 ± 7.3 × 10 <sup>7</sup>	IC	50% ± 8.2%	7%	6 mo	G-CSF for 5 d; ↓ LV ESV, ↓ LV wall motion score

NR: Not recorded or equals no change; CPC: Circulating progenitor cells; BMC: Bone marrow cells; ICM: Ischemic cardiomyopathy; IC: Intracoronary injection; IM: Intramyocardial injection; AMI: Acute myocardial infarction; OMI: Old myocardial infarction; G-CSF: Granulocyte colony stimulating factor; ESV: LV end-systolic volume; SPECT: Single photon emission computer tomography. Adapted from Henning<sup>[213]</sup>.

BMCs were isolated by density gradient centrifugation at a centralized processing facility and contained 1% to 1.3% CD34<sup>+</sup> hematopoietic cells. However, the median percentage of mononuclear cells that exhibit migration capacity was only 29%<sup>[241]</sup>.

Cardiac magnetic resonance imaging with gadolinium was performed on patients at baseline prior to cell infusion and at 4 mo after the injection of BMCs into the infarct-related coronary artery and were

compared with MRIs of control patients treated with best medical care. At 4 mo after coronary infusion, there were no significant differences in infarct scar size or LV myocardial wall thickening in patients treated with BMCs at either 5-7 d or 3-4 wk after AMI in comparison with control patients. Moreover, LV function did not significantly improve at 4 mo after the intracoronary infusion of autologous BMCs in either the early or late treated groups in comparison with the placebo group.

**Table 6 Stem cells in the treatment of patients with acute myocardial infarction**

Ref.	n	Random-ized	Time post MI	Cell dose	Baseline	LVEF	Duration	Other findings
Strauer <i>et al</i> <sup>[232]</sup>	20	Cohort	8 d	$2.8 \pm 2.2 \times 10^7$	$57\% \pm 8\%$	5%	3 mo	↑ Regional but not global LVEF ↓ LV ESV and infarct size
Bartunek <i>et al</i> <sup>[216]</sup>	35	Cohort	10 d	$12.6 \pm 2.2 \times 10^6$	$45\% \pm 2.5\%$	7%	4 mo	↑ LV regional function, ↑ perfusion; ↑↑ restenosis
Li <i>et al</i> <sup>[234]</sup>	70	Yes	6 d	$7.3 \pm 7.3 \times 10^7$	$50 \pm 8.2$	7%	6 mo	↓ LV ESV, LV wall motion score
Janssens <i>et al</i> <sup>[221]</sup>	67	Yes	1 d	$172 \times 10^6$	$48.5 \pm 7.2$	3.30%	4 mo	↓ Infarct size
Meyer <i>et al</i> <sup>[226]</sup> and Wollert <i>et al</i> <sup>[237]</sup>	60	Yes	4.8 d	$24.6 \times 10^8$	$50.0 \pm 10.0$	=	6-18 mo	↑ LVEF at 6 but not at 18 mo
Kang <i>et al</i> <sup>[222]</sup>	96	Yes	4 d	$1-2 \times 10^9$	$52.0 \pm 9.9$	5.1% AMI	6 mo	↓ LV ESV and infarction in acute MI; = ESV and = old MI
Lunde <i>et al</i> <sup>[224,225]</sup>	100	Yes	6 d	$68 \times 10^6$	$41.3 \pm 11.0$	=	6-12 mo	LVEF ↑ in treated and controls; = EDV and infarct size
Ge <i>et al</i> <sup>[219]</sup>	20	Yes	1 d	$4 \times 10^7$	$53.8 \pm 9.2$	4.80%	6 mo	↑ LV regional wall perfusion by SPECT
Meluzin <i>et al</i> <sup>[235,236]</sup>	66	Yes	5-9 d	$10^7-10^8$	$42 \pm 0.0$	3-5	3-12 mo	↑ LVEF 3% @10 <sup>7</sup> ↑ LVEF 5%-7% @ 10 <sup>8</sup> 3-12 mo
Schächinger <i>et al</i> <sup>[230,231]</sup>	204	Yes	3-8 d	$2.4 \times 10^8$	$48.3 \pm 9.2$	6-7	4-12 mo	↑ EF when Rx > 4 d post MI and when EF < 48.9%; ↑ LV perfusion

Adapted from Henning<sup>[213]</sup>. MI: Myocardial infarctions; LVEF: Left ventricular ejection fraction.

However, patients with NT-proBNP levels at baseline above 1437 ng/L experienced a greater increase in LVEF of 7.1% in the early group and 9% for the late BMC group. In all cell and placebo treatment groups, LV scar as determined by late gadolinium enhancement on MRI decreased by more than 10 g with a 4%-5% decrease in the ratio of myocardial scar to myocardial mass.

## ASSESSMENT OF LATETIME, TIME, AND SWISS TRIALS

Several variables in these studies contributed to the lack of significant improvement of AMI patients treated with BMCs in comparison with placebo treated patients.

### Early percutaneous coronary intervention

Patients with AMIs in the LateTIME, TIME, and Swiss Multicenter Trials were treated with PCI within a median of 4 to 5 h of the onset of chest pain. Thereafter, the patients were treated with American and European Heart Association guided best medical therapy. Consequently, AMI sizes and the extent of LV remodeling in the different trial patients were significantly limited and the differences between BMC treated patients and placebo treated patients were small. Although the initial qualifying LV ejection fractions by echocardiography after PCI in the LateTIME and TIME trial patients were  $\leq 45\%$ , the LVEFs by MRI at the time of BMC injection were greater than 45%. Bone marrow mononuclear cells are much less effective in patients with small myocardial infarctions with near normal LVEFs. In addition, placebo treated patients continue to improve with best medical therapy after AMIs as demonstrated by the control patients in the Bone Marrow Transfer to Enhance ST-elevation

Infarct Regeneration (BOOST) trial in which the LVEFs equaled or exceeded the increases in the LVEFs in the BMC treated patients at 18 mo after AMI<sup>[242]</sup>. In addition, the Valsartan in Acute Myocardial infarction Trial and trials of neuro-hormonal blockade of patients with AMIs have demonstrated that optimal medical therapy can increase LVEF by a mean of 2.7% at 20 mo<sup>[243]</sup>. Consequently much larger numbers of patients will be required in clinical trials to demonstrate statistically significant differences between BMC treated patients and placebo treated patients who receive PCI early after the onset of AMI and guideline directed optimal medical therapy.

### Heterogeneous bone marrow cell populations

Unfractionated adult BMCs contain less than 3% CD34<sup>+</sup> and 1% CD34<sup>+</sup>/CD133<sup>+</sup> hematopoietic progenitor cells and  $\leq 1\%$  CD105<sup>+</sup> mesenchymal stem cells in healthy subjects when marrow cells are separated by Ficoll density gradient-based separation. However, both CD34<sup>+</sup> endothelial colony number and the mesenchymal cell colony number were significantly decreased amongst subjects that participated in the LateTime and Time Trials<sup>[244]</sup>. In addition, the marrow aspirates in the LateTIME and TIME Trials were separated by an automated cell process system (Sepax, Biosafe), which recovered only 23.6% of the total nucleated cells<sup>[245]</sup>. Consequently, the BMCs delivered in the LateTIME and TIME trials contained smaller numbers of CD34<sup>+</sup> and CD105<sup>+</sup> cells than earlier BMC studies. Moreover, stem cell motility can decline by as much as 68% 72 h after harvest from the bone marrow<sup>[246]</sup>. In addition,  $140-150 \times 10^6$  unfractionated BMCs may not be the most optimal dose of BMCs for stem cell treatment of patients with AMI. In this regard, BMCs from patients with advanced age and patients with chronic diseases, such as ischemic heart disease or diabetes mellitus, are often functionally impaired, propagate poorly,

and have a shortened life span<sup>[246-248]</sup>. Consequently, BMC colony forming units and cell migration capability must be determined in addition to bone marrow cell number and viability prior to use in the treatment of AMI. Furthermore, BMCs produced only a modest increase in the LVEF of approximately 2%-3% in earlier analyses of stem cell trials of patients with AMIs or ischemic cardiomyopathies<sup>[213,214]</sup>. Despite well conducted clinical trials, unfractionated BMCs selectively infused into an infarct related coronary artery have a small therapeutic effect and may not be the most optimal cells for the treatment of patients with AMIs.

### **Red blood cell contamination of stem cells**

Red blood cell contamination of bone marrow mononuclear cells can significantly decrease the migration ability and the efficacy of BMCs. Large numbers of red blood cells in the cell preparations cause reduced BMC viability, decreased colony forming capacity, and are associated with reduced recovery of LVEF in patients with AMIs<sup>[249]</sup>. In patients in the REPAIR-AMI Trial, contamination of the bone marrow cells with red blood cells prior to infusion into patients with AMI independently predicted reduced recovery of LVEF<sup>[249]</sup>. Moreover, the addition of red blood cells to BMCs dose-dependently decreased neovascularization in ischemic hind-limbs compared to treatment with BMCs without red blood cells<sup>[249]</sup>. The mechanism by which red blood cells interfere with bone marrow cell propagation, migration and neovascularization involves a dose-dependent reduction of BMC mitochondrial membrane potential and a decrease in BMC mitochondrial adenosine triphosphate (ATP) production<sup>[249]</sup>. As a consequence of decreased mitochondrial metabolism and function, stem cell self-renewal and differentiation are decreased.

### **Heparin decreases stem cell migration**

Heparin can bind to the chemoattractant stromal derived factor-1 (SDF-1), which is released from ischemic myocardium, and also bind to its receptor CXCR4 on stem cells and thereby block CXCR4 signaling and stem cell migration to injured myocardium<sup>[250]</sup>. Heparin, in a dose-dependent manner, can inhibit SDF-1 induced BMC migration and homing of BMCs to areas of myocardial ischemia<sup>[250-253]</sup>. Incubation of BMCs with 20 U/mL of heparin for 30 min abrogates SDF-1 BMC migration by 84% *in-vitro* and significantly reduces the homing of injected BMCs to injured and infarcted myocardium by 50% in research animals<sup>[250]</sup>. Decreased migratory capacity of BMCs also correlates with reduced neovascularization and decreased functional capacity in subjects with limb ischemia<sup>[251]</sup>. In addition, heparin decreases the concentration of vascular endothelial growth factor (VEGF) in ischemic tissue and thereby decreases neovascularization<sup>[252]</sup>. Heparin also interferes with activation of the cell survival factor Akt (Protein Kinase B) by SDF-1-CXCR4 signaling and in this manner interferes with cell survival and growth. In contrast, the thrombin inhibitor bivalirudin does not interfere with

BMC homing or SDF-1/CXCR4 signaling and does not decrease VEGF<sup>[252]</sup>.

### **Stem cell expulsion from myocardium**

Ninety to 97% of unfractionated BMCs leave the myocardium in less than 2 h after injection directly into the myocardium or into the coronary arteries<sup>[254,255]</sup>. Most of the cells are ejected out of the myocardium through the myocardial injection sites or through the coronary veins and lymphatics into the right heart due to the massaging action of the contracting myocardium. The cells are ultimately lodged in the lungs, liver, spleen and kidneys. In addition, approximately 12% of BMCs are retained in the catheter delivery system after injection<sup>[255]</sup>. With the intravenous injection of BMCs or other cells for cardiac repair the majority of the cells become entrapped in the lungs. Consequently, fourfold greater numbers of cells are required above that required for intramyocardial or intracoronary injection for repair of myocardial infarctions<sup>[256]</sup>.

### **Future bone marrow cell studies**

The BAMI Trial (The effect of intracoronary reinfusion of bone marrow derived mononuclear cells on all-cause mortality in acute myocardial infarction) is recruiting 3000 patients with LVEFs  $\leq$  45% within 7 d of AMIs, who have undergone successful coronary reperfusion therapy, for randomization into treatment with either intracoronary autologous unfractionated bone marrow mononuclear cells or placebo<sup>[257]</sup>. Hopefully the BAMI Trial will avoid the important variables that have been described in this paper and will provide definitive answers to the questions whether BMCs can significantly decrease patient mortality due to myocardial infarction, substantially reduce infarct size and increase LVEF over three years in comparison with patients treated with best medical therapy.

## **CARDIAC STEM CELLS**

Cardiovascular investigators have sought alternatives to BMCs for cardiac repair in patients with ischemic heart disease. Cardiac stem cells, which are multipotent progenitor cells, are present in niches in the heart and contribute to the physiological turnover of myocytes and vascular endothelial cells in the heart. The number of cardiac stem cells in the heart is estimated at one cardiac stem cell per 10000 cardiac myocytes<sup>[258]</sup>. Consequently, endogenous cardiac stem cells are not normally able to reverse heart damage due to myocardial infarctions. The turnover of cardiac myocytes occurs at rates estimated to be 1% to as much as 22% per year and is dependent on the age, sex, and the health of the individual<sup>[259,260]</sup>. Two major types of autologous cardiac stem cells have been investigated in patients with injured and infarcted myocardium in the SCIPIO and CADUCEUS clinical trials: C-kit + lineage negative cardiac stem cells



isolated from right atrial appendages and cardiosphere derived cells (CDCs) grown from right ventricular cardiac muscle biopsies.

## C-KIT + STEM CELLS

C-kit is a receptor for stem cell factor, which is released from the ischemic myocardium, and is important in the chemoattraction of stem cells to apoptotic, injured and necrotic myocardium. C-kit + stem cells have the capacity for self-renewal, clonogenicity and multi-potency<sup>[261,262]</sup>. These stem cells can express the cardiac transcription factors GATA-4, Nkx2.5 and MEF2 and can differentiate into myogenic, vascular endothelial and smooth muscles cells *in-vitro*<sup>[261,262]</sup>. In research animals with AMIs, cardiac stem cells can form new myocardium<sup>[262]</sup>.

Autologous C-kit cardiac stem cells from right atrial appendages have recently been used for the treatment of patients with myocardial infarctions and ischemic cardiomyopathies in the open labeled Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCIPIO) Trial<sup>[263-265]</sup>. In this trial C-kit positive stem cells were isolated during coronary artery bypass surgery from the right atrial appendages of patients with LVEFs < 40%. The cells were then propagated in the laboratory. Four mo later, a maximum of one million cardiac stem cells were injected directly into the patient's saphenous vein grafts and coronary arteries supplying infarcted myocardium. The two year results of this trial have been presented at the American Heart Association Scientific Sessions in November of 2012 and 2013. In the SCIPIO trial the LVEF, measured by three-dimensional echocardiography and by MRI with gadolinium in patients who received cardiac stem cells, increased in 12 patients in absolute units by 11.9% at 2 years<sup>[265]</sup>. Left ventricular scar, determined by MRI, decreased by as much as 20.4 g at 2 years ( $n = 6$ ) and was associated with an increase in viable myocardium of 17.9 g ( $n = 6$ ) at 2 years<sup>[265]</sup>. New York Heart Association Functional Class score decreased in these patients by 0.77 at 2 years ( $n = 13$ ). A left internal mammary graft dissection occurred in one treated patient, which was treated with a graft stent, and a peri-procedural myocardial infarction occurred in a second treated patient<sup>[264]</sup>. In this study, C-kit cardiac stem cells were proposed to chemoattract patients' native stem cells to areas of myocardial injury and also to transdifferentiate to myocytes for cardiac repair. A Phase 2 trial of safety and efficacy of C-kit cardiac stem cells in a larger group of patients is currently being planned.

## CARDIOSPHERE DERIVED CELLS

Percutaneous endomyocardial biopsy specimens of the right ventricular septal wall and apex in patients, when grown in culture, can yield spherical multicellular

clusters termed "cardiospheres". Cardiospheres are a mixture of stromal, mesenchymal and hematopoietic progenitor cells that contain cells that express CD 105 (commonly associated with mesenchymal stem cells) and partially express C-kit<sup>[266,267]</sup>. Cardiosphere derived cells (CDCs), when injected into the border of myocardial infarctions in mice, engraft and increase viable myocardium<sup>[268]</sup>. The functional benefit of CDCs is predominantly due to the secretion of growth factors and the recruitment of endogenous stem cells to injured and infarcted myocardium for myocyte generation. In this regard, cardiospheres and CDCs secrete the growth factors angiopoietin-2, basic fibroblastic growth factor, hepatocyte growth factor, insulin-like growth factor 1, stromal derived factor-1 and vascular endothelial growth factor which are beneficial in repair of injured myocardium<sup>[267,268]</sup>.

Autologous CDCs have been investigated in the open labeled Cardiosphere-derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) Trial<sup>[269,270]</sup>. In this trial, 17 patients, post myocardial infarction with LVEFs of 25%-45%, underwent endomyocardial biopsies of the right ventricular septum. Cardiosphere derived cells were obtained from cultures of the endomyocardial biopsies and the cells were propagated. In this trial, between 12.5 and 25 million CDCs were then given directly into the infarct related coronary artery of each of the 17 patients 1.5 to 3 mo after their myocardial infarctions. The one year followup of 12 of the 17 patients treated with autologous CDCs and 8 control patients have been reported<sup>[270]</sup>. Left ventricular scar by MRI significantly decreased by a mean of 11.9 g in CDC-treated patients and by 1.7 g in control patients. Left ventricular viable mass increased by a mean of 22.6 g in treated patients in comparison with 1.8 g in control patients. Left ventricular ejection fractions did not significantly increase but the regional wall function of infarcted segments did increase and correlated with the decrease in LV myocardial scar size<sup>[269,270]</sup>. Covariate statistical analysis demonstrated that the lower percentage of infused CD90<sup>+</sup> cells caused the greatest reductions in scar size in patients with large infarctions. A Phase 2 study of CDCs (ALLSTAR Trial) is currently in progress that involves allogeneic CDCs for the treatment of patients after myocardial infarction.

## ASSESSMENT OF THE SCIPIO AND CADUCEUS TRIALS

In the SCIPIO trial 1545 patients were evaluated. Two hundred thirteen patients had LVEFs < 40% and 20 patients were treated with C-kit + stem cells. Twelve of 20 patients had MRI determinations of left ventricular function whereas the control patients did not have MRI determinations of left ventricular function. In the CADUCEUS Trial 436 patients were evaluated and 17 patients received CDCs. Consequently, these

trials report a highly selected patient population and the results of these trials cannot be applied to all patients with myocardial infarctions and ischemic cardiomyopathies. Much larger trials are necessary of each of these cell types in patients with myocardial infarctions.

In each of these studies, LV infarction was defined by MRI of delayed enhancement of myocardium in the region of coronary artery occlusion/reperfusion due to gadolinium that leaked from myocardial capillaries and pooled in the myocardial interstitial spaces and intracellular spaces. In these patients the gadolinium volume of distribution was increased and washout from the myocardium was reduced. However, cardiac stem cells can incorporate into damaged blood vessels, chemoattract endogenous stem cells that can form entirely new blood vessels, and can also secrete angiogenic growth factors that stimulate new blood vessels from preexisting vessels. Consequently, the blood vessels in the damaged myocardium of patients treated with these stem cells were less permeable to gadolinium<sup>[271]</sup>. Infarct scars can potentially appear smaller on MRI due to less gadolinium leak as well as contracture of the myocardial infarction. Moreover inter-scan variability and intra- and inter-observer variability in infarct measurements and interpreting MRI scans can account for some myocardial changes between pre- and post-stem cell infusion<sup>[272]</sup>. Rebuttals to these arguments against the use of contrast enhanced MRI in estimating infarct size and myocardial regeneration after stem cell treatment have been published<sup>[273]</sup>. The rebuttal is based on a porcine myocardial infarction study in which allogeneic CDCs decreased infarct scar size and led to cardiomyocyte hyperplasia on MRI and also on histological examination<sup>[273]</sup>. Nevertheless, anatomical and histological examinations of myocardial biopsies of infarcted hearts of patients or myocardial autopsy examinations of patients treated with these stem cells are necessary to determine if infarct fibrosis is significantly decreased and if substantial generation of new myocytes occurs. Trials of larger numbers of patients treated with C-kit + cardiac stem cells and cardiosphere derived cells for longer times are warranted to determine the precise mechanisms of action of these stem cells and their clinical benefit.

## FUTURE DIRECTIONS

The LateTIME, TIME, Swiss, SCIPO and CAUDUCEUS Trials demonstrate that stem cells can be safely administered to patients with acute myocardial infarctions and ischemic cardiomyopathies and do not have significant adverse effects. Specific bone marrow cell subsets, such as unconditioned or conditioned mesenchymal cells or CD34<sup>+</sup> hematopoietic cells, may prove to be more efficacious in myocardial infarction repair than unfractionated BMCs<sup>[274,275]</sup>. In this regard, bone marrow mesenchymal stem cells or mesenchymal stem cells conditioned with

cardiogenic growth factors have been reported to be beneficial in increasing LV function and functional capacity in patients with ischemic cardiomyopathies<sup>[275]</sup>. In addition, mesenchymal stem cells may enhance the beneficial effects of C-kit cardiac stem cells when these cells are administered together<sup>[276]</sup>. The large size of mesenchymal stem cells, however, requires that these cells be most safely delivered into the heart by direct myocardial injection rather than intracoronary injection in order to avoid problems of cell clumping and coronary occlusion. Mesenchymal stem cells and also umbilical cord stem cells are reported to be “immunoprivileged” and lack Class II human leukocyte antigens<sup>[277,278]</sup>. If allogeneic stem cells prove to be safe and effective in limiting myocardial damage and LV remodeling after myocardial infarction in patients, then these cells might become an “off the shelf” product that surpasses the significant limitations of inter-patient variability of unfractionated bone marrow mononuclear cells. Since the functional benefit of stem cells appears to be predominantly due to the secretion of biologically active factors, the ultimate rejection of allogeneic stem cells may not be of major concern if the rejection is delayed long enough to allow these cells to exert their paracrine effects. Nevertheless, stem cell trials must be performed in patients with large myocardial infarctions and LVEFs by MRI less than 40% at the time of stem cell administration because stem cells may not be efficacious in patients with small infarctions and near normal or normal LVEFs. In these studies, substantial stem cell viability, colony forming and migration capabilities must be established prior to infusion in patients.

A major problem with all stem cell trials is the short term engraftment and survival of stem cells in injured and infarcted myocardium. The cells that remain in the myocardium do not survive due to ischemia, inflammation, or anoikis or migrate from the myocardium in one to two weeks<sup>[254,255]</sup>. Consequently, stem engraftment in the heart must be increased in order to significantly enhance their beneficial effects. Possible treatment options include “conditioning” of the myocardium prior to stem cell delivery or co-delivery of stem cells directly into the myocardium with extracellular matrix molecules, nanofibers, hydrogels, or fibrin glues<sup>[213,214,279]</sup>. Co-delivery of stem cells with other molecules will require direct intramyocardial cell injection at the time of cardiac surgery or cardiac catheterization which appears to produce the greatest functional benefit<sup>[280]</sup>. Alternatively, stem cells can be administered in patches that are applied directly to the epicardial surface of the damaged myocardium at the time of cardiac surgery<sup>[213,214]</sup>. Direct stem cell to myocyte contact and interactions may be crucial in eliciting beneficial myocyte functional effects. In addition, genetic engineering of stem cells must be developed that facilitate the homing of stem cells to ischemic myocardium and the retention of the stem cells within the myocardium after intracoronary or intravenous injection.

A major mechanism of action of stem cells studied

to date in myocardial repair is the secretion of growth factors, chemokines, anti-inflammatory cytokines and exosomes or microparticles, which contain proteins, messenger ribonucleic acids and micro-ribonucleic acids. Hypoxic stress appears to increase the paracrine effects of stem cells<sup>[281,282]</sup>. Biologically active factors from stem cells can suppress inflammatory cytokines and inflammatory cells in the injured myocardium, improve myocardial metabolism, promote angiogenesis, inhibit myocyte and endothelial cell apoptosis, recruit endogenous progenitor cells to injured myocardium, and possibly stimulate surviving myocytes to re-enter the cell cycle and proliferate. The most efficacious stem cell biologically active factors must be identified, purified, and the pharmacologic effects established in research animals and ultimately in patients with injured myocardium.

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## Mitochondrial function and regulation of macrophage sterol metabolism and inflammatory responses

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to the inner mitochondrial membrane, *via* a complex of cholesterol trafficking proteins. Oxysterols are key signalling molecules, regulating the transcriptional activity of LXRs which coordinate macrophage sterol metabolism and cytokine production, key features influencing the impact of these cells within atherosclerotic lesions. The precise identity of the complex of proteins mediating mitochondrial cholesterol trafficking in macrophages remains a matter of debate, but may include steroidogenic acute regulatory protein and translocator protein. There is clear evidence that targeting either of these proteins enhances removal of cholesterol *via* LXR $\alpha$ -dependent induction of ATP binding cassette transporters (ABCA1, ABCG1) and limits the production of inflammatory cytokines; interventions which influence mitochondrial structure and bioenergetics also impact on removal of cholesterol from macrophages. Thus, molecules which can sustain or improve mitochondrial structure, the function of the electron transport chain, or increase the activity of components of the protein complex involved in cholesterol transfer, may therefore have utility in limiting or regressing atheroma development, reducing the incidence of coronary heart disease and myocardial infarction.

**Key words:** Atherosclerosis; Macrophage; Cholesterol; High density lipoproteins; Apolipoproteins; ATP binding cassette transporters; Scavenger receptor B1; Mitochondria (dys)function; Sterol 27-hydroxylase; Liver X receptors

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**Core tip:** Mitochondrial cholesterol trafficking to CYP27A1 located on the inner mitochondrial membrane regulates the formation of oxysterol ligands for liver X receptors (LXRs) in sterol-laden macrophage "foam" cells. In turn, ligation of LXR $\alpha$  has profound implications for sterol removal and inflammatory responses in macrophage "foam" cells, both factors which may contribute to the effective resolution of atherosclerotic lesions and reductions in the incidence of coronary heart disease and its sequelae.

### Abstract

The aim of this review is to explore the role of mitochondria in regulating macrophage sterol homeostasis and inflammatory responses within the aetiology of atherosclerosis. Macrophage generation of oxysterol activators of liver X receptors (LXRs), *via* sterol 27-hydroxylase, is regulated by the rate of flux of cholesterol

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## INTRODUCTION

Coronary heart disease (CHD) is the major cause of morbidity and mortality worldwide, and the single largest cause of disease burden, determined according to disability-adjusted life years, the sum of life lost and years lived with disability<sup>[1,2]</sup>. Genetic factors contribute to coronary heart disease, fuelled by behavioural (smoking, physical inactivity, unhealthy diet, excess alcohol intake), metabolic (hypertension, diabetes, elevated serum cholesterol, overweight and obesity) and environmental (poverty, stress, educational status) factors<sup>[1-3]</sup>.

Atherosclerosis is the primary cause of coronary heart disease characterised by chronic and unresolved inflammatory responses at sites of perturbed laminar blood flow in large and medium-sized arteries<sup>[4-6]</sup>. Activation of the arterial endothelial layer allows the accumulation of low density lipoprotein (LDL) within the intima of the vessel, where it can become modified *via* oxidation or crosslinking, triggering the recruitment of monocytes, neutrophils, lymphocytes and circulating stem cells to sites of inflammation<sup>[4-6]</sup>. Within this complex microenvironment, monocytes differentiate into macrophages which lie within a broad phenotypic spectrum, ranging from pro- (M1) to anti-inflammatory (M2)<sup>[6]</sup>.

Arterial macrophages become laden with excess cholesterol and cholesteryl esters, part *via* the unregulated uptake of modified LDL by scavenger receptors (*e.g.*, CD36, CD68, LOX-1 and SR-AI/AII), and by phagocytosis of apoptotic cells, resulting in formation of "foam cells", a hallmark of early "fatty streak", developing, and unstable atherosclerotic lesions<sup>[7-10]</sup>. During the early phase of lesion development, this process may represent a protective mechanism; however, in more advanced lesions, cholesterol-laden macrophages, by releasing inflammatory cytokines and matrix metalloproteinases, contribute to chronic unresolved inflammation<sup>[10]</sup>, accelerating the disease process and acute thrombotic events such as cerebrovascular stroke or myocardial infarction.

Thus, removal of cholesterol from macrophage "foam cells" may achieve successful regression and stabilisation of atheroma, and the importance of this pathway in protecting against CHD is supported by epidemiological studies in humans, and in genetically modified mice in which components of this pathway have been overexpressed or deleted. For example, HDL-cholesterol (HDL-C) emerged as an independent risk factor for cardiovascular disease in the Framingham

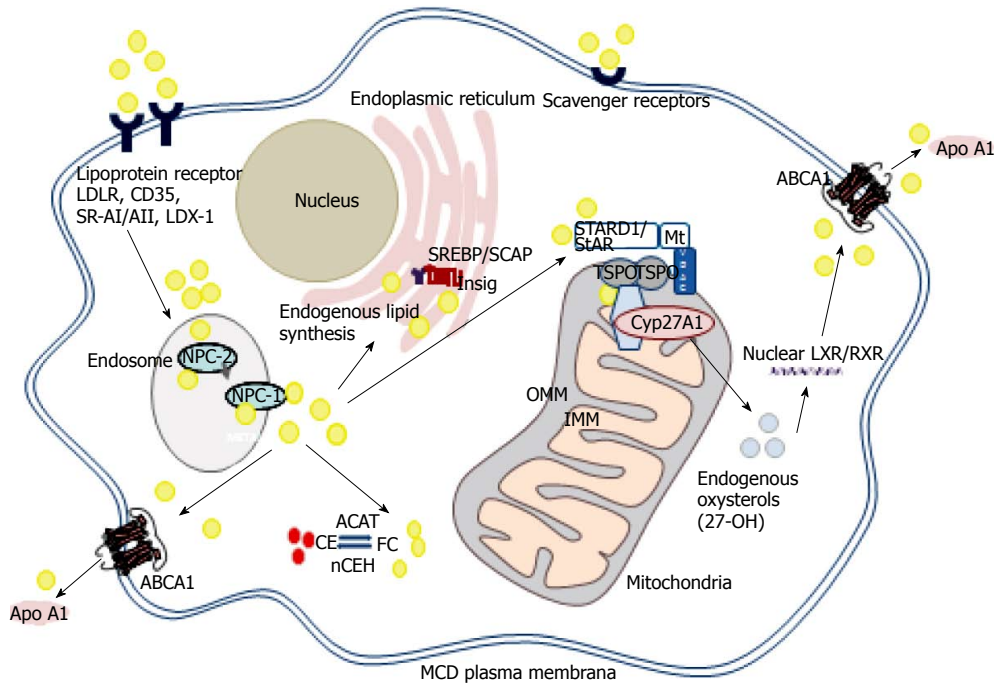
Heart Study, offering a risk reduction of 2%-3% for each 1 mg/dL increase in HDL-C concentration<sup>[11,12]</sup>. HDL particles also possess antioxidant, anti-thrombotic and pro-fibrinolytic properties, and can counteract the chronic inflammation<sup>[13-16]</sup>, proliferation of haematopoietic stem cells<sup>[17]</sup> and leucocytosis<sup>[10,18]</sup> which promote atherosclerosis. However, increasing the level of HDL-C, with niacin<sup>[19,20]</sup>, fibrates<sup>[20]</sup> or dalcetrapib (dal-OUTCOMES III trial)<sup>[20,21]</sup>, does not necessarily confer protection against CHD<sup>[19-21]</sup> and in patients with systemic inflammation, coronary heart disease, chronic renal disease or diabetes, the protective properties of HDL are lost, and the particles transformed into those with pro-atherogenic potential<sup>[22-24]</sup>. Thus, it is not just the level, but the quality, composition (including levels of cargo molecules such as sphingosine-1-phosphate)<sup>[25]</sup> and function of HDL particles that are important.

Some, but not all, of the beneficial effects associated with HDL are mediated *via* the interaction of ATP binding cassette (ABC) transporters, such as ABCA1, ABCG1 and ABCG4, with apolipoproteins and HDL (Figure 1). While ABCA1 promotes efflux of cholesterol and phospholipids to lipid-poor apolipoproteins, such as apoA-I and apoE<sup>[13]</sup>, ABCG1 and ABCG4 promote efflux of cholesterol, oxysterols and desmosterol to HDL<sup>[26]</sup>. Thus, these transporters together in a sequential manner to generate nascent HDL, which can then mature to HDL<sub>3</sub> and HDL<sub>2</sub> within the reverse cholesterol transport pathway in the bloodstream<sup>[25]</sup>.

Both rare and common genetic variations in ABCA1 influence the levels of HDL-C<sup>[26]</sup> and risk of ischaemic heart disease (IHD). However, the association between ABCA1 variants and coronary disease seem to be independent of the plasma level of HDL-C<sup>[27]</sup>. Instead, cholesterol efflux from macrophages is strongly linked to atherosclerosis and provides a novel way of assessing cardiovascular risk that provides a greater level of prediction than HDL-C<sup>[28]</sup>. Thus the expression and activity of the ABCA1 protein, and the quality and functionality of the nascent HDL generated, may prove valuable discriminants of the risk of cardiovascular disease<sup>[29]</sup>.

Importantly, macrophage ABCA1 expression and cholesterol accumulation are intrinsically linked to the inflammatory status of these cells. Excess cholesterol proves cytotoxic and pro-inflammatory if recycling *via* ABCA1 is disrupted in macrophages<sup>[30-33]</sup>. Enhanced Toll-like receptor signalling is noted in ABCA1/ABCG1 null macrophages, resulting in increased expression of pro-inflammatory genes, and free cholesterol accumulation<sup>[34]</sup>, while activation of Toll-like receptors 3 and 4 represses induction of ABCA1 and reduces macrophage cholesterol efflux<sup>[35]</sup>. Conversely, interleukin-6 (IL-6) attenuates pro-inflammatory responses and stimulates efflux of cholesterol *via* ABCA1 in human macrophages<sup>[36]</sup>. In good agreement with this integrated paradigm, macrophage ABCA1 limits inflammatory responses *via* ApoA-I dependent activation of the Jak2/Stat3 pathway<sup>[37,38]</sup>, while macrophage sterol





**Figure 1 The role of mitochondrial cholesterol trafficking in regulation of macrophage sterol metabolism.** Increased expression of steroidogenic acute regulatory protein (StAR, STARD1) or 18 kDa translocator protein (TSPO) drive cholesterol trafficking to mitochondrial sterol 27-hydroxylase (CYP27A1), enhancing endogenous production of oxysterols (24-, 25- and 27-hydroxycholesterol), in turn activating liver X receptors (LXR) and enhancing cholesterol efflux to apolipoprotein A-I (Apo A1) via ATP binding cassette transporter A1 (ABCA1). One current model for cholesterol transfer from the outer (OMM) to inner (IMM) mitochondrial membrane, derived from studies in steroidogenic cells, involves a complex of proteins, including StAR, TSPO, voltage-dependent anion channel (VDAC), regulatory subunits of protein kinase A (PKA-R1 $\alpha$ ), acyl CoA binding domains-1 and -3, ATPase family AAA domain-containing protein 3A (ASTAD3A) and optic atrophy type 1 proteins. Exogenous cholesterol delivered to the endocytic pathway via lipoprotein or scavenger receptors is transported either to the plasma membrane, enhancing cholesterol efflux via ABCA1, to lipid poor acceptors such as Apo A1 or Apo E, or delivered to the endoplasmic reticulum (ER), retaining the Sterol Regulatory Element Binding Protein (SREBP)/SREBP-cleavage activating protein (SCAP) complex, in turn reducing cholesterol biosynthesis. Oxysterols enhance this process by binding to Insig-1/2 (insulin-induced gene-1 or -2). Excess cholesterol is esterified via Acyl CoA: Cholesterol Acyltransferase-1 (ACAT-1), and stored in lipid droplets within the cytoplasm as "foamy" droplets. nCEH: Neutral cholesteryl ester hydrolase; FC: Free cholesterol; CE: Cholesteryl ester; NPC-1/NPC-2: Niemann-Pick C1/C2 protein; StAR: Steroidogenic acute regulatory protein; RXR: Retinoic acid receptor.

accumulation activates Liver X Receptor nuclear (LXR) transcription factors, achieving induction of ABCA1 and ABCG1 and repression of inflammation (below)<sup>[39,40]</sup>.

## MACROPHAGE LIPID METABOLISM AND INFLAMMATION ARE REGULATED BY LIVER X RECEPTORS

Activation of nuclear LXRs (LXR $\alpha/\beta$ ) is marshals cellular responses to increasing levels of sterol, promoting cholesterol efflux (above)<sup>[39-43]</sup>. Liver X receptors form heterodimeric complexes with retinoic acid receptors (RXRs), and bind to imperfect direct repeats of the nuclear receptor half-site TGACCT<sup>[39-43]</sup>. Ligand binding dissociates co-repressor proteins, destined for ubiquitination and proteasomal degradation, and engages co-activator proteins such as histone demethylases and G-protein pathway suppressor-2 (GPS2), stimulating target gene transcription<sup>[44]</sup>.

Activation of LXR $\alpha$  also represses cholesterol biosynthesis via novel negative LXR DNA-response elements within the promoter region of squalene synthase and lanosterol 14 $\alpha$ -demethylase and suppresses uptake

of LDL<sup>[45,46]</sup>. Oxysterols also bind to Insig-1/2, facilitating sequestration of sterol-regulatory element binding proteins (SREBPs) at the endoplasmic reticulum, ensuring repression of cholesterol biosynthesis and uptake<sup>[45]</sup>. Deletions of LXR $\alpha$  and LXR $\beta$  in murine models of atheroma cause lipid accumulation within the aortic root, even in the absence of an atherogenic diet<sup>[47,48]</sup>.

It is also evident that LXRs modulate innate and adaptive immune responses mediated by macrophages, neutrophils, lymphocytes, neutrophils and dendritic cells<sup>[45]</sup>, decreasing cytokine-mediated expression of a range of pro-inflammatory genes. This is achieved via a mechanism involving nuclear receptor co-repressor (NCoR), silencing mediator of retinoid and thyroid receptors (SMRT) and inhibition of nuclear factor kappa B (NF $\kappa$ B) signalling<sup>[45,48,49]</sup>. Activation of LXRs is also achieved by phagocytosis of apoptotic cells by macrophages increasing expression of receptor tyrosine kinase (*Mertk*), amplifying phagocytosis and cell clearance, and reducing production of inflammatory mediators<sup>[50]</sup>. Absence of LXR signalling enhances the apoptosis of macrophages challenged with *Listeria monocytogenes*, *Escherichia coli* or *Salmonella typhimurium*, via loss of the anti-apoptotic factor AIM/

Spa<sup>[51,52]</sup>.

## MACROPHAGE GENERATION OF OXYSTEROL LIGANDS FOR LIVER X RECEPTORS

High levels of mitochondrial sterol 27-hydroxylase (CYP27A1) are found in human macrophages, and this enzyme can produce modified sterols, proven to act as LXR ligands *in vitro* and *in vivo*<sup>[53-56]</sup>. Loss of CYP27A1 leads to the lipid storage disease, cerebrotendinous xanthomatosis (CTX), which triggers accumulation of cholesterol and cholestanol in brain and tendons, progressive neurological deterioration, xanthomas and, as a secondary complication, accelerated atherosclerosis<sup>[57,58]</sup>.

The rate-limiting step controlling CYP27A1 activity is the flux of cholesterol from the outer to the inner mitochondrial membrane, *via* a mitochondrial cholesterol trafficking complex (discussed below)<sup>[59]</sup>. Mitochondrial oxysterols therefore act as key cell signalling molecules, the levels of which can be moderated by sulfation (SULT2B1b), esterification (ACAT-1) or metabolism to soluble bile acid derivatives<sup>[53]</sup>. Conceivably, this process could be “uncoupled” by accumulation of free cholesterol at the interface between endoplasmic reticulum (ER) and mitochondrial membranes, triggering ER stress and proteasomal degradation of ABCA1, and opening of the permeability transition pore in mitochondria<sup>[53]</sup>. Esterification of excess oxysterols may then result: over 85% of the 27-hydroxycholesterol in human atherosclerotic lesions is esterified and incapable of activating LXRs and its downstream pathways<sup>[60,61]</sup>. Loss of this protective pathway predicates mitochondrial damage, apoptosis and cytotoxicity, features associated with addition of exogenous atheroma-relevant oxysterols ( $\geq 20$   $\mu\text{mol/L}$ ) to cultured cells<sup>[62]</sup>.

Thus, it is clear that the biological impact of oxysterols are not solely restricted to LXR activation<sup>[63-67]</sup>. For example, oxysterols also serve as endogenous ligands for G-protein coupled receptor 183 (Epstein-Barr virus-induced gene 2, *EBI2*)<sup>[63]</sup>, function as selective estrogen receptor modulators<sup>[64]</sup>, bind to the Smoothened molecule to modulate Hedgehog signalling<sup>[65]</sup>, while CYP27A1-derived  $7\alpha$  and  $7\beta$ , 27-hydroxycholesterol modify innate and adaptive immune responses by acting as agonists of retinoic acid-related (RAR) orphan receptor gamma t (ROPR $\gamma$ )<sup>[66]</sup>.

Acute exposure of macrophages to exogenous oxysterols induce rapid (< second) oscillations in cytoplasmic  $[\text{Ca}^{2+}]$  triggered by influx from the extracellular medium, followed by sustained increases in  $[\text{Ca}^{2+}]$  mediated by translocation of TRPC1 (transient receptor potential, canonical) channels into lipid rafts in the plasma membrane<sup>[68]</sup>. Calcium transfer between ER and mitochondria is facilitated by mitochondria-associated membranes, which act as a hub for lipid transfer, regulation of mitochondrial morphology (fission, fusion and trafficking), apoptosis, autophagy

and ER stress<sup>[69]</sup>, although the role of endogenously generated oxysterols in these processes remains unknown at present. Certainly, chronic exposure to exogenous oxysterol congeners can activate calcium release from the ER, increasing dephosphorylation of Bcl-2 antagonist of cell death by the calcium-dependent phosphatase calcineurin, and promoting apoptosis<sup>[68]</sup>.

## TARGETING PROTEIN CONSTITUENTS OF THE MITOCHONDRIAL CHOLESTEROL TRAFFICKING COMPLEX: IMPACT ON MACROPHAGE STEROL METABOLISM AND INFLAMMATION

Despite intensive investigations in steroidogenic cells and tissues, the nature of the mitochondrial cholesterol trafficking complex remains a matter of debate. One recent model suggests a basal complex, forming contact sites between the outer and inner mitochondrial membranes, composed of the 18 kDa translocator protein (TSPO), adenine nucleotide transporter (ANT) and voltage-dependent anion channel (VDAC)<sup>[70-72]</sup>. In hormone-stimulated steroidogenic tissues, a “transduceosome” complex is formed, involving recruitment of the regulatory subunits of protein kinase A (PKA-R1 $\alpha$ ) and acyl CoA binding domain proteins-1 and -3. Elevated levels of cyclic adenosine monophosphate (cAMP) release PKA catalytic subunits to phosphorylate 37 kDa steroidogenic acute regulatory protein at the outer mitochondrial membrane; import of both StAR and cholesterol into the inner mitochondrial membrane and matrix facilitate both proteolytic processing of StAR to its 30 kDa form, and conversion of cholesterol into pregnenolone by CYP11A1<sup>[70-72]</sup>. However, a dynamic 800 kDa bioactive protein complex in steroidogenic cells has also been described, which does not involve ANT, but is composed of TSPO, VDAC, CYP11A1, ATPase family AAA domain-containing protein 3A (ASTAD3A) and optic atrophy type 1 proteins<sup>[73]</sup>; in this model, StAR facilitated binding of cholesterol to the 800 kDa complex, enhancing steroidogenesis.

Importantly, there is a growing realisation that key mitochondrial cholesterol trafficking proteins, such as StAR, play an important role in non-steroidogenic tissues<sup>[74]</sup>. This, combined with conflicting results regarding the impact of genetic deletion of TSPO on steroidogenesis and viability in mice<sup>[75-78]</sup>, may lead to increased consideration of alternate functions for these proteins<sup>[74]</sup>. For example, StAR is expressed in endothelial cells, monocytes and macrophages<sup>[79-82]</sup>, albeit at levels far lower than those found in adrenal or gonadal tissues<sup>[74]</sup>. By contrast, other components of the mitochondrial trafficking complex, such as TSPO, are widely expressed in a variety of tissues, including macrophages<sup>[78,81]</sup>.

Importantly, both StAR and TSPO appear to impact on macrophage lipid and inflammatory phenotype, in

part *via* the pathway involving sterol 27-hydroxylase, activation of LXR $\alpha$  and upregulation of ABCA1/ABCG1 mRNA and protein<sup>[81-83]</sup>, arguing a functional role for these proteins in mediating cholesterol supply to CYP27A1. Overexpression of StAR decreased macrophage lipid content<sup>[82,83]</sup>, repressed inflammation<sup>[82]</sup> and apoptosis<sup>[84]</sup> and increased macrophage cholesterol efflux<sup>[82,83]</sup>, while a viral vector expressing StAR reduced aortic lipids and atheroma in apoE<sup>-/-</sup> mice<sup>[85]</sup>. However, exploiting any putative anti-atherogenic properties of StAR could prove problematic, due to the associated induction of lipogenesis in macrophages<sup>[83,86]</sup>, presumably *via* LXR $\alpha$  dependent induction of *Srebp1c*<sup>[87]</sup>.

This led to focus on other components of the mitochondrial cholesterol trafficking complex and, in particular, TSPO<sup>[81]</sup>. Transient overexpression of TSPO in human (THP-1) macrophages increased the levels of ABCA1 mRNA and protein, and enhanced efflux of cholesterol to apoA-I, HDL and human serum, a finding reversed by gene knockdown of TSPO. Small molecule TSPO ligands also increased cholesterol efflux, an effect that was amplified in macrophages genetically engineered to overexpress TSPO<sup>[81]</sup>. Notably, TSPO overexpression caused a decline in macrophage total neutral lipid mass, without induction of lipogenesis, and effectively prevented "foam cell" formation following exposure to modified LDL<sup>[81]</sup>. These effects were associated with induction of both LXR $\alpha$  and PPAR $\alpha$  the latter providing a plausible mechanism for the observed reductions in macrophage lipid mass<sup>[81]</sup>. Notably, overexpression of some of the other proposed components of the mitochondrial cholesterol trafficking complex, such as VDAC, ANT and ACBD1, discussed above, exerted minimal effects on the macrophage cholesterol efflux pathway<sup>[81]</sup>.

Expression of TSPO is upregulated by exposure to modified LDL in human macrophages<sup>[81]</sup>, and TSPO ligands have been used to image vascular inflammation in CD68 positive macrophages at sites of disturbed flow in murine carotid arteries<sup>[88]</sup>, and macrophage burden<sup>[89]</sup> and intraplaque inflammation<sup>[90]</sup> within human carotid atherosclerotic lesions. Despite this evident association with inflammation, it appears that upregulation of TSPO, or signalling *via* this protein, may represent an adaptive mechanism designed to limit tissue damage. Overexpression of TSPO in microglia decreased production of pro-inflammatory cytokines, reflected in increased expression of alternately activated M2 stage-related genes and mediated *via* repression of NF- $\kappa$ B activation<sup>[91]</sup>. Similarly, TSPO ligands inhibited the proliferation of retinal microglial cells, and repressed the output of reactive oxygen species and TNF $\alpha$ <sup>[92]</sup>. In good agreement, levels of TSPO are higher in dystrophic murine retina, and in microglia treated with LPS, while TSPO ligand XBD173 repressed the expression of chemokine (C-C motif) ligand 2 (CCL2), IL-6 and iNOS<sup>[93]</sup>. The TSPO ligand, PK11195 has proved effective in ameliorating the severity of disease in an experimental murine model of multiple sclerosis, by reducing inflammatory responses and promoting oligodendroglial regeneration<sup>[94]</sup>. TSPO has also been

posited as a novel target for Alzheimer's disease<sup>[95]</sup>, anxiety, psychiatric and neurologic disorders<sup>[96-99]</sup>, pain<sup>[100]</sup>, cancer<sup>[101]</sup> and vascular dysfunction<sup>[88-90,102]</sup>. At present, it is not known how many of these effects are related to the cholesterol trafficking function of TSPO, although LXRs influence expression of an array of genes involved in cholesterol homeostasis, glucose metabolism, inflammation and Alzheimer's disease<sup>[103]</sup>. It is also clear that some of the reported effects of TSPO and its ligands may require re-evaluation, given the lack of phenotype recently reported in healthy TSPO<sup>-/-</sup> mice<sup>[75,76]</sup>.

## MITOCHONDRIAL STRUCTURE AND BIOENERGETICS: IMPACT ON CHOLESTEROL HOMEOSTASIS

Mitochondria exhibit constant movement, fusion and fission<sup>[104]</sup>. The mitochondrial membrane protein mitofusin (Mfn2) is involved in maintaining mitochondrial morphology, energy provision, and cellular growth and apoptosis<sup>[105-107]</sup>. Recently, Mfn2 has emerged as a regulator of macrophage cholesterol efflux, *via* upregulation of peroxisome proliferator activated receptor- $\gamma$  (PPAR $\gamma$ ) ABCA1, ABCG1 and scavenger receptor-B1 (SR-B1), reflected in marked reductions in cholesterol mass<sup>[107]</sup>. Overexpression of Mfn2 attenuates the formation of atherosclerotic lesions in rabbit carotid arteries, and levels of Mfn2 are progressively reduced during lesion formation in apoE<sup>-/-</sup> mice during atherogenesis; levels of Mfn2 are also reduced in atherosclerotic, compared with non-atherosclerotic, human arteries<sup>[107]</sup>.

Remodelling of the inner mitochondrial membrane by optic atrophy 1 (OPA1) also alters the efficiency of mitochondrial cholesterol trafficking, at least in steroidogenic cells<sup>[108,109]</sup>. Increased steroidogenesis is reported in trophoblasts undergoing syncytialisation, which express increased levels of the pro-fission mitochondrial shaping protein Drp1 increased, and decreased levels of Opa1 and mitofusin. An inverse relationship between levels of Opa1 and steroidogenesis were also evidenced in cells genetically manipulated to express higher levels of Opa1, while accumulation of cholesterol at the inner mitochondrial membrane was observed in mitochondria lacking Opa1<sup>[108,109]</sup>.

Finally, it is self-evident that ATP is needed to mount an effective non-adaptive immune response, and to fuel cholesterol biosynthesis and the activity of ABC transporters that determine the rate of macrophage cholesterol efflux. However, more subtle changes in mitochondrial function or loss of bioenergetic capacity, the emerging concept of the Bioenergetic Health Index (BHI)<sup>[110]</sup>, have been shown to reduce the efficiency of mitochondrial cholesterol trafficking and hormone biosynthesis in steroidogenic tissues<sup>[111,112]</sup>. Dissipation of the mitochondrial membrane potential ( $\Delta\psi_m$  using carbonyl cyanide *m*-chlorophenylhydrazone), inhibition of electron transport at complex III (using antimycin), reduction of pH (nigericin) and inhibition



of ATP synthase (oligomycin) blocked the formation of progesterone and synthesis or import of StAR protein in Leydig cells<sup>[111,112]</sup>.

A parallel study in macrophages supports the notion that acute loss of mitochondrial function is also associated with dysregulated cholesterol homeostasis<sup>[113]</sup>. Cholesterol efflux was inhibited by nigericin and oligomycin in RAW 264.7 macrophages; levels of ABCA1 protein decreased in response to oligomycin treatment, despite paradoxical increases in *Abca1* mRNA<sup>[113,114]</sup>, reflecting findings in carotid atherosclerotic lesions<sup>[114]</sup>. Further, while oligomycin treatment did not alter cholesterol biosynthesis, cholesterol esterification was significantly inhibited, promoting apoptosis. Oligomycin induced expression of genes involved in cholesterol efflux (*Abca1*, *Abcg4*, *Stard1*) and cholesterol biosynthesis (*Hmgcr*, *Mvk*, *Scap*, *Srebp2*) arguing that loss of coordinated regulation of sterol homeostasis is caused by loss of mitochondrial ATP generation<sup>[113]</sup>. In turn, accumulation of free cholesterol or fatty acids can trigger mitochondrial dysfunction, which could promote inflammation *via* loss of LXR $\alpha$ -dependent repression of NF- $\kappa$ B (above) and upregulation of cytokine expression, but also by NLRP3 inflammasome-dependent and -independent pathways<sup>[115]</sup>.

## QUESTIONS FOR THE FUTURE

This review summarizes the current evidence that, in part, macrophage sterol homeostasis, and inflammatory responses, can be linked to mitochondrial cholesterol trafficking, and mitochondrial structure and bioenergetics. Whether proteins involved in mitochondrial structure, fission, fusion or organelle dynamics can also impact on these processes is currently uninvestigated and an area of keen interest. More particularly, mitochondria-mediated hormetic effects in aging<sup>[116,117]</sup> suggest a retrograde signalling pathway by which mitochondrial dysfunction in a single distinct tissue elicits the mitochondrial stress response in some (but not all) distal tissues. In turn, this suggests that loss of effective mitochondrial function, such as that caused by hepatic insulin resistance for example, may be transmitted *via* "mitokines" to peripheral tissues, promoting vascular dysfunction and cardiovascular disease. These exciting findings offer some intriguing possibilities for therapeutic strategies aimed at sustaining or improving mitochondrial function.

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Case Control Study

## Quantification of epicardial fat: Which method can predict significant coronary artery disease?

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**Author contributions:** Saad Z and Donkol RH designed the study, performed CCTA studies, and wrote the manuscript; El-Rawy M and Donkol RH shared selection of cases, clinical and echocardiographic assessment as well as collection of data and interpreted CCTA scans; Boghattas S analyzed the data.

**Ethics approval:** The study was approved by institutional ethics committee.

**Informed consent:** All participants provided written informed consent prior to study enrollment.

**Conflict-of-interest:** Corresponding author with no conflict of interest regarding the study.

**Data sharing:** Technical appendix, statistical code, and dataset are available. Participants gave informed consent for data sharing. No additional data are available.

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### Abstract

**AIM:** To compare the predictive value of three methods of epicardial fat (EF) assessment for presence of significant coronary artery disease (CAD) [*i.e.*, epicardial fat volume (EFV), EFV indexed with body surface area (EFV/BSA) and EFV indexed with body mass index (EFV/BMI)].

**METHODS:** The study was performed on 170 patients (85 women and 85 men) with clinical suspicion of CAD. They aged 26-89 years with a median age of 54 years. The patients were classified into three groups: Group 1: 58 patients with normal coronary arteries; group 2: 48 patients with non-significant CAD and group 3: 64 patients with significant CAD. The three methods for assessment of epicardial fat were retrospectively studied to determine the best method to predict the presence of significant CAD.

**RESULTS:** The three methods for epicardial fat quantification and measurements, *i.e.*, EFV, EFV/BSA and EFV/BMI with post-hoc analysis showed a significant difference between patients with significant coronary artery disease compared to the normal group. Receiver operating characteristic curve analysis showed no significant difference between the three methods of epicardial fat measurements, the area under curve ranging between 0.6 and 0.62. The optimal cut-off was 80.3 cm<sup>3</sup> for EFV, 2.4 cm<sup>3</sup>/m<sup>2</sup> for EFV indexed with BMI and 41.7 cm<sup>3</sup>/(kg/m<sup>2</sup>) for EFV indexed with BSA. For this cut-off the sensitivity ranged between 0.92 and 0.94, while specificity varied from 0.31 to 0.35.

**CONCLUSION:** Any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

**Key words:** Quantification of epicardial fat; Coronary heart disease; Epicardial fat volume

**Core tip:** There is a great correlation between the volume of epicardial fat and presence of significant coronary artery disease. There are different methods for quantification of epicardial fat volume (EFV). The aim of the study is to compare the predictive value of the three methods used for quantification of epicardial fat (EFV, EFV indexed with body surface area and EFV indexed with body mass index) for presence of significant coronary artery disease. The study concluded the three methods for assessment of epicardial fat have the same equivalent predictive value for significant coronary artery disease and any one of them can be used as a sensitive predictor for significant coronary artery disease.

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## INTRODUCTION

Epicardial fat (EF) is the visceral fat of the heart deposited under the visceral layer of the pericardium. Under normal physiological conditions, EF tissue displays biochemical, mechanical and thermogenic cardioprotective properties. Under pathological circumstances, EF can be strongly related to the development of coronary artery disease (CAD). The accumulation of EF is known to be a rich source of free fatty acids and a number of proinflammatory cytokines<sup>[1-3]</sup>.

It has been hypothesized that EF may act as a paracrine (immunological) organ that influences the coronary arteries by promoting chronic inflammation and endothelial dysfunction<sup>[4-6]</sup>.

Several imaging modalities can be used to quantify EF volume (EFV) such as echocardiography, computed tomography, and magnetic resonance imaging<sup>[7]</sup>. Due to distinct low attenuation values of fat on computed tomography (CT). ECG-gated cardiac CT with its high spatial resolution and true volume coverage of the heart, allows accurate measurement of epicardial and thoracic fat distances and volumes<sup>[6,7]</sup>.

Besides EFV, some authors derived a second parameter, the body surface area indexed EFV (EFV/BSA)<sup>[8,9]</sup>; however, comparison between these two parameters has not been considered. Moreover, despite the predictive value of higher body mass index for cardiovascular event<sup>[10,11]</sup>, the added value of body mass index (BMI)-adjusted EFV (EFV/BMI) also has not been previously assessed as well.

The aim of the current study is to compare the predictive value of the three methods for EF assessment (EFV, EFV/BSA and EFV/BMI) for presence of significant

CAD.

## MATERIALS AND METHODS

### Patients

A total of 170 consecutive patients with clinical suspicion of coronary artery disease (CAD) aged 26-89 years with a median age of 54 years (85 women and 85 men) were included in our study From November 2012 through February 2014. Patients underwent 128-MDCT according to appropriate use criteria for cardiac computed tomography<sup>[12]</sup>. All participants provided written informed consent and the study was approved by institutional ethics committee.

The patients were divided into 3 groups according to the severity of coronary artery stenosis assessed by quantitative coronary angiography. Patients in group 1 have normal coronary arteries. Patients in group 2 were have non-significant coronary artery stenosis with percent-diameter stenosis less than 50%. Patients in group 3 have significant CAD with percent-diameter stenosis more than 50% or occlusion<sup>[13]</sup>.

### Multidetector computed tomography

Our patients were scanned with a 128-multidetector computed tomography (MDCT) dual source scanner (SOMATOM Flash; Siemens Medical Solutions, Erlangen, Germany). Patients with uncontrolled heart rate (> 65 beats per minute) received oral beta blocker (metoprolol 50 mg) before the CT scan. Sublingual nitroglycerin 0.5 mg was administered before the scan to achieve coronary vasodilation. A non-contrast CT scan was performed to determine the total calcium burden of the coronary tree (sequential scan with 32 Å~ 0.6-mm collimation, tube current 60 mA at 120 kV). Contrast-enhanced CT angiography data were acquired with the use of a spiral scan with 32 Å~ 0.6-mm collimation, 330-ms gantry rotation, pitch of 0.2, and tube voltage at 120 kV.

Sequential ECG-triggering scans were performed in 142 patients with controlled heart rate and retrospective ECG-gating scans with tube current modulation were performed in 28 patients due to heart rate variability. Intravenous contrast agent (60-90 mL; 350 mg iodine/mL) was injected with flow rate of 5.0 mL/s followed by a 30-mL saline chaser<sup>[1]</sup>.

**Image interpretation:** The total calcium score was calculated and interpretation of CCTA was analysed for all patients using commercially available software packages "Syngo Via", Siemens Healthcare, Forchheim, Germany.

### Measurement of EFV

EFV was measured blindly by two observers for all patients using an offline workstation (Aquarius NetStation; TeraRecon Inc., San Mateo, CA). Using the 3.0-mm-thick axial slices used for calcium scoring, the parietal

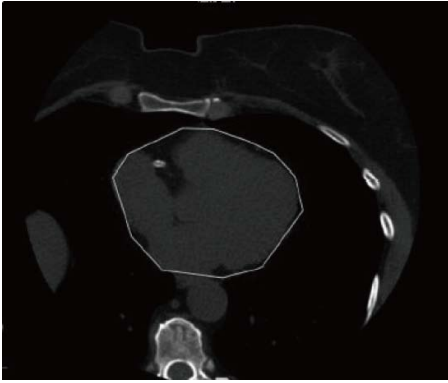


Figure 1 Measurement of epicardial fat volume; the electronic line delineates the contour of epicardium in noncontrast computed tomography.

pericardium was traced manually in every 4<sup>th</sup> slice starting from the aortic root to the cardiac apex<sup>[1]</sup> (Figure 1). The computer software then automatically interpolated and traced the parietal pericardium in all slices interposed between the manually traced slices. The automatically traced slices were examined and verified for accuracy. Fat voxels were identified using threshold attenuation values of -30 to -190 HU (Figure 2).

Division the estimated EFV of every patient by patient's BSA calculated the indexed EFV to BSA (EFV/BSA). Also, division of EFV by patient's BMI calculated the indexed EFV to BMI (EFV/BMI).

### Statistical analysis

The statistical analysis was performed by StatDirect 2.8.0 and Mecal 9.2.1.0 (for ROC curve analysis and comparison). Because the analyzed quantitative variables (EFV, EFV/BMI, EFV/BSA, BMI, BSA and age) were not normally distributed (Shapiro and Wilk test), the values are expressed as medians with interquartile range. Dichotomous data are presented as frequency (percent).

Differences in characteristics of patients were compared using Pearson's  $\chi^2$  test for dichotomous variables (or Fisher's exact test when appropriate) and Kruskal-Wallis test for continuous test.

To compare the predictive performance for risk of significant CAD between the three EF parameters, we plotted receiver operating characteristic (ROC) curve from which the optimal cutoff was derived and we calculated the area under curve (AUC). AUC, optimal cutoff, sensitivity and specificity for each EFP and pairwise comparison of AUC have been determined. A two-tailed *P*-value less than 0.05 were considered as statistically significant; significant differences are presented with asterisk in the tables. The statistical methods of this study were reviewed by a biostatistician.

## RESULTS

### General characteristics

Table 1 illustrated the important clinical findings and measurements of the patients among the three groups

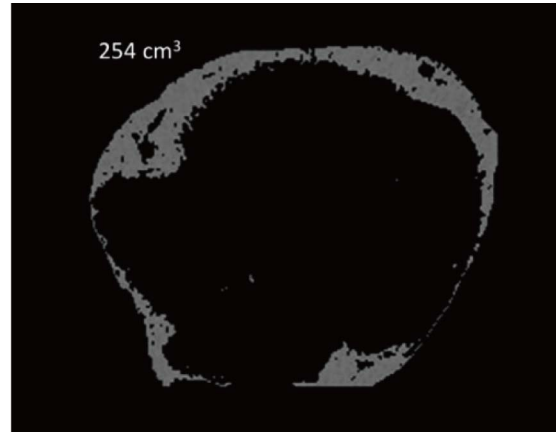


Figure 2 Measurement of epicardial fat volume; the fat voxels were identified using threshold attenuation values (-30 to -190 HU) and the volume of epicardial fat is measures.

of patients.

### Correlation of EFP with CT coronary angiography results

Comparison of EF parameters with CT coronary angiography results is presented in Table 2. Comparison of EFP medians revealed a progressive increase from group 1 to group 3. The medians were 81, 104 and 113 for EFV (cm<sup>3</sup>), 2.7, 3.7 and 3.8 for EFV/BMI (cm<sup>3</sup>/m<sup>2</sup>) and 45.6, 53.9 and 61.8 for EFV/BSA [cm<sup>3</sup>/(kg/m<sup>2</sup>)]. Nevertheless, the post-hoc analysis showed significant difference only between groups 1 and group 3 for all EFP: *P* = 0.002 for EFV, *P* = 0.012 for EFV/BMI, and *P* = 0.007 for EFV/BSA.

### ROC curves analysis

ROC curve for the three the EFP is presented in Figure 3. AUC was 0.62 for EFV, 0.6 for EFV/BMI and 0.61 for EFV/BSA and pairwise comparison failed to show significant difference. The optimal cut-off was 80.3 cm<sup>3</sup> for EFV, 2.4 cm<sup>3</sup>/m<sup>2</sup> for EFV/BMI and 41.7 cm<sup>3</sup>/(kg/m<sup>2</sup>) for EFV/BSA. Sensitivity and specificity were respectively 0.92 and 0.35 for EFV, 0.93 and 0.31 for EFV/BMI, and 0.94 and 0.32 for EFV/BSA.

## DISCUSSION

Noninvasive quantitative measurement of epicardial fat volume from CT is feasible, and may play a clinical role in cardiovascular risk assessment<sup>[6]</sup>. It have shown its reproducibility and correlation to CAD presence, severity, and prognosis<sup>[14-16]</sup>.

Recently, multiple studies have shown a deleterious relationship between epicardial fat burden and coronary atherosclerosis, arrhythmogenesis and major adverse cardiovascular events (MACE)<sup>[17,18]</sup>. In this study, the epicardial fat volume is correlated with the presence of coronary artery stenosis. In agreement with this findings, Alexopoulos *et al.*<sup>[19]</sup> observed on coronary CT angiography a significant increase in epicardial fat

**Table 1** General characteristics of the patients among the three groups (total number of patients 170) *n* (%)

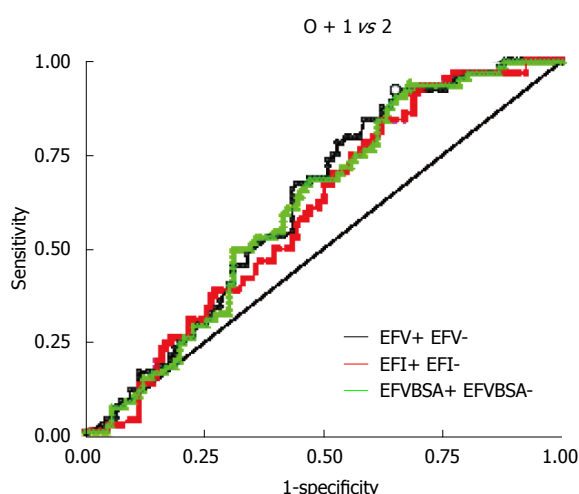
	Group 1 <i>n</i> = 58	Group 2 <i>n</i> = 48	Group 3 <i>n</i> = 64	<i>P</i>
Age	48.5 (40.3-50.0)	56 (48.5-64.5)	55 (50-62)	0.004
Male	21 (36.2)	32 (57.1)	32 (50)	0.008
Hypertension	24 (41.4)	29 (60.4)	37 (57.8)	0.090
Diabetes mellitus	23 (39.7)	34 (70.8)	43 (67.2)	0.001
Smoking	5 (8.6)	4 (8.3)	14 (21.8)	0.065
Familial history	7 (12.1)	2 (4.2)	6 (9.4)	0.351
BMI	28.4 (25.9-33.9)	29.5 (25.9-33.1)	31.6 (29.3-33.1)	0.007
BSA	1.80 (1.74-1.88)	1.85 (1.78-1.94)	1.88 (1.81-1.95)	0.093

BMI: Body mass index; BSA: Body surface area.

**Table 2** Comparison of three parameters for measuring the epicardial fat with computed tomography coronary angiography results

	Group 1	Group 2	Group 3	<i>P</i>
EFV (cm <sup>3</sup> )	81 (59.4-124)	104 (83.5-126)	113 (92-138)	0.004
EFVBMI (cm <sup>3</sup> /m <sup>2</sup> )	2.7 (1.9-3.9)	3.7 (2.5-4.3)	3.8 (3.0-4.6)	0.014
EFVBSA [cm <sup>3</sup> /(kg/m <sup>2</sup> )]	45.6 (35.3-66.9)	53.9 (44.4-68.6)	61.8 (48.1-75.4)	0.011

EFV: Epicardial fat volume; EFVBMI: EFV indexed with body mass index; EFVBSA: EFV indexed with body surface area.



**Figure 3** Receiver operating characteristic curves compare the three parameters for measuring the epicardial fat. AUC was 0.62 for EFV, 0.6 for EFVBMI, and 0.61 for EFVBSA (non-significant pairwise comparison). EFV: Epicardial fat volume; EFVBMI: EFV indexed with body mass index; EFVBSA: EFV indexed with body surface area.

volume with increasing coronary luminal stenosis; epicardial fat volume was also larger in patients with mixed or noncalcified plaques.

A recent study by Nakazato *et al.*<sup>[20]</sup> assessed the relationship of epicardial fat volume to weight, BMI and waist circumference, and evaluated whether changes in these parameters over a 4-year period influenced epicardial fat volume measured by noncontrast CT in a relatively healthy asymptomatic population. They found that weight, BMI and waist circumference demonstrated moderate cross-sectional relationships to epicardial fat volume, and that changes in these parameters were related to epicardial fat volume change.

In another study, Shmilovich *et al.*<sup>[9]</sup> assessed the body surface area indexed EVF in a healthy population and validated it as a predictor of major adverse cardiovascular events.

By reviewing the literature, the two parameters EFV and EFV/BSA, have no clear evidence of differences in their accuracies and predictive values<sup>[9,18-20]</sup>. In the current study we compared the predictive value of three EFP for the presence of significant CAD. To our knowledge, no similar studies have been performed before and EFV/BMI has not previously assessed also, the predictive value of EF/VBSA for CAD has not been compared with EFV.

The major outcome of the present study, derived from the ROC curve analysis, is that the three methods for assessing the EF have an equivalent predictive value for significant CAD and any one of them can be used as a predictor for significant CAD. Therefore, the clinical impact of the indexed EFV seems to be limited. To note that in the series using the indexed EFV to BSA, the benefit of such indexing has not been evaluated and ROC curve analysis has not been applied for assessment of possible increase in the predictive value for total occlusion of coronary arteries<sup>[16]</sup> or major adverse cardiovascular events<sup>[9]</sup>.

According to the traditional academic point system, the accuracies of the three methods for measuring EF are classified as poor considering that the AUC are in the range 0.6-0.7. This limited accuracy is related mainly to the low specificity, ranging from 0.31 to 0.35, while the sensitivity is high, varying from 0.92 to 0.94, therefore the three methods can be considered as sensitive but poorly specific predictors for significant CAD. Consequently, indexing EFV by BSA or BMI doesn't improve significantly the sensitivity and, more



importantly, the specificity of EF for significant CAD.

In the current study, the optimal cut-off was 80.3 cm<sup>3</sup> for EFV, 2.4 cm<sup>3</sup>/m<sup>2</sup> for EFVBMI and 41.7 cm<sup>3</sup>/(kg/m<sup>2</sup>) for EFVBSA. The optimal cut-off for EFVBSA was lower than the value 50 cm<sup>3</sup>/m<sup>2</sup> reported by Ueno *et al*<sup>[16]</sup> (labeled VEAT in their series), despite a higher median value in our series: 53.7 cm<sup>3</sup>/m<sup>2</sup> vs 47.1 cm<sup>3</sup>/m<sup>2</sup>. This difference is likely related to the definition of the end-point itself: significant CAD in the current study vs total occlusion of the coronary arteries in the series of Ueno *et al*<sup>[16]</sup>. More recently, Shmilovich *et al*<sup>[9]</sup> derived the threshold for the upper normal limit of indexed EF to BSA in a healthy population. The indexed EFV was also non-normally distributed, and the 75<sup>th</sup>-percentile was 47.1 cm<sup>3</sup>/m<sup>2</sup>, while in our series the 75<sup>th</sup>-percentile for the group 1 (composed of patients without CAD) was 66.9 cm<sup>3</sup>/m<sup>2</sup>. Clearly, these values cannot be compared considering the major differences in the design of the two studies, the series of Shmilovich *et al*<sup>[9]</sup> including only asymptomatic patients with low risk and without clinical or biological risk factors for CAD.

Analysis of the correlation between the EFP and CT coronary angiography results revealed a trend of increase from group 1 to group 3. This result is similar to the conclusion of Taguchi *et al*<sup>[21]</sup> that pericardial fat is the strongest independent variable for the severity of CAD. Nevertheless, only groups 1 and 3 differed significantly. The overlap of values, for all EFP, between groups 2 and 3 may explain in great part the low specificity observed for the optimal cut-off. This point merits further analysis to achieve a higher predictive value of EF for significant CAD.

Relatively small number of studied patients with no available follow up data to observe the outcome or prognostic value of EFP considered a study limitation. Additionally our study may be affected by selection bias as we evaluate patients with clinical suspicion of CAD.

In conclusion, the major outcome of the present study that the three methods for assessment of EF have the same equivalent predictive value for significant CAD and any one of them can be used as a sensitive predictor for significant CAD. Additionally, we provided a threshold for each one of the three EFP. For further validation of this threshold additional larger study is recommended.

## COMMENTS

### Background

Epicardial fat (EF) under normal physiological conditions, EF tissue displays biochemical, mechanical and thermogenic cardio protective properties. Under pathological circumstances, EF can be strongly related to the development of coronary artery disease (CAD). Several imaging modalities can be used to quantify EF volume (EFV) such as echocardiography, computed tomography, and magnetic resonance imaging.

### Research frontiers

ECG-gated cardiac computed tomography with its high spatial resolution and true volume coverage of the heart, allows accurate measurement of epicardial fat volumes. Besides EFV, some authors derived a second parameter, the body

surface area indexed EFV (EFV/BSA); however, comparison between these two parameters has not been considered. Moreover, despite the predictive value of higher body mass index for cardiovascular event, the added value of body mass index (BMI)-adjusted EFV (EFV/BMI) also has not been previously assessed as well. The current study compares the predictive value of the three methods for EF assessment (EFV, EFV/BSA and EFV/BMI) for presence of significant CAD.

### Innovations and breakthroughs

In the current study the authors compared the predictive value of three EFP for the presence of significant CAD. To our knowledge, no similar studies have been performed before and EFV/BMI has not previously assessed also, the predictive value of EFV/BSA for CAD has not been compared with EFV. The major outcome of the present study is that the three methods for assessing the EF have an equivalent predictive value for significant CAD. Furthermore the authors provided a threshold for each one of the three EF parameters.

### Applications

The study results suggested that; any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

### Terminology

EF is the visceral fat of the heart deposited under the visceral layer of the pericardium and has the same origin as abdominal visceral fat.

### Peer-review

This is a good study in which the authors compare the predictive value of three methods used for quantification of epicardial fat [i.e., EFV, EFV indexed with body surface area (EFV/BSA) and EFV indexed with body mass index (EFV/BMI)] for presence of significant CAD. The results are interesting and suggest that any one of the three methods for assessment of epicardial fat can be used to predict significant CAD since all have the same equivalent predictive value.

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## Myocarditis in athlete and myocardial bridge: An innocent bystander?

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often unnoticed or misdiagnosed. Athletes with myocarditis must stop practicing their activity since International medical Literature described some cases of sudden death. In the present report, we describe a case of an asymptomatic, apparently healthy, competitive athletes, who was diagnosed a myocarditis and as incidental finding a myocardial bridging. We focused the attention on the importance of anamnesis, electrocardiogram and athletes' entourage for the diagnosis of such insidious pathologies and we evaluated the follow up, focusing the attention on electrocardiogram changes as well as on restitution ad integrum and prognosis, especially for the athletes.

**Key words:** Myocarditis; Sudden cardiac death; Pre-participation screening

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**Core tip:** Pre participation screening allows sports physicians to diagnose potential causes of sudden death in athletes. We describe a case of an athlete with a previous myocarditis, and an incidental myocardial bridging, suspended from competition and followed in the later time. We also pointed out the question of the possible scarring of myocarditis in relation to the restart of physical activity and training.

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### Abstract

Myocarditis is a bacterial or viral inflammatory disease,

### INTRODUCTION

Myocarditis is an inflammatory disease of the myocardium,

possibly determined by both bacterial and viral infections. Coxsackievirus B, enteroviruses adenovirus and parvovirus B19 are often involved in acute processes<sup>[1]</sup>. Myocardial involvement caused by granulomatous or (auto) immune processes with unknown pathogenetic mechanisms, but this is less common than virus-induced myocarditis or post-infectious inflammatory cardiomyopathy.

Myocarditis is frequently anticipated by flu-like signs and symptoms like chills, fever, headache, muscle aches, general malaise. Moreover, gastrointestinal symptoms such as decreased appetite, nausea, vomiting, and diarrhoea are commonly reported. Cardiac signs and symptoms may appear a few hours to a few days since the beginning of myocardial inflammation and consist in chest pain, palpitations, dyspnea, hypotension, gallop rhythm, rales, jugular venous dilatation, and cardiac tamponade.

In rare cases, the inflammatory condition causes acute hemodynamic compromise, possibly leading to heart failure, complex arrhythmias and fatal events<sup>[2]</sup>. In reported cases, Myocarditis scenario can overlap Ischemic disease<sup>[3]</sup>, and acute coronary syndrome, even an ST-segment elevation myocardial infarction.

Nevertheless, myocarditis can be undiagnosed and completely asymptomatic with not perceived short lasting fever (5-7 d).

In the absence of structural heart diseases myocarditis accounts for approximately 10% of recent-onset cardiomyopathy in adults. Early fulminant disease is still associated with a high mortality rate. Patients who survive the critical phase have a fairly good prognosis and survival from myocarditis is approximately 60%-70%. In the remaining patients progressive chronic heart failure and unpredictable sudden cardiac death remain a serious concern, often occurring years after the initial clinical event and sometimes despite complete recovery of the myocardial function.

Diagnosis is commonly carried out by integrating different methods<sup>[4]</sup>.

Electrocardiogram (ECG): a sensitive cheap tool of diagnosis. Abnormal ST-T waves and conduction block are frequently observed in myocarditis as well as isolated or complex/polymorphic ventricular arrhythmias present at rest or during stress test<sup>[5]</sup>. A gradual increase in the width of the QRS complex can be a sign of exacerbation of myocarditis. Continuous ECG monitoring is crucial to detect arrhythmic burden and presence of threatening rhythm disorder.

Echocardiography: in case of echo alterations, myocarditis can be confirmed by transient wall thickening, reduced wall motion and reduced cardiac chamber size in addition to pericardial effusion on echocardiography and increase of echo reflection<sup>[6]</sup>.

Cardiac magnetic resonance imaging (MRI): in addition to the cinematic mode on MRI, T1-weighted early signal enhancement and gadolinium-delayed imaging of the heart are useful to make a diagnosis of myocarditis<sup>[7]</sup>. T2-weighted images reveal the regions

of the heart affected by inflammation. Cardiac magnetic resonance (CMR) has emerged as a leading modality in the noninvasive diagnosis of myocarditis due to its ability to detect myocardial edema, hyperaemia, necrosis and fibrosis in a safe and reproducible fashion. The presence of late enhancement gadolinium (LGE) has been reported in 24%-95% of patients with myocarditis, typically being multifocal and located in the epicardium of the lateral wall and mid wall of the septum. Moreover, the long term prognosis can be determined by the magnitude of damage at MRI, since the left ventricle end diastolic volume, the presence of LGE in the septum and the total amount of LGE are the strongest independent predictors of impaired ventricular function and ventricular dilatation at follow up<sup>[8]</sup>.

Blood Biochemistry: during the acute phase of myocarditis, it is possible to observe a plasma increased elevation of C-reactive protein, aspartate aminotransferase, lactate dehydrogenase, MB form of creatine kinase, and cardiac troponin T<sup>[9]</sup>.

Other techniques may include computed tomography (CT) of the heart to assess possible artery disease<sup>[2,10]</sup>, chest X-ray (qualitative quick assessment of cardiac enlargement and pulmonary congestion); cardiac catheterization, including endomyocardial biopsy (the gold standard for diagnosis but an invasive method not risk free and usually performed only after the acute phase and in clinical stability of the patient)<sup>[11]</sup>.

Myocarditis is reported as a cause of Sudden death in athletes<sup>[2]</sup>.

Italian guidelines for the pre-participation screening sustain that "subjects with certain diagnosis of myocarditis cannot be involved in any kind of sport activity, until the illness process is completely resolved, and however not before of at least 6 mo since the diagnosis was made. The competitive sport activity can be restarted only if clinical evaluation and instrumental evaluations demonstrate the lack of alterations in myocardial contractile function as well as the absence of relevant arrhythmias"<sup>[12]</sup>.

## CASE REPORT

A 41-year-old male, regularly involved in competitive sport (American football and weightlifting) came, on march 2013, to our Sport's Medicine laboratory, for the annual mandatory pre-participation screening. Football screening protocol include Cardiological examination, basal ECG, Stress Test. Family and personal history was negative. Clinical examination was unremarkable.

Rest-ECG showed deep T-wave inversion in  $\geq 2$  contiguous anterior, inferior or lateral leads, particularly in leads I, II, III, aVF and in V4-V5-V6. This pattern was absent in all the previous ECGs the patient made for previous screening, and in particular it was absent recently, when he underwent job medical exam (ECG January 2013) (Figure 1).

The patient underwent echocardiography which



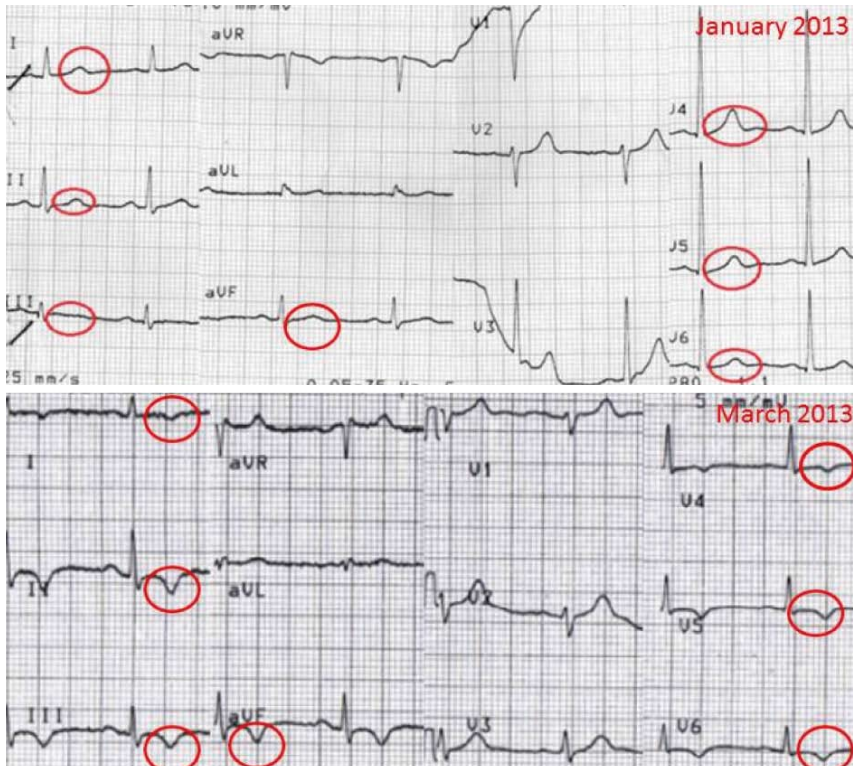


Figure 1 Electrocardiogram changes: Ventricular.



Figure 2 Echocardiogram (March 2013): Echo-free space in the lateral wall and hyper echogenicity of the lateral portion of the left ventricle.



Figure 3 Cardiac magnetic resonance: Areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis.

showed a mild echo-free space with pericardial effusion in the lateral wall and a slight increase in the hyper echogenicity level of the lateral portion of the left ventricle (Figure 2).

As the patient was completely asymptomatic and actually training and competing a cardiac exercise stress test was performed. T-waves inversion was stable all along the duration of the test. Patient reached 168 beats per minute at peak exercise heart rate and 200 watt of peak mechanical Power. Blood pressure kinetics was normal. No presence of Arrhythmias. No symptoms were reported. Holter ECG monitoring showed absence of ventricular arrhythmias.

The patients underwent through blood examination and biochemistry, which didn't show any alterations.

Through anamnesis the patient remembered having experienced a "persistent flu" on January 2013,

characterized by elevated fever (40 °C) and atypical chest pain which he considered aspecific and not to be referred to his General practitioner, for several days. He was administered a broad-spectrum antibiotic therapy (Cephalexin) and symptoms completely disappeared within 4 d.

As a third level examination we performed a cardiac MRI which showed the presence of some areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis, in a context of normal dimensions and function (Figure 3). The characteristic pattern of LGE in myocarditis is patchy or multifocal in a subepicardial or intramyocardial distribution, often involving the lateral wall. This feature is not pathognomonic but is clearly distinct from ischemic heart disease, which typically presents with subendocardial or transmural LGE within a

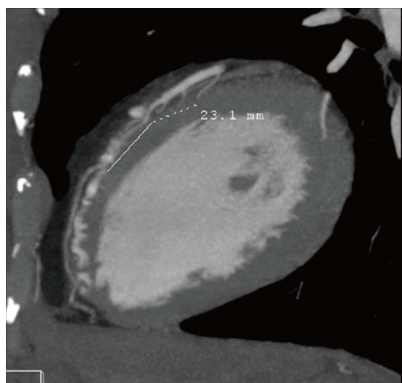


Figure 4 Tomographic computed scan: Myocardial bridge (23 mm).

coronary artery territory.

Despite it, considering the age of the athlete and possible overlapping with ischemic disease even if in absence of wall abnormalities at echo scan of the heart, we excluded coronary involvement through a CT scan of coronary arteries. As a collateral finding a coronary abnormal 23 mm superficial intramycardial course in the medium tract of Left descending artery was found.

The intramuscular course of coronary arteries can be detected and characterized by Computed Coronary Tomographic Angiography (CCTA). CCTA is an easy and reliable tool for comprehensive *in vivo* diagnosis of the intramuscular course of coronary arteries. It is generally estimated that the myocardial bridging can be detected in about one-third of the adults in autopsy series, whereas the reported incidence in angiographic series is much lower < 5%. The incidence of myocardial bridging in CCTA studies (30.5%) is in concordance with the reported incidence in autopsy series<sup>[13]</sup>.

We concluded for Myocarditis in association with incidental finding of Myocardial bridge (Figure 4).

According to cardiologic Italian guidelines for competitive sports, we recommended to avoid physical overload and sport practice. A therapy with low dose Bisoprolol was started and a follow up was based on monthly ECG and a repetition of echocardiography and stress-test at three months' time.

T wave inversion pattern showed a gradual reduction in amplitude up to complete reversal after 4 mo (Figure 5).

Echocardiogram performed in October 2013 was normal and no sign of effusion was present. Stress test was within normal limits and no repolarization abnormalities could be found.

Clinical evaluation, normalization of basal ECG and echocardiogram demonstrated the complete electrical recovery of the athlete from myocarditis. He continued to be excluded from competitive sports, according to cardiologic Italian guidelines for competitive sports for the presence of intramycardial Coronary bridge.

After one year of follow up the patient did not

experience any cardiological symptoms, clinical examination was unremarkable and electrocardiographic and echocardiographic findings as well as stress test were within normal limits.

## DISCUSSION

Myocarditis is a rare recognised cause of sudden death in athletes, its signs and symptoms may overlap ischemic disease as well as cardiomyopathy and it can be minimally symptomatic or able to determine rare fatal events<sup>[14]</sup>. Even in asymptomatic subjects, recognition of this disease can modify prognosis since inflammatory cardiomyopathy can determine adverse event also many years after apparently total restitution ad integrum, this is the reason why indication for clinical follow up and for adequate exercise prescription can change the patient "scenario".

Intramycardial bridge is a recognised cause of sudden death in athletes too<sup>[15]</sup>. The prevalence of this abnormality shows a wide range of frequency, up to 44% with 64-slice coronary CT scan<sup>[16]</sup>. In the last years Computed Scan has allowed diagnosis even in asymptomatic subjects. This finding requires, accordingly to cardiological Italian guidelines for competitive sports, to avoid competition and high intensity training.

With this report, we firstly intend to remark the relevance of anamnesis and combined techniques such as basal ECG<sup>[17]</sup>, echocardiography, MRI and Computed Scan of the heart, in the diagnosis or exclusion of myocarditis when clearing athletes for competition. ECG is reported to be normal in over 32% of subjects with acute myocarditis. This can also be due to the time at which the ECG is performed, for the transitory nature of abnormalities<sup>[18]</sup>. MRI has a sensitivity of 81%, specificity of 71% and accuracy of 81% for diagnosing acute myocarditis<sup>[5]</sup>.

Moreover, T wave inversion were in the same location of increased echo reflex and in proximity of mild echo free area interpreted as mild pericardial effusion, in lateral portion of left ventricle. Such data was also confirmed at MRI and at Computed Scan of the heart, with no sign of oedema, but with presence of fibrosis, indicating a past inflammatory based necrosis. This information is crucial to possibly diagnose Myocarditis as well as for the prognosis.

Secondly, we would like to underline the importance of a proper dialogue between sport physicians and the athletes, the medical team and even, in some cases, the family doctor. A focal point is that even if mandatory pre-participation screening in our country is annually based, it seems essential that sports physicians recommend to re-evaluate athletes experiencing persistent flues or not common signs and symptoms such as atypical thoracic pain, asthenia and palpitations. This information is to be extended to athletes and general practitioner.

## ECG changes

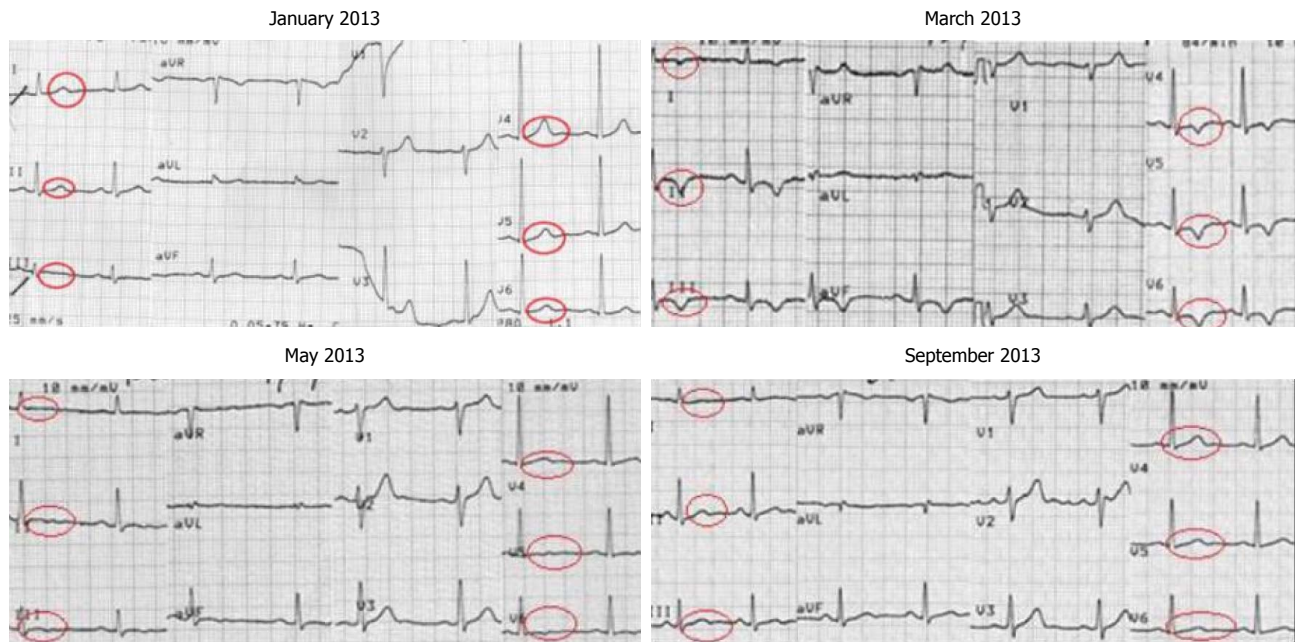


Figure 5 Electrocardiogram follow up.

According to actual Italian guidelines, a period of 6 mo following restitution ad integrum is sufficient to be again eligible for sport competition. With MRI, myocardial damage, previously not detected can be well appreciated as fibrosis or sub epicardial or intramural damage.

It seems important to note that the presence of delayed enhancement and fibrosis is not considered as a risk predictor in previous Italian Cardiological Guidelines for pre participation screening, in fact a complete reversal of T waves abnormalities, with preserved function and absence of symptoms and arrhythmias is considered sufficient for a restitution and integrum. Are now this concept still valid? Which is the role of Myocardial scar in determining a good prognosis? Is an ECG reversal to normality also a sign of better prognosis?

## COMMENTS

### Case characteristics

The athlete during the pre-participation screening was asymptomatic, he reported elevated fever (40 °C), atypical chest pain with asthenia and persistent flu, 2 mo before.

### Clinical diagnosis

The athlete, with a previously normal electrocardiogram (ECG), showed T wave inversion in most leads, after a "persistent flu" with elevated fever 2 mo later.

### Differential diagnosis

Differential diagnosis with myocardial infarction was performed through computed tomography (CT) scan which didn't show coronary artery disease as well as through echocardiography which showed normal systolic function.

### Laboratory diagnosis

The patient underwent blood examination (C-reactive protein, aspartate aminotransferase, lactate dehydrogenase, MB form of creatine kinase, and cardiac troponin T) which didn't show any alterations.

### Imaging diagnosis

The authors performed the following instrumental examinations: (1) ECG: T

wave inversion in leads I, II, III, aVF and in V4-V5-V6; (2) Echocardiogram: mild echo-free space with pericardial effusion in the lateral wall and a slight increase in the hyper echogenicity level of the lateral portion of the left ventricle; (3) magnetic resonance imaging (MRI): areas of intramural and subepicardial delayed enhancement in the lateral wall, suggestive of fibrosis; and (4) CT scan: coronary abnormal 23 mm superficial intramural course in the medium tract of left descending artery.

### Pathological diagnosis

The authors didn't perform any histological examination.

### Treatment

After the detection of the intramural bridge the authors started a therapy with low dose bisoprolol and the authors suspended the athlete from competition and training. A monthly follow up was started.

### Term explanation

Pre participation screening is the annual medical evaluation mandatory for competitive athlete in Italy.

### Experiences and lessons

It would be desirable that sports physicians recommend to re-evaluate athletes experiencing persistent flues or not common signs and symptoms such as atypical thoracic pain, asthenia and palpitations, during the sports season; this information is to be extended to athletes and general practitioner.

### Peer-review

This case report highlights the importance of anamnesis and 12 leads ECG examination for the athletes in diagnosis of subtle transitory myocardial alterations due to infections. Moreover, despite a normalization of ECG during follow up, after a myocarditis, the authors emphasized the eventuality to focus on the possibility of myocardial scars, which can be easily detected with MRI.

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