

# World Journal of *Cardiology*

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2014-2017

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### TOPIC HIGHLIGHT

- 517 Antihypertensive drugs and glucose metabolism  
*Rizos CV, Elisaf MS*
- 531 Hypertension and medical expenditure in the Japanese population: Review of prospective studies  
*Nakamura K, Okamura T, Miura K, Okayama A*
- 539 Adipose tissue and vascular inflammation in coronary artery disease  
*Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Russo PE, Riegler L, Bianchi R, Crisci M, Di Palma G, Golino P, Russo MG, Calabrò R, Calabrò P*
- 555 Risks and diagnosis of coronary artery disease in Hodgkin lymphoma survivors  
*Kupeli S*
- 562 Non-invasive evaluation of arrhythmic risk in dilated cardiomyopathy: From imaging to electrocardiographic measures  
*Iacoviello M, Monitillo F*
- 577 Mechanisms underlying the impaired contractility of diabetic cardiomyopathy  
*Ward ML, Crossman DJ*
- 585 Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis  
*Satoh H, Sano M, Suwa K, Saitoh T, Nobuhara M, Saotome M, Urushida T, Katoh H, Hayashi H*
- 602 Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment  
*Komamura K, Fukui M, Iwasaku T, Hirotsu S, Masuyama T*
- 610 Stem cell mechanisms during left ventricular remodeling post-myocardial infarction: Repair and regeneration  
*Zamilpa R, Navarro MM, Flores I, Griffey S*
- 621 Chronic total occlusion: To treat or not to treat  
*Bardaji A, Rodriguez-López J, Torres-Sánchez M*

- 630 Significance of lead aVR in acute coronary syndrome  
*Tamura A*

**REVIEW**

- 638 Calpain system and its involvement in myocardial ischemia and reperfusion injury  
*Neuhof C, Neuhof H*
- 653 Neuregulin-1/erbB activities with focus on the susceptibility of the heart to anthracyclines  
*Vasti C, Hertig CM*

**MINIREVIEWS**

- 663 Assessment of coronary artery disease using coronary computed tomography angiography and biochemical markers  
*Gitsioudis G, Katus HA, Korosoglou G*

**CASE CONTROL STUDY**

- 671 Lipid profile in children with coronary artery disease in Sindh, Pakistan  
*Baloch S, Devrajani BR, Baloch MA, Pir MA*

**RETROSPECTIVE STUDY**

- 675 Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis?  
*Chellamuthu S, Smith AM, Thomas SM, Hill C, Brown PWG, Al-Mohammad A*

**CASE REPORT**

- 682 Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon  
*Barsoum EA, Saiful FB, Asti D, Morcus R, Khoeiry G, Lafferty J, McCord DA*
- 685 Worsening of coronary spasm during the perioperative period: A case report  
*Teragawa H, Nishioka K, Fujii Y, Idei N, Hata T, Kurushima S, Shokawa T, Kihara Y*

**LETTER TO THE EDITOR**

- 689 3D-echo in preoperative assessment of aortic cusps effective height  
*Nijs J, Gelsomino S, Kietselaer BBLJH, Parise O, Lucà F, Maessen JG, La Meir M*

## Contents

*World Journal of Cardiology*  
Volume 6 Number 7 July 26, 2014

**APPENDIX** I-V Instructions to authors

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## WJC 6<sup>th</sup> Anniversary Special Issues (1): Hypertension

# Antihypertensive drugs and glucose metabolism

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## Abstract

Hypertension plays a major role in the development and progression of micro- and macrovascular disease. Moreover, increased blood pressure often coexists with additional cardiovascular risk factors such as insulin resistance. As a result the need for a comprehensive management of hypertensive patients is critical. However, the various antihypertensive drug categories have different effects on glucose metabolism. Indeed, angiotensin receptor blockers as well as angiotensin converting enzyme inhibitors have been associated with beneficial effects on glucose homeostasis. Calcium channel blockers (CCBs) have an overall neutral effect on glucose metabolism. However, some members of the CCBs class such as amlodipine and nifedipine have been shown to have advantageous effects on glucose homeostasis. On the other hand, diuretics and  $\beta$ -blockers have an overall disadvantageous effect on glucose metabolism. Of note, carvedilol as well as nebivolol seem to differentiate themselves from the rest of the  $\beta$ -blockers class, being more attractive options regarding their effect on glucose homeostasis. The adverse effects of some blood pressure lowering drugs on glucose metabolism may, to an extent, compromise their cardiovascular protective role. As a result the effects on glucose homeostasis of the various blood pressure lowering drugs should be taken into account when

selecting an antihypertensive treatment, especially in patients which are at high risk for developing diabetes.

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**Key words:** Hypertension; Glucose metabolism; Antihypertensive drugs

**Core tip:** Hypertension is a major contributor to the development and progression of cardiovascular disease. Increased blood pressure often coexists with insulin resistance. The various antihypertensive drugs have different effect on glucose metabolism. Indeed, angiotensin receptor blockers as well as angiotensin converting enzyme inhibitors have been associated with beneficial effects on glucose homeostasis. Calcium channel blockers are considered to have neutral metabolic effects. On the other hand, diuretics and  $\beta$ -blockers have an overall disadvantageous effect on glucose metabolism. As a result the metabolic effects of the various blood pressure lowering drugs should be taken into account when selecting an antihypertensive treatment.

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## INTRODUCTION

Hypertension is a growing epidemic affecting an important percentage of the population<sup>[1]</sup>. Hypertensive patients have increased risk for the development and progression of both microvascular and macrovascular complications. As a result the need for a comprehensive management of high blood pressure is essential.

Hypertension is strongly associated with risk factors that impair glucose homeostasis and is often presented as a component of the metabolic syndrome. Indeed,

hypertension is related with obesity, insulin resistance as well as diabetes mellitus<sup>[2,3]</sup>. As a result, hypertensive patients have a 2.5-fold higher risk of type 2 diabetes mellitus (T2DM) onset compared with normotensive subjects<sup>[4]</sup>. The various classes of antihypertensive drugs have different effects on blood glucose metabolism. Indeed, antihypertensive agents, such as  $\beta$ -blockers and thiazide diuretics have been associated with negative effects on blood glucose in contrast to other classes, such as angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACE-I). As a result, the treatment of hypertensive subjects should be carefully selected as to not further deteriorate an already at risk glucose homeostasis.

## RESEARCH

We searched PubMed up to December 2013 using combinations of the following keywords: hypertension, glucose metabolism, glucose homeostasis, antihypertensive drugs, angiotensin converting enzyme inhibitors, ARBs, calcium channel blockers (CCB),  $\beta$  blockers, renin inhibitors, alpha blockers, diuretics. Major randomized controlled trials, original papers, review articles and case reports were included. The references of these articles were scrutinized for relevant articles. For articles not written in English, only the abstracts were considered. A minor limitation of this review is that our literature search was exclusively based on the PubMed database.

## RENIN ANGIOTENSIN ALDOSTERONE SYSTEM

The renin angiotensin aldosterone system (RAAS) is strongly associated with glucose homeostasis. A number of studies have identified antihypertensive drugs that act by intervening in the RAAS as overall having beneficial effects on glucose metabolism.

### Angiotensin converting enzyme inhibitors

The majority of clinical trials evaluating the effects of ACE-I on glucose metabolism have showed a positive outcome. Large clinical trials have revealed that ACE-I are associated with a lower incidence of new-onset T2DM in hypertensive subjects. The heart outcomes prevention evaluation (HOPE) study demonstrated the favorable influence of ramipril on cardiovascular (CV) disease (CVD) incidence in high risk patients<sup>[5]</sup>. Patients recruited were  $\geq 55$  years old, had a history of coronary artery disease, stroke, peripheral vascular disease, or diabetes plus at least one other CV risk factor [hypertension, elevated total cholesterol levels, low high-density lipoprotein cholesterol (HDL-C) levels, cigarette smoking, or documented microalbuminuria]. For a mean period of 5 years the HOPE trial randomized the above high-risk patients ( $n = 9279$ ) to ramipril (10 mg/d) or placebo. Ramipril reduced new onset diabetes by 34% ( $P < 0.001$  *vs* placebo)<sup>[5]</sup>. However, there are some limitations of

the HOPE results regarding new onset diabetes. Indeed, diabetes development in HOPE was not a pre-specified endpoint of the study. Moreover, the diagnosis of diabetes was patient reported.

Similarly, the Captopril Prevention Project (CAPPP) study was a prospective, randomized trial which compared the effect of captopril *vs* antihypertensive treatment with diuretics,  $\beta$ -blockers, or both in hypertensive patients ( $n = 10985$ )<sup>[6]</sup>. Treatment with captopril was associated with fewer patients developing diabetes compared with the control group (OR = 0.79; 95%CI: 0.67-0.94;  $P = 0.007$ ). However, because of the design of the study, the query arises as to whether the differences in development of T2DM in the CAPPP trial were due to a protective effect of ACE-I or a deleterious effect of  $\beta$ -blockers and diuretics. Another limitation of the study was that blood pressure as well as diabetes mellitus at baseline was more common in the captopril group than in the group that received conventional treatment. In addition, in the captopril group a diuretic or a CCB was added to treatment in order to achieve the blood pressure goal.

The Antihypertensive and Lipid-Lowering Treatment to prevent heart attack trial (ALLHAT) was a randomized, double-blind, trial which evaluated whether treatment with a CCB or an ACE-I lowers the incidence of coronary heart disease (CHD) or other CVD events *vs* treatment with a diuretic<sup>[7]</sup>. Patients ( $n = 33357$ ) with hypertension and at least one other cardiac heart disease risk factor were randomized to chlorthalidone, amlodipine, or lisinopril for a mean follow-up of 4.9 years. Lisinopril treatment reduced the relative risk of developing T2DM by 30% (95%CI: 23%-37%;  $P < 0.001$ ) compared with patients treated with chlorthalidone and by 17% (95%CI: 7%-26%;  $P < 0.01$ ) compared with patients treated with the amlodipine<sup>[7]</sup>.

The studies of left ventricular dysfunction (SOLVD) was a double-blind trial which randomized patients with asymptomatic left ventricular (LV) dysfunction to receive enalapril or placebo for a mean follow-up of 37.4 mo<sup>[8]</sup>. Enalapril significantly reduced the incidence of heart failure and the rate of related hospitalizations compared with placebo<sup>[8]</sup>. A retrospective study evaluated the effect of enalapril on the incidence of diabetes in patients from the SOLVD trial<sup>[9]</sup>. Enalapril significantly reduced the incidence of diabetes compared with placebo (HR = 0.22; 95%CI: 0.10-0.46;  $P < 0.0001$ )<sup>[9]</sup>.

On the other hand, some studies have shown that ACE-I have a neutral effect on glucose metabolism. A study in patients with T2DM and hypertension ( $n = 24$ ) resulted in no change in insulin sensitivity aftertrandolapril treatment<sup>[10]</sup>. Similarly, enalapril treatment did not affect insulin sensitivity in patients with essential hypertension ( $n = 20$ )<sup>[11]</sup>. Moreover, lisinopril did not affect insulin sensitivity in healthy volunteers ( $n = 22$ )<sup>[12]</sup>. The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication (DREAM) study evaluated the effects of ramipril or placebo in patients ( $n = 5269$ ) without CVD but with impaired fasting glucose levels or impaired glucose tolerance. Patients received ramipril (up to 15 mg

per day) or placebo (and rosiglitazone or placebo) for a median of 3 years<sup>[13]</sup>. Although ramipril treatment did not reduce the incidence of diabetes, it increased regression to normoglycemia in the study population ( $P = 0.001$ ). The Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication Ongoing Follow-up (DREAM On) study followed patients from the DREAM trial for a median 1.6 years after the end of the trial<sup>[14]</sup>. Ramipril did not influence diabetes occurrence. Similarly, regression to normoglycemia was not altered by ramipril.

A meta-analysis of randomized control trials associated ACE-I treatment with a reduction of new-onset T2DM (RR = 0.73; 95%CI: 0.63-0.84)<sup>[15]</sup>. Similar were the results of another meta-analysis of randomized clinical trials where ACE-I had a smaller incidence of new-onset T2DM (OR = 0.77; 95%CI: 0.72-0.82;  $P < 0.0006$ ) compared with control groups<sup>[16]</sup>.

### ARBs

Treatment with ARBs has also been associated with an overall beneficial effect on glucose homeostasis. Indeed, large clinical trials have associated ARB treatment with lower incidence of new-onset T2DM. The losartan intervention for endpoint reduction (LIFE) in hypertension study was a double-blinded, randomized, parallel-group trial in patients ( $n = 9193$ ) aged 55-80 years with essential hypertension (sitting blood pressure 160-200/95-115 mmHg) and LV hypertrophy<sup>[17]</sup>. Patients were randomized to losartan or atenolol based antihypertensive treatment for a mean follow-up of 4.8 years<sup>[17]</sup>. Losartan treatment was associated with a reduction of new-onset T2DM compared with the control group (HR = 0.75; 95%CI: 0.63-0.88;  $P = 0.001$ ).

The Antihypertensive treatment and Lipid Profile In a North of Sweden Efficacy Evaluation (ALPINE) study compared the effect of hydrochlorothiazide, alone or in combination with atenolol, against candesartan, alone or in combination with felodipine, in newly diagnosed patients with primary hypertension ( $n = 342$ )<sup>[18]</sup>. After 12 mo, fasting plasma glucose and fasting serum insulin increased in the diuretic group, while a decrease was observed in the candesartan group ( $P < 0.001$  for the comparison of the 2 groups). The incidence of new-onset T2DM was higher in the hydrochlorothiazide (4.1%) group compared with the candesartan group (0.5%;  $P = 0.03$ )<sup>[18]</sup>.

The Valsartan Antihypertensive Long-term Use Evaluation (VALUE) was a prospective, double-blind, randomized trial that recruited hypertensive patients with additional CV risk factors<sup>[19]</sup>. Study subjects were randomized to either valsartan or amlodipine based regimen. Drug up-titration or the addition of further antihypertensive drugs, excluding ARBs, was allowed to achieve BP control. The valsartan based group had a smaller incidence of new-onset T2DM compared with the amlodipine group (HR = 0.77; 95%CI: 0.69-0.86;  $P < 0.0001$ )<sup>[19]</sup>.

The Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) study was a double-blind randomized control trial which

evaluated candesartan *vs* placebo in patients with heart failure ( $n = 7599$ ) for a median follow-up of 37.7 mo<sup>[20]</sup>. Among patients without a history of diabetes, new-onset T2DM was significantly lower in the candesartan group compared with the placebo group (HR = 0.78; 95%CI: 0.64-0.96;  $P = 0.020$ )<sup>[20]</sup>. The CHARM program consisted of 3 component trials, each comparing candesartan with placebo in a distinct population of patients with symptomatic heart failure: (1) the CHARM-Alternative which included patients with LV ejection fraction (LVEF)  $\leq 40\%$  and intolerant of ACE-I; (2) the CHARM-Added which included patients with LVEF  $\leq 40\%$  who were treated with an ACE-I; and (3) the CHARM-Preserved which included patients with LVEF  $> 40\%$ . The candesartan group had a smaller incidence of T2DM compared with placebo only in the CHARM-Preserved trial (OR = 0.60; 95%CI: 0.41-0.86;  $P = 0.005$ ).

The nateglinide and valsartan in impaired glucose tolerance outcomes research (NAVIGATOR) was a double-blind, randomized clinical trial in subjects with impaired glucose tolerance with known CVD or with CV risk factors<sup>[21]</sup>. Patients ( $n = 9518$ ) were randomized to receive valsartan (up to 160 mg daily) or placebo for a median of 5.0 years. The valsartan group had a smaller incidence of T2DM compared with placebo (HR = 0.86; 95%CI: 0.80-0.92;  $P < 0.001$ )<sup>[21]</sup>. Despite the reduction of T2DM incidence, valsartan treatment did not reduce the rate of CV events.

On the other hand, the Study on Cognition and Prognosis in the Elderly (SCOPE) evaluated the effects of candesartan *vs* placebo in elderly patients aged 70-89 years ( $n = 4964$ ) with hypertension for a mean follow-up of 3.7 years<sup>[22]</sup>. Open-label active antihypertensive therapy was added as needed. There was not a significant difference regarding new-onset T2DM between the 2 groups<sup>[22]</sup>. Similarly the CHARM-Added as well as the CHARM-Alternative studies did not show a difference regarding new-onset T2DM with candesartan treatment<sup>[20]</sup>.

A number of meta-analyses indicate the protective role of ARB treatment regarding T2DM development. Geng *et al*<sup>[23]</sup> in a meta-analysis of 11 randomized control trials with 79773 patients (59862 non-diabetic patients at baseline) showed a beneficial effect of ARBs on T2DM development. Incidence of new-onset diabetes was significantly reduced in the ARBs group compared with controls (OR = 0.79; 95%CI: 0.74-0.84). This reduction of T2DM incidence was apparent in the comparison of ARBs to placebo (OR = 0.83; 95%CI: 0.78-0.89),  $\beta$ -blockers (OR = 0.73; 95%CI: 0.62-0.87), CCBs (OR = 0.76; 95%CI: 0.68-0.85) and non-ARBs (OR = 0.57; 95%CI: 0.36-0.91)<sup>[23]</sup>. ARBs were associated with significant reduction in the risk of new-onset diabetes in patients with hypertension (OR = 0.74; 95%CI: 0.68-0.81), heart failure (OR = 0.70; 95%CI: 0.50-0.96), impaired glucose tolerance (OR = 0.85; 95%CI: 0.78-0.92) or cardiocerebrovascular diseases (OR = 0.84; 95%CI: 0.72-0.97). A meta-analysis by Abuissa *et al*<sup>[15]</sup> of randomized controlled trials associated ARBs treatment with



a reduction of new-onset T2DM [RR = 0.77 (95%CI: 0.71-0.83)]<sup>[15]</sup>. Another meta-analysis of randomized clinical trials showed that ARBs had a smaller risk of new-onset T2DM (OR = 0.79; 95%CI: 0.73-0.85;  $P < 0.00001$ ) compared with control groups<sup>[16]</sup>. Similarly, Cheung et al in a meta-analysis of studies with ARBs showed that sartans were associated with a decrease of new-onset diabetes<sup>[24]</sup>.

**Telmisartan:** Among members of the ARB family, some have the ability to partially activate PPAR $\gamma$ . Indeed, when various ARBs were evaluated regarding their PPAR $\gamma$  activating capacity, telmisartan was identified as the most prominent one<sup>[25,26]</sup>. Irbesartan was also associated with a milder activation of PPAR $\gamma$ . However only telmisartan retained its PPAR $\gamma$ -activating ability in lower concentrations usually attained during oral drug treatment<sup>[25]</sup>. This capacity of telmisartan can be attributed, at least partially, to its unique structure which differentiates it from other ARBs as well as to its structural resemblance with pioglitazone, a full PPAR $\gamma$  agonist<sup>[25]</sup>. Telmisartan in contrast to thiazolidinediones is only a partial PPAR $\gamma$  agonist. This leads to a diverse but overlapping gene expression compared with full activation of PPAR $\gamma$  and thus bestowing upon telmisartan unique pleiotropic effects<sup>[27]</sup>.

A number of studies have identified telmisartan as having beneficial effects on glucose homeostasis both in non-diabetic subjects<sup>[28,29]</sup> as well as diabetic patients<sup>[30,31]</sup>. Furthermore, studies comparing telmisartan with other ARBs have shown that telmisartan had more favorable effects on glycemic profile<sup>[32,33]</sup>. Hypertension often co-exists with dyslipidemia as commonly seen in metabolic syndrome. Moreover, there have been studies associating statin treatment with deteriorating effects on glucose metabolism<sup>[34-37]</sup>. Indeed, in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial (JUPITER)<sup>[38]</sup> rosuvastatin was associated with an increase in physician-reported newly diagnosed diabetes ( $P = 0.01$ ) and an increase in glycated hemoglobin (HbA1c) *vs* placebo ( $P = 0.001$ ). We have shown that telmisartan not only retains its beneficial effects on glucose homeostasis when co-administered with a statin, but also seems to negate any adverse effect of statin therapy on glycemic indices<sup>[39]</sup>. Patients ( $n = 151$ ) with mixed dyslipidemia, stage 1 hypertension and prediabetes were randomized to receive rosuvastatin (10 mg/d) plus telmisartan 80 mg/d or irbesartan 300 mg/d or olmesartan 20 mg/d<sup>[39]</sup>. After 6 mo, the homeostasis Model Assessment Insulin Resistance (HOMA-IR) index improved only in the telmisartan group (-29%) compared with either irbesartan (+16%;  $P < 0.01$  *vs* RT) or olmesartan group (+14%;  $P < 0.05$  *vs* RT) ( $P < 0.05$  for all *vs* baseline).

A number of large clinical trials have evaluated the effect of telmisartan on the incidence of new-onset T2DM. The Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET) evaluated the effects of telmisartan on hard clinical endpoints<sup>[40]</sup>. High risk patients ( $n = 25620$ ) with

coronary, peripheral or cerebrovascular disease or diabetes with end-organ damage were randomized to 3 groups and were followed for a median period of 56 mo. The first group received telmisartan (80 mg/d), the second group ramipril (10 mg/d) and the third group telmisartan plus ramipril (80/10 mg/d). The ONTARGET trial did not reveal any difference between ramipril (6.7%) and telmisartan (7.5%; HR = 1.12; 95%CI: 0.97-1.29) regarding new onset diabetes<sup>[40]</sup>.

The Telmisartan Randomised Assessment Study in ACE intolerant subjects with CVD (TRANSCEND) came as a complementary study to ONTARGET<sup>[41]</sup>. High risk patients ( $n = 5926$ ) intolerant to ACE-I with coronary, peripheral or cerebrovascular disease or diabetes with end-organ damage were randomized to telmisartan (80 mg/d) or placebo on top of any current therapy. TRANSCEND had the same primary endpoint as ONTARGET. A clear trend in reducing new clinical diagnosis of diabetes with telmisartan was seen in the TRANSCEND trial. The telmisartan group had lower new diabetes incidence (11%) *vs* placebo (12.8%;  $P = 0.081$ ).

The prevention regimen for effectively avoiding second strokes (PROFESS) study evaluated the effects of telmisartan on stroke incidence after a mean period of 30 mo<sup>[42]</sup>. Patients ( $n = 20332$ ) with a history of recent ischemic stroke were randomly assigned ( $2 \times 2$ ) to receive either both aspirin (25 mg/twice daily) and extended-release dipyridamole (200 mg/twice daily) or clopidogrel (75 mg/d); and telmisartan (80 mg/d) or placebo. Similarly, in the PROFESS a trend was seen in reducing new onset diabetes with telmisartan (1.2%) *vs* placebo (1.5%;  $P = 0.1$ ).

Although the TRANSCEND and PROFESS both only showed trends for the reduction of new-onset T2DM, it should be noted that both of them had some limitations regarding their power to identify beneficial effects of telmisartan on diabetes onset. Indeed, more than one third of the TRANSCEND population had already a history of diabetes, thus decreasing the power of the remaining study population to detect any mild beneficial effect on T2DM development. Moreover, a great percentage (37%) of the PROFESS population was already treated with ACE-Is, which have an established overall positive effect regarding new onset diabetes prevention<sup>[43]</sup>. Therefore, again any benefits of telmisartan would be harder to detect on-top of an ACE-I therapy. Moreover, the PROFESS had a much smaller follow up period in contrast to studies with ARBs that showed benefits in new onset diabetes like the LIFE<sup>[17]</sup> and VALUE<sup>[19]</sup>. Indeed, the PROFESS population was monitored for 2.5 years *vs* 4.8 and 4.2 years for the LIFE and VALUE populations, respectively. This difference could explain why telmisartan showed only a trend for reduction of new onset diabetes.

### Renin inhibitors

Aliskiren is the first approved renin inhibitor which acts by directly inhibiting the renin enzyme at the point of



RAAS activation, blocking the conversion of angiotensinogen to angiotensin I and decreasing levels of angiotensin I and angiotensin II<sup>[44]</sup>. A limited number of studies have evaluated the capacity of aliskiren to affect glucose metabolism. In a recent study, hypertensive patients with abnormal LV diastolic dysfunction but with normal LV systolic function ( $n = 78$ ) were randomized to aliskiren (up to 300 mg/d) treatment or control group which was treated with  $\beta$ -blockers or CCBs<sup>[45]</sup>. Fasting insulin and glucose remained unchanged in the aliskiren group, in contrast to the control group where an increase in both fasting insulin ( $P = 0.03$ ) and glucose ( $P = 0.003$ ) were observed. In another double-blind trial, patients with diabetes mellitus and hypertension ( $n = 837$ ) were randomized to once-daily aliskiren (150 mg titrated to 300 mg after four weeks), ramipril (5 mg titrated to 10 mg) or the combination for eight weeks<sup>[46]</sup>. No changes in HbA1c and fasting plasma glucose were observed in any treatment group. Another study randomized hypertensive patients with metabolic syndrome to aliskiren (300 mg/d) or losartan (100 mg/d)<sup>[47]</sup>. At study end patients performed an euglycemic hyperinsulinemic clamp and insulin sensitivity was assessed by glucose infusion rate. Insulin resistance improved only in the aliskiren group compared with losartan group ( $P < 0.05$  between groups).

### Mechanisms

The RAAS plays a major role both in the pathogenesis of hypertension as well as glucose homeostasis. As a result, a number of mechanisms have been suggested that can play a role in the overall beneficial effect that drugs which effect RAAS have on glucose metabolism.

Bradykinin may play an important role towards a beneficial effect on glucose homeostasis. The ACE beyond the conversion of angiotensin I to angiotensin II can also decrease bradykinin levels<sup>[48]</sup>. Indeed, ACE promotes the degradation of bradykinin to inactive fragments *via* a kininase II - mediated mechanism<sup>[49]</sup>. As a result, ACE-I can increase bradykinin levels<sup>[50]</sup>. Bradykinin has been shown to promote insulin sensitivity at the skeletal muscle level<sup>[51,52]</sup>.

The principal glucose transporter protein that mediates insulin-stimulated glucose transport into muscle and adipose tissues is the glucose transporter type 4 (GLUT4), thus playing a key role in the regulation of glucose homeostasis<sup>[53]</sup>. Angiotensin II decreases GLUT-4 translocation to the cell membrane<sup>[54,55]</sup>. As a result the RAAS inhibition could promote insulin sensitivity. Indeed, the inhibition of AT1 receptors prevented the decline of GLUT-4 in a diabetic rat heart model<sup>[56]</sup>. Moreover, both ACEIs and ARBs have been associated with increase of GLUT-4 protein expression in skeletal muscle and myocardium in insulin-resistant animal models<sup>[57]</sup>.

Moreover, angiotensin II inhibits adipogenic differentiation of human adipocytes *via* the AT1 receptor<sup>[58]</sup>. Angiotensin II may inhibit preadipocytes recruitment, resulting in the storage of lipids in muscle and other tissues, thus increasing insulin resistance<sup>[59]</sup>. As a result, the

blockade of RAAS would promote the recruitment of preadipocytes thereby increasing the number of small insulin-sensitive adipocytes leading to improved insulin sensitivity.

Furthermore, angiotensin II can promote the production of inflammatory cytokines<sup>[60]</sup>. Inflammatory cytokines promote oxidative stress thus also leading to increased insulin resistance. In addition, endothelial dysfunction is also associated with insulin resistance<sup>[61]</sup>. Angiotensin converting enzyme inhibitors have also been shown to improve vascular function and insulin-mediated vascular responses<sup>[61]</sup>. Furthermore, ACE-I may also have direct beneficial effects on pancreatic  $\beta$  cells<sup>[62]</sup>.

In addition ACE inhibition can lead to vasodilation of blood vessels<sup>[63]</sup>. This vasodilation has as a result the increment of total perfused area and thus increases glucose uptake and insulin sensitivity<sup>[64,65]</sup>. The activation of the sympathetic nervous system has also been associated with insulin resistance<sup>[66]</sup>. Both ACE-I<sup>[67]</sup> as well as ARBs<sup>[68]</sup> have been shown to decrease levels of catecholamines such as norepinephrine and epinephrine, thus further contributing to amelioration of insulin resistance.

Potassium levels play a significant role in insulin secretion since hypokalemia substantially impairs the insulin secretory response to glucose. As a result the increase of potassium levels by inhibiting the RAAS may also contribute to the improvement of glucose levels. Moreover, magnesium has also been shown to affect glucose homeostasis. Indeed, magnesium deficiency is associated with both a reduced cellular insulin action<sup>[69]</sup> and impaired insulin secretion<sup>[70]</sup>. The inhibition of the RAAS system leads to increased magnesium levels. A pooled analysis of studies using ACEIs in patients ( $n = 96$ ) with essential hypertension found that changes in insulin sensitivity index (ISI) were directly correlated to alterations in serum magnesium levels<sup>[71]</sup>.

### CCBS

CCBs are generally considered as having an overall neutral metabolic profile. Indeed, a recent meta-analysis of 10 randomized clinical trials evaluated the effect of CCB treatment on new onset T2DM<sup>[72]</sup>. The overall risk of diabetes among subjects taking CCBs was not significant (RR = 0.99; 95%CI: 0.85-1.15). Compared with other classes of antihypertensive drugs, CCBs were associated with a higher incidence of diabetes than ACEIs (pooled risk ratio 1.23; 95%CI: 1.01-1.51) or ARBs (1.27; 95%CI: 1.14-1.42) and a lower incidence compared with  $\beta$ -blockers (RR = 0.83; 95%CI: 0.73-0.94) or diuretics (RR = 0.82; 95%CI: 0.69-0.98).

Another recent meta-analysis of 5 clinical trials compared the efficacy of ARBs and CCBs regarding their effect on insulin resistance as assessed using the HOMA-IR index in non-diabetic patients<sup>[73]</sup>. Both ARBs and CCBs had a similar effect on blood pressure reduction. However, ARBs reduced the HOMA-IR index (weighted mean difference -0.65, 95%CI: -0.93--0.38) and fasting plasma insulin (weighted mean difference -2.01, 95%CI:

-3.27--0.74) significantly more than CCBs. A recent re-analysis of data from the NAVIGATOR trial showed that CCBs were not associated with new onset diabetes (HR = 0.95; 95%CI: 0.79-1.13)<sup>[74]</sup>.

Of note overdose of CCB has been associated with hyperglycemia primarily due to the blockade of pancreatic L-type calcium channels and insulin resistance on the cellular level<sup>[75]</sup>.

However, not all members of the CCB class have the same effect on glucose homeostasis. Indeed, azelnidipine has been associated with beneficial effect on glucose homeostasis in a diabetic animal model<sup>[76]</sup>. Moreover, similar beneficial effects were seen in a small study in non-diabetic patients ( $n = 17$ ) with essential hypertension who had controlled blood pressure levels using amlodipine (5 mg/d)<sup>[77]</sup>. Azelnidipine (16 mg/d) or amlodipine (5 mg/d) was administered in a crossover design for 12-wk. Despite similar blood pressure reduction, azelnidipine significantly decreased levels of glucose and insulin 120 min after the 75 g oral glucose tolerance test (OGTT) ( $P < 0.05$  *vs* amlodipine). This effect may be associated with the anti-inflammatory effects of azelnidipine<sup>[78]</sup>, since proinflammatory cytokines have been associated with impaired glucose tolerance<sup>[79]</sup>. Furthermore, azelnidipine inhibits the enhancement of sympathetic nervous activity<sup>[80]</sup>. Since the activation of the sympathetic nervous system has been associated with insulin resistance<sup>[66]</sup>, azelnidipine treatment may contribute to the amelioration of insulin resistance.

Another interesting member of the CCB class is manidipine<sup>[81]</sup>. A beneficial effect on insulin resistance has been shown with manidipine treatment<sup>[82]</sup>. The beneficial effects of manidipine have been observed in both non-diabetic and T2DM patients<sup>[83,84]</sup>. Furthermore, we have recently shown that manidipine can ameliorate the possible statin-associated increase in insulin resistance<sup>[85]</sup>. In a prospective, randomized, open-label, blinded endpoint study a total of 40 patients with impaired fasting glucose, mixed dyslipidemia, and stage 1 hypertension were included. Patients were randomly allocated to rosuvastatin (10 mg/d) plus olmesartan (20 mg/d) or manidipine (20 mg/d). After 3 mo, a significant increase in HOMA-IR index by 14% ( $P = 0.02$  *vs* baseline) was seen in the olmesartan plus rosuvastatin group. On the other hand, HOMA-IR index did not change in the manidipine plus rosuvastatin group ( $P = \text{NS}$  *vs* baseline;  $P = 0.04$  *vs* olmesartan plus rosuvastatin group). This favorable effect of manidipine may be linked to the drug's capacity to partially activate the PPAR $\gamma$  which plays a major role in glucose metabolism<sup>[82]</sup>. Indeed, the effect of manidipine to activate PPAR $\gamma$  is about two-thirds of that of pioglitazone, a full PPAR $\gamma$  activator<sup>[82]</sup>. This partial activation of PPAR $\gamma$  may contribute to the avoidance of side effects commonly associated with thiazolidinediones treatment. Moreover, an increase of adiponectin levels (which are inversely associated with the development of insulin resistance and metabolic syndrome) has been observed with manidipine<sup>[86]</sup>. Furthermore, manidipine induces a smaller activation of the sympathetic nervous

system, which can also play a role in the beneficial effects on glucose homeostasis. Indeed, when compared with other CCBs, manidipine is associated with lower levels of plasma norepinephrine<sup>[87]</sup>.

## **$\beta$ -BLOCKERS**

A number of studies have associated treatment with  $\beta$ -blockers as having a disadvantageous effect on glucose homeostasis<sup>[88-91]</sup>. Indeed, a prospective study of three cohorts, namely the Nurses' Health Study (NHS) I and II and the Health Professionals Follow-up Study evaluated the association between the use of different classes of antihypertensive medications and the risk of T2DM incident<sup>[92]</sup>. Treatment with a  $\beta$ -blocker was associated with a greater risk for the development of diabetes. Similarly, in the Atherosclerosis Risk in Communities study  $\beta$ -blockers led to an increase of risk for new-onset T2DM (RR = 1.28; 95%CI: 1.04-1.57)<sup>[4]</sup>. A large meta-analysis of patients with hypertension ( $n = 94492$ ) treated with beta blockers evaluated the risk for the development of T2DM<sup>[93]</sup>. Beta-blocker therapy resulted in a 22% increased risk for new-onset T2DM (RR = 1.22, 95%CI: 1.12-1.33) compared with non-diuretic antihypertensive agents. On the other hand, a recent reanalysis of data from the NAVIGATOR trial showed that  $\beta$ -blockers were not associated with new onset diabetes (HR = 1.10, 95%CI: 0.92-1.31)<sup>[74]</sup>.

However, not all members of the  $\beta$  blocker class have similar effect on glucose homeostasis. Indeed, carvedilol as well as nebivolol have shown a differentiation from the rest of the class<sup>[94,95]</sup>. The Glycemic Effects in Diabetes Mellitus: Carvedilol-Metoprolol Comparison in Hypertensives (GEMINI) study was a randomized, double-blind, parallel-group trial that compared the effects of carvedilol and metoprolol tartrate on glycemic control<sup>[96]</sup>. Patients ( $n = 1235$ ) with hypertension ( $> 130/80$  mmHg) and T2DM that were already receiving RAS blockers were randomized to receive carvedilol (6.25-25 mg/twice daily) or metoprolol (50-200 mg/twice daily). Open-label hydrochlorothiazide and a dihydropyridine calcium antagonist were added, if needed, to achieve blood pressure target. While blood pressure control was similar between groups, a difference was seen regarding glucose effects. The HbA1c increased with metoprolol (by 0.15%;  $P < 0.001$ ) but not carvedilol (by 0.02%;  $P = 0.65$ ). Moreover, insulin sensitivity improved with carvedilol (9.1%;  $P = 0.004$ ) but not metoprolol (2.0%;  $P = 0.48$  *vs* baseline;  $P = 0.004$  between groups). Similarly, a study in subjects with metabolic syndrome compared nebivolol (5 mg/d) with metoprolol (100 mg/d)<sup>[97]</sup>. After 12-wk treatment both nebivolol and metoprolol had similarly decreased blood pressure and heart rate. However, metoprolol decreased insulin sensitivity compared with nebivolol ( $P = 0.03$ ).

## **Mechanisms**

Several possible mechanisms that may be responsible

for the disadvantageous effect of  $\beta$ -blockers have been described. Treatment with conventional  $\beta$ -blockers leads to an unopposed  $\alpha$ 1-activity which causes vasoconstriction and decreased blood flow to the muscles, which are an important organ in the regulation of glucose homeostasis<sup>[98,99]</sup>. As a result a decrease in insulin-stimulated glucose uptake would occur, leading to insulin resistance. Furthermore,  $\beta$ -blockers can also decrease the first phase of insulin secretion from pancreatic  $\beta$  cells<sup>[88,89]</sup>. In addition, treatment with  $\beta$ -blockers can also lead to weight gain<sup>[100]</sup>. Since increased body weight is strongly associated with insulin resistance<sup>[101]</sup>, this effect of  $\beta$ -blockers can further deteriorate glucose homeostasis.

## DIURETICS

An important class of antihypertensive drugs is diuretics. This class includes loop diuretics such as furosemide, thiazide diuretics such as hydrochlorothiazide, thiazide-like diuretics such as chlorthalidone and potassium-sparing diuretics, such as amiloride, eplerenone and spironolactone.

A number of studies have associated diuretic treatment of hypertension as having a negative effect on glucose homeostasis<sup>[18,102]</sup>. Indeed, a meta-analysis of 22 clinical trials with 143153 nondiabetic patients evaluated the effects of various antihypertensive drug classes on diabetes incidence<sup>[43]</sup>. Treatment with diuretic was associated with increased risk for new onset diabetes compared with other antihypertensive treatments as well as placebo<sup>[43]</sup>. A long-term cohort study with initially untreated hypertensive subjects ( $n = 795$ ) evaluated new-onset diabetes incidence according to antihypertensive treatment<sup>[103]</sup>. Diuretic treatment was present in 53.5% of subjects that developed T2DM, compared with 30.4% of patients that did not develop diabetes ( $P = 0.002$ ). Moreover, diuretic treatment was an independent predictor of new onset diabetes ( $P = 0.004$ ). Furthermore, a recent reanalysis of data from the NAVIGATOR trial showed that diuretics were associated with an increased risk of new onset diabetes (HR = 1.23, 95%CI: 1.06-1.44)<sup>[74]</sup>.

A post hoc subgroup analyses of the ALLHAT study among nondiabetic participants of the study who were randomized to receive chlorthalidone ( $n = 8419$ ), amlodipine ( $n = 4958$ ), or lisinopril ( $n = 5034$ ) evaluated the effects of antihypertensive treatment on glucose levels as well as new-onset diabetes<sup>[104]</sup>. Chlorthalidone treatment was associated with a greater risk for developing diabetes compared with the other 2 treatment regimens ( $P < 0.001$ )<sup>[104]</sup>. The Systolic Hypertension in the Elderly Program (SHEP) was a placebo-controlled, double-blind, randomized, multicenter clinical trial that evaluated the efficacy of chlorthalidone in patients ( $n = 4736$ ) with isolated systolic hypertension<sup>[105]</sup>. After 3 years of treatment, the incidence of new-onset diabetes was similar between the chlorthalidone (8.6%) and placebo group (7.5%;  $P = 0.25$  between groups)<sup>[105]</sup>. However, when study participants were re-evaluated after a mean follow-up of 14.3 years, 13.0% of patients developed diabetes in

the chlorthalidone group *vs* 8.7% in the placebo group ( $P < 0.0001$ )<sup>[106]</sup>.

Of note, chlorthalidone seems to be differentiated from the rest of the thiazide diuretics class<sup>[107]</sup>. Indeed, chlorthalidone has a different chemical structure compared with the rest of thiazide diuretics<sup>[107]</sup> as well as the ability to inhibit carbonic anhydrase<sup>[108]</sup>. Carbonic anhydrase regulates a number of CV related risk factors<sup>[109,110]</sup> and its activity is also directly proportional to increasing blood glucose concentration<sup>[111]</sup>. As a result, chlorthalidone may have a more favorable metabolic profile compared with the other thiazide diuretics<sup>[107]</sup>.

The effects of amiloride on blood glucose levels were evaluated in a study by Stears *et al*<sup>[112]</sup>. Patients with essential hypertension ( $n = 37$ ) received, in random order, 4 wk of once-daily treatment with hydrochlorothiazide (25-50 mg), nebivolol (5-10 mg), combination (hydrochlorothiazide 25-50 mg and nebivolol 5-10 mg), amiloride (10-20 mg), and placebo. Each drug was force titrated at 2 wk and separated by a 4-wk placebo washout. Both amiloride and hydrochlorothiazide had similar changes in blood pressure reduction. However, an increase of glucose levels after a 2 h OGTT was observed with hydrochlorothiazide treatment, while no change was seen with amiloride ( $P < 0.0001$ ).

The Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) evaluated the effects of eplerenone on new-onset diabetes mellitus in patients ( $n = 1846$ ) with mild heart failure<sup>[113]</sup>. After a follow-up of 21 mo, eplerenone had no effect on new-onset diabetes mellitus (HR = 0.94, 95%CI: 0.59-1.52). Another study compared the effects of eplerenone with spironolactone in patients ( $n = 107$ ) with mild chronic heart failure<sup>[114]</sup>. Spironolactone increased levels of HbA1c ( $P < 0.0001$ ), while no change was observed in the eplerenone group.

## Mechanisms

Among the possible mechanisms through which thiazide diuretics may affect glucose homeostasis, hypokalemia may play an important role<sup>[115]</sup>. Indeed, hypokalemia can lead to decreased insulin secretion by  $\beta$  cells in response to glucose, as well as decrease in blood flow in muscles. A quantitative review evaluating studies that used thiazide diuretics, found an inverse relationship between glucose and potassium with thiazide use<sup>[116]</sup>. Similar results were observed in an analysis of data from the SHEP study<sup>[117]</sup>. In the first year of the study among 3790 nondiabetic participants each 0.5-mEq/L decrease in serum potassium was independently associated with a 45% higher adjusted diabetes risk (95%CI: 24%-70%;  $P < 0.001$ ). However, a prespecified subgroup analysis of metabolic parameter data from patients participating in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study did not confirm a relationship between hypokalemia and deterioration of serum glucose levels<sup>[118]</sup>.

Moreover, a decrease in magnesium can be seen with diuretic treatment. This could also contribute to the dis-



advantageous effects of diuretics on glucose homeostasis, since hypomagnesaemia is an independent predictor of T2DM<sup>[119,120]</sup>. Furthermore, thiazide treatment is also associated with visceral fat redistribution, liver fat accumulation and low-grade inflammation, which in turn increase the risk of new-onset diabetes<sup>[121]</sup>.

## OTHER ANTIHYPERTENSIVE DRUGS

There is little evidence about the effects of other, less used, antihypertensive drugs on glucose homeostasis. A randomized, double-blind multicenter study compared moxonidine (0.2-0.6 mg/d) with metoprolol (50-150 mg/d) in hypertensive subjects ( $n = 127$ ) with T2DM<sup>[122]</sup>. After 12 wk of treatment both groups had similar blood pressure reductions as well as similar HbA1c values. However, fasting plasma glucose decreased in the moxonidine group, while an increase was seen in the metoprolol group ( $P < 0.05$ ). Furthermore, the HOMA-IR decreased with moxonidine in contrast to the increase observed with metoprolol. Another multicenter, prospective, randomized study compared moxonidine with metformin<sup>[123]</sup>. Patients older than 40 years old, with impaired glucose tolerance (or diabetes mellitus treated with diet alone) and a body mass index (BMI) of at least 27 kg/m<sup>2</sup> were treated twice daily with moxonidine 0.2 mg or metformin 500 mg for 16 wk. Compared with metformin, moxonidine decreased the area under the curve for insulin ( $P = 0.049$ ). On the other hand, only metformin significantly decreased fasting plasma glucose ( $P < 0.05$  *vs* baseline and *vs* moxonidine) as well as HbA1c ( $P < 0.005$  *vs* baseline). Both treatments similarly increased the Matsuda ISI from baseline to a comparable degree ( $P < 0.05$  *vs* baseline for both groups). Another randomized open parallel study evaluated the chronic effects of moxonidine *vs* amlodipine in obese hypertensive patients ( $n = 40$ )<sup>[124]</sup>. Plasma levels of insulin 120 min after glucose load, decreased with moxonidine treatment ( $P < 0.05$ ) while no change was seen with amlodipine. A multinational, open-label, observational study, the Moxonidine Efficacy on blood pressure Reduction revealed in a metabolic SYndrom population (MERSY) study evaluated the effects of moxonidine on serum metabolic parameters<sup>[125]</sup>. Patients with hypertension received moxonidine (0.2-0.4 mg/d) either as monotherapy or as adjunctive therapy for 6 mo. A beneficial trend in metabolic parameters such as fasting plasma glucose and body weight was observed with moxonidine.

A small study evaluated the effects of doxazosin in hypertensive non-insulin depended diabetic patients<sup>[126]</sup>. Doxazosin significantly improved insulin sensitivity during the euglycemic insulin clamp and enhanced OGTT. Similarly another small study showed a beneficial effect of doxazosin (2 mg or 4 mg daily for 3 mo) on insulin resistance indices in hypertensive patients ( $n = 21$ ) with T2DM.

## CONCLUSION

Hypertension is associated with increased morbidity and

mortality. Furthermore, hypertensive patients have an increased prevalence of insulin resistance as often is the case with metabolic syndrome subjects. This disturbance in glucose homeostasis further increases the risk for the development of CVD as well as the development of diabetes. The various antihypertensive drug categories have different effects on glucose metabolism. Indeed, ACE-I and ARBs have the most favorable effect on insulin resistance and the development of T2DM. Moreover, CCBs have an overall neutral metabolic effect. However, both azelnidipine and manidipine have been associated with beneficial glucose effects. On the other hand, diuretics as well as  $\beta$ -blockers have been associated with detrimental effects on glucose metabolism.

An interesting query is whether the adverse effects of some antihypertensive drug categories on glucose metabolism and their potency to increase new-onset diabetes mellitus incidence is also associated with an increase in CVD events. It would be reasonable to assume that the drug-induced increases in glucose levels and T2DM incidence would have increased CVD risk similarly to traditional risk factors for new-onset diabetes. However, no such increase in CVD risk was seen in the ALLHAT study in those who developed diabetes in the chlorthalidone treatment arm<sup>[127]</sup>. Similarly were the results from the SHEP study<sup>[106]</sup>. Diabetes at baseline was associated with increased CV mortality rate (adjusted HR = 1.659, 95%CI: 1.413-1.949) and total mortality rate (adjusted HR = 1.510, 95%CI: 1.347-1.693). Furthermore, diabetes that developed during the trial among subjects on placebo was also associated with increased CV adverse outcome (adjusted HR = 1.562, 95%CI: 1.117-2.184) and total mortality rate (adjusted HR = 1.348, 95%CI: 1.051-1.727). However, diabetes that developed among subjects during diuretic therapy did not have statistically significant associations with CV mortality rate (adjusted HR = 1.043, 95%CI: 0.745-1.459) or total mortality rate (adjusted HR = 1.151, 95%CI: 0.925-1.433). In addition, diuretic treatment in diabetic patients was strongly associated with lower long-term CV mortality rate (adjusted HR = 0.688, 95%CI: 0.526-0.848) and total mortality rate (adjusted HR = 0.805, 95%CI: 0.680-0.952). Of note, even if new-onset T2DM after diuretic or  $\beta$ -blocker is not associated with increased CVD morbidity and mortality, the health care cost should be considered. Indeed, the management and treatment costs of a hypertensive patient with diabetes are far greater compared with a non-diabetic patient.

On the other hand, the Progetto Ipertensione Umbria Monitoraggio Ambulatoriale study, a long-term cohort study in initially untreated hypertensive subjects with a median follow up of 6 years, identified diuretic treatment as an independent predictor of new onset diabetes ( $P = 0.004$ )<sup>[103]</sup>. Of interest, CV event risk was similar between diabetic subjects at study baseline and subjects that developed new-onset T2DM during the study. An interesting study, evaluated hypertensive subjects ( $n = 754$ ) and followed them long term for 25-28 years<sup>[128]</sup>. Patients were treated with thiazide diuretics and beta-adrenergic blocking drugs with the addition of hydralazine during



the first decade. Calcium antagonists were substituted for hydralazin and, if needed, ACE-I were added when these drugs became available. After 25 years, treatment with  $\beta$ -blockers was associated with new-onset T2DM. New-onset diabetes was associated with an increased risk for stroke (HR = 1.67; 95%CI: 1.1-2.6;  $P < 0.05$ ), myocardial infarction (OR = 1.66; 95%CI: 1.1-2.5;  $P < 0.05$ ) and mortality (OR = 1.42; 95%CI: 1.1-1.9;  $P < 0.05$ ). The mean observation time from onset of diabetes mellitus to a first stroke was 9.1 years and to a first myocardial infarction 9.3 years.

Despite the various effects of different antihypertensive drugs on glucose homeostasis, the overall expected benefits *vs* the potential risks should always be carefully weighted for each individual patient. As a result, when the benefits for a patient that should receive a treatment with an antihypertensive class with unfavorable glucose profile are greater than the risk of increased insulin resistance, then the glycemic effects of the antihypertensive drug should not disqualify the patient from treatment. Furthermore, there is often some diversity among the members of an antihypertensive class regarding their effect on glucose. As a result, the antihypertensive drug with the least adverse effect on glucose can be selected. Indeed, despite the overall adverse effect of the  $\beta$ -blockers families on glucose homeostasis, newer members of the class, such as carvedilol and nebivolol, have shown that they are clearly different from the rest regarding glucose effects.

Overall, when treating hypertensive patients the physician should carefully assess the individual patient's medical history which often dictates a particular treatment. When there are no contraindications, an antihypertensive drug with a favorable or at least neutral effect on glucose homeostasis should be selected. This way, any beneficial effects of lowering blood pressure would not be shadowed in any way by a worsening of the metabolic profile. Patients with a strong indication for receiving a  $\beta$ -blocker or a diuretic should not be disqualified only because of the negative effect of these drug categories on glucose homeostasis. When a drug with negative effects on glucose homeostasis is selected, the physician should have in mind the possible deterioration of glucose metabolism and increased risk for new-onset diabetes and thus follow-up the patient accordingly.

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## Hypertension and medical expenditure in the Japanese population: Review of prospective studies

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creased further in cases of hypertensive patients who have another concomitant cardiovascular risk factor. In particular, hypertension, especially moderate-to-severe untreated hypertension, increases the risk of long-term hospitalization resulting in considerably higher medical expenditure, compared with non-hospitalized cases. Therefore, assuming that the use of antihypertensive medication is essential for hypertensive patients to prevent serious vascular diseases, a cost-effective high-risk strategy needs to be considered to reduce both ill-health and the economic burden due to hypertension. However, from a population perspective, medical expenditure attributable to hypertension comes mainly from pre-to-mild hypertension. Therefore, there is also a need to consider a population strategy that aims to shift the entire population to lower levels of blood pressure.

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**Key words:** Hypertension; Medical expenditure; Japan; Cohort study

### Abstract

Hypertension is a major determinant of health and is likely to have an effect on medical economics. The economic burden due to hypertension may be attributable not only to antihypertensive medication but also to the very expensive procedures required for cases of cardiovascular disease that occur more frequently in hypertensive compared with normotensive individuals. The objective of this article was to review articles published on prospective cohort studies that measured medical expenditure attributable to hypertension in community-dwelling populations in Japan. Many medical services in these populations are provided under the medical insurance system that requires the enrolment of all Japanese residents. Personal medical expenditure attributable to hypertension increases with worsening severity of the condition. Medical expenditure was in-

**Core tip:** Hypertension is likely to affect medical economics. We reviewed articles published on prospective cohort studies that measured medical expenditure attributable to hypertension in community-dwelling populations in Japan. Personal medical expenditure attributable to hypertension increased with worsening severity of the condition. Medical expenditure was increased further in hypertensive patients who had another concomitant cardiovascular risk factor. In particular, hypertension, especially moderate-to-severe untreated hypertension, increased the risk of long-term hospitalization. This resulted in considerably higher medical expenditure, compared with non-hospitalized cases. However, from a population perspective, medical expenditure attributable to hypertension is mainly from pre-to-mild hypertension.

Nakamura K, Okamura T, Miura K, Okayama A. Hypertension and medical expenditure in the Japanese population: Review of prospective studies. *World J Cardiol* 2014; 6(7): 531-538 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/531.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.531>

## INTRODUCTION

Hypertension is a major cause of premature death and disability in the world mainly as a result of cardiovascular disease including coronary heart disease and stroke, and other vascular diseases<sup>[1,2]</sup>. This burden of ill-health represents an economic burden, which is attributable not only to antihypertensive medication but also to very expensive procedures such as percutaneous coronary intervention, coronary artery bypass grafts, neurosurgical treatment, or hemodialysis that are required in cases of serious vascular diseases that occur more frequently in hypertensive than normotensive individuals. Therefore, only prospective cohort studies can measure medical expenditure attributable solely to hypertension in the general population. This fundamental information is required when considering the cost-effectiveness of treating and preventing hypertension.

Japan provides an ideal situation to measure medical expenditure attributable to hypertension, as it is possible to use epidemiological methods to analyse data on health checkups and medical expenditure. Health checkups are commonly conducted in communities and worksites in Japan, whereas data on medical expenditure are available from the medical insurance system that controls medical cost nationwide and is compulsory for all Japanese residents (see ACKNOWLEDGMENTS)<sup>[3-5]</sup>. Several epidemiological studies have used these merits to examine the relationship between hypertension status and medical expenditure after a follow-up period in Japanese populations. The objective of this article is to review articles published on these epidemiological studies in Japan.

## SEARCH STRATEGY AND SELECTION

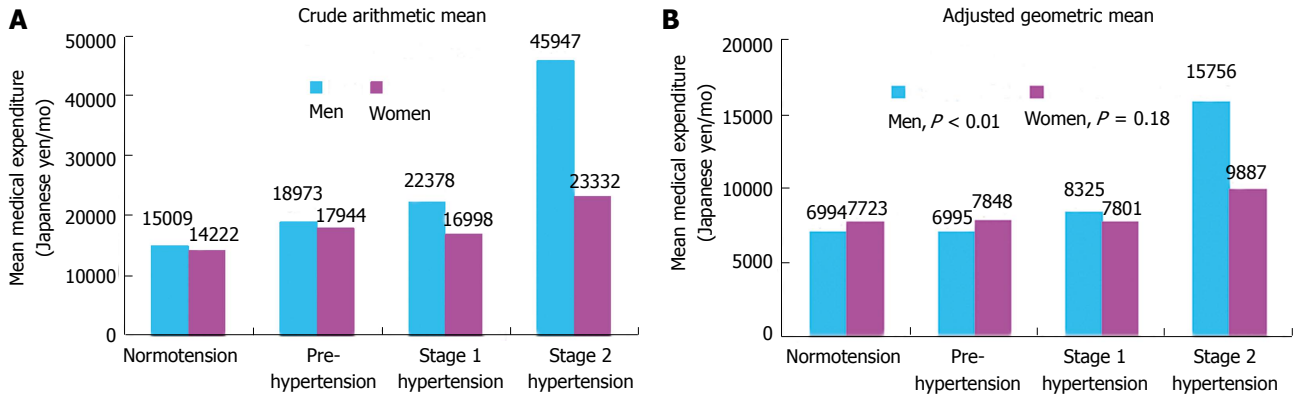
We performed a systematic search on Medline for relevant articles published between January 1966 and January 2014. We searched using medical subject headings (MeSH) terms and text words: {(hypertension [MeSH term], including MeSH terms found below this term in the MeSH tree) or (hypertension [text word]) or (high blood pressure [text word])} and {(costs and cost analysis [MeSH term], including MeSH terms found below this term in the MeSH tree) or (cost [text word]) or (expenditure [text word]) or (expense [text word])} and {(Japan [text word])}. We restricted the search to English language articles so that everyone could read the full texts if necessary. Using this search strategy we identified a total of 163 articles. We set the following inclusion criteria that suited the objectives of our study: (1) prospective cohort, but not cross-sectional studies, that examined the

relationship between hypertension status and subsequent medical expenditure; (2) studies conducted in a general Japanese population, but not a population that consisted solely of individuals with a particular high-risk condition or hospital patients; (3) hypertension status assessed by blood pressure measurement and/or medical history of taking antihypertensive medication, with medical expenditure being measured using insurance claim history files of the Japanese medical insurance system; and (4) studies that provided evidence about how much medical expenditure is incurred by hypertension and/or evidence on any relevant topics. We read the titles and abstracts of all the articles identified in the Medline search to exclude any articles that seemed irrelevant. The full texts of the remaining articles were read to determine if they met our inclusion criteria. Of the 163 articles identified, only six articles were considered as relevant and met our inclusion criteria. Although we manually searched for extra relevant articles in the reference lists of the identified articles and other publications, no additional relevant article was identified from these sources. Of the six relevant articles, three articles were from the same cohort study, but each dealt with different topics without duplicate publication<sup>[6-8]</sup>. The remaining three articles were all different<sup>[9-11]</sup>.

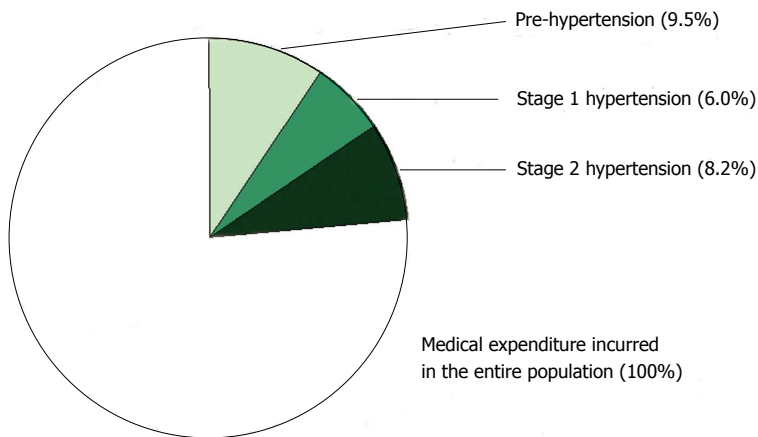
## HYPERTENSION AND MEDICAL EXPENDITURE

The first study to report on the relationship between hypertension status and subsequent medical expenditure was the Shiga National Health Insurance (NHI) cohort study<sup>[6]</sup>. This study was conducted in seven towns and one village in Shiga prefecture in the central part of Japan, and included 4191 community-dwelling beneficiaries of NHI, an insurance group for self-employed individuals (*e.g.*, farmers and fishermen) and retirees and their dependants. The study participants were aged between 40-69 years and were not taking antihypertensive medication and did not have a history of cardiovascular disease. They were classified into four sex-specific categories according to their blood pressure measured at a baseline survey in 1989-1991. The four blood pressure categories were defined as follows according to the 7<sup>th</sup> report of the Joint National Committee in the United States<sup>[12]</sup>: “normotension” (systolic blood pressure (SBP) < 120 mmHg and diastolic blood pressure (DBP) < 80 mmHg); “prehypertension” (SBP 120-139 mmHg and/or DBP 80-89 mmHg); “stage 1 hypertension” (SBP 140-159 mmHg and/or DBP 90-99 mmHg); and “stage 2 hypertension” (SBP ≥ 160 mmHg and/or DBP ≥ 100 mmHg). The participants were followed up for 10 years from 1990 to calculate the mean medical expenditure per month during the follow-up period. The cumulative hospitalization rate and all-cause mortality for each blood pressure category were also recorded. If a participant withdrew or died, the follow-up period was terminated at that point. The medical expenditure recorded in this study was confined to the fee schedule range used in the medical insurance system





**Figure 1** Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in male and female Japanese medical insurance beneficiaries aged 40-69 years, grouped according to sex and hypertension status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, body mass index, smoking habit, drinking habit, serum total cholesterol, and a history of diabetes. From Nakamura *et al.*<sup>[6]</sup>

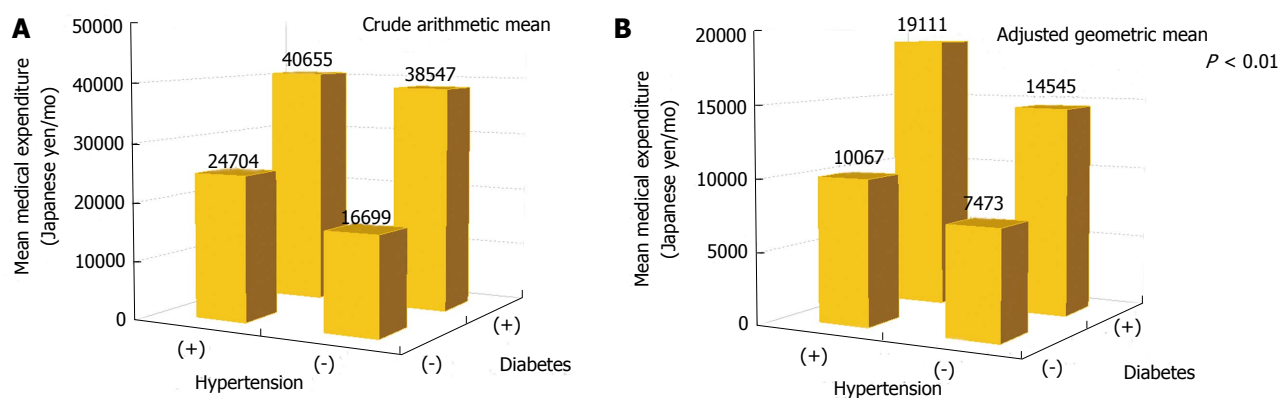


**Figure 2** Percentage of medical expenditure attributable to pre-, stage 1, and stage 2 hypertension relative to medical expenditure incurred by the entire population of Japanese medical insurance beneficiaries aged 40-69 years (100%). From Nakamura *et al.*<sup>[6]</sup>

in Japan, and was calculated as the sum of expenditure from the insurance organization and the beneficiary. The crude arithmetic mean of medical expenditure increased with worsening severity of hypertension, especially in men (Figure 1A). The adjusted geometric mean of medical expenditure, calculated using analysis of covariance that incorporated logarithmically-transformed values of medical expenditure as the dependent variable and major cardiovascular risk factors as covariates, also correlated positively with blood pressure levels (Figure 1B). The odds ratio for cumulative hospitalization (1.96, 95%CI: 1.29-2.98) and hazard ratio for all-cause mortality (3.19, 95%CI: 1.67-6.08) in stage 2 hypertensive men were also higher than those in normotensive men after adjustment for potential confounding factors. This study estimated medical expenditure attributable to the three grades of hypertension (*i.e.*, “pre-hypertension”, “stage 1 hypertension”, and “stage 2 hypertension”) from a population perspective. The medical expenditure attributable to these three hypertension grades accounted for 23.7% of the medical expenditure incurred in the combined male and female study participants (Figure 2). The percentage for each-hypertension-related medical expenditure was 9.5% for “pre-hypertension”, 6.0% for “stage 1 hypertension”, and 8.2% for “stage 2 hypertension”.

The Ohsaki NHI cohort study<sup>[9]</sup> was conducted sub-

sequently in Ohsaki city, Miyagi prefecture in the north-east part of Japan using a similar method. This study included 12340 community-dwelling NHI beneficiaries aged 40-79 years without a history of cardiovascular disease or cancer. The study participants were classified into the following two categories according to their blood pressure and antihypertensive medication status assessed in 1994-1995: “normotension” (SBP < 140 mmHg, DBP < 90 mmHg, and not taking antihypertensive medication); and “hypertension” (SBP ≥ 140 mmHg, DBP ≥ 90 mmHg and/or taking antihypertensive medication). The arithmetic mean of medical expenditure per month during the 6-year follow-up period from 1996 was higher in hypertensive participants than in normotensive participants even after adjustment for age, sex, smoking and alcohol drinking habits, and obesity, hyperglycaemia, and dyslipidemia status: 275.9 United States dollars/mo *vs* 203.5 United States dollars/mo, respectively (1 United States dollar = 115 Japanese yen at the foreign exchange rate given in the article). When the hypertensive participants were divided further into untreated and treated hypertensive subjects, the mean medical expenditure was increased further in the treated hypertensive group than in the untreated hypertensive and normotensive groups: 317.7 United States dollars/mo *vs* 223.0 United States dollars/mo *vs* 202.9 United States dollars/mo, respec-



**Figure 3** Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in Japanese medical insurance beneficiaries aged 40-69 years, grouped according to hypertension and diabetes status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, sex, body mass index, smoking habit, drinking habit, and serum total cholesterol. From Nakamura *et al.*<sup>[7]</sup>

tively.

The Ibaraki NHI cohort study<sup>[10]</sup>, which was conducted over a wide area in Ibaraki prefecture in the eastern part of Japan, used a similar, but partially different method. This study included 42426 community-dwelling NHI beneficiaries aged 40-69 years without a history of cardiovascular disease. The study measured medical expenditure for just one year (2006), four years after the baseline survey in 2002 that assessed hypertension status. Monthly medical expenditure was compared for the same four blood pressure categories as those used in the Shiga NHI cohort study, although stage 2 hypertension included both participants who had a SBP  $\geq 160$  mmHg and/or DBP  $\geq 100$  mmHg free from antihypertensive medication and those on antihypertensive medication. The median medical expenditure increased with more severe hypertension in every stratum of age (*i.e.*, 40-54 years and 55-69 years) and sex.

## HYPERTENSION COMBINED WITH ANOTHER RISK FACTOR AND MEDICAL EXPENDITURE

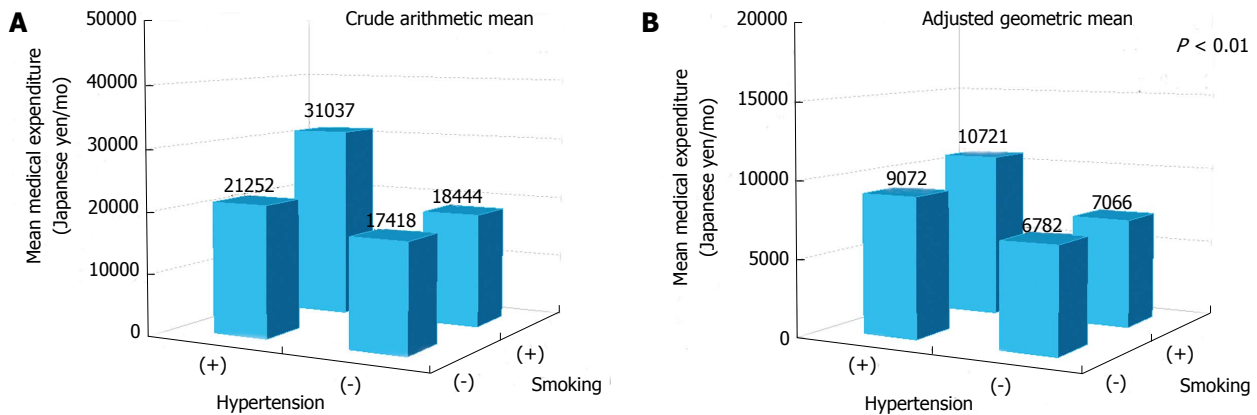
The Shiga NHI cohort study<sup>[7]</sup> examined the relationship between the combination of hypertension status and diabetes status and subsequent medical expenditure in 4535 participants. This patient group was selected as the coexistence of hypertension and diabetes is often due mainly to insulin resistance accompanied by compensatory hyperinsulinemia<sup>[13,14]</sup>, which occurs more frequently in obese than in non-obese individuals<sup>[15,16]</sup>. The mean of medical expenditure per month over a 10-year follow-up period was compared in the following four categories: “neither hypertension nor diabetes”; “hypertension alone”; “diabetes alone”; and “both hypertension and diabetes”. Hypertension was defined as a SBP  $\geq 140$  mmHg, a DBP  $\geq 90$  mmHg, and/or taking antihypertensive medication, while diabetes was defined as having a history of diabetes assessed by a self-reported questionnaire. The participants with both hypertension and

diabetes, who accounted for 1.3% of the study population, incurred on average, higher medical expenditure compared with those without hypertension, diabetes, or their combination, even after adjustment for confounding factors (Figure 3). Similarly, the “both hypertension and diabetes” group had the highest risk of all-cause mortality among the four categories, with an adjusted hazard ratio of 2.21 (95%CI: 1.11-4.42), relative to the “neither hypertension nor diabetes” group.

The Ohsaki NHI cohort study<sup>[9]</sup> compared mean medical expenditure per month over a 6-year follow-up period in four similar categories in participants stratified according to the presence or absence of obesity defined as a body mass index  $\geq 25.0$  kg/m<sup>2</sup>. Hypertension was defined as described earlier, whereas hyperglycemia was defined as a plasma glucose  $\geq 150$  mg/dL and/or having a self-reported history of diabetes. The results of this study showed a pattern similar to those of the Shiga NHI cohort study for both obese and non-obese participants, although obesity resulted in additional medical expenditure in each of the four blood pressure and plasma glucose categories. In short, non-obese participants with both hypertension and hyperglycemia, with hypertension alone and with hyperglycemia alone had increased medical expenditure of 85.2%, 33.0% and 48.3%, respectively, compared with non-obese participants with neither hypertension nor hyperglycemia. In contrast, obese participants with both hypertension and hyperglycemia had a 91.0% increase in expenditure compared with the same reference group. The medical expenditure attributable to both hypertension and hyperglycemia with and without obesity accounted for 1.4% and 1.8% of the medical expenditure incurred in the entire population, respectively.

The Shiga NHI cohort study<sup>[8]</sup> examined the relationship between the combination of hypertension status and smoking status and subsequent medical expenditure in 1708 male participants after excluding male ex-smokers and all females, as smoking is more prevalent in Japanese men than in Japanese women<sup>[17]</sup>. Mean medical expenditure per month over a 10-year follow-up period was compared in the following four categories: “neither hyper-





**Figure 4** Crude arithmetic mean (A) and adjusted geometric mean (B) of medical expenditure per month over 10 years of follow-up in male Japanese medical insurance beneficiaries aged 40-69 years, grouped according to hypertension and smoking status. Analysis of covariance was used to compare log-transformed monthly medical expenditure in each blood pressure category, after adjustment for age, body mass index, drinking habit, serum total cholesterol, and a history of diabetes. From Nakamura *et al.*<sup>[8]</sup>

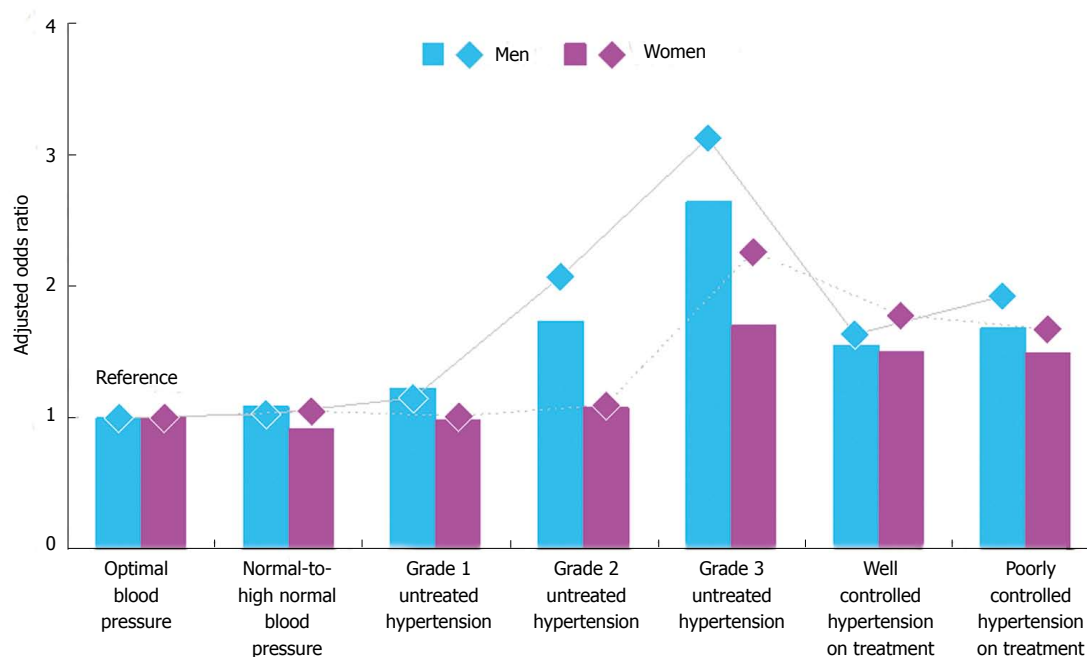
tension nor smoking”; “hypertension alone”; “smoking alone”; and “both hypertension and smoking”. Hypertension was defined as described earlier, while smoking was defined as currently smoking. Participants with both hypertension and smoking, who accounted for 24.9% of the study population, incurred on average, higher medical expenditure compared with those without hypertension, smoking, or their combination, even after adjustment for confounding factors (Figure 4).

## OTHER RELEVANT TOPICS

The latest cohort study collected similar data throughout Japan, and reported interesting results which revealed how hospitalization influenced the causality between hypertension and increased medical expenditure<sup>[11]</sup>. Unlike the three previous cohort studies, this study included both NHI beneficiaries (12 local organizations) and beneficiaries in the Employee’s Health Insurance scheme (nine local organizations), which is available for employees and their dependants. Currently, all Japanese people younger than 75 years should be enrolled in either NHI or Employee’s Health Insurance schemes (enrolment ratio, 1:2)<sup>[3]</sup>. A total of 314622 participants aged 40-69 years without a history of cardiovascular disease or end-stage renal disease were included in the final analyses. The study participants were age and sex-specifically classified into seven categories according to their blood pressure and antihypertensive medication status assessed at the baseline survey in 2008. The seven blood pressure categories were defined according to the 2007 criteria of the European Society of Hypertension and the European Society of Cardiology<sup>[18]</sup>. Participants who were not taking antihypertensive medication were classified into one of the following five categories: “optimal blood pressure” (SBP < 120 mmHg and DBP < 80 mmHg); “normal-to-high normal blood pressure” (SBP 120–139 mmHg and/or DBP 80–89 mmHg); “grade 1 hypertension” (SBP 140–159 mmHg and/or DBP 90–99 mmHg); “grade 2 hypertension” (SBP 160–179 mmHg and/or DBP 100–109 mmHg); and “grade 3 hypertension” (SBP

≥ 180 mmHg and/or DBP ≥ 110 mmHg). The remaining participants, who were taking antihypertensive medication, were classified into one of the following two categories: “well controlled hypertension on treatment” (SBP < 140 mmHg and DBP < 90 mmHg on medication); and “poorly controlled hypertension on treatment” (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg on medication). This study first compared the risk of undergoing hospitalization one year (2009) after the baseline survey in each blood pressure category. In men aged 40-54 or 55-69 years, the risk of undergoing hospitalization in 2009, especially long-term hospitalization, increased with worsening severity of untreated hypertension (bars in Figure 5, results presented only for men and women aged 40-54 years). The “grade 2-to-3 untreated hypertension” group appeared to have a further increased risk of being hospitalized for at least 14 cumulative days than the “well controlled hypertension on treatment” group. The results derived from the female cohorts need to be interpreted with caution because of the lower prevalence of hypertension and the small number of hospitalizations in females compared with males. However, in women aged 40-54 years, the “grade 3 untreated hypertension” group appeared to have a further increase in hospitalization risk compared with the “well controlled hypertension on treatment” group. Participants who were hospitalized, especially long-term, incurred considerably higher medical expenditure compared with non-hospitalized participants, regardless of their hypertension status, age, or sex. Hypertensive participants on medication appeared to incur less than half of the medical expenditure of hospitalized participants, as long as they remained out of hospital for treatment of hypertension alone. However, this study did not clarify whether the use of antihypertensive medication could offset long-term medical expenditure.

The study also compared the risk of incurring extremely high medical expenditure, defined as at least 99<sup>th</sup> percentile values of the sex-specific distribution of medical expenditure in the year after the baseline survey in each of the blood pressure categories. This comparison was made because of the fact that a very small percent-



**Figure 5** Adjusted odds ratios for two kinds of events over one year of follow-up in male and female Japanese medical insurance beneficiaries aged 40-54 years, grouped according to hypertension status. The bars represent the risk of undergoing hospitalization for  $\geq 14$  cumulative days, while the diamonds represent the risk of falling into the top 1% group of medical expenditure. A logistic regression model was used to calculate odds ratios after adjustment for age, body mass index, smoking habit, serum low-density lipoprotein cholesterol, log-transformed fasting plasma glucose, and medications for hypercholesterolemia and diabetes, with the “optimal blood pressure” group acting as the reference. Male and female participants who fell into the sex-specific top 1% medical expenditure group each incurred  $\geq 1571$  euros/mo and  $\geq 1249$  euros/mo, respectively (1 euro = 95.91 Japanese yen). From Nakamura *et al.*<sup>[11]</sup>.

age of patients accounts for a substantial percentage of the medical expenditure in the entire population<sup>[19]</sup>. Male and female participants who fell into the top 1% group of medical expenditure incurred at least 1571 euros/mo and 1249 euros/mo, respectively (1 euro = 95.91 Japanese yen at the foreign exchange rate given in the article), with the corresponding median cumulative hospitalization periods being 38 and 32 d. The sum of medical expenditure in these top 1% male and female groups accounted for 25.6% and 21.2% of medical expenditure in the entire population, respectively. The risk of incurring such extremely high medical expenditure increased with more severe untreated hypertension in men aged 40-54 or 55-69 years and in women aged 40-54 years (diamonds in Figure 5, results presented only for men and women aged 40-54 years). In men and women aged 40-54 years, the “grade 2-to-3 untreated hypertension” group had a further increased risk of incurring greater medical expenditure compared with the “well controlled hypertension on treatment” group. These results were consistent with the results regarding the risk of being hospitalized for at least 14 cumulative days.

## CONCLUSION

Epidemiological studies demonstrated that hypertension caused increased medical expenditure in community-dwelling populations in Japan. Medical expenditure was increased further in hypertensive subjects who had another concomitant cardiovascular risk factor. These studies therefore show that the treatment of hypertension itself is costly. However, attention should be paid to

evidence that hypertension, especially moderate-to-severe untreated hypertension, increased the risk of long-term hospitalization, which resulted in considerably higher medical expenditure compared with non-hospitalized cases. Furthermore, hypertension, especially moderate-to-severe untreated hypertension, increased the risk of surges in medical expenditure, due mainly to long-term hospitalization. Therefore, based on the assumption that use of antihypertensive medication is essential for hypertensive subjects to prevent serious vascular diseases<sup>[20,21]</sup>, a cost-effective high-risk strategy needs to be considered in order to reduce both ill-health and the economic burden due to hypertension. It should also be noted that from a population perspective, medical expenditure attributable to hypertension appears to result largely from pre-to-mild hypertension, although personal hypertension-related medical expenditure is higher with more severe hypertension. This is in accordance with Rose’s theory that “a large number of people exposed to a small risk may generate many more cases than a small number exposed to high risk”<sup>[22]</sup>. Too much focus on hypertensive subjects, especially those with moderate-to-severe hypertension may result in failure to comprehensively reduce the burden of interest. Therefore, there is also a need to consider a population strategy, which aims to shift the entire population to lower levels of blood pressure.

## ACKNOWLEDGMENTS

### *The medical insurance system in Japan*

This system requires the enrolment of all Japanese resi-

dents (*i.e.*, “health-insurance-for-all”). Every Japanese resident is able to receive medical services at all clinics and hospitals given approval to provide outpatient medical services and hospitalization. This system consists of three insurance groups (previously two insurance groups) with eligibility for each group depending on the individual’s age and occupation. The fee schedule set by the National Government is uniform across the insurance groups and applies to all the approved clinics and hospitals. Prices are controlled strictly by a fee schedule and are determined on a “fee-for-service” basis. However, recently approximately 20% of acute care hospitals have changed to a flat-fee per day payment system for hospitalized patients according to the diagnosis and procedures undertaken (Diagnosis Procedure Combination/Per-Diem Payment System). The clinic or hospital requests medical expenditure from the insurance organization in which the beneficiary is enrolled and also the beneficiary himself/herself, with the insurance organization paying 70%-90% and the beneficiary paying the balance. However, the medical insurance system does not cover some medical services including health checkups for asymptomatic individuals or inoculations, with annual health checkups available free or at fairly low charges in communities and worksites.

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## Adipose tissue and vascular inflammation in coronary artery disease

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response, which characterizes obesity and metabolic syndrome. This might represent an important pathophysiological link with atherosclerotic complications and cardiovascular events. A great number of adipocytokines have been described recently, linking inflammatory milieu and vascular pathology. The understanding of these pathways is crucial not only from a pathophysiological point of view, but also to a better cardiovascular disease risk stratification and to the identification of possible therapeutic targets. The aim of this paper is to review the role of Adipocytokines as a possible link between obesity and vascular disease.

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**Key words:** Adipocytokines; Obesity; Metabolic syndrome; Coronary artery disease; Inflammation

**Core tip:** Our article, provide a comprehensive review of the evidence about adipose tissue and cardiovascular risk, focusing on the pathophysiological and clinical role of fat-derived mediators, the so-called adipocytokines.

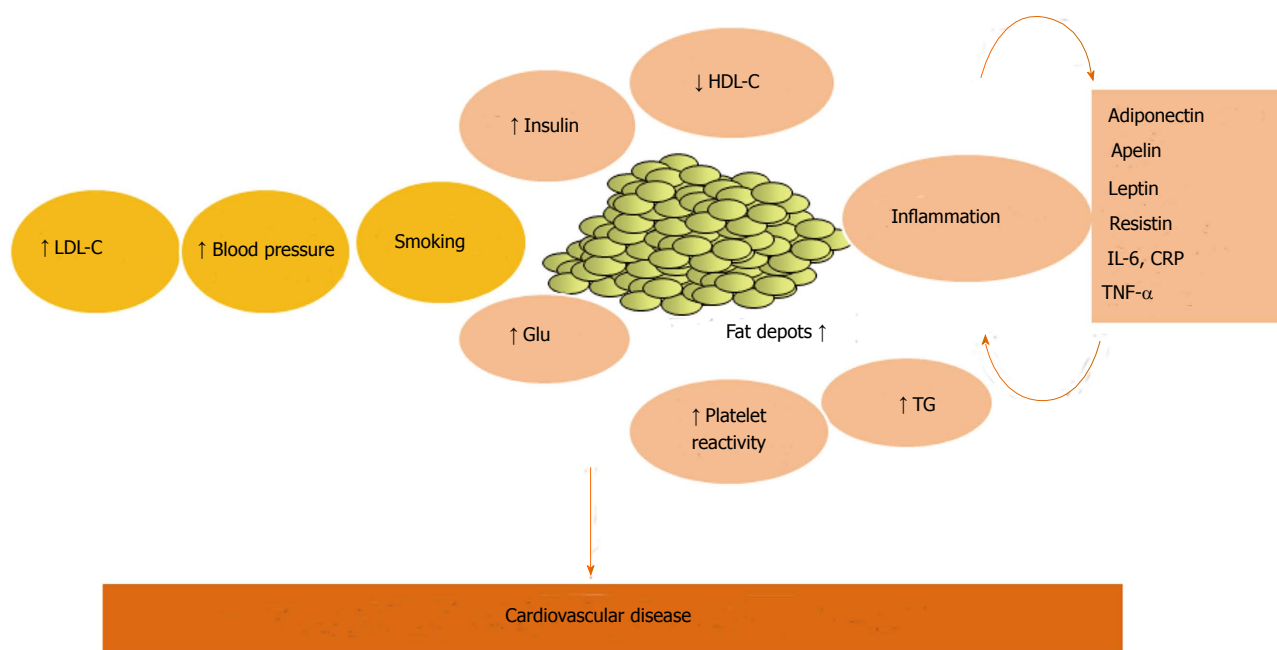
### Abstract

Obesity has become an important public health issue in Western and developing countries, with well known metabolic and cardiovascular complications. In the last decades, evidence have been growing about the active role of adipose tissue as an endocrine organ in determining these pathological consequences. As a consequence of the expansion of fat depots, in obese subjects, adipose tissue cells develop a phenotypic modification, which turns into a change of the secretory output. Adipocytokines produced by both adipocytes and adipose stromal cells are involved in the modulation of glucose and lipid handling, vascular biology and, moreover, participate to the systemic inflammatory

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### INTRODUCTION

Obesity is rapidly spreading to epidemic levels in Western and developing countries, becoming a serious health issue. It is associated with increasing morbidity and mortal-



**Figure 1** A cartoon illustrating the complex interplay between traditional and non-traditional risk factors in the pathophysiological continuum which leads to cardiovascular disease, with the emerging role of inflammatory pathways. LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; CRP: C-reactive protein; TG: Triglycerides; Glu: Glucose; IL-6: Interleukin-6; TNF: Tumor necrosis factor.

ity<sup>[1]</sup>. Obesity shares several features with metabolic syndrome (MetS) and both are associated with well known risk factors for cardiovascular disease (CVD)<sup>[2]</sup>, such as glucose and lipid metabolism impairment, endothelial dysfunction and atherosclerosis, finally leading an increased risk of cardiovascular events<sup>[2]</sup>.

Recent evidence claimed that inflammation might represent the pathophysiological link between obesity and MetS (Figure 1), and increasing interest has been developed in the role of adipose tissue as an active trigger of this systemic inflammatory response<sup>[3]</sup>.

Since adipose tissue is capable of releasing several mediators, the so-called adipocytokines, it is now considered an endocrine organ, affecting metabolism and vascular function. The understanding of these pathways is crucial not only from a pathophysiological point of view, but also to a better cardiovascular disease (CVD) risk stratification and to the identification of possible therapeutic targets.

## ADIPOSYTY AND CARDIOVASCULAR RISK

There is consistent epidemiologic evidence for an independent association between obesity and CVD<sup>[4]</sup>. In several large, prospective, long-term studies, obesity was independently associated with all-cause mortality and death from CVD in both women and men. The Nurses' Health Study evaluated the relationship between body mass index (BMI) and mortality in 115195 women<sup>[5]</sup>. A significant trend for increasing risk of death with increasing BMI and an association between BMI and cardiovascular mortality were found.

In the Framingham Heart Study, obesity was an in-

dependent risk factor for all-cause mortality among male participants, followed up for 30 years<sup>[6]</sup>. However, recent data have demonstrated an "obesity paradox", with obese patients suffering from CVD showing a better short- and long-term prognosis than leaner matched subjects<sup>[7]</sup>. In particular, in a large meta-analysis<sup>[8]</sup>, the overall obesity population, defined by BMI, showed an increased risk of mortality compared with normal BMI. However, overweight patients (BMI 25-30 kg/m<sup>2</sup>) had a significantly 6% lower mortality than patients with normal BMI. This finding was more pronounced in older cohorts<sup>[9]</sup>. Interestingly, several studies<sup>[7]</sup> suggested an influence of body fitness and on the relationship between adiposity and CVD prognosis. Thus obesity paradox seemed evident in patients with low fitness.

### Adipose tissue depots

The so-called visceral obesity is now well-known to be associated to a higher mortality than the peripheral obesity, since it is linked to a higher prevalence of dysmetabolism and hypertension and endothelial dysfunction<sup>[3]</sup>. This evidence highlighted the importance of adipose tissue location in determining its unfavourable effects.

Obesity is characterized by an expanded adipose tissue mass<sup>[10]</sup> and, interestingly, as noted above, in overweight subjects a typical pattern of adipokines is present, which negatively affect metabolic situation and cardiovascular function<sup>[11]</sup>.

Typically, the source of these substances are organs (the liver), and immune cells<sup>[12-14]</sup>. Most notably, several studies indicate that fat could either regulate the synthesis of these molecules or could be itself an immediate source.

The complexity of adipose tissue as an endocrine organ has been highlighted in several studies which have reported important differences among adipocytes from different depots. These characteristics may account for a differential contribution to obesity-related disorders<sup>[15]</sup>.

Adipocytes from brown adipose tissue (BAT) are mainly represented in fetuses and newborn and are implicated in thermogenesis<sup>[16,17]</sup>. A small amount of BAT persists in adults human AT<sup>[17]</sup>.

On the other hand, white adipose tissue (WAT) represents a main kind of adipose tissue in adults<sup>[15]</sup>, largely present in the subcutaneous region (SAT) or close and within the abdominal viscera (VAT).

Vague<sup>[18]</sup> first described a link between increased VAT and atherosclerosis, diabetes, and other diseases. This association might be explained by the increased production of mediators, acting with endocrine, autocrine and paracrine mechanisms<sup>[3]</sup>. Bigger VAT adipocytes in obese subjects are related to a higher mediators release, comparing to SAT<sup>[19,20]</sup>. These factors, adipocytokines include molecules like tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), leptin, adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1), apelin, interleukin-6 (IL-6), resistin, angiotensinogen, serum amyloid A (SAA), and C-reactive protein (CRP)<sup>[21]</sup>.

Moreover, several inflammatory cells<sup>[19]</sup> are largely represented in the WAT stroma. These cells play an important role in tissue homeostasis, such as the clearance of necrotic adipocytes<sup>[18]</sup>, increased in obesity. In particular, macrophages produce the majority of TNF- $\alpha$  and increase IL-6 and inducible-Nitric Oxide synthase expression. Then they were thought to be the primary source of the cytokines in adipose tissue<sup>[19,22,23]</sup>. However, we have demonstrated with an *in vitro* model, that the mature adipocytes fraction isolated from human adipose tissue is directly involved in both CRP and SAA release<sup>[24]</sup>.

Interestingly, among visceral fat depots, another specific local fat depot has been studied in the last decade for its relationship with MetS and coronary artery disease (CAD), *i.e.*, epicardial adipose tissue (EAT). EAT surrounds the heart, within the pericardium, it is in contact with coronary vessels (*i.e.*, perivascular) and the surface of ventricles. It shows a higher rate of lipolysis and lipogenesis comparing to other fat depots<sup>[25]</sup>. EAT is involved in myocardial energy supply, thermoregulation, and protection of the cardiac autonomic nervous system as well as in the regulation of coronary vessel motion and lumen diameter<sup>[26]</sup>.

In post-mortem series, epicardial fat have been reported to account for 20% of total heart weight<sup>[27]</sup>. Obesity is associated with an increase in the amount of EAT. Both total weight of epicardial fat and epicardial fat cells size correlate with body weight<sup>[28]</sup>. Moreover, epicardial fat assessed by echocardiography correlated significantly in multivariable analysis with key components of the MetS, including waist circumference, which is known to parallel the increase in VAT seen in MetS<sup>[29,30]</sup>. Then, the cardioprotective features of EAT might be somewhat blunted by the increase in cardiovascular risk carried by its pathological enlargement in obesity<sup>[31]</sup>.

From a clinical point of view, several clinical and epidemiological studies have found an association between EAT and cardiometabolic risk factors and early stages of atherogenesis. The echocardiographic EAT evaluation was found to be associated with arterial stiffness and intima-media thickness (IMT), two indexes of subclinical atherosclerosis, in hypertensive patients in a study from our group<sup>[32]</sup>. Among the 459 patients examined, subjects with epicardial fat > 7 mm were older, had higher systolic, diastolic, and pulse pressure, increased left ventricular mass index, carotid IMT, diastolic and stiffness parameters compared with those with epicardial fat < 7 mm. Age, carotid IMT, and stiffness parameters were independently related to epicardial fat. These findings have been confirmed in a more recent study by Choi *et al.*<sup>[33]</sup>.

Importantly, the Framingham Heart Study<sup>[34]</sup> and Multi-Ethnic Study of Atherosclerosis<sup>[35]</sup> identified EAT volume as independent risk markers for CVD. Moreover, recently results of the Heinz Nixdorf Recall study, a population-based prospective cohort study, have been published<sup>[36]</sup>. Among the 4093 participants incidence of coronary events increased by quartile of EAT. Doubling of the EAT, measured by computed tomography (CT) scan, carried a 1.5-fold risk of coronary events after adjusting for cardiovascular risk factors and coronary artery calcium score.

Comparing to subcutaneous fat, EAT shows a more dense inflammatory cell infiltrate, predominantly represented by macrophages<sup>[37]</sup>, and it produces highly atherogenic and inflammatory adipocytokines in patients with CAD<sup>[38]</sup>.

EAT might then participate also in the inflammatory process within the atherosclerotic plaque. The incidence and severity of CAD and coronary calcification have been in fact associated with EAT thickness and volume<sup>[39]</sup>.

A recent study assessed the relationship between EAT volume and plaque vulnerability in significant coronary stenosis using intravascular ultrasound. Authors found a positive correlation between EAT volume, measured by CT scan and the percentage of necrotic plaque tissue, and an inverse correlation between with the percentage of fibrous tissue<sup>[40]</sup>. Low-density lipoprotein (LDL) cholesterol level and EAT volume were independently associated with the percentage of necrotic plaque tissue. These findings are consistent with previous reports<sup>[41]</sup>.

Moreover, EAT is associated also with microvascular dysfunction in the absence of obstructive CAD. In a recent study, patients underwent Rb-82 positron emission tomography to obtain standard myocardial perfusion index and myocardial flow reserve (MFR). EAT thickness, EAT volume and coronary calcium score values were higher in patients with impaired flow reserve, with EAT thickness showing the strongest negative correlation with MFR<sup>[42]</sup>.

## OBESITY, VASCULAR INFLAMMATION AND ATHEROSCLEROSIS

In last decades, it became clear that atherosclerosis is

**Table 1** Possible link of resistin, C-reactive protein and apelin with vascular inflammation, atherosclerosis and cardiovascular disease

Adipokine	Modulation of inflammation	Association with CVD
Leptin	↑ T cell activation and Th lymphocyte response ↑ cytokine release ↑ NK cell activation ↑ macrophages' cytokine release Activates neutrophils ↑ chemotaxis ↑ oxidative stress ↑ CRP production from endothelium	↑ blood pressure ↑ atherothrombosis: ↑ cholesterol accumulation in vessel wall ↑ adhesion molecules (ICAM, VCAM) expression ↑ endothelial dysfunction (increasing eNOS production, ↓ NO, ↑ET-1) ↑ proliferation and migration of EC and VSMC ↑ ROS accumulation ↑ VSMC apoptosis ↓ angiogenesis ↑ platelet activity ↓ fibrinolysis ↑ PAI-1 ↑TF release from mononuclear cells Induce insulin resistance Associated with HDL-C and inversely with LDL-C ↓ atherothrombosis: ↓ ICAM-1, VCAM-1, and E-selectin ↓ Transformation of macrophages to foam cells ↓ vascular muscular smooth cells proliferation and migration ↑ TIMP, through the increase in IL-10 ↑ oxidation of free fatty acids in several tissue ↑ insulin sensitivity
Adiponectin	↓ T cell activation and proliferation ↓ NF-κB Increases IL-10 Inhibits CRP and IL-6 release	Associated with HDL-C and inversely with LDL-C ↓ atherothrombosis: ↓ ICAM-1, VCAM-1, and E-selectin ↓ Transformation of macrophages to foam cells ↓ vascular muscular smooth cells proliferation and migration ↑ TIMP, through the increase in IL-10 ↑ oxidation of free fatty acids in several tissue ↑ insulin sensitivity

CVD: Cardiovascular disease; CRP: C-reactive protein; EC: Endothelial cells; ET-1: Endothelin-1; ICAM: Intercellular adhesion molecule; HDL-C: High density lipoprotein cholesterol; IL: Interleukin; LDL-C: Low density lipoprotein cholesterol; NF-κB: Nuclear factor κB; NK: Natural killer; NO: Nitric oxide; PAI-I: Plasminogen activator inhibitor-I; TF: Tissue factor; TIMP: Tissue inhibitor of metalloproteinase; VCAM: Vascular cell adhesion molecule; VSMC: Vascular smooth muscle cells.

other than a cholesterol storage disease. A large body of literature highlighted the possible role of inflammation as causal factor in atherogenesis, from endothelial dysfunction to clinical events<sup>[43]</sup>. One of the first recognized stages of the atherosclerotic process consists of LDL intimal deposition and endothelial dysfunction. This is caused by the imbalance between nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>)-mediated vasorelaxation and the increase in endogenous vasoconstrictors, such as endothelin-1 (ET-1)<sup>[44]</sup>. LDL become then oxidized (ox-LDL) by local reactive oxygen species (ROS) and subsequently induce endothelial cells to express adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule 1, and selectins<sup>[45]</sup>. This together with the secretion of chemoattractant mediators, such as complement factors, IL-8, monocyte chemoattractant protein-1, determines mononuclear cells recruitment into the vascular wall. Thus, macrophages recruited to intima become “foam cells”, *via* ox-LDL phagocytosis<sup>[44]</sup>. Subsequent stages include the transition of the atherosclerotic plaque from the “fatty streak” to a more fibrous lesion. Main actors in this stage are vascular smooth muscle cells, which accumulate in the intima and produce extracellular matrix (ECM)<sup>[44]</sup>.

Inflammation plays a key role in plaque destabilization and rupture which cause acute vascular events. High rate of vascular inflammation interferes with fibrous cap formation, induces apoptosis and degradation of the ECM, *via* an upregulation of metalloproteinase. The subsequent activation of the coagulation cascade leads to intravascular thrombus formation and the acute clinical events. In this setting, tissue factor (TF) plays a pivotal role in the

pathophysiology of acute coronary syndromes by triggering the formation of intracoronary thrombi following endothelial injury<sup>[45]</sup>.

As noted above, from a pathophysiological point of view, adipose tissue is not an innocent bystander in this process since it is found to be capable of producing enzyme, cytokines, growth factors and hormones which might affect each one of the stages described above. Moreover, even if the majority of patients suffering of cardiovascular diseases had at least one traditional risk factor<sup>[46,47]</sup>, almost 25% of subjects did not show any of those<sup>[48]</sup>. In this setting, identifying new risk factors might increase our ability to discover and take care of high-risk patients. Within novel prediction factors, adipocytokines have been studied.

### Leptin

Leptin has been the first adipocytokine identified as the product of the *ob* gene in obese *ob/ob* mice<sup>[49]</sup>, which participate in the signalling of fat stores<sup>[50]</sup>.

Then, early studies on leptin focused on its role in obesity and potential therapies to control it. However, soon it became evident a broader biological role for leptin, including its potential implication in leading to cardiovascular complications in obese patients (Table 1). However, conflicting results on leptin role in CVD have been reported.

In animal and *in vitro* models, leptin promotes atherogenesis, through an increase in oxidative stress in endothelial cells<sup>[51]</sup>, an increased platelet reactivity and thrombosis<sup>[52]</sup>. On the other hand, several studies reported opposite effects such as induction of nitric oxide



production, with anti atherogenic properties<sup>[53]</sup>.

Most *in vivo* studies in humans demonstrated that leptin levels are linked with cardiovascular risk factors, like hyperlipidemia or hypertension<sup>[54-57]</sup>, and markers of endothelial dysfunction, thrombophilia and inflammation. Finally, several prospective trials described a link of leptin levels with atherosclerosis and myocardial infarction (MI)<sup>[58-61]</sup>.

In the West of Scotland Coronary Prevention Study, including 377 male participants and 738 controls<sup>[59]</sup>, a 20% increase in the incidence of CAD was associated with each standard deviation increase in leptin levels, even after adjustment for potential confounders. Sattar *et al*<sup>[54]</sup> found a moderate association, although non significant, between leptin levels and CAD risk. This association was attenuated by BMI, but continued to be significant with other validated risk factors, including several markers of inflammation. Also in female population no significant link between leptin plasma levels and CAD was noted<sup>[61]</sup>. On the other hand, Kappelle *et al*<sup>[62]</sup>, in healthy men, demonstrated a positive association of leptinemia and leptin/adiponectin ratio with incident CVD, even after additional adjustment for several potential confounders, such as clinical risk factors, lipid and microalbuminuria.

In patients with known CAD, leptin might predict future cardiovascular events independent of other risk factors, including lipid status and CRP, according to several reports. Wolk *et al*<sup>[60]</sup> followed up 504 patients who had undergone coronary angiography for both stable angina (SA) and ACS for up to 4 years. In the field of ACS, admission plasma leptin levels is associated with the success of thrombolytic therapy in patients with STEMI presenting < 6 h<sup>[63]</sup>. In particular, authors found higher rates of thrombolysis failure in patients with basal plasma leptin levels  $\geq 14$  ng/mL, in comparison with patients with levels less than 14 ng/mL.

The association between plasma leptin and adiponectin levels and recurrent cardiovascular events after an ACS has been investigated in The Long-Term Intervention with Pravastatin in Ischaemic Disease study<sup>[64]</sup>. Leptin was a significant and independent predictor of recurrent cardiovascular events.

Consistent with this report, Rajendran *et al*<sup>[65]</sup> measured leptin levels, high-sensitivity C Reactive protein (hs-CRP) and IL-6 levels in patients with acute myocardial infarction (AMI), showing higher levels of leptin than control subjects.

The relationship between serial serum leptin levels measurement after thrombolysis for AMI and the degree of coronary atherosclerosis, coronary reperfusion, echocardiographic findings, and clinical outcome was investigated by Khafaji *et al*<sup>[66]</sup> in a small study. Leptin concentrations peaked 36 h after admission. Significant correlation of mean serum leptin with reduced ejection fraction and a trend for an increase in the mean serum leptin levels with increasing number of diseased vessels were found. However, there was no correlation between serum leptin levels and outcome or myocardial reperfusion.

Karakas *et al*<sup>[67]</sup> in a population-based case-cohort

study within the MONICA/KORA studies. After adjustment for various confounding factors, neither increased leptin levels or low adiponectin were associated with the incidence of coronary events in healthy subjects. Moreover, the leptin/adiponectin ratio didn't improve the ability of the single adipocytokine to predict incident CAD. In another study conducted among patients with ACS and controls, lipid profiles, leptin, pregnancy associated plasma protein A (PAPP-A) and CRP levels were assessed as markers of plaque vulnerability<sup>[68]</sup>. Significantly higher levels of leptin, PAPP-A and high-sensitivity CRP (hs-CRP) were observed in cases. At the multivariable analysis, leptin was not independently associated with ACS, while a positive correlation between CRP and leptin concentrations was noted.

Higher adiponectin and lower leptin levels were found to be associated with a high incidence of adverse events also in a Japanese cohort after successful emergency percutaneous coronary intervention for AMI<sup>[69]</sup>. A low leptin to adiponectin ratio remained a significant independent predictor of adverse events during long-term follow-up at the multivariable analysis.

Similar observations came earlier from Ku *et al*<sup>[70]</sup>. They found that subjects with low baseline leptin levels had higher subsequent CV events and death. Interestingly, although subjects with low leptin had fewer co-morbidities and more favorable metabolic and inflammatory profiles, they showed a worse prognosis than subjects with higher leptin levels. This could be an example of "reverse epidemiology"<sup>[71,72]</sup>, whereby a predictor of disease becomes inversely associated with prognosis once the disease has developed. According with this idea, a second paper described an association between low leptin levels and cardiovascular death in patients with chronic kidney disease<sup>[73]</sup>. Moreover, Leptin is elevated in chronic CAD. Multiple reports have shown that leptin causes coronary vasodilatation, activates endothelial progenitor cells, prevents lipid accumulation, and protects against ischemia-reperfusion injury<sup>[53,60,74,75]</sup>. Then a relative leptin deficiency might explain poorer prognosis seen in subjects with established CAD. Finally, the lack of association between leptin and mortality, especially in patients with higher BMI, could be otherwise explained by leptin resistance<sup>[76]</sup>.

### Adiponectin

Adiponectin is a well-described adipocytokine, traditionally reported as a protective factor with an anti inflammatory effect (Table 1)<sup>[11]</sup>. It is clear that its circulating levels decrease with weight gain and are inversely correlated to the amount of VAT, as illustrated in CT scan studies<sup>[11]</sup>. Interestingly, decreased adiponectin was associated with enhanced pro-inflammatory phenotype in EAT in patients with CAD<sup>[77]</sup>. As noted above about leptin, growing conflicting data on adiponectin levels are emerging, suggesting higher complexity of its role, than previously thought. This is particularly evident in the balance between obesity, cardiovascular effects, and prognosis.

Consistent with a putative protective role, Ouchi *et al*<sup>[78]</sup>

first detected lower adiponectin levels in subjects with established CAD. Following this experience, several cross-sectional and prospective studies have confirmed an inverse correlation between plasma adiponectin levels and incidence, severity and outcome of CAD<sup>[79-82]</sup>.

Recent studies, however, failed to demonstrate this correlation<sup>[83,84]</sup>, or even showed a paradoxical link between higher adiponectin levels and negative events, especially in patients with known CAD or at high cardiovascular risk<sup>[85]</sup>. Moreover, Zhang *et al.*<sup>[86]</sup> demonstrated higher adiponectin levels in patients with stable CAD and inducible ischemia.

In the Pravastatin Or atorVastatin Evaluation and Infection Trial-Thrombolysis in Myocardial Infarction 22<sup>[87]</sup>, plasma adiponectin was measured in 3931 subjects with Acute Coronary Syndrome. Adiponectin was negatively associated with age, diabetes, BMI, and triglycerides, while a positive link was noted with the risk of death, MI, and heart failure.

Seven hundred and thirty-five consecutive patients with STEMI treated with primary percutaneous coronary intervention were included in a report by Lindberg *et al.*<sup>[88]</sup>. Plasma adiponectin was measured immediately before the procedure. Patients with highest adiponectin quartile had increased mortality compared to patients with low adiponectin. After adjustment for conventional risk factors high adiponectin concentration remained an independent predictor of all-cause mortality.

Also in a cohort from the Jackson Heart Study, conducted among African Americans, Adiponectin was associated with a higher risk of incident stroke in women<sup>[89]</sup>.

This conflicting evidence has highlighted the complex role of adiponectin in the pathogenesis of CVD. Interestingly, the paradoxical association of adiponectin with a worse outcome was found in a population with a more advanced disease status, which might trigger a compensatory increase in adiponectin levels<sup>[71]</sup>. Otherwise, the worse long-term outcome could be the result of the more advanced disease status *per se*. Another possible explanation of these findings might be a condition of relative resistance to adiponectin, as suggested by animal models<sup>[85]</sup>.

In two recent meta-analysis of prospective studies, it has been shown that plasma adiponectin levels are not related to the risk of CAD or stroke in apparently healthy<sup>[90]</sup> and diabetic patients<sup>[91]</sup>. Another reason why this association could not be found might lie in adiponectin isoforms. In particular, several studies now consider the high molecular weight (HMW) form to be biologically active<sup>[92]</sup>. However, only few cohort studies have prospectively evaluated the association of HMW adiponectin with CAD<sup>[92-94]</sup>.

Among 30111 women from the Nurses' Health Study, high levels of total and HMW adiponectin, and HMW/total adiponectin ratio were associated with a lower risk of CAD. These associations were largely mediated by parameters related to glucose and lipid metabolism and inflammation, especially HDL-cholesterol levels<sup>[95]</sup>. In a study by Kunita *et al.*<sup>[96]</sup>, in 394 patients referred for com-

puted tomography angiography (CTA), levels of plasma HMW adiponectin were evaluated. In patients with obstructive CAD HMW adiponectin was significantly lower than that in patients without. Furthermore, it was significantly associated with the disease extent and with characteristics of plaque instability, such as positive remodeling, low CT density and adjacent spotty calcium. In a recent nested case-control study conducted among 15566 free of CVD subjects, baseline total and HMW adiponectin and their ratio were examined<sup>[97]</sup>. After adjustment for matched variables and traditional risk factors, total and HMW adiponectin and their ratio were not associated with overall risk of CVD. However, the highest quartile for HMW adiponectin and HMW/total adiponectin ratio decreased risk of CVD compared with the lowest quartile among middle-aged individuals with high blood glucose, while this association was not seen among the elderly.

## Resistin

Resistin was discovered as a fat-derived molecule in obese mice, for its capacity of inducing insulin resistance<sup>[98,99]</sup>. The main source in animals were adipocytes, in particular, from abdominal depots<sup>[98]</sup>. The substantial lack of homology between human and mouse resistin genes made difficult to confirm these observation in humans. In particular, in humans resistin is produced mainly by stromal vascular cells, rather than adipocytes<sup>[100]</sup>. However, resistin expression has been reported in human WAT by preadipocytes<sup>[101]</sup>. Even the relationship between resistin and insulin resistance, overweight and DM type 2 was extensively reported in human with non consistent conclusions<sup>[99,102-104]</sup>.

Considering that macrophages are its main source in humans, a main pro-inflammatory role has been hypothesized for Resistin. Thus, several *in vitro* studies was conducted (Table 2), which illustrated that Resistin levels increased in response to endotoxin and proinflammatory cytokines administration<sup>[104]</sup>. Moreover, Chen *et al.*<sup>[105]</sup> recently reported that ox-LDL induced resistin mRNA expression in cultured adipocytes. In a recent study from our group<sup>[106]</sup>, resistin induced prothrombotic phenotype of human coronary artery endothelial cells (HCAECs). HCAECs incubated with resistin showed an upregulation of TF expression, and its activity was induced in a dose-dependent manner through the activation of NF- $\kappa$ B pathway.

In light of these evidences, resistin has been studied for its implications in CAD.

In apparently healthy individuals from the European Investigation into Cancer and Nutrition-Potsdam Study, individuals in the highest quartile of resistin levels, compared with the lowest quartile, had a relevant increased risk of myocardial infarction but not of ischemic stroke<sup>[107]</sup>. This association persisted even after adjustment for CRP levels. In contrast, subsequent study described the independent link between resistin and the incidence of ischemic stroke within post menopausal women<sup>[108]</sup>.

In a recent study of 6636 adults recruited from general population, after 3.5 years of follow-up, the group in

**Table 2** Possible link of resistin, C-reactive protein and apelin with vascular inflammation, atherosclerosis and cardiovascular disease

Adipokine	Modulation of inflammation	Association with CVD
Resistin	↑NFκB dependent cytokine release and adhesion molecule expression (including TNF-α/IL-6) on endothelial cells ↑ proliferation of vascular smooth muscle cells through ERK and PI3K pathways	Endothelial dysfunction: ↓ NO and EDHF ↑ ET-1 release ↑ VEGF and MMP ↑ expression of adhesion molecules and chemokines ↓ TRAF-3 Controversial effects in humans on insulin resistance and type 2 diabetes
CRP	↑ expression of ICAM, VCAM, E selectin recruitment of mononuclear cells through MCP 1 VSMC proliferation, migration ↑ CD4 <sup>+</sup> Lymphocytes ↑ gamma - INF production ↑ ET-1 release ↑ CD40/CD40L on endothelium ↑ complement activation	↑ endothelial dysfunction through ↓ NO vasodilatation ↑ oxidized LDL opsonization and macrophages uptake with subsequent foam cells formation ↑ in vessel wall oxidative stress ↑ TF and PAI 1 ↑ MMP
Apelin	↓ superoxide radicals ↓ NADPHO ↓ oxidative injury ↑ NO ↑ vascular progenitor cells mobilization	↑ inotropism ↑ neoangiogenesis ↓ endothelial dysfunction ↓ Ang II and BP ↓ myocardial damage after infarction ↑ cholesterol efflux from macrophages

Ang: Angiotensin; BP: Blood pressure; CRP: C-reactive protein; CVD: Cardiovascular disease; EDHF: Endothelium-derived hyperpolarizing factor; ERK: Extracellular signal-regulated kinase; ET-1: Endothelin-1; HDL-C: High density lipoprotein cholesterol; ICAM: Intercellular adhesion molecule; IL: Interleukin; INF: Interferon; LDL-C: Low density lipoprotein cholesterol; MCP: Monocyte chemoattractant protein; MMP: Metalloproteinase; NADPHO: Nicotinamide adenosine dinucleotide phosphate oxidase; NO: Nitric oxide; PI3K: Phosphatidylinositol-3-kinase; TF: Tissue factor; TNF: Tumor necrosis factor; TRAF: Tumor necrosis factor receptor-associated factor; VCAM: Vascular cell adhesion molecule; VEGF: Vascular endothelial cell growth factor; VSMC: Vascular smooth muscle cells.

the highest quintile of resistin plasma levels had a higher incidence of AMI<sup>[109]</sup>. The serum resistin concentrations were higher in women, and the associated increase in the risk of AMI based on the resistin level was also higher in women than in men.

In patients with known CAD, some cross-sectional and case-control studies showed higher plasma resistin levels than controls. Subject referring angina which had CAD at the coronary angiography had higher Resistin levels than patients without CAD<sup>[110]</sup>. Besides, resistin was also associated with coronary artery calcification at the CT scan<sup>[111]</sup>. Pischon *et al*<sup>[112]</sup> documented higher resistin levels in women with CAD compared with healthy subjects from the CORA study. This link remained significant even after adjustment for several risk factors except the hs-CRP levels.

In contrast, no relationship between Resistin levels and CAD was found among 1161 subjects in the LURIC study<sup>[113]</sup>. Moreover, Resistin was not associated with cardiovascular mortality. Then, high resistin levels could simply mirror the presence of other established cardiovascular risk factors. However in the same study, an enhanced expression of adhesion molecules was found in association with increased resistin levels, highlighting a pathophysiological role in atherogenesis.

In a perspective study<sup>[114]</sup> comparing subjects with stable angina and subjects with unstable angina (UA), NSTEMI, and STEMI, higher resistin levels were found within subjects with ACS. Interestingly, an early rise in resistin levels was reported, at 3-6 h after symptoms onset. This increase lasted 6 and 12 h after.

## CRP

CRP is an acute phase protein, member of the *pentraxin family*. Since it is a well-known marker of systemic inflammation<sup>[45]</sup>, CRP was one of the first studied protein from its potential role in both pathogenesis and risk prediction of atherosclerosis. Subsequent studies showed that CRP is other than an innocent bystander of the inflammatory response associated with atherogenesis<sup>[45,115]</sup> (Table 2). In particular, together with the Adipocytokines, CRP characterizes the chronic inflammatory status associated with obesity and MetS.

Interestingly, the adipose tissue has been described as producer of CRP<sup>[23,116]</sup>. In particular, we found that mature adipocytes are able to produce CRP, under inflammatory stimuli<sup>[23]</sup>. This finding was confirmed later in the experience by Anty *et al*<sup>[117]</sup>, which demonstrated the expression of the CRP gene in adipocytes of obese subjects.

From a clinical point of view, high sensitivity assays are available to detect even low CRP concentration. Then high-sensitivity (hs) CRP has been largely evaluated as a suitable candidate for cardiovascular risk prediction. This idea was first supported by pioneering studies by Ridker *et al*<sup>[118]</sup>, which demonstrated higher hs-CRP levels in apparently healthy subject who developed CV events during follow-up. In light of these results, Ridker *et al*<sup>[119]</sup> evaluated several risk prediction algorithms to improve the cardiovascular risk classification in apparently healthy American women. In particular, a simplified score, including hs-CRP (Reynolds risk score), was validated in this study, and subsequently in a male population<sup>[120]</sup>.



Then the American Heart Association (AHA) and the CDC Consensus incorporated hs-CRP into the risk prediction strategy of cardiovascular diseases<sup>[121]</sup>. Measurement of hs-CRP is considered reasonable in the assessment of absolute risk for CAD in intermediate-risk individuals, with a Framingham risk score of 10% to 20%. This recommendation was confirmed in ACCF/AHA Guidelines in 2010<sup>[122]</sup> and in the recently published European Society of Cardiology Guidelines on Prevention<sup>[123]</sup>.

Moreover, in a metaanalysis<sup>[124]</sup> confirmed a role of hs-CRP for a better risk stratification of subjects at intermediate risk for CVD. In particular, for every 400 to 500 people screened for hs-CRP or fibrinogen level, one additional cardiovascular event could be prevented over a period of 10 years.

Results from the Justification for the Use of Statins in Primary Prevention: an International Trial Evaluating Rosuvastatin (JUPITER)<sup>[125]</sup> provided robust evidence of the association between inflammation and cardiovascular risk. subjects with LDL cholesterol below 130 mg/dL were treated with rosuvastatin *vs* placebo; patients at higher cardiovascular risk were identified by a hs-CRP level of 2.0 mg/L or higher. The Steering Committee stopped the trial after a median follow-up of 1.9 years due to striking benefit in patients treated with rosuvastatin (44% relative risk reduction of the primary end point and of hard outcomes).

Tehrani *et al*<sup>[126]</sup>, recently investigate whether inflammatory markers had an impact on the association of high density lipoprotein (HDL) cholesterol with CVD. In 3888 older adults without known CVD, authors evaluated CRP, IL-6, and lipoprotein-associated phospholipase A2 levels. CAD incidence was higher for higher levels of CRP, IL-6, and lower for higher levels of HDL-C. Compared to high HDL-C/low-inflammation categories, incident CAD was increased for those with high HDL-C and high CRP or highest IL-6 tertile. Then the protective relation of high HDL-C for incident CAD appears to be attenuated by greater inflammation.

Hs-CRP has also been studied for its potential role in the prediction of adverse outcome in patients with established CVD. Several studies clearly demonstrated an association between hs-CRP and future acute coronary events in patients with SA<sup>[42]</sup>.

However, conflicting report exists about the additive benefit of measurement of hs-CRP. While data from Sinning *et al*<sup>[127]</sup> suggest that, in patients with established CVD, traditional risk factors are the most powerful predictors with only little information added by inflammatory markers (including CRP), on the other hand several studies<sup>[45,128]</sup> showed that hs-CRP independently predicts cardiac events in patients with ACS<sup>[45]</sup>.

Moreover, patients with higher hs-CRP on admission for ACS had higher rate of impaired myocardial perfusion<sup>[129]</sup> and death.

Nakachi *et al*<sup>[130]</sup> reported that an hs-CRP elevation at admission and increase independently predicted 30-d events. In contrast, Bogaty *et al*<sup>[131]</sup> found that serial mea-

surements of hs-CRP in ACS patients have only a modest predictive ability, which disappeared after adjustment for common clinical variables. However authors did not exclude patients with acute or chronic inflammatory diseases.

Among ACS patients of the FAST-MI, authors found that low fetuin-A and high hs-CRP concentrations were associated with cardiovascular death, even after adjustment for GRACE risk score<sup>[132]</sup>. In another study by Schaub *et al*<sup>[133]</sup> in 398 consecutive patients presenting with acute chest pain novel biomarkers like myeloperoxidase, MRP-8/14 and hs-CRP, provided incremental value in the risk stratification of these patients.

## Apelin

Apelin was first discovered in 1998<sup>[134]</sup>, as the ligand of the so-called APJ receptor, a G-protein-coupled receptor (GPCR) identified in 1993 from a human genomic library. It is produced as *preproapelin*, then cleaved by an AT-converting enzyme to form several shorter C-terminal active peptides, *i.e.*, apelin-13, -16, -17, -19, -36 and a pyroglutamate form (Pyr1 apelin-13)<sup>[135]</sup>. Since the absence of an immediately apparent ligand, APJ was first classified as an *orphan GPCR*. It shares 31% sequence homology with the human angiotensin II (AT II) type 1 receptor, which led to further characterization of the Apelin-APJ system.

Overall, the apelin system has several physiological roles, most notably in the cardiovascular system, hypothalamus and the adipo-insular axis<sup>[136]</sup>, such as fluid homeostasis, glucose homeostasis, feeding behaviour, regulation of vascular tone, cardiac inotropism and immunity. First studies about apelin-APJ system found both similar and opposite functions to those of the AT system<sup>[137]</sup>. The distribution of both receptors and peptides overlaps in the hypothalamus and vasculature<sup>[138]</sup>. Moreover, Apelin has been detected in adipose tissue<sup>[139]</sup> and it was found that it was both produced and secreted by adipocytes. Apelin has been then considered as a novel adipokine. Also APJ is present in human and mouse adipose tissue, both in isolated adipocytes and in the stromal vascular fraction<sup>[140]</sup>.

Apelin expression in adipose tissue is regulated by nutritional status. In obese subjects, APJ-apelin expression is increased and this up-regulation could be reversed after diet or surgery-induced weight loss<sup>[141]</sup>. Moreover, changes in insulin levels might be involved for both apelin and APJ regulations in adipose tissue, according to the severity of insulin resistance<sup>[140]</sup>. A close relationship between apelin and insulin has been demonstrated both *in vivo* and *in vitro*. In cultured adipocytes, insulin treatment increased expression and secretion of apelin. Apelin expression in adipocytes is increased in various mouse models of obesity associated with hyperinsulinemia<sup>[139,142]</sup>. Interestingly, in highly insulin-resistant mice, such as db/db ones, APJ expression isn't increased<sup>[143]</sup> and in studies conducted in type 2 diabetic subjects, the effect of insulin resulted completely blunted in adipose tissue<sup>[140]</sup>.

Moreover, Apelin expression in adipose tissue is regulated also by TNF- $\alpha$ , gastro-intestinal inflammation, per-



oxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) coactivator-1  $\alpha$  (PGC1 $\alpha$ ), Eicosapentaenoic acid- $\alpha$  polyunsaturated fatty acid from the omega-3 family, which all increase the apelin expression and secretion<sup>[142,144-146]</sup>. AT II exerts different effects on the expression of apelin, depending on the receptor involved: type 1 AT receptor mediates an increase of the apelin secretion, while type 2 receptor may reduce its production<sup>[147]</sup>. Interestingly, glucocorticoids modulate the production of apelin and its secretion from fat cells, simultaneously increasing AT II and suppressing apelin expression, suggesting a possible pathogenetic mechanism in obesity-related hypertension<sup>[148]</sup>.

Since the increase in vascular density is essential for adipose tissue expansion, with endothelial cells actively promoting the development of preadipocytes and growth of mature adipocytes, apelin has been proposed to contribute to the development of new vasculature in expanding fat depot<sup>[149]</sup>. Several studies have demonstrated that apelin is a potent angiogenic factor<sup>[149]</sup>, grossly equivalent to vascular endothelial cell growth factor and, like other angiogenic factors, its gene is upregulated under hypoxia condition<sup>[150]</sup>. Hypoxia induces expression and secretion of apelin in both human and murine adipocytes, through the hypoxia-inducible transcription factor 1 $\alpha$ .

The first report in humans of plasma apelin concentrations was shown in obese and hyperinsulinemic subjects<sup>[139,141]</sup> where plasma apelin levels are increased. In morbidly obese patients with or without diabetes, apelin levels were only higher in the diabetic morbidly obese subjects<sup>[151]</sup>. However, reduced plasma apelin levels were described in obese subjects with untreated type 2 diabetes, compared to non-diabetic subjects and anti-diabetic treatment (rosiglitazone and metformin) was found to increase apelin concentration, with the improvement of glycemic profile<sup>[152,153]</sup>.

Changes in apelin levels after weight loss or bariatric surgery in obese individuals were also investigated. Diet-induced weight loss decreases apelin levels in moderate obese women<sup>[154]</sup> but not significantly in patients with the MetS<sup>[155]</sup> or in obese children<sup>[156]</sup>. Bariatric surgery resulted in a significant decrease in apelin levels only in morbidly obese patients exhibiting impaired fasting glucose or type 2 diabetes before surgery<sup>[151]</sup>. Probably, obesity *per se* is not the main determinant of increased plasma apelin concentrations since circulating apelin levels are not necessarily significantly correlated to the BMI in all the published studies<sup>[140]</sup>.

The possible role of Apelin in the atherosclerotic process has been investigated. In studies by Liu *et al.*<sup>[157]</sup>, Apelin-13, the predominant circulating apelin isoform, significantly promoted intracellular cholesterol efflux and reduces macrophage foam cell formation, indicating a potential antiatherogenic function. Moreover, Kadoglou *et al.*<sup>[158]</sup> have demonstrated lower apelin levels in patients with carotid atherosclerosis as compared to healthy controls, and that apelin increment is independently associated with atorvastatin-related carotid plaque stabilization. The same

group reported considerably lower apelin concentrations in CAD patients in comparison with healthy controls<sup>[159]</sup>. This finding was confirmed in other studies conducted among subjects with stable angina (SA), and the plasma apelin levels were found to be negatively correlated with the severity of the disease<sup>[160]</sup>.

A decrease of Apelin levels early after AMI has also been reported, with a progressive elevation over time, however reaching values lower than control subjects at 24 wk<sup>[161]</sup>. These observations were confirmed in patients with a first STEMI, where the reduction in apelin levels was independent from left ventricular dysfunction and outcome<sup>[162]</sup>. In comparison to asymptomatic CAD patients, plasmatic apelin were lower in ACS patients on admission, with a negative correlation with the severity of CAD<sup>[163]</sup>.

A myocardial protective effect has been suggested from studies on the possible therapeutic use of apelin in CAD in animal models. Azizi *et al.*<sup>[164]</sup> demonstrated in rat models of MI that post-infarct treatment with [Pyr1]-apelin-13 significantly attenuates myocardial damage, *via* the reduction of oxidative injury and enhancement of NO production. In addition, apelin-13 has been found to promote angiogenesis and ameliorate cardiac repair after AMI by a mechanism involving vascular progenitor cells<sup>[165]</sup>.

However from a pathophysiological point of view, some conflicting data have been reported. For example, Rittig *et al.*<sup>[166]</sup> observed that plasma apelin levels are not associated with early stages of atherosclerosis in young subjects prone to atherosclerosis and type 2 diabetes. Interestingly, other studies in animal models suggested a role of apelin-APJ system in vasculature oxidative stress. Furthermore, Apelin is upregulated in human atherosclerotic coronary artery and potentially constricts human coronary artery<sup>[167]</sup>. Data by Jin *et al.*<sup>[168]</sup> show that genetic defects in apelin/APJ pathway may confer a potential risk for CAD in Chinese hypertensive patients. These evidences underline the complex role of Apelin and its receptor in atherosclerosis.

## CONCLUSION

Vascular inflammation represents a fundamental link between obesity, MetS and their detrimental complications. Conflicting evidence about the *in vitro* and *in vivo* effects of Adipocytokines suggests the high complexity of these mediators interplay in the pathogenesis of atherosclerosis and, moreover, in the risk stratification of CAD patients. Even large evidence about the use of hs-CRP for primary and secondary prevention of CVD has been questioning for its real additive value.

However, the involvement of adipocytokines in the pathogenesis of atherothrombosis and dysmetabolism remains clear, although it appears to be way more complex than previously thought. The understanding of these pathways may lead to the development of targeted treatment of obesity-related disorders. In this setting, the

JUPITER trial provided some clue about the association between inflammation and the risk of CVD, even though it was not designed to evaluate the role of the pharmacological modulation of inflammation<sup>[124]</sup>. In this context, only two trials are ongoing, the Cardiovascular Inflammation Reduction Trial<sup>[169]</sup> and The canakinumab anti-inflammatory thrombosis outcomes study (CANTOS)<sup>[170]</sup>. The first is investigating the role of low-dose methotrexate on incident heart attacks, strokes, or death in people with type 2 diabetes or MetS that have had a heart attack or multiple coronary stenoses. CANTOS is studying the effect of Canakinumab, a human monoclonal antibody that neutralizes interleukin-1beta, in secondary prevention<sup>[170]</sup>.

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## WJC 6<sup>th</sup> Anniversary Special Issues (2): Coronary artery disease

# Risks and diagnosis of coronary artery disease in Hodgkin lymphoma survivors

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## Abstract

Higher mortality rates are reported because of cardiovascular diseases in individuals living in industrialized areas of the World. In cancer patients, cardiotoxic chemotherapeutic agents and/or mediastinal radiotherapy are additional risk factors for the development of coronary artery disease. An improved survival rate for patients with Hodgkin lymphoma was reported in recent decades. Determining and handling the long-term effects of cancer treatment have become more important nowadays, parallel to the good results reached in survival rates. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are routinely used to treat Hodgkin lymphoma but are commonly associated with a variety of cardiovascular complications. Drugs used in cancer treatment and radiotherapy may cause deleterious effects on contractile capacity and conduction system of the heart. Approximately ten years after the completion of all therapies, the cardiovascular disease risk peaks in patients who survived from Hodgkin lymphoma. The value of coronary computed tomography angiography as a diagnostic tool in determining coronary artery disease as early as possible is underlined in this review, in patients who are in remission and carry the risk of coronary artery disease probably because of

chemo/radiotherapy used in their treatment. Survivors of Hodgkin lymphoma especially treated with combined chemoradiotherapy at younger ages are candidates for coronary computed tomography angiography.

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**Key words:** Coronary artery disease; Hodgkin lymphoma; Computed tomography angiography; Cardiotoxicity; Survivors

**Core tip:** With substantial increase in survival rates from cancer, late adverse effects of cancer therapy have become extremely important. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are routinely used to treat Hodgkin lymphoma (HL) but are commonly associated with a variety of cardiovascular complications including coronary artery disease (CAD). For surviving individuals after HL treatment, coronary computed tomography angiography is a non-invasive and useful method in detecting CAD at an early stage. Survivors of HL especially treated with combined chemo-radiotherapy at young ages, who carry the risk of CAD development are candidates for coronary computed tomography angiography.

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## INTRODUCTION

Surviving individuals after treatment of malignant diseases have markedly increased in last decades probably because of advanced diagnostic abilities and effective cancer treatment. Long-term unintended effects of aggressive treatments, unfortunately have emerged as a

serious problem at the same time. The adverse effects on heart are among the deadliest effects having high rate of morbidity and mortality. Cardiotoxic chemotherapeutic agents, such as doxorubicin, daunorubicin and epirubicin can decrease the cardiac functioning and contractility of myocardium and the signs of malfunction may even emerge many years after ceseation of cancer treatment<sup>[1-5]</sup>. The degree of cardiac dysfunction depends basically on cumulative drug doses of anthracyclines<sup>[6-11]</sup>. Mediastinal radiotherapy delivered at the same time with cardiotoxic antineoplastic drugs can also affects the normal functioning of the heart in that population<sup>[12,13]</sup>. Screening these individuals for treatment related cardiac toxicity, diagnosing and treating them as early as possible are cornerstones of proper management of cardiovascular disorders. Therefore, screening of cardiac functions of these individuals after ceseation of cancer treatment is particularly important and the principles for the following-up of these patients have been published<sup>[14]</sup>.

Some researchers have reported higher relative risks of myocardial infarction mortality in patients treated at younger ages than in patients treated at older ages and in men than in women<sup>[15-19]</sup>. Other researchers have reported valvular dysfunction, carotid, subclavian and coronary artery disease and even fatality from cardiac infarction at early childhood after radiation therapy for the treatment of Hodgkin lymphoma (HL)<sup>[20-23]</sup>. In contrast to numerous papers dealing with cardiac functions in cancer survivors, articles investigating the status of heart and its vasculature in survivors of HL treated in pediatric age group are scarce<sup>[11-13,24]</sup>.

## CORONARY HEART DISEASE

In industrialized Western countries, coronary heart disease is among the leading causes of mortality<sup>[25,26]</sup>. Coronary artery disease (CAD) is diagnosed more often in middle-aged males and it is also one of the major causes of mortality in women after menopause<sup>[27]</sup>. Advanced biological age, hypertension, increased body-mass index, hyperlipidemia, diabetes mellitus, smoking or use of tobacco products, and presence of CAD among the family members are among the traditional risk factors for CAD<sup>[28]</sup>. Researchers are trying to find out genes that creating predisposition to CAD<sup>[29-31]</sup>. Beside these, cardiotoxic chemotherapeutic agents and/or mediastinal radiotherapy are additional risk factors for cancer survivors.

## RISK FACTORS IN PATIENTS WITH HODGKIN LYMPHOMA

### *Hodgkin lymphoma*

In developed countries, lymphomas are the third most frequent tumors among the pediatric cancers following leukemias and central nervous system tumors. In contrast, in our country and most of the developing parts of the World lymphomas just follow the leukemias in frequency<sup>[32]</sup>. With advanced diagnostic and therapeutic fa-

cilities the survival rates in low and high risk patients with HL increased to 95% and 90% respectively<sup>[33,34]</sup>. Similarly, improved results after HL treatment were also published in articles from Turkey in recent years<sup>[35,36]</sup>. To diagnose earlier and proper treatment of long-term unwanted effects have become one of the main issues in practice of both Pediatric and Medical Oncology parallel to the good results taken in cancer treatment. The frequency of cardiovascular disease peaks generally five to ten years after the completion of HL treatment<sup>[35,36]</sup>.

### *Treatment toxicity in HL*

In HL combined chemotherapy with low dose involved field radiotherapy (1500-2500 cGy) is the preferred treatment. The mostly used chemotherapeutic regimens in HL are mechlorethamine, vincristine, prednisone and procarbazine (MOPP), cyclophosphamide, vincristine, prednisone and procarbazine (COPP), doxorubicin, bleomycin, vinblastine, dacarbazine (DBVD), OPPA (vincristine, procarbazine, prednisone, adriamycin), and MOPP/DBVD alternating protocols<sup>[33,34]</sup>. Among the acute side effects of multiagent chemotherapy protocols nausea and vomiting are the leading ones. Many chemotherapy schemes produce bone marrow suppression and reversible alopecia. Bleomycine-related pulmonary toxicity, vincristine-related neurotoxicity, doxorubicin-related cardiotoxicity are the other side effects of chemotherapy. Radiation pneumonitis, pulmonary fibrosis, spontaneous pneumothorax, abnormalities in growing soft tissues and bones, cardiovascular, and endocrine abnormalities constitute the late effects of treatment. Second malignant neoplasms, especially ALL, are also among the late effects of therapy<sup>[33,34,37-39]</sup>.

Antineoplastic drugs, especially anthracyclines and mediastinal radiotherapy can cause decrease in cardiac contractility, heart insufficiency, pericardial effusion, constrictive pericarditis, coronary artery disease, myocardial infarction, and arrhythmias<sup>[15-19,40]</sup>. Vascular narrowing and cerebrovascular accidents are also among the late complications. Late subclinical cardiovascular side effects are apparent especially in patients 30 to 50 years of age<sup>[37]</sup>. The most common chemotherapeutic agents implicated in the development of cardiovascular complications include the anthracyclines, alkylating agents, and vinca alkaloids<sup>[41,42]</sup>. Alkylating agents such as cyclophosphamide may exacerbate anthracycline or radiation induced cardiac injury. In adults, the frequency of congestive heart failure increases with the cumulative doxorubicin doses greater than 550 mg/m<sup>2</sup><sup>[37]</sup>. Mediastinal radiation and other chemotherapeutic drugs are thought to lower the threshold. Since then, all patients treated with anthracycline-containing protocols and mediastinal radiotherapy must be followed up for cardiac injury.

### *Effect of radiation on vessels*

In the treatment of HL, anthracyclines and delivering irradiation to the nodal areas affected are routinely administered. Although not more often, deaths because of myocardial infarction at early ages after HL treatment

were reported<sup>[20-23]</sup>. It is impossible to find out exact figures in literature for the frequency of heart diseases in HL survivors. Radiation arteritis may occur as a result of the previous radiation therapy<sup>[43]</sup>. Arteries of young children are more susceptible than those in adults. Stenosis and occlusion can be detected angiographically in arteries in the area of radiation. Additionally computed tomography angiography (CTA) can show arterial wall thickening and radiation effects in other soft tissues.

The effects of radiation in tissues received radiation can be classified into a few groups: occurring in epithelial and parenchymal organs, in blood vessels and in stroma<sup>[43]</sup>. The vessels having the shortest diameter are the most radiosensitive ones. The reason behind this sensitivity is mostly arising from vulnerable character of endothelium layering the vessels. The changes of radiation in tissues are best studied and documented in animal trials and include irregularity of cytoplasm with the formation of pseudopodia or swelling of cytoplasm often obstructing the lumen, detachment of endothelial cells from the basal lamina, cell pyknosis, rupture of plasma membrane, thrombosis, and rupture of the capillary wall<sup>[44]</sup>.

Arteritis occurs basically in vessel wall and inflammation progresses to thickening in arterial wall resembling the process of atherosclerosis<sup>[45]</sup>. Foam cell plaques in medium and small arteries are suggestive of irradiation. Recent studies confirm that acute vasculitis can be induced by ionizing radiation. Some researchers determined acute vasculitis in small arteries next to coronary arteries or iliac arteries exposed to local radiation therapy. The estimated doses received at the sites of vasculitis varies between 600 and 4000 cGy. Large arteries are less often affected from radiation because of their large lumen and thick wall. Some experimental evidence indicates that arterial perforations may occur due to high dose irradiation<sup>[43]</sup>.

### HL and CAD

Heart diseases are among the frequently seen long-term effects of chemo/radiotherapy used in HL treatment. Mediastinal radiotherapy and cardiotoxic chemotherapeutic agents are commonly associated with a variety of cardiovascular complications including CAD. The mechanism of injury is multifactorial and likely involves endothelial damage of the coronary arteries and secretion of multiple inflammatory and profibrotic cytokines<sup>[46-48]</sup>. Heidenreich *et al*<sup>[23]</sup> have reported unexpected early deaths from myocardial infarction at young ages after HL<sup>[21-23]</sup>.

Taken into consideration the relation between the degree of HL treatment and treatment related risks on heart, studies conducted with the aim of giving smaller doses of radiotherapy and lower doses or shortened duration of cardiotoxic agents can limit heart toxicity. Monitoring the patients for classical and generally accepted risk factors for CAD is another important method in lowering the incidence of heart diseases in HL survivors. Rademaker *et al*<sup>[13]</sup> reported that coronary CTA and calcium scores are useful methods for the evaluation of irradiation-related CAD in their nine patient series. In a recent study, we investigated CAD by using CTA in 119

HL survivors treated at the pediatric age group<sup>[12]</sup>. Hodgkin lymphoma survivors who are in remission at least 2 years after cessation of treatment were investigated. They were questioned about the coronary artery risk factors. Complete blood count, general biochemistry, lipid profile, cardiac troponin-T (cTT) and creatinine kinase myocardial band have been studied. Additionally electrocardiogram (ECG), telecardiography, echocardiography, and coronary CTA were undertaken in all patients. Using a multiplanar reformat, intensity projection, and volume rendering reformat techniques, CTA images were reviewed and mediastinal and cardiac vascular abnormalities were investigated. In 19 (16%) of the patients we determined coronary artery abnormalities. We found statistically significant relation between radiation therapy delivered to the mediastinum and development of an abnormality in coronaries. Probability of developing a coronary abnormality was 6 to 8 times higher in group of patients receiving mediastinal radiotherapy more than 2000 cGy in comparison with the other group receiving radiotherapy less than 2000 cGy by multivariate analysis ( $P = 0.009$ )<sup>[12]</sup>. This study confirms the detrimental late effects of mediastinal radiotherapy on coronary arteries of growing children.

## DIAGNOSIS OF CARDIOVASCULAR DISEASES AFTER TREATMENT OF HODGKIN LYMPHOMA

### Screening for cardiovascular complications

Screening the long-term survivors of a malignant disease for chemo/radiotherapy related toxicity on heart and managing the abnormalities as early as possible are obviously vital strategies in good management of cardiovascular complications. For this reason, cardiac monitoring of surviving patients after completion of treatment is an obligation.

It is ideal to find out minimally invasive and accurate methods of diagnosis to describe cardiac toxicity similar to other late-effect studies. Currently, most of the centers use echocardiography (ECHO) for periodic follow-up of the heart condition. cTT, an appropriate serological marker to suspect from damage in myocardium was suggested for earlier detection of anthracycline related toxicity after animal studies<sup>[49]</sup>. However, no elevation of serum cTT after cessation of adriamycin was reported, although insignificant increases were scored in individuals receiving adriamycin<sup>[50]</sup>. Kismet *et al*<sup>[11]</sup> have found no correlation between serum cTT values, cumulative dose of adriamycin, and systolic or diastolic functions of the heart and concluded that screening with ECHO is more appropriate than cTT for determining subclinical cardiotoxicity.

Echocardiography is the most commonly used diagnostic facility to follow cardiac functions of cancer survivors<sup>[1]</sup>. The traditional approach of screening cardiac toxicity comprises a baseline examination before the start of the cardiotoxic chemotherapy and serial measurements of contractile capacity of the heart (*e.g.*, ejection frac-

tion and fractional shortening) during the course of the treatment. However, the measurement of only ejection fraction as an indicator of left ventricular (LV) function is not reliable to determine subclinical disorders of myocardium<sup>[51,52]</sup>. Additionally, conventional Doppler ECHO has some limitations, basically because its dependence on loading conditions, and frequently has negative influence on the interpretation of the findings.

Tissue doppler imaging (TDI) is recently used commonly to evaluate the velocity of myocardial segments with the use of Doppler effect. TDI is superior to traditional Doppler studies in that it can overcome the dependence of loading and detect the abnormalities in LV. This new technique can be employed in evaluation of LV functioning in part or in whole. TDI has some advantages on conventional Doppler ECHO in the evaluation of global or regional diastolic functional capacity of LV<sup>[53]</sup>. Alehan *et al*<sup>[24]</sup> showed that subtle systolic and diastolic malfunction occurs in long-term survivors of HL by using TDI. Survivors treated with anthracycline based chemotherapy and/or mediastinal radiotherapy may suffer from heart toxicity many years after the cessation of treatment. Malfunction in cardiac systole generally follows the dysfunction in cardiac diastole and prophylactic administration of medications such as angiotensin converting enzyme inhibitors can help preventing the deterioration of heart damage. Obviously, more investigation is necessary to find out accurate strategy for monitoring heart toxicity, but it seems at least today, serial examinations of contractile capacity with TDI in individuals who are in remission after HL treatment can help determining patients under risk of cardiac disorders<sup>[24]</sup>.

### Screening for coronary artery disease with CTA in survivors of Hodgkin lymphoma

CTA employs X-ray to screen blood flow in vasculature in whole body<sup>[54]</sup>. X-ray bundles are scattered from a spinning device into the body part which is examined, and they form cross-sectional images that are collected by the computer to give a 3D Picture of the study region. Compared to catheter angiography, the gold standard procedure for evaluation of arteries, involving placement of a catheter and injection of some amounts of contrasted medium into a large vessel, CTA is a minimally invasive procedure. Major and minor complications can be seen in conventional angiography<sup>[55]</sup>. Contrasted material is injected into a small vein in CTA, and for most of the patients hospitalization is not necessary. Apart from cost advantage compared to conventional angiography CTA provides information about the vascular wall and soft tissues besides vessel lumen, helps determining the pathologic vessels and additional extra vascular abnormalities in advance<sup>[12,13,56]</sup>.

In the cardiac CT, predicted radiation exposure is 2-2.5 Rem and this is higher than 1.5-2 Rem that is exposed in diagnostic pediatric cardiac catheterization<sup>[54]</sup>. With contemporary modern detectors, the exposure can be decreased by using the ECG dose modulation technique by using higher X-ray doses to evaluate coronary arteries in

diastolic phases and lowered doses in systolic phases<sup>[21]</sup>. In normal coronaries, it is unusual to find calcification in an arterial wall. CTA is also sensitive in detecting calcium in arterial wall<sup>[13]</sup>. The increase in calcium scores can be halted with the use of hypolipidemic drugs in patients with high calcium scores in their coronaries<sup>[57]</sup>. A conventional angiography, however, cannot be indicated solely based on coronary calcium scoring due to its low specificity<sup>[55]</sup>. In the presence of massive coronary calcification, a CTA cannot show the thickening in the vessels because of signal changes<sup>[54]</sup>.

Although the CTA has found a place of application in many fields and clinical situations<sup>[58-61]</sup> it currently has some limitations. Blocked blood vessels make difficult the interpretation of the images<sup>[55]</sup>. The CTA is not yet reliable for visualization of small, vessels in rapidly moving organs. CTA images can be blurred because of movements during the examination or because of the heart that is not beating properly. High-density objects such as metal clips, stents, and calcified plaques prevent the proper visualization of the neighboring tissues by the attenuation they created<sup>[55]</sup>. The dose of radiation exposed during the examination is also a limiting parameter. With a 64-detector computerized tomography, the dose of radiation given to the patients is approximately 6.5 to 15 mSv and this is much more than that used in conventional angiography<sup>[54]</sup>. The examination brings some risks such as allergic reaction to the contrast material and it must not be performed in renal disease, severe diabetes, pregnant or breastfeeding women.

The above mentioned study is the unique study in which CTA was used for determination of abnormalities in coronary arteries in HL survivors treated in childhood<sup>[12]</sup>. The capability of CTA in early detection of CAD was shown for the first time in this patient population. Based on our findings we concluded that individuals at the age of 17-28 years, treated in childhood for HL and carry the risk of CAD and specifically treated with radiation therapy into the mediastinum, are candidates for coronary CTA.

## CONCLUSION

Serial follow-up including screening for valvular disease with TDI and coronary artery disease with CTA and coronary artery calcium scoring, must be applied to the survivors of HL who have been treated with anthracycline including regimens and/or mediastinal radiotherapy like a great majority of the patients with HL.

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## WJC 6<sup>th</sup> Anniversary Special Issues (3): Cardiomyopathy

# Non-invasive evaluation of arrhythmic risk in dilated cardiomyopathy: From imaging to electrocardiographic measures

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**Core tip:** Arrhythmic risk stratification and decision making towards implantation of a cardioverter defibrillator in dilated cardiomyopathy patients are still open challenges. This review critically revises the possible clinical usefulness of available non-invasive diagnostic tools employed to stratify arrhythmic risk prognosis in dilated cardiomyopathy patients.

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## Abstract

Malignant ventricular arrhythmias are a major adverse event and worsen the prognosis of patients affected by ischemic and non-ischemic dilated cardiomyopathy. The main parameter currently used to stratify arrhythmic risk and guide decision making towards the implantation of a cardioverter defibrillator is the evaluation of the left ventricular ejection fraction. However, this strategy is characterized by several limitations and consequently additional parameters have been suggested in order to improve arrhythmic risk stratification. The aim of this review is to critically revise the prognostic significance of non-invasive diagnostic tools in order to better stratify the arrhythmic risk prognosis of dilated cardiomyopathy patients.

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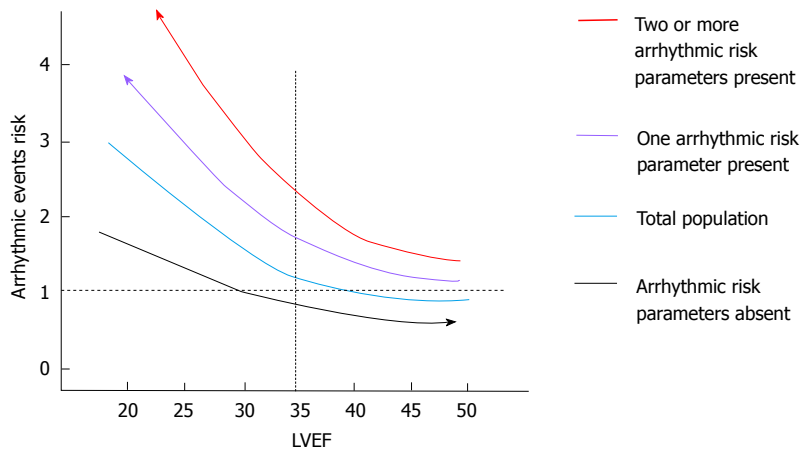
**Key words:** Dilated cardiomyopathy; Major ventricular arrhythmias; Prognosis; Ventricular repolarization; Left ventricular systolic function

## INTRODUCTION

The main adverse events affecting the prognosis for both ischemic (IDCM) and non-ischemic (NIDCM) dilated cardiomyopathy patients are the occurrence of malignant ventricular arrhythmias and sudden death and the progression towards heart failure<sup>[1]</sup>. In order to reduce the incidence of sudden death due to ventricular arrhythmias, the best therapeutic strategy to date is cardioverter defibrillator implantation (ICD)<sup>[1-3]</sup>. Both for NIDCM and IDCM, the decision to implant an ICD is mainly guided by the evaluation of left ventricular systolic function, *i.e.*, by the calculation of left ventricular ejection fraction (LVEF)<sup>[4]</sup>. However, its use in defining eligible patients has a number of limitations.

In particular, there are a large number of patients who do not benefit from ICD<sup>[5]</sup>. In fact, the majority of patients with low LVEF who were enrolled in the main trials evaluating the effect of ICD did not suffer from malignant ventricular arrhythmias. For example, only 26% of the MADIT II patients had malignant ventricu-





**Figure 1** The effect of a better arrhythmic risk stratification are shown. The presence of one or more arrhythmic risk factor allows detection of a population at higher risk of arrhythmic events across all the values of left ventricular ejection fraction. On the other hand, the absence of arrhythmic risk factors is associated with the detection of the group of patients at lower risk of events. LVEF: Left ventricular ejection fraction.

lar arrhythmias during a 24 mo follow-up<sup>[2]</sup>. Only 31% of the 829 patients enrolled in the ICD group of the SCD-HeFT trial received shocks from their device for any cause and only 177 (21%) received shocks to arrest rapid ventricular tachycardia or ventricular fibrillation. During a five year follow-up, the annual average rate of ICD shocks was 7.5%; however, the annual average rate for appropriate ICD shocks (*i.e.*, shocks for rapid, sustained ventricular tachycardia or fibrillation) was 5.1%. Moreover, in the SCD-HeFT trial, 32 (4%) patients had their ICD removed during follow-up and ICD complications, defined as clinical events requiring surgical correction, hospitalization, or new and otherwise unanticipated drug therapy, occurred in 5% of patients at the time of implantation and in 9% at a later stage in the trial<sup>[3]</sup>.

It is clear from these data that the need to better assess arrhythmic risk is still a challenge<sup>[5]</sup>. Better characterization of patients using additional parameters should be able to detect those with a higher or lower risk of arrhythmic events, thus avoiding ICD implantation in patients with low LVEF at low risk and facilitating the implantation of patients with good LVEF at higher risk (Figure 1).

The aim of this review is to critically revise the possible clinical usefulness of the available non-invasive parameters related to the pathophysiology of ventricular arrhythmias (Figure 2) which have been proposed in order to better stratify the arrhythmic risk of dilated cardiomyopathy patients.

## THE IMAGING TO DETECT ARRHYTHMIC SUBSTRATES

### *The assessment of left ventricular systolic function*

As previously stated, the use of LVEF to guide decisions on whether to implant ICD leads to only a small percentage that will suffer from ventricular arrhythmias in a selection of a large population. However, the limitation of this approach is also related to several technical and biological aspects.

Firstly, in repeated evaluations, the LVEF calculation is characterized by a wide variability, particularly when an echocardiographic approach is considered. This is even

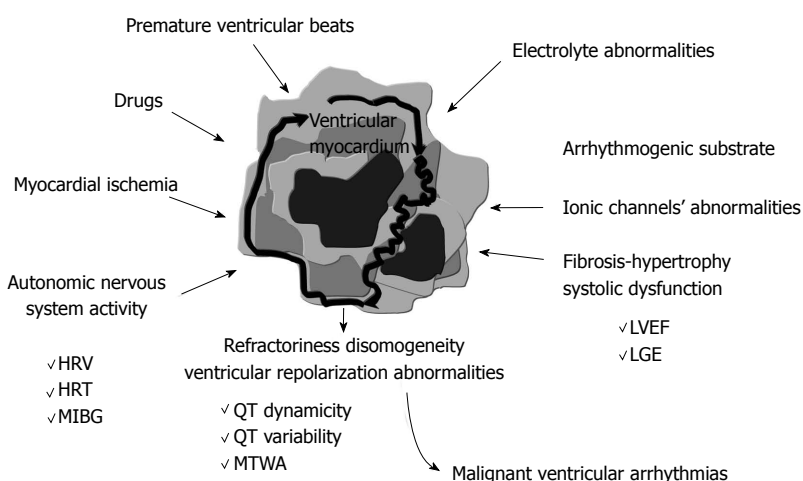
more pronounced when different readers perform the calculation<sup>[6]</sup>. An improvement in the accuracy of LVEF calculation by echocardiography could be obtained using contrast echocardiography<sup>[7]</sup> or the 3-dimensional (3D) approach<sup>[8]</sup>, but the gold standard for a more accurate and reproducible 3-D quantification of left ventricular (LV) volumes is cardiac magnetic resonance (CMR)<sup>[7,9]</sup>.

Apart from the technical limitations in LVEF assessment, variability of the measure may also be influenced by biological factors. In particular, LVEF can vary in the different loading conditions due to changes in intravascular volumes and/or adrenergic drive<sup>[5,10]</sup>. Moreover, LVEF can change over time in response to conventional medical therapy<sup>[11]</sup>.

In this setting, the new echocardiographic measures to evaluate left ventricular systolic function, which are less loading dependent, could be a new, useful tool to improve arrhythmic risk stratification by echocardiography<sup>[10]</sup>. Among these, two-dimensional (2-D) speckle tracking analysis<sup>[12]</sup> seems to be a particularly promising technique as it has been validated by sonomicrometry and tagged magnetic resonance imaging<sup>[13]</sup> and can quantify global and regional cardiac function more accurately and objectively by detecting mild ventricular function abnormalities in both left and right ventricular cardiomyopathies<sup>[14-15]</sup>.

2-D speckle-tracking analysis is based on the detection and the motion tracking of natural acoustic myocardial reflections and interference patterns within an ultrasonic window. The tracking system analyses of echocardiographic grayscale B-mode images permits measurement of the entity of myocardial deformation (strain). Strain parameters can be individualized for each of the myocardial segments or can be expressed as global strain when all the segmental values are averaged. The global longitudinal strain (GLS) is the mean values of myocardial segmental deformation, evaluated using standard apical views. From a technical point of view, the use of 2-D strain measures offers some advantages over routine echocardiographic assessment of LVEF using Simpson's rule. In particular, strain analysis is not based on any geometrical assumption and should depend less on loading conditions. Moreover, in regional contractility

Triggering and/or favouring factors



**Figure 2** The main parameters proposed in order to better characterize arrhythmic risk are shown. These parameters can reflect arrhythmic substrate by functional (left ventricular systolic function) or anatomical (myocardial fibrosis) information. The parameters assessing sympathetic nervous system activity are also reported, as well as those reflecting the dispersion of ventricular refractoriness, *i.e.*, those based on the analysis of ventricular repolarization. HRV: Heart rate variability; HRT: Heart rate turbulence; LGE: Late gadolinium enhancement; LVEF: Left ventricular ejection fraction; MIBG: Iodine-123 metaiodobenzylguanidine; MTWA: Microvolt T-wave alternans.

dysfunction, strain measures better correlate with LVEF as assessed by magnetic resonance<sup>[16]</sup>. Finally, GLS is easy to compute and less dependent on specific training to ensure reproducibility<sup>[17]</sup>.

In order to evaluate the role of this novel technique in stratifying arrhythmic risk prognosis, we recently studied a group of heart failure (HF) outpatients affected by IDCM and NIDCM who had never previously experienced sustained ventricular arrhythmias<sup>[18]</sup>. During a mean follow-up of  $26 \pm 13$  mo, 31 of 230 patients experienced entricular ventricular tachycardia (VT)/fibrillation (VF) or sudden death. At multivariate analysis, after correction for the univariate predictors, *i.e.*, NYHA class, NT-proBNP and non-sustained ventricular tachycardia (NSVT), GLS remained significantly associated with ventricular arrhythmic events. The best GLS cut-off value detected by ROC curves for the 1 year occurrence of events was  $-10.0\%$ , with a 73% sensitivity and a 61% specificity in detecting patients prone to experiencing major ventricular arrhythmias. Interestingly, the annual incidence rates of arrhythmic events were significantly greater in the 24 patients with a LVEF  $> 35\%$  and a GLS above  $-10\%$  than in the 114 patients with GLS below  $-10\%$ , whereas no additive value was observed among patients with a LVEF  $\leq 35\%$ .

### Assessment of myocardial fibrosis

In arrhythmic risk stratification, the usefulness of CMR is related not only to the possibility of more accurately estimating LVEF<sup>[19-22]</sup>, but also to its ability to detect the presence of myocardial replacement fibrosis<sup>[23]</sup>. CMR assessment of fibrosis is made possible by using late gadolinium enhancement. Gadolinium is a contrast agent that has been shown to be extremely safe. It is an extracellular agent, accumulating in areas of interstitial expansion due to myocardial fibrosis, edema or infiltration. After gadolinium administration, it is possible to assess three phases: the first provides immediate images at rest or during stress, followed by early enhancement after 5 min and late enhancement 5 to 20 min after administration<sup>[22]</sup>. Late gadolinium enhancement (LGE) imaging allows the detection of contrast accumulation in areas of

infarction or fibrosis due to slower contrast kinetics and greater volume or distribution in extracellular matrix. The extent and pattern of LGE enhancement varies according to the underlying pathological process. Fibrosis extent can be quantified as a percentage of total LV mass using dedicated software<sup>[22-23]</sup>. Moreover, the relative safety of gadolinium agents and tissue characterization sequences allows repeated imaging, follow-up, family screening and serial risk stratification<sup>[24]</sup>.

The presence of fibrosis, as assessed by LGE, is associated with a greater probability of inducible ventricular tachycardia<sup>[25]</sup>. Moreover, there is considerable evidence that it is also associated with a worse prognosis and an increased arrhythmic risk. Table 1 summarizes the main studies with this evidence<sup>[26-33]</sup>.

Assomull *et al.*<sup>[26]</sup> first evaluated the prognostic impact of midwall fibrosis in patients diagnosed with NIDCM, prospectively followed up for  $658 \pm 355$  d. Midwall fibrosis was present in 35% of patients and was associated with a higher rate of all-cause death and hospitalization for a cardiovascular event. Multivariate analysis showed that it was the only significant predictor of death or hospitalization. Midwall fibrosis also predicted sudden cardiac death (SCD) or VT and remained predictive of SCD/VT after correction for baseline LVEF.

Iles *et al.*<sup>[28]</sup> prospectively evaluated 103 patients meeting criteria for ICD implantation for primary prevention of SCD who were affected by both IDCM and NIDCM. Regional fibrosis was identified with LGE in 71% of patients, in all patients with a diagnosis of IDCM and in 51% of those affected by NIDCM. Interestingly, among NIDCM patients, LGE was associated with arrhythmic events during follow-up in 29%, whereas no NIDCM patients without LGE experienced arrhythmic events.

Finally, the relevant role played by LGE in arrhythmic risk stratification has been supported by a study evaluating a large sample of NIDCM patients<sup>[33]</sup>. In this series, 30% of patients had fibrosis and were characterized by a lower LVEF and a more severe functional limitation. The presence of fibrosis was independently associated with an increased arrhythmic risk as well as an increased prob-

**Table 1** The main studies evaluating the association between myocardial fibrosis assessed by cardiac magnetic resonance and the risk of arrhythmic and non-arrhythmic events

Ref.	Clinical setting	Number of patients	CMR parameters	End-points (mean follow-up)	Results
Assomoul <i>et al</i> <sup>[26]</sup> , 2006	NIDCM	101	Midwall fibrosis (LGE)	All-cause death and hospitalization (follow-up 658 ± 355 d)	Independent association with death and hospitalization
Wu <i>et al</i> <sup>[27]</sup> , 2008	NIDCM and LVEF ≤ 35%	65	Presence and extent of LGE	Composite end-point (hospitalization for heart failure, appropriate ICD firing, cardiac death) (Follow-up median 24 mo)	Presence of LGE was associated with a greater risk of primary outcome
Iles <i>et al</i> <sup>[28]</sup> , 2011	IDCM/NIDCM before ICD implantation	103	Regional fibrosis with LGE	Arrhythmic events and appropriate ICD therapy (follow-up 573 d)	LGE was associated with arrhythmic events and appropriate ICD therapy during follow-up
Lehrke <i>et al</i> <sup>[29]</sup> , 2011	NIDCM	184	Presence of LGE	Composite end-point (hospitalization for decompensated heart failure, cardiac death, cardioverter defibrillator discharge) (follow-up 31 mo)	Presence of LGE was associated with composite endpoint
Gao <i>et al</i> <sup>[30]</sup> , 2012	IDCM/NIDCM	124	Presence and quantification of LGE	Primary composite outcome: occurrence of appropriate ICD therapy, SCA, SCD (follow-up 632 ± 262 d)	Myocardial scar quantification by LGE-CMR predicts arrhythmic events in patients being evaluated for ICD eligibility
Neilan <i>et al</i> <sup>[31]</sup> , 2013	NIDCM	162	Presence and quantification of LGE	Major adverse cardiac events (cardiovascular death and appropriate ICD therapy) (follow-up: 29 ± 18 mo)	Presence of LGE was a strong predictor of major cardiac events
Li <i>et al</i> <sup>[32]</sup> , 2013	NIDCM	293	Presence and extent of LGE	All-cause mortality (follow-up: 3.2 yr)	Presence of LGE is an independent predictor of increased all-cause mortality Diffuse LGE is associated with higher mortality
Gulati <i>et al</i> <sup>[33]</sup> , 2013	NIDCM	472	Presence and extent of midwall fibrosis	Primary end-point: all cause mortality Secondary end-point: cardiovascular mortality or cardiac transplantation Arrhythmic and HF secondary end-points (follow-up 5.3 yr)	Midwall fibrosis assessed with LGE-CMR provided independent prognostic information and improved risk stratification beyond LVEF for all-cause mortality and SCD

CMR: Cardiac magnetic resonance; IDCM: Ischemic dilated cardiomyopathy; LGE: Late gadolinium enhancement; NIDCM: Non ischemic dilated cardiomyopathy; SCA Survived cardiac arrest; SCD: Sudden cardiac death; ICD: Implantable cardioverter defibrillator.

ability of death. Moreover, whether fibrosis was present or not, it was possible to detect the group of patients at higher and lower risk across the LVEF spectrum. For example, patients with a LVEF of 35% and fibrosis had a 19.9% estimated risk of death *vs* 9.4% of patients with the same LVEF but without fibrosis.

Although there is considerable evidence to suggest the relevance of LGE in arrhythmic risk stratification, particularly in NIDCM, this technique has not been recommended yet by current guidelines for the selection of patients who will benefit from ICD implantation.

## ELECTROCARDIOGRAPHIC MEASURES OF ARRHYTHMIC RISK

### Fragmented QRS

Prolonged QRS duration prevalence in patients with congestive heart failure varies between 20% and 50%<sup>[34]</sup>. Left bundle branch block and, in general, QRS prolongation (> 120 ms) in heart failure patients independently predict increased overall mortality and SCD<sup>[35-36]</sup>.

However, fragmented QRS complexes (f-QRS) on a routine 12-lead electrocardiogram have also been pro-

posed as a marker of depolarization abnormality<sup>[37]</sup>.

Various studies have suggested that the region of a myocardial scar is associated with alteration in QRS morphology, leading to a terminal conduction delay or a fragmentation of QRS complexes on the 12-lead ECG<sup>[38-39]</sup>.

Fragmented QRS includes various RSR' patterns with different morphologies of the QRS interval (QRS duration < 120 ms), with or without the Q wave. It is defined by the presence of an additional R wave (R') or notching in the nadir of the S wave, or the presence of > 1 R' wave (fragmentation) in 2 contiguous leads, corresponding to a major coronary artery territory<sup>[40]</sup>.

Brenyo *et al*<sup>[41]</sup> observed that fragmented QRS (f-QRS), particularly when present in inferior leads, is predictive of SCD, SCD or appropriate ICD shock and all-cause mortality in patients with IDCM.

Sha *et al*<sup>[42]</sup> evaluated a population of 128 patients with NIDCM and left ventricular dysfunction (ejection fraction, EF ≤ 40%). They observed that in the group with f-QRS, all-cause mortality and ventricular tachyarrhythmias were significantly more frequent than those observed in the non-fQRS group.

Finally, Das *et al*<sup>[43]</sup> tried to assess the prognostic

**Table 2** Main studies evaluating the role of dynamic ventricular repolarization measures in predicting arrhythmic and non arrhythmic events

Ref.	Clinical setting	Number of patients	Parameter evaluated	Cut-off suggested	End-points (mean follow-up)	Results
Chevalier <i>et al</i> <sup>[46]</sup> , 2003	Acute myocardial infarction	265	QT dynamicity and HRV (24-h Holter) LVEF Late potential	QTe slope: 0.18	Sudden death and total mortality (follow-up 81 ± 27 mo)	Increased diurnal QTe dynamicity independently associated with sudden death
Haigney <i>et al</i> <sup>[47]</sup> , 2004	Postinfarction patients (low LVEF)	871	QT variability (QTVN) QTVI (QTVN adjusted for heart rate variance)		Arrhythmic events (VT or VF) (follow-up 2 yr)	Increased QT variability associated with an increased risk for VT/VF
Jensen <i>et al</i> <sup>[48]</sup> , 2005	Postinfarction patients	481	QT/RR slope and intercept QT/RR VR LVEF VPB and VT		All-cause mortality (follow-up 3 yr)	VR, LVEF, VPB and age made up the optimal Cox model for risk stratification. VR was a promising risk factor for identifying sudden arrhythmic death
Iacoviello <i>et al</i> <sup>[49]</sup> , 2007	NIDCM (no history of SVT/VF)	179	QTe slope (24 h Holter) LVEF NSVT QRS duration QTe and QTd at ECG	QTe-slope: 0.19	Major arrhythmic events, (VT or VF or SCD) (follow-up 39 ± 22 mo)	Increased QTe slope is associated with occurrence of major arrhythmic events. The presence of NSVT and/or QTe slope > 0.19 showed 90% sensitivity and 60% specificity in identifying patients with arrhythmic events
Cygankiewicz <i>et al</i> <sup>[50]</sup> , 2009	CHF patients. IDCM/NIDCM LVEF ≥ 35%	294	QTe slope SDNN TS LVEF	QTe slope: 0.21	Primary endpoint: total mortality Secondary endpoint: sudden death (follow-up 44-mo)	Combination of SDNN, TS, and QTe slope is a predictor of increased risk of mortality and sudden death

BRS: Baroreflex sensitivity; CHF chronic heart failure; EPS Electrophysiological study; ICD Implantable cardioverter defibrillator; IDCM: Ischemic dilated cardiomyopathy; HR Heart rate; HRV: Heart rate variability; LVEF: Left ventricular ejection fraction; MCE: Major cardiac events; NIDCM: Non ischemic dilated cardiomyopathy; NSVT: Non sustained ventricular tachycardia; OHR: Onset heart rate; QTc: QT interval corrected for heart rate; QTe: QT interval calculated at the end of T-wave; SCD: Sudden cardiac death; SDNN: Standard deviation of RR intervals; SR: Sinus rhythm; TS: Turbulence slope; PVB: Premature ventricular beats; VT: Ventricular tachycardia; VR: Variability ratio.

significance of fQRS for an arrhythmic event in 368 patients with IDCM and NIDCM who underwent ICD implantation for primary or secondary prevention of SCD. The authors concluded that fQRS on a 12-lead ECG is a predictor of arrhythmic events but is not associated with a greater probability of death.

### Analysis of ventricular repolarization

The analysis of ventricular repolarization is an intriguing way to implement risk stratification of major arrhythmic events. However, in a large study evaluating NIDCM, the electrocardiographic measure of QT intervals and their dispersion at ECG failed to demonstrate any role in predicting arrhythmic events<sup>[44]</sup>.

Compared to the “static” evaluation of QT interval and dispersion at ECG, the possibility of evaluating QT dynamicity and/or variability during a short-term or 24 h period offer a more complete assessment of ventricular depolarization, the expression of the complex interaction between arrhythmic substrate, heart rate and autonomic nervous system activity<sup>[45]</sup>. Table 2 summarizes the main studies evaluating the prognostic role of QT-dynamicity or variability measures<sup>[46-50]</sup>.

Recently, we studied a series of patients affected by

NIDCM to evaluate the role of QT dynamicity in predicting major arrhythmic events as assessed by 24-h ECG recordings<sup>[49]</sup>. The QT dynamicity index proposed was QT-slope, *i.e.*, the slope of the regression line between QT end and RR during a 24 h period. At univariate analysis, QTe-slope was significantly associated with major arrhythmic events as well as LVEF, NSVT and standard deviation of RR intervals (SDNN). At multivariate analysis, only the QTe-slope, LVEF and NSVT were significant predictors of events, regardless of SDNN, a QRS duration >120 ms or beta-blocker therapy.

The analysis of QT dynamicity has also been found to be associated with an increased arrhythmic risk in patients with IDCM. Chevalier *et al*<sup>[46]</sup> demonstrated that QTe slope compared with LVEF, HRV and late potentials was the strongest independent predictor of sudden death in patients with myocardial infarction. In 871 postinfarction patients with severe left ventricular dysfunction enrolled in the MADIT study, Haigney *et al*<sup>[47]</sup> demonstrated an increased incidence of malignant ventricular arrhythmias in those with increased QT variability. In this study, QT variability was assessed using a semiautomated algorithm that measured beat-to-beat QT duration. Similarly, in a population of postinfarction patients, Jensen *et al*<sup>[48]</sup>



demonstrated the prognostic usefulness of a novel QT dynamics parameter: the QT/RR variability ratio (VR), defined as the ratio between the standard deviation of all QT intervals and the standard deviation of all RR intervals. It was evaluated in 481 patients and found to be associated with the occurrence of sudden arrhythmic death.

Finally, the potential usefulness of QT-e slope has also been demonstrated in a large population of 294 patients affected by CHF due to both IDCM and NIDCM and relatively preserved LVEF  $> 35\%$ <sup>[50]</sup>.

### Microvolt T-wave alternans

Microvolt T-wave alternans (MTWA) analysis involves the detection of changes in T-wave morphology occurring on an every-other-beat basis. A wide electrical alternans of T-wave was an ECG abnormality, first described 50 years ago as being associated with cardiac mortality<sup>[51-52]</sup>. Discordant alternans is responsible for dispersion of repolarization of sufficient magnitude to cause unidirectional block and re-entry. A critical dispersion of repolarization is an important condition for development of re-entrant arrhythmias<sup>[53]</sup>.

Since MTWA is heart rate dependent, it is generally assessed by increasing heart rate with atrial pacing or by exercise stress. The analysis is based on the alignment of ECG cycles to the QRS complex and on the measurement of T-wave amplitude. The beat-to-beat fluctuations of T-wave are then analyzed using fast Fourier transformation and MTWA is represented by the pronounced peak visible in the spectrum at 0.5 cycles/beat. A significant MTWA is present if the alternans voltage is over a threshold (generally 1.9 microV) and if the alternans ratio  $K \geq 3$ . Generally, an alternans which is longer than 1 min occurring at a heart rate  $\leq 110$  beats/min is considered positive<sup>[54]</sup>.

In 1994, Rosenbaum *et al.*<sup>[55]</sup> was the first to demonstrate the efficacy of MTWA in stratifying patients for the risk of ventricular tachyarrhythmic events. However, the studies published to date are not concordant, as summarized in Table 3<sup>[56-64]</sup>.

The meta-analysis carried out by Hohnloser *et al.*<sup>[65]</sup> suggested that MTWA assessed by spectral analysis provides an accurate means of predicting major ventricular arrhythmias. Moreover, the event rate was very low among patients with a negative MTWA test. These results were concordant with the meta-analysis by Calò *et al.*<sup>[66]</sup> who analyzed fifteen studies involving 5681 patients. A positive MTWA determined an approximately 2.5-fold higher risk of cardiac death and life-threatening arrhythmia and showed a very high NPV in both ischemic and non-ischemic patients. An abnormal MTWA test was associated with a 5-fold increased risk for cardiac mortality in the low-indeterminate group and about a 6-fold increased risk in the beta-blocker group. The potential usefulness of MTWA has also been confirmed by Merchant *et al.*<sup>[67]</sup> who analyzed the data of five studies with 2883 patients without ICDs. Among patients with an LVEF of  $\leq 35\%$ , a negative MTWA test result was associated with a low risk

for SCD. Conversely, in patients with a LVEF of  $> 35\%$ , a positive MTWA test result identified those at a significantly heightened SCD risk. Finally, the Alternans Before Cardioverter Defibrillator (ABCD) trial<sup>[64]</sup> was the first to use electrophysiological study (EPS) or MTWA to guide prophylactic ICD implantation in patients with a LVEF  $\leq 40\%$ , coronary artery disease and NSVT. The authors demonstrated that risk stratification strategies using the non-invasive MTWA are comparable to invasive strategy.

These results seem to encourage the use of MTWA testing in patients who do not have ICDs in order to identify those at higher risk of ventricular arrhythmic events. However, the meta-analysis of Gupta *et al.*<sup>[68]</sup> concluded that spectrally derived MTWA testing does not sufficiently modify the risk of VTE to change clinical decisions. Moreover, the MTWA technique is characterized by limitation in its feasibility. In an unselected population of 1003 patients with HF, Kraaier *et al.*<sup>[69]</sup> showed that only half were eligible for MTWA testing and the most common result was an indeterminate test. They concluded that MTWA treadmill testing is not widely applicable in typical HF patients and is unlikely to refine risk stratification for sudden death on a population level.

## ASSESSMENT OF AUTONOMIC NERVOUS SYSTEM ACTIVITY

In the genesis of malignant arrhythmias, apart from the presence of a vulnerable substrate, an altered sympathetic nervous activity and the presence of trigger factors, such as ventricular beats, play a fundamental role. The importance of autonomic dysfunction in increasing the risk of death in patients with heart disease may be applicable to all patients with cardiac disease regardless of etiology<sup>[70,71]</sup>. The pro-arrhythmic effects of the sympathetic nervous system in the normal and ischemic heart are mainly related to the indirect and direct effects of beta-adrenergic receptor activity, but also to the direct effects of alpha-1 adrenergic receptors activity<sup>[72]</sup>.

The direct effects on myocardiocytes are mediated by the activation of cyclic nucleotide and protein kinase regulatory cascade, which can alter spatial heterogeneity of calcium transients and consequently increase the dispersion of repolarization<sup>[73]</sup>. The major indirect effect of beta-receptors activity is the impairment of oxygen supply caused by increased metabolic activity, coronary vasoconstriction, especially in vessels with damaged endothelium, and changes in preload and afterload. On the other hand, the increase in parasympathetic activity is able to modulate ventricular arrhythmias by means of one of the following three effects: a reduction in sinus heart rate, a direct influence on myocardial electrophysiology and a reduction in myocardial oxygen demand due to the negative inotropic action. However, vagal and sympathetic effects cannot be considered in isolation. Sympathovagal interactions are critical in order to understand the electrophysiological function of the heart. Processes disturbing sympathovagal balance have the potential to facilitate cardiovascular instability, leading to cardiac arrhythmias or

**Table 3** Main studies evaluating the role of microvolt T-wave alternans in predicting arrhythmic and non arrhythmic events

Ref.	Clinical setting	Number of patients	Parameter evaluated	End-points (mean follow-up)	Results
Adachi <i>et al</i> <sup>[56]</sup> , 1999	NIDCM	57	TWA, LVEF, NYHA, Signal average ECG, QT dispersion	Ventricular tachycardia	MTWA associated with VT
Klingenheben <i>et al</i> <sup>[57]</sup> , 2000	CHF (no history SVT/VF)	107	TWA	Arrhythmic events (follow-up 18 mo)	MTWA is an independent predictor of arrhythmic events
Kitamura <i>et al</i> <sup>[58]</sup> , 2002	NIDCM	146	Onset heart rate for TWA	SCD, documented sustained ventricular tachycardia/ventricular fibrillation (follow-up 21 ± 14 mo)	TWA and LVEF were independent predictors of arrhythmic events
Hohnloser <i>et al</i> <sup>[59]</sup> , 2003	NIDCM (LVEF 29 ± 11%)	137	MTWA, FEVS, mean RR interval, HRV, BRS.	SCD, SCA, SVT or VF (follow-up 14 ± 6 mo)	MTWA is an independent predictor of ventricular tachyarrhythmic events
Bloomfield <i>et al</i> <sup>[60]</sup> , 2004	IDCM (LVEF ≤ 30%)	177	MTWA, QRS measurement	All-cause mortality. (follow-up 20 ± 6 mo)	Compared to QRS duration, an abnormal MTWA is a stronger predictor of death
Salerno-Uriate <i>et al</i> <sup>[61]</sup> , 2007	NIDCM (NYHA II-III LVEF ≤ 40%)	446	TWA, VO2 peak	Combined primary endpoint of cardiac death and life-threatening ventricular arrhythmias Secondary endpoint: total mortality, combination of arrhythmic death and life-threatening arrhythmias. (follow-up 18 to 24 mo)	Abnormal TWA associated with a 4-fold higher risk of cardiac death and life-threatening arrhythmias
Baravelli <i>et al</i> <sup>[62]</sup> , 2007	NIDCM (NYHA II-III LVEF 29 ± 6.4%)	70	MTWA, VO2 peak	Combined primary endpoint of MCE: total cardiac death or VT/VF (including appropriate ICD shock) Secondary endpoint: MAE: SCD or SVT/VF (follow-up 19.2 ± 10.7 mo)	MTWA and peak VO2, but not the two single tests, were significant prognostic markers of both MCE and MA
Gold <i>et al</i> <sup>[63]</sup> , 2008	CHF (IDCM/NIDCM, 71% NYHA II, LVEF 24 ± 7%)	490	TWA	Composite primary end point: SCD, SVT / VF, or appropriate ICD discharge (follow-up 30 mo)	MTWA not predictive of MAE or mortality
Costantini <i>et al</i> <sup>[64]</sup> , 2009	IDCM LVEF ≤ 40%	566	TWA, EPS	Primary endpoint: appropriate ICD discharge or SCD at 1 yr follow-up (follow-up 1.6 ± 0.6 yr)	Strategies employing MTWA, EPS, or both might identify the subset of patients least likely to benefit from ICD implantation

BRS: Baroreflex sensitivity; CHF: Chronic heart failure; EPS: Electrophysiological study; HR: Heart rate; HRV: Heart rate variability; ICD: Implantable cardioverter defibrillator; LVEF: Left ventricular ejection fraction; MCE: Major cardiac events; MTWA: Microvolt T-Wave alternans; NYHA: New York Heart Association; NSVT: Non sustained ventricular tachycardia; OHR: Onset heart rate; SR: Sinus rhythm; SCA: Sudden cardiac arrest; SCD: Sudden cardiac death; SDNN: Standard deviation of RR intervals; VF: Ventricular fibrillation; VT: Ventricular tachycardia.

even sudden death.

It is clear that every marker of autonomic activity may be used as a clinical prognostic factor. The evaluation of sympathetic nervous system activity can be based on electrocardiographic measures reflecting autonomic control of heart rate, such as the beat-to-beat heart variability (HRV), heart rate turbulence (HRT) and the reflex chronotropic response to a blood pressure change; *i.e.*, baroreflex activity (BRS). Moreover, nuclear imaging techniques can estimate cardiac denervation.

### Measures of autonomic control of heart rate

The prognostic role of measures evaluating autonomic control of heart rate has been widely investigated.

HRV is a term which includes a large number of different indices evaluating the beat-to-beat variability by using either time domain or frequency domain analysis<sup>[74]</sup>. Time domain analysis is based on the detection of

each QRS complex and on measurement of all intervals between adjacent QRS complexes, resulting from sinus rhythm, as NN intervals or as instantaneous heart rate. Among the statistical time domain indices, SDNN is the simplest and is the standard deviation of NN intervals generally assessed in 24 h Holter recordings. The prognostic significance of SDNN has been evaluated both in patients with ischemic and non-ischemic diseases, as well as in heart failure patients, but the results are controversial.

Brower *et al*<sup>[75]</sup> assessed the prognostic value of HRV measures in patients with mild or moderate chronic heart failure (NYHA class II-III). Ninety-five patients were followed-up for 4 years. None of the conventional time and frequency domains were related to survival. Szabò *et al*<sup>[76]</sup> followed-up a group of 159 patients with idiopathic or ischemic dilated cardiomyopathy, selected on the basis of a left ventricular ejection fraction of < 40%. During follow-up, cardiac mortality was subdivided into sudden

cardiac death and death due to progressive pump failure. SDNN was found to have an independent predictive value for all cause mortality, while not being related to the type of the death. Fauchier *et al.*<sup>[77]</sup> designed a study to evaluate HRV in patients with idiopathic dilated cardiomyopathy to determinate its prognostic value. The group of patients with depressed SDNN ( $< 100$  ms.) had an increased risk of cardiac death or heart transplantation during the follow-up ( $49.5 \pm 35.6$  mo).

In patients with mild-to-moderate ventricular dysfunction and NIDCM, a low SDNN, combined with an increased QT dynamicity, has been found to be associated with an increased risk of arrhythmic events<sup>[50]</sup>. However, in other studies, no independent association with arrhythmic events has been found<sup>[44]</sup>.

HRT is another parameter reflecting autonomic control of heart rate. It is the expression of the baroreflex-mediated transient acceleration-deceleration response of the sinus node triggered by a premature ventricular beat (PVB)<sup>[78]</sup>. HRT is a baroreflex-mediated biphasic reaction of heart rate in response to premature ventricular beats. It is quantified by: turbulence onset (TO) reflecting the initial acceleration of heart rate following premature beat; and turbulence slope (TS) describing subsequent deceleration of heart rate following a premature ventricular beat. TO is the percentage of relative change in the mean of 2 RR intervals after a PVB. TS is the slope of the steepest regression line computed over the sequence of every 5 consecutive RR intervals following a PVB within 15 RR and is expressed in ms/RR. HRT can be calculated only in patients with sinus rhythm presenting with eligible PVBs<sup>[79]</sup>. Abnormal HRT identifies patients with an autonomic dysfunction or impaired baroreflex sensitivity due to a variety of disorders, but may also reflect changes in the autonomic nervous system induced by different therapeutic modalities such as drugs, revascularization or cardiac resynchronization therapy<sup>[80]</sup>. HRT has been introduced as an autonomic predictor for cardiac events in heart failure patients and in large cohorts of postinfarction patients<sup>[80-91]</sup>, as summarized in Table 4. The retrospective analysis of the ATRAMI trial<sup>[81]</sup> showed that HRT identified postinfarction patients at risk of both all-cause death and arrhythmic events. Other large trials confirmed the prognostic role of abnormal HRT for predicting mortality and arrhythmic events in postinfarction patients<sup>[85,89]</sup> as well as in both NIDCM and IDCM patients<sup>[88,90]</sup>. However, the results of the studies, particularly in NIDCM, are conflicting. In the Marburg study, Grimm *et al.*<sup>[84]</sup> observed that in 242 patients with idiopathic cardiomyopathy, HRT onset is a significant predictor of transplant-free survival, but for arrhythmia risk stratification, only LVEF remained a significant risk predictor on multivariate analysis. Moreover, analysis of the Frankfurt DCM database showed that HRT and HRV did not yield predictive power for arrhythmic events<sup>[87]</sup>.

### Cardiac denervation assessed by nuclear imaging

In the pathophysiology of malignant ventricular arrhythmias, a relevant role is played not only by sympathetic au-

tonomic nervous system hyperactivity but also by cardiac sympathetic denervation. The presence of cardiac denervation can cause heterogeneity in a refractory period of the ventricular myocardium, thus favoring the onset and the persistence of ventricular arrhythmias. A scintigraphic approach using <sup>123</sup>I-labeled metaiodobenzylguanidine (MIBG) can explore the presence of abnormalities in cardiac sympathetic innervation<sup>[92-96]</sup>.

This radiotracer is administered at rest and planar and single-photon emission computerized tomography images are then acquired after 15 min (early) and 3-5 h (delayed). Generally, the analysis of MIBG distribution is based on the delayed images which reflect overall cardiac sympathetic function, including uptake, re-uptake, storage and release processes of norepinephrine at presynaptic nerve terminals, rather than real time, beat-by-beat sympathetic drive<sup>[96]</sup>. The quantitative index calculated after MIBG injection is the heart/mediastinal ratio (H/M). This is derived by the mean counts per pixel of the region of interest drawn over the heart and that drawn over the upper mediastinum<sup>[97]</sup>. The value of H/M range is from 1.9 to 2.8 in a normal subject. A normal H/M ratio reflects the density of receptors and the integrity of presynaptic nerve terminals and uptake function. A low H/M ratio reflects a reduced myocardial uptake and a poor cardiac adrenergic receptor density<sup>[95,98]</sup>.

Besides global myocardial uptake (heart-to-mediastinum ratio), other markers have been used, including washout kinetics and regional uptake heterogeneity. The myocardial washout rate (WR) is expressed as the rate of decrease in myocardial counts over time between early and late imaging, reflecting the neuronal integrity or sympathetic tone<sup>[98]</sup>. In HF patients, high myocardial WR and low early and delayed H/M are detectable<sup>[99-101]</sup>.

The presence of an altered distribution of MIBG can also be found in NIDCM patients<sup>[102]</sup> and has been associated with other parameters reflecting arrhythmic risk<sup>[103-104]</sup>.

Over the last three decades a number of studies have reported the relevance of an altered MIBG distribution in predicting increased risk of death and arrhythmic events<sup>[105-115]</sup>. In a group of patients with heart failure, Nakata *et al.*<sup>[101]</sup> revealed that impaired cardiac sympathetic innervation assessed by MIBG activity has an incremental and prognostic role for predicting cardiac death and may be useful for identifying a threshold level for selecting patients at risk for death by heart failure, sudden cardiac death and fatal myocardial infarction.

The largest trial evaluating the prognostic role of cardiac denervation assessed by MIBG is the ADMIRE study<sup>[108]</sup>, in which a total of 961 subjects with NYHA functional class II/III HF and LVEF  $\leq 35\%$  were evaluated. Time to first occurrence of NYHA functional class progression, a potentially life-threatening arrhythmic event, and cardiac death were the end-points considered. For H/M  $< 1.60$ , 2 year probabilities of cardiac death and all-cause mortality were 11.2% and 16.1% *vs* 1.8% and 3% for the group with H/M  $\geq 1.60$ . Moreover, non-fatal arrhythmic events or sudden cardiac death

**Table 4** The main studies evaluating the prognostic significance of heart rate turbulence and risk stratification

Ref.	Clinical setting	Number of patients	Cut-off proposed	End-points (mean follow-up)	Results
Schmidt <i>et al</i> <sup>[80]</sup> , 1999	Postinfarction patients	577	TO 0% TS 2.5 ms/RR	All-cause mortality (follow-up 22 mo)	HRT2 predictive for all-cause mortality
Ghuran <i>et al</i> <sup>[81]</sup> , 2002	Postinfarction patients (ATRAMI)	1212	TO 0% TS 2.5 ms/RR	Combined end-point of fatal and non fatal cardiac arrhythmias (follow-up 21 mo)	HRT associated with endpoints
Barthel <i>et al</i> <sup>[83]</sup> , 2003	Postinfarction patients (ISAR-HRT)	1455	TO 0% TS 2.5 ms/RR	All-cause mortality (follow-up 22 mo)	HRT independent predictor of mortality in patients with LVEF $\geq 30\%$
Grimm <i>et al</i> <sup>[84]</sup> , 2003	NIDCM, LVEF $\leq 30\%$	242	TO 0% TS 2.5 ms/RR	Transplant-free survival (follow-up: 41 mo)	TO predictor of transplant-free survival. TO and TS only as univariate predictor of MCE
Exner <i>et al</i> <sup>[85]</sup> , 2007	Myocardial infarction (REFINE)	322	TO 0% TS 2.5 ms/RR	Cardiac death or resuscitated cardiac arrest (follow-up 47 mo)	HRT (10-14 wk after MI) predictive for cardiac death or resuscitated cardiac arrest
Cygankiewicz <i>et al</i> <sup>[86]</sup> , 2008	CHF (IDCM/and NIDCM)	607	TO 0% TS 2.5 ms/RR	All-cause mortality, sudden death and heart failure death (follow-up: 44 mo)	Abnormal TS predictive for all-cause mortality, sudden death and heart failure death
Klingenheben <i>et al</i> <sup>[87]</sup> , 2008	NIDCM (Mean LVEF 28%)	114	TO 0% TS 2.5 ms/RR	Arrhythmic events (follow-up 22 mo)	HRT non predictive for arrhythmic events
Miwa <i>et al</i> <sup>[88]</sup> , 2009	IDCM (241) and NIDCM (134)	375	TO 0% TS 2.5 ms/RR	Cardiac mortality Combined endpoint of cardiac death and/or stable sustained VT (follow-up 15 mo)	Abnormal HRT predictive for cardiac mortality and combined endpoint Prognostic value observed in both ischemic and non-ischemic cardiomyopathy
Huikuri <i>et al</i> <sup>[89]</sup> , 2009	Postinfarction CARISMA	312	TS 2.5 ms/RR	Primary endpoint of documented VT/TV (follow-up 2 yr)	TS evaluated at 6 wk after MI predictive for primary endpoint No prognostic value for HRT evaluated 1 wk after MI
Ikedo <i>et al</i> <sup>[90]</sup> , 2011	NIDC	134	TO 0% TS 2.5 ms/RR	Combined endpoint of cardiac mortality and sustained VT (follow-up 15 mo)	Abnormal HRT predictive for combined endpoint
Miwa <i>et al</i> <sup>[91]</sup> , 2012	IDCM / NIDCM (LVEF $\leq 40\%$ )	299	TO 0% TS 2.5 ms/RR	Combined endpoint of sudden cardiac death and sustained VT (follow-up 32 mo)	Abnormal HRT predictive for combined endpoint

HRT: Heart rate turbulence; NIDCM: Non-ischemic dilated cardiomyopathy; TO: Turbulence onset; TS: Turbulence slope; MCE: Major cardiac events; LVEF: Left ventricular ejection fraction; CHF: Chronic heart failure; NYHA ICD: Implantable cardioverter defibrillator.

were observed in patients with  $H/M < 1.60$ . ADMIRE-HF provided prospective validation of the independent prognostic value of MIBG in the assessment of patients with HF, in identifying patients at high risk of arrhythmic events, sudden cardiac death and ICD discharge.

Finally, it is worth noting that the prognostic significance of MIBG in predicting sudden death has also been demonstrated in a small population of patients with mild-to-moderate CHF<sup>[112]</sup>.

## THE MULTIPARAMETRIC APPROACH TO ARRHYTHMIC RISK STRATIFICATION

Different studies evaluating the role of non-invasive diagnostic tools in predicting arrhythmic events have demonstrated that the combination of the different parameters could be a useful approach in order to better improve arrhythmic risk stratification. Generally, the combination of the different parameters allows the identification of

a smaller group of patients at higher risk of arrhythmic events.

In our series of patients<sup>[49]</sup>, by combining LVEF ( $< 35\%$  *vs*  $> 35\%$ ), NSVT and QTe-slope ( $> 0.19$  *vs*  $< 0.19$ ), arrhythmic events were more frequently observed in patients with NSVT and a low LVEF and in those with a low LVEF and steeper QTe slope. No significantly higher risk was observed in patients with a higher LVEF and NSVT or steeper QTe slope. When all three variables were considered together, the patients with a low LVEF and NSVT or a steeper QTe slope were found to have a higher arrhythmic risk. In the subgroup of patients with LVEF  $< 35\%$ , the presence of NSVT and QTe slope  $> 0.19$  defined a small population with the highest probability of events.

Also, among HF patients with a LVEF  $> 35\%$ , the combination of different arrhythmic risk parameters improved prognostic stratification. Cygankiewicz *et al*<sup>[50]</sup> demonstrated that in this population of patients, the presence of two or more independent risk parameters



(SDNN  $\leq$  86 ms, HRT  $<$  2.5 ms/RR and QTc slope  $>$  0.21) detected a population at higher risk of death (30% 3 year mortality) and sudden death (12%), with a rate of events similar to that observed among patients with LVEF  $\leq$  35%.

Merchant *et al.*<sup>[114]</sup> tried to assess whether a multi-marker strategy would provide more robust SCD risk stratification than LVEF alone. The authors observed that a multivariable model based on the presence of coronary artery disease, LVEF and MTWA status provides a significantly more robust SCD risk prediction than LVEF as a single risk marker. These findings suggest that multi-marker strategies based on different aspects of the electroanatomic substrate may be capable of improving primary prevention implantable cardioverter-defibrillator treatment algorithms.

Finally, Yukinaka *et al.*<sup>[115]</sup> correlated the incidence of ventricular arrhythmias with mismatches in myocardial <sup>99m</sup>Tc-methoxyisobutylisonitrile/MIBG accumulation and late ventricular potentials. Patients with late ventricular potentials had greater I-123 MIBG defect scores. The combination of late ventricular potentials and I-123 MIBG uptake could improve the prediction of ventricular arrhythmias after myocardial infarction.

## LIMITATIONS OF ALTERNATIVE NON-INVASIVE ARRHYTHMIC RISK PARAMETERS

Although the above mentioned studies have provided evidence about the independent association among a number of parameters and the risk of malignant ventricular arrhythmias, their routine use is still limited for different reasons. In particular, most of the parameters have shown conflicting results, probably related to the methodological differences, such as the studied population (NIDCM or IDCM), the follow-up duration, the end-points considered and the pharmacological treatment at the enrolment. Moreover, all measures are affected by both technical and biological limitations. Finally, almost all these studies were aimed at only evaluating the associations between the studied parameters and the occurrence of ventricular arrhythmias, but not to demonstrate their ability to select patient populations who could benefit from ICD implantation. This ability could be demonstrated only by randomized studies that, to date, are still lacking.

## CONCLUSION

Malignant ventricular arrhythmias and sudden death are the main adverse events affecting the prognosis of both NIDCM and IDCM. ICD implantation, *i.e.*, the best therapeutic strategy to reduce the incidence of sudden death, is currently mainly guided by the estimation of LVEF. However, this measure is affected by a number of technical and biological limitations. For these reasons, the best assessment of arrhythmic risk is still a challenge. The use

of other non-invasive parameters reflecting functional or anatomical arrhythmic substrate (LGE), sympathetic nervous activity (HRT, SDNN, the presence of sympathetic denervation by MIBG) and the abnormalities in myocardial refractoriness (QT dynamicity/variability, MTWA) could be useful in order to better characterize both patients with reduced and preserved LVEF at higher risk of arrhythmic events.

Although several studies have shown these parameters to be independently associated with events, their routine use is still limited due to the lack of randomized studies demonstrating their ability to select patient populations who could benefit from ICD implantation. Future prospective studies should aim to reduce this gap in the evidence in order to justify the indication of these techniques in daily clinical practice.

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## WJC 6<sup>th</sup> Anniversary Special Issues (3): Cardiomyopathy

# Mechanisms underlying the impaired contractility of diabetic cardiomyopathy

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## Abstract

Cardiac dysfunction is a well-known consequence of diabetes, with sustained hyperglycaemia leading to the development of a cardiomyopathy that is independent of cardiovascular disease or hypertension. Animal models of diabetes are commonly used to study the pathophysiology of diabetic cardiomyopathy, with the hope that increased knowledge will lead ultimately to better therapeutic strategies being developed. At physiological temperature, left ventricular trabeculae isolated from the streptozotocin rat model of type 1 diabetes showed decreased stress and prolonged relaxation, but with no evidence that decreased contractility was a result of altered myocardial  $\text{Ca}^{2+}$  handling. Although sarcoplasmic reticulum (SR)  $\text{Ca}^{2+}$  reuptake appeared slower in diabetic trabeculae, it was offset by an increase in action-potential duration, thereby maintaining SR  $\text{Ca}^{2+}$  content and favouring increased contraction force. Frequency analysis of t-tubule distribution by confocal imaging of ventricular tissue labeled with wheat germ agglutinin or ryanodine receptor antibodies showed a reduced T-power for diabetic tissue, but the differences were minor in comparison to other models of heart failure.

The contractile dysfunction appeared to be the result of disrupted F-actin in conjunction with the increased type I collagen, with decreased myofilament  $\text{Ca}^{2+}$  sensitivity contributing to the slowed relaxation.

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**Key words:** Diabetic cardiomyopathy; Heart failure; Contractility; T-tubules; Excitation-contraction coupling; Calcium homeostasis

**Core tip:** Diabetic patients develop a cardiomyopathy that is independent of vascular disease, and is thought to develop as a direct result of the prolonged hyperglycaemia. Animal models of diabetes can help us understand the cellular mechanisms that lead ultimately to contractile dysfunction of diabetic cardiomyopathy. The streptozotocin rat model of type 1 diabetes has slowed  $\text{Ca}^{2+}$  transients and twitch force kinetics, with reduced myofilament  $\text{Ca}^{2+}$  sensitivity. Myocytes are decreased in volume in diabetic hearts, with reduced and disrupted F-actin, and type 1 collagen is increased. Together, these changes all contribute to the reduced contractility of diabetic cardiomyopathy.

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## INTRODUCTION

Patients with diabetes develop a cardiomyopathy that is independent of coronary artery disease and hypertension<sup>[1]</sup>, and contributes to the increased mortality and morbidity of the disease<sup>[2,3]</sup>. The mechanisms that lead to

development of the diabetic cardiomyopathy are poorly understood, although they appear to be a direct result of cellular damage from the hyperglycaemia. The early stages of the cardiomyopathy are associated with reduced diastolic function, with 27%-70% of asymptomatic diabetic patients showing some form of diastolic abnormality<sup>[4-6]</sup>. Later this progresses to include systolic dysfunction and heart failure<sup>[7,8]</sup>. Diabetes manifests in two forms, both of which are a result of abnormal glucose metabolism. Type I diabetes usually has its onset early in life and is characterized by insufficient insulin production, whereas type II diabetes has its origin downstream of insulin binding to its receptor, and is therefore known as insulin-resistant diabetes. Diabetic cardiomyopathy develops in both type I and type II forms of the disease<sup>[9,10]</sup>.

Although the heart contains many different cell types, it is the cardiac myocytes that perform the work that enables the heart to function as a pump. With each cardiac cycle, the myocytes experience rapid changes in intracellular ion concentrations that are crucial to the hearts inotropy, lusitropy, and energy metabolism. This review will outline the ultrastructural and functional changes that contribute to the impaired contraction and relaxation characteristic of diabetic cardiomyopathy.

## MECHANISMS CONTRIBUTING TO DIABETIC CARDIOMYOPATHY

### *Streptozotocin rat model of diabetes*

Animal models have frequently been used in research into the cellular mechanisms associated with diabetes<sup>[11]</sup>, with the insulin-deficient streptozotocin rat (STZ) commonly studied. Type-1 diabetes in humans is characterized by the destruction of the pancreatic  $\beta$ -cells, as occurs in the STZ. Streptozotocin is a naturally occurring glucose analog that is particularly toxic to the insulin-producing beta cells of the pancreatic islets. The chemical is transported into cells *via* the glucose transporter-2 (GLUT-2)<sup>[12]</sup>. Since the pancreatic beta cells have high levels of GLUT-2, they accumulate streptozotocin in large quantities, resulting in their destruction and the onset of a diabetic state. Rats treated with a single dose of streptozotocin (60 mg/kg) rapidly develop biochemical and functional myocardial abnormalities. They exhibit increased water consumption (180 mL/d compared to 43 mL/d for sham-injected control) and elevated plasma glucose levels (31 mmol/L compared to 4 mmol/L for control) that are sustained. Isolated cardiac muscle preparations from diabetic rats 8 wk post-injection show depressed contractility, diminished compliance and decreased inotropic drug responses<sup>[13]</sup>. Abnormalities in contraction and metabolism have been reported both *in vivo* and *in vitro* in the STZ diabetic rat model, reflecting changes at the cardiac myocyte level as a result of the sustained hyperglycaemia. The STZ rat has proved an invaluable model for investigation of the pathogenesis of type 1 diabetes and its complications, and in the development of potential new treatments for the disease<sup>[14-16]</sup>.

### *The reduced contractility of diabetic hearts*

Contraction in cardiac muscle is brought about by an increase in the myocyte intracellular  $\text{Ca}^{2+}$  concentration (the “ $\text{Ca}^{2+}$  transient”). Propagation of the action potential across the surface sarcolemma and throughout the transverse tubule system (t-tubules) opens voltage-gated L-type  $\text{Ca}^{2+}$  channels causing a synchronised influx of  $\text{Ca}^{2+}$  into the myocytes (the “ $\text{Ca}^{2+}$  current”). This  $\text{Ca}^{2+}$  current then triggers release of  $\text{Ca}^{2+}$  from the junctional region of the sarcoplasmic reticulum (SR) *via* the ryanodine receptors (RyRs) in a process termed “ $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release”<sup>[17,18]</sup>. In this way the intracellular  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  is rapidly increased to approximately 10 times the resting level.  $\text{Ca}^{2+}$  then diffuses to the contractile proteins where it binds to troponin C, initiating cross-bridge cycling and force development. Excitation-contraction coupling has therefore been a major focus of those investigating the cellular mechanisms that underlie the reduced contractility of failing hearts.

### *Intracellular calcium transients in diabetic hearts*

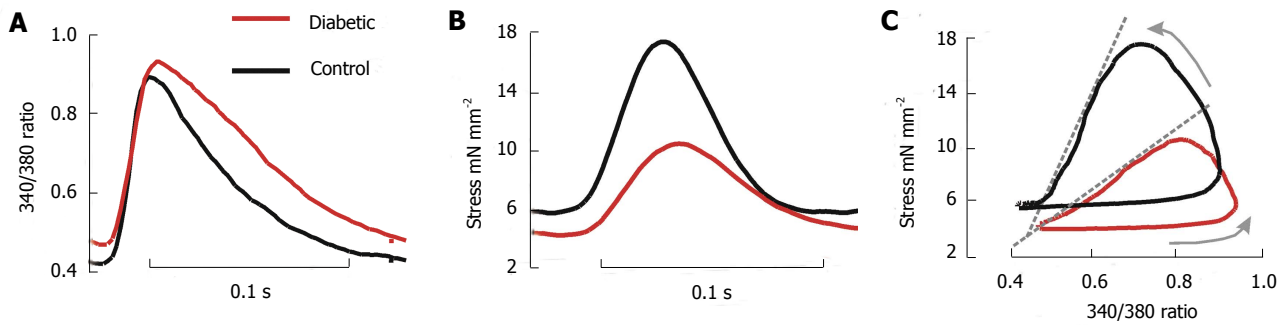
Measurements carried out on multicellular trabeculae isolated from the left ventricle under near physiological conditions (1.5 mmol/L  $[\text{Ca}^{2+}]_o$ , 37 °C and 5 Hz) showed trabeculae from diabetic rats had depressed contractility with prolonged contraction and relaxation in comparison to their controls, consistent with other studies<sup>[19-21]</sup>.

An alteration of intracellular  $\text{Ca}^{2+}$  homeostasis has previously been suggested as underlying the diabetic cardiac dysfunction (for review see<sup>[22]</sup>) although, as noted, results are often contradictory. While some of these discrepancies might be attributable to the extent of disease progression (diabetic stage) and experimental conditions, very few studies have examined the  $[\text{Ca}^{2+}]_i$  control of contractility under near-physiological temperatures and rates of stimulation. Our study showed that diabetic rats had an unchanged resting  $[\text{Ca}^{2+}]_i$  level and amplitude of  $\text{Ca}^{2+}$  transient, despite a reduced contractility<sup>[23]</sup>. Averaged  $\text{Ca}^{2+}$  transients and isometric twitches at 5 Hz stimulation are shown in Figure 1 for trabeculae from control (solid line) and diabetic (dotted line) rats, superimposed for comparison. Figure 1C shows the  $[\text{Ca}^{2+}]_i$ -stress phase plot, with a right shifted relaxation phase for diabetic trabeculae which suggests diminished myofibrillar  $\text{Ca}^{2+}$  sensitivity.

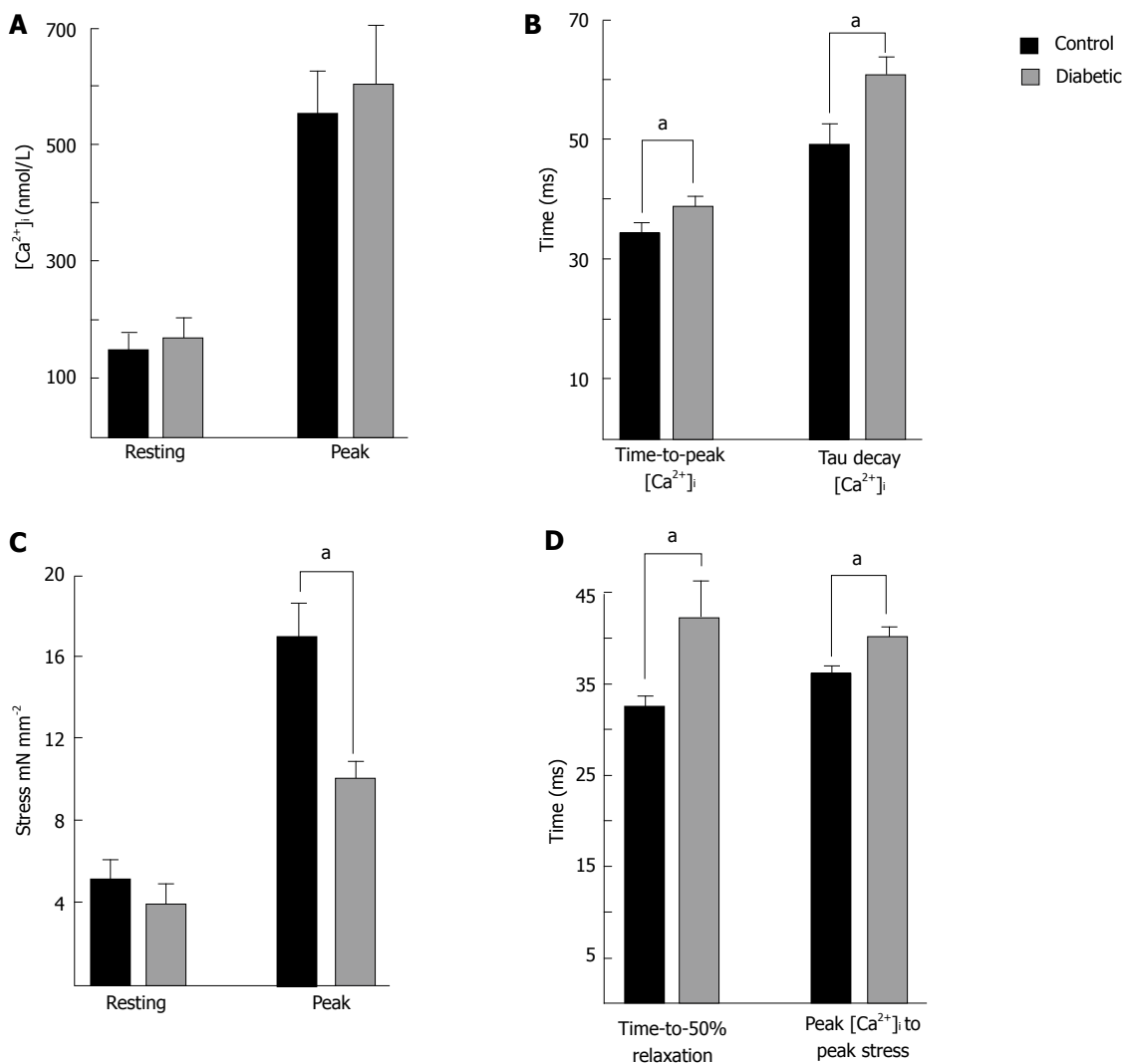
Figure 2 shows averaged data from trabeculae at 5 Hz stimulation and at 37 °C. Diabetic rats had prolonged time-to-peak  $[\text{Ca}^{2+}]_i$  and a prolonged time constant of  $\text{Ca}^{2+}$  transient decay, consistent with some other reports<sup>[20,24-26]</sup>. The slower kinetics of  $\text{Ca}^{2+}$  transient would contribute to the prolonged time course of cardiac contraction and relaxation in diabetic rats, but it is unclear if the reduced rate of the decay in the  $\text{Ca}^{2+}$  transient is sufficient to explain the slowed mechanical relaxation.

Our study showed that contractility was reduced in trabeculae from diabetic hearts, even when peak  $[\text{Ca}^{2+}]_i$  was matched between diabetic and control trabeculae by altering stimulation rate<sup>[23]</sup>, suggesting that altered  $[\text{Ca}^{2+}]_i$  handling was not the primary mechanism of contractile





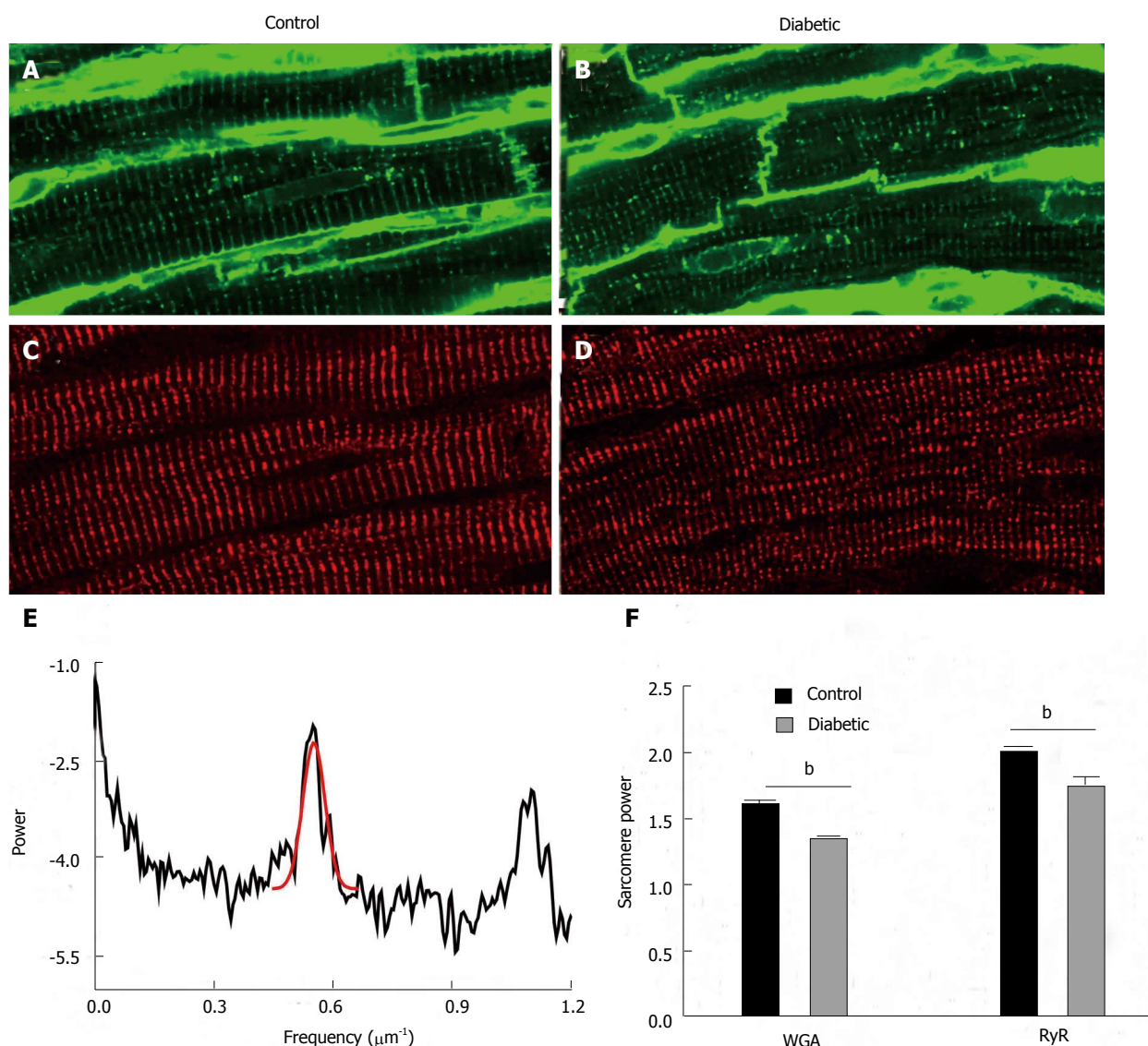
**Figure 1 Average intracellular  $\text{Ca}^{2+}$  transients and isometric stress.** Data were recorded from left ventricular trabeculae of diabetic (red lines) and control (black lines) hearts at 5 Hz, 37 °C, and 1.5 mmol  $[\text{Ca}^{2+}]_o$ , 7 trabeculae per group. A:  $\text{Ca}^{2+}$  transient (340/380 fluorescence ratio); B: Stress; C: Phase plots of the relationship between fluorescence and stress. The arrows indicate the direction of time, and the dashed grey lines accentuate the slope of the relaxation component. (Modified from Zhang *et al*<sup>[23]</sup>).



**Figure 2 Summary of intracellular  $\text{Ca}^{2+}$  and isometric stress parameters.** Data were recorded from left ventricular trabeculae at 37 °C, 5 Hz stimulation frequency, and 1.5 mmol  $[\text{Ca}^{2+}]_o$ . Data are mean  $\pm$  SE 8 wk post injection for control ( $n = 7$ ) and diabetic ( $n = 8$ ). A: Shows resting and peak  $[\text{Ca}^{2+}]_i$ . The  $\text{Ca}^{2+}$  transients were prolonged in diabetic trabeculae; B: Shows the time to reach peak  $[\text{Ca}^{2+}]_i$ , and the time constant of the  $\text{Ca}^{2+}$  transient decay; C: Shows no difference in resting stress, but peak stress was reduced in diabetic trabeculae; D: Shows the time to 50% relaxation of stress was prolonged in diabetic, as was the time from the peak of the  $\text{Ca}^{2+}$  transient to the peak of the twitch. \* $P < 0.05$ , diabetic vs control.

dysfunction. The mechanical relaxation was intrinsically slower in diabetic rat hearts, which was exacerbated by the reduced rate of decrease of  $[\text{Ca}^{2+}]_i$ . In support of this

idea, Figure 2D shows that the interval between the time-to-peak  $[\text{Ca}^{2+}]_i$  and the time-to-peak stress in diabetic rats was increased in comparison to control.



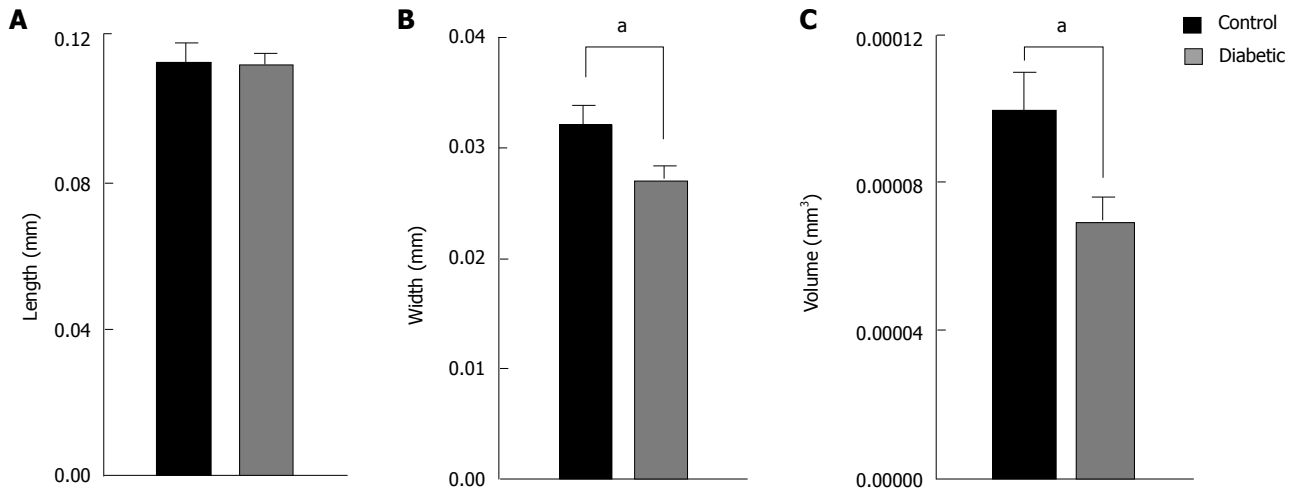
**Figure 3 Structural changes in proteins associated with excitation-contraction coupling.** Transverse tubules were visualised by labelling with wheat germ agglutinin in (A) control and (B) diabetic tissue. The same tissue sections were dual labelled with antibodies against ryanodine receptors (RyR) in (C) control and (D) diabetic tissue. The periodicity or regularity of labelling was assessed using a fast Fourier transform. An example of this analysis is shown in (E) which is the plot of the FFT in control myocyte labelled with RyR. The peak associated with sarcomeric periodicity (approximately  $0.55 \mu\text{m}^{-1}$ ) is fitted with a Gaussian in red. The height of this peak is used as a metric to assess the regularity of sarcomere labelling termed "sarcomere" power. (F) This shows the mean sarcomere power for both wheat germ agglutinin and ryanodine receptor labelling from 18 cells from 3 control animals and 18 cells from 3 diabetic animals. Both wheat germ agglutinin and ryanodine receptor sarcomere power were modestly but highly significantly reduced in cells from diabetic hearts (Bonferroni corrected *t* test,  $^bP < 0.01$ , diabetic vs control.).

Analysis of electrocardiogram (ECG) in lightly anesthetized diabetic rats prior to experimentation showed that the normalized QT interval was prolonged, implying the cardiac action potential was slower<sup>[23]</sup>. This would contribute to the prolonged  $\text{Ca}^{2+}$  transients observed in diabetes, but cannot explain the observed  $\text{Ca}^{2+}$  transient changes in full. Logarithmic plots of  $\text{Ca}^{2+}$  transients from control and diabetic trabeculae in Zhang *et al*<sup>[23]</sup> (2008) show that the linear portion of the  $\text{Ca}^{2+}$  fluorescence decay was delayed in trabeculae from diabetic hearts, consistent with the increase in the time-to-50% repolarization of the ventricular action potential reported in their study. Prolonged depolarization during the plateau phase of the action potential will lead also to increased L-type  $\text{Ca}^{2+}$  influx, although this was not shown in the Zhang *et al*<sup>[23]</sup>

(2008) study. Frequently studies have reported changes in SERCA protein expression in explanation of observed changes to the time course of the  $\text{Ca}^{2+}$  transients<sup>[27,28]</sup>, but decreased SERCA activity and/or expression may only contribute in part to the prolonged  $\text{Ca}^{2+}$  transient decay. Action potential duration is also important in determining the duration of the  $\text{Ca}^{2+}$  transient, and therefore the SR  $\text{Ca}^{2+}$  load, which in turn determines SR  $\text{Ca}^{2+}$  release *via* the RyRs<sup>[29]</sup>. ECG measurements in insulin-treated type 1 diabetic patients also show abnormal repolarization with the reports of increased QT interval and increased QT dispersion<sup>[30]</sup>.

### T-tubule system structure

The t-tubules are an important component of the excita-



**Figure 4** Average dimensions of isolated ventricular myocytes from diabetic and control rat hearts. Cell length (A) was not different between diabetic ( $n = 35$ ) and control ( $n = 19$ ) hearts, whereas cell width (B) and cell volume (C) was reduced. <sup>a</sup> $P < 0.05$ , diabetic vs control.

tion-contraction coupling system in cardiac myocytes<sup>[31]</sup>. T-tubules are an extension of the sarcolemma that project transversely into the interior of the cell adjacent to the z-line, although numerous axial connections between sarcomeres are observed<sup>[32]</sup>. This structure facilitates synchronous contraction by conducting the action potential deep within the myocyte and triggering  $\text{Ca}^{2+}$  release from the SR in regions located away from the cell surface. There is evidence that loss of normal transverse tubule structure is a key feature of both animal<sup>[33,34]</sup> and human heart failure<sup>[35,36]</sup>. Frequency analysis of t-tubule distribution at the z-line has been used to quantify the structural changes in t-system labelling of myocytes from rodents at different stages of heart failure<sup>[33]</sup>. This analysis exploits the periodic nature of t-tubule distribution at the z-line of sarcomere. By converting t-tubule images into the frequency domain with a fast Fourier transform, a peak associated with sarcomere spacing of  $2\ \mu\text{m}$  is observed in the power spectrum<sup>[37,38]</sup>. In failing myocytes the periodic pattern of t-tubule labelling is disrupted resulting in reduced sarcomere peak. This peak is termed “T-power” and provides a useful metric to quantify t-tubule structure.

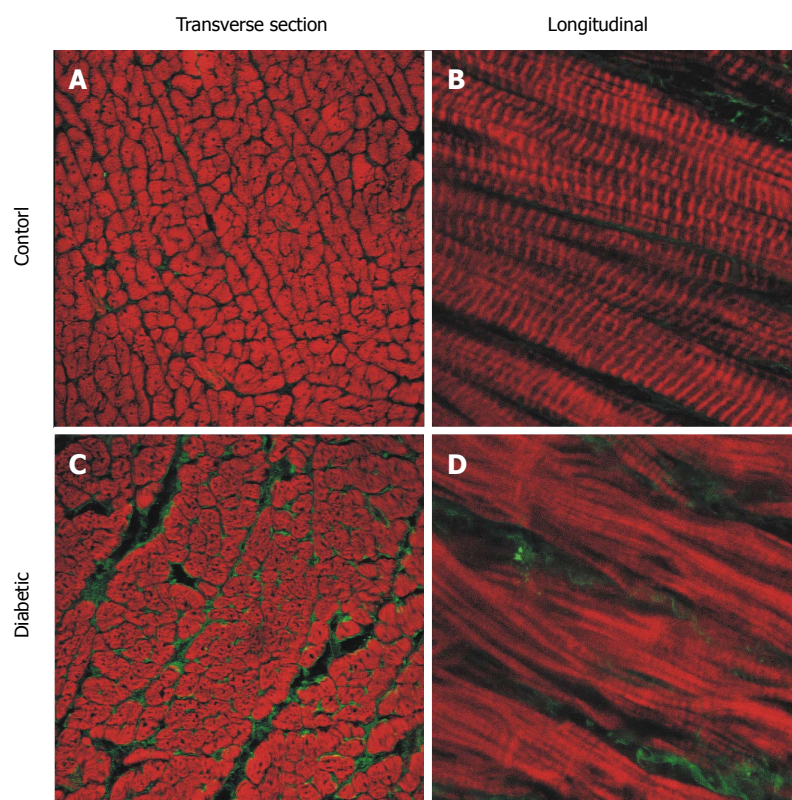
Currently there is lack of comparable data for changes in t-tubules in the diabetic heart. To address this gap in knowledge we have used confocal laser scanning microscopy to examine the labelling of the t-tubules [wheat germ agglutinin (WGA)] and the ryanodine receptors (RyR), in the hearts of STZ rats with end stage heart failure as shown in Figure 3. Analysis of this labelling in the frequency domain has shown a significant but surprisingly modest decrease in T-power (or sarcomere power) in the t-tubule system of diabetic myocytes. A similar analysis of RyR labelling showed a comparable decrease in sarcomere power in diabetic myocytes. Visual inspection of the labelling in Figure 3 shows that both the structure of t-system (WGA) and the SR (RyR) are largely intact in myocytes from diabetic rat hearts, which is consistent with the comparatively normal calcium transients measured in the cardiac trabeculae of this animal

model<sup>[23]</sup>. This contrasts with the dramatic loss of the t-system structure reported in non-diabetic animal heart failure. For example the t-system is dramatically remodelled in spontaneously hypertensive rat, while the labelling of RyR is largely intact<sup>[34,38]</sup>. A similar situation is seen in non-diabetic human heart failure<sup>[36]</sup>. This may turn out to be a key point of difference between diabetic and other forms of heart failure, and it remains to be seen if a similar pattern of t-system preservation is seen in the diabetic human heart. Alternatively, the lack of obvious changes in the t-tubule distribution of STZ-induced diabetic rat hearts 8 wk post injection may reflect the relatively short duration of the disease. Figure 2B shows an increase in the time-to-peak of the  $\text{Ca}^{2+}$  transients in longitudinal section (LV) trabeculae from diabetic hearts, which may reflect changes in excitation-contraction coupling from hyperglycaemia-induced loss of t-tubule structure.

#### Ventricular remodeling of diabetic hearts

Although intracellular  $\text{Ca}^{2+}$  cycling is essential to the contraction and relaxation of cardiac myocytes, the extracellular matrix and the myofilaments within the myocytes are essential also. The contractile proteins that make up the myofilaments are the end effectors of excitation-contraction coupling, and their responsiveness to  $\text{Ca}^{2+}$  directly determines myocyte contractility (for reviews see<sup>[39,40]</sup>). Changes in the contractile proteins of diabetic hearts have been reported, and are likely to contribute substantially to the observed changes in contraction and relaxation. Figure 2C and D show both reduced contraction (peak stress) and slowed relaxation in LV trabeculae from diabetic rat hearts. The slower time course of contraction in trabeculae from diabetic hearts could be explained, in part, by a shift in the myosin isoenzyme distribution from the faster alpha heavy chain to the beta form as previously reported<sup>[41]</sup> (for review see<sup>[42]</sup>). Changes in other aspects of the contractile protein system have also been described in diabetic hearts. The thin filament regulatory troponin-tropomyosin complex shows decreased  $\text{Ca}^{2+}$  sensitivity in skinned<sup>[43,44]</sup> and intact<sup>[16]</sup> cardiac muscle





**Figure 5** Representative confocal images of longitudinal section free wall immuno-labelled for type I collagen (green) and f-actin (red). Sections from the endocardium of control (A and B) and diabetic (C and D) rat hearts. Left hand side panels: Transverse sections from endocardium (25 × objective). Right hand side panels: Longitudinal sections (63 × objective, zoom × 3). (Modified from Zhang *et al.*<sup>[23]</sup>).

preparations. The consequence of reduced  $\text{Ca}^{2+}$  sensitivity is increased force production for any given cytosolic  $\text{Ca}^{2+}$  concentration, favouring force production during systole, but decreasing relaxation which would contribute to diastolic failure.

Ultra-structural analysis by electron microscopy has revealed loss and disorganisation of actin filaments in STZ diabetic hearts<sup>[21]</sup>, which was supported by confocal analysis of phalloidin labelled ventricular tissue with disorganisation and a reduction of f-actin labelling evident<sup>[16,21,23]</sup>. We have also observed that myocyte cell diameter is reduced in the STZ diabetic rat, suggesting that amount of myofilament protein per myocyte is reduced. Figure 4 shows mean  $\pm$  SE data from enzymatically isolated ventricular myocytes from diabetic and control rats. Cell length was not different between groups, but both width and volume were markedly reduced in myocytes from diabetic hearts. Similar changes in f-actin content and myocyte size in the STZ diabetic rat have been reported by Kawaguchi *et al.*<sup>[45]</sup> (1999). Pertinently these authors also identified a decrease in myocyte diameter in the diabetic human heart<sup>[45]</sup>. Changes in myocyte volume have been shown to occur as early as one week after induction of diabetes in the STZ<sup>[46]</sup>. The decreased myocyte volume is evident in the left hand side (LHS) panel of Figure 5C and D where representative tissue from the LV free wall of diabetic rat hearts shows reduced myocyte diameter. It appears then that the diabetic myocytes are atrophied. Ultrastructural changes in mitochondrial morphology have been shown by electron microscopy of diabetic rat heart, with likely consequences not only for myocyte volume but also energy metabolism<sup>[21]</sup>. Proteomic analysis of diabetic rat heart identified multitude

of changes in the mitochondrial proteome<sup>[47]</sup>. The most notable changes are an increase in enzymes involved in long chain fatty acids oxidation and decrease in enzymes involved in catabolism. Metabolism of the diabetic heart is shifted from a mix of carbohydrates and fatty acids for energy supply to relying almost solely on fatty acids, with a resultant increase in the production of oxygen free radical end products<sup>[48]</sup>. Significantly the proteomic analysis also showed changes in proteins involved with oxidative stress, suggesting that impaired energy metabolism might lead to myocytes being unable to meet the energetic needs producing changes in the structure and function of the contractile machinery.

Diabetic cardiomyopathy is also associated with increased stiffness in the left ventricle<sup>[49]</sup>, and a decreased maximum rate-of-rise in developed stress<sup>[23]</sup>, suggesting that cardiac compliance is reduced in diabetic rats. The extracellular matrix in healthy hearts provides a scaffolding that supports the myocytes and other tissue components, enabling the coordinated transduction of force that is necessary for the heart to function as a pump. Collagen is an important component of the extracellular matrix, with type I and type III collagens the most abundant types in ventricular tissue forming 90% of the total collagen content<sup>[50]</sup>. Figure 5 shows type I collagen is increased in diabetic rat hearts, which would contribute to the decreased ventricular compliance, with no change in type III collagen<sup>[23]</sup>. Myocardial echodensity has been reported as increased in asymptomatic diabetic patients, thought to be a result of increased collagen deposition<sup>[51]</sup>. It is proposed that increased echodensity might therefore act as an early indicator of the subsequent development of diabetic cardiomyopathy.



## CONCLUSION

In conclusion, diabetic cardiomyopathy arises as a result of the sustained hyperglycaemia and the damaging effects this has on the heart. Ventricular myocytes from untreated diabetic rat hearts show contractile dysfunction after 8 wk of hyperglycaemia, with prolonged action potential duration, slower  $\text{Ca}^{2+}$  transient decay and reduced myofilament  $\text{Ca}^{2+}$  sensitivity. Gross structural changes to the myocardium are evident at this stage of the disease. Extracellular type 1 collagen is increased, t-tubules are less regular in appearance, and F-actin within myocytes is reduced in content and disrupted in appearance. We conclude that it is these structural changes that are the main contributors to the contractile dysfunction of diabetic cardiomyopathy, along with mitochondrial changes that compromise energy supply. We suggest that consideration should therefore be given in future studies to the contribution of these observed structural changes to the contractile deficit in the diabetic hearts, rather than focusing on myocyte  $\text{Ca}^{2+}$  handling in searching for effective treatments for diabetic cardiomyopathy.

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WJC 6<sup>th</sup> Anniversary Special Issues (3): Cardiomyopathy

## Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis

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### Abstract

The recent development of cardiac magnetic resonance (CMR) techniques has allowed detailed analyses of cardiac function and tissue characterization with high spatial resolution. We review characteristic CMR features in ischemic and non-ischemic cardiomyopathies (ICM and NICM), especially in terms of the location and distribution of late gadolinium enhancement (LGE). CMR in ICM shows segmental wall motion abnormalities or wall thinning in a particular coronary arterial territory, and the subendocardial or transmural LGE. LGE in NICM generally does not correspond to any particular coronary artery distribution and is located mostly in the mid-wall to subepicardial layer. The analysis of LGE distribution is valuable to differentiate NICM with diffusely impaired systolic function, including dilated cardiomyopathy, end-stage hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, and myocarditis, and those with diffuse left ventricular (LV) hypertrophy including HCM, cardiac amyloidosis and Anderson-Fabry disease. A transient low signal intensity LGE in regions of severe

LV dysfunction is a particular feature of stress cardiomyopathy. In arrhythmogenic right ventricular cardiomyopathy/dysplasia, an enhancement of right ventricular (RV) wall with functional and morphological changes of RV becomes apparent. Finally, the analyses of LGE distribution have potentials to predict cardiac outcomes and response to treatments.

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**Key words:** Cardiomyopathy; Cardiac magnetic resonance; Late gadolinium enhancement; Cardiac function; Clinical features; Prognosis

**Core tip:** We review characteristic cardiac magnetic resonance (CMR) features in ischemic and non-ischemic cardiomyopathies (NICM), especially in terms of location and distribution of late gadolinium enhancement (LGE). LGE in NICM does not correspond to any particular coronary artery distribution and is located mostly in the mid-wall to subepicardial layer. The analysis of LGE distribution is valuable to differentiate NICM with diffusely impaired systolic function; dilated cardiomyopathy, end-stage hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, and myocarditis, and those with diffuse LV hypertrophy; HCM, cardiac amyloidosis and Anderson-Fabry disease. The analyses of LGE distribution have potentials to predict cardiac outcomes and response to treatments.

Satoh H, Sano M, Suwa K, Saitoh T, Nobuhara M, Saotome M, Urushida T, Katoh H, Hayashi H. Distribution of late gadolinium enhancement in various types of cardiomyopathies: Significance in differential diagnosis, clinical features and prognosis. *World J Cardiol* 2014; 6(7): 585-601 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/585.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.585>



## INTRODUCTION

The management of patients with left ventricular (LV) dysfunction starts from the identification of underlying myocardial disorders. The primary diagnostic issue is the differentiation between ischemic and non-ischemic cardiomyopathies (ICM and NICM). NICM include several disorders, such as dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), cardiac sarcoidosis, stress cardiomyopathy, and others<sup>[1,2]</sup>, but often show similar clinical presentations which lead to progressive heart failure, a high risk of fatal arrhythmias, and a high mortality rate<sup>[3]</sup>.

NICM have been traditionally diagnosed non-invasively with chest roentgenography, standard 12-lead electrocardiography (ECG), transthoracic and/or transesophageal echocardiography and nuclear imaging, and invasively with coronary angiography, left ventriculography, and endomyocardial biopsy.

Imaging with cardiac magnetic resonance (CMR) is non-invasive, uses no ionizing radiation, and has high spatial resolution. Recent advantage of CMR has enabled us to assess cardiac morphology, function and tissue characteristics both in ICM and NICM<sup>[4,5]</sup>. Thus, CMR is capable of identifying cardiac abnormalities not readily recognized by conventional imaging modalities<sup>[6-8]</sup>.

We have been studying the late gadolinium enhancement (LGE) in various NICM and attempting to verify the values for differential diagnosis, clinical features, and prognosis<sup>[9-13]</sup>. This review article focuses on various types of NICM, and discusses initially about CMR techniques and differential diagnosis from ICM, and then about the usefulness of CMR, especially the clinical significance of location and distribution of LGE.

## RECENT DEVELOPMENT OF CMR

CMR imaging comprises several techniques of magnetic resonance imaging (MRI) sequences. Cine-CMR, which is based on the steady state free precession sequence, provides accurate information about cardiac morphology and function. First-pass contrast enhanced perfusion-CMR with and without vasodilators can provide assessment of myocardial perfusion reserve<sup>[14]</sup>.

LGE-CMR relies on the delivery of intravenous gadolinium chelate to the myocardium, which is a biologically inert tracer that freely distributes in extracellular space but does not cross the intact cell membrane. Due to a combination of increased extracellular volume and slower washout kinetics, there is a relative accumulation of gadolinium in areas of necrosis, fibrosis, infiltration, and inflammation in the late washout phase. Since gadolinium shortens T1 relaxation time, it produces brighter signal intensity, and this technique is sensitive and reproducible in the detection of myocardial scarring both in

ICM and NICM<sup>[15,16]</sup>. However, since LGE is ascribed to relative accumulation of gadolinium in areas of damaged myocardium, LGE-CMR techniques may miss a diffuse type of fibrosis<sup>[16,17]</sup>. Recently, T1 mapping with a Look-Locker sequence after injection of gadolinium has become a promising tool to quantify interstitial myocardial fibrosis<sup>[18]</sup>.

There are also special sequences that are used less often to clarify the cause of NICM. These include fat suppression black blood for detection of fatty infiltration, T2-weighted imaging for myocardial edema, and T2-star (T2\*) for the assessment of myocardial iron<sup>[19-21]</sup>.

Thus, the combination of multiple CMR sequences helps clinicians differentially diagnose NICM. The characteristic features in each CMR sequence will be discussed below under each specific NICM.

## DIFFERENTIAL DIAGNOSIS OF ICM AND NICM

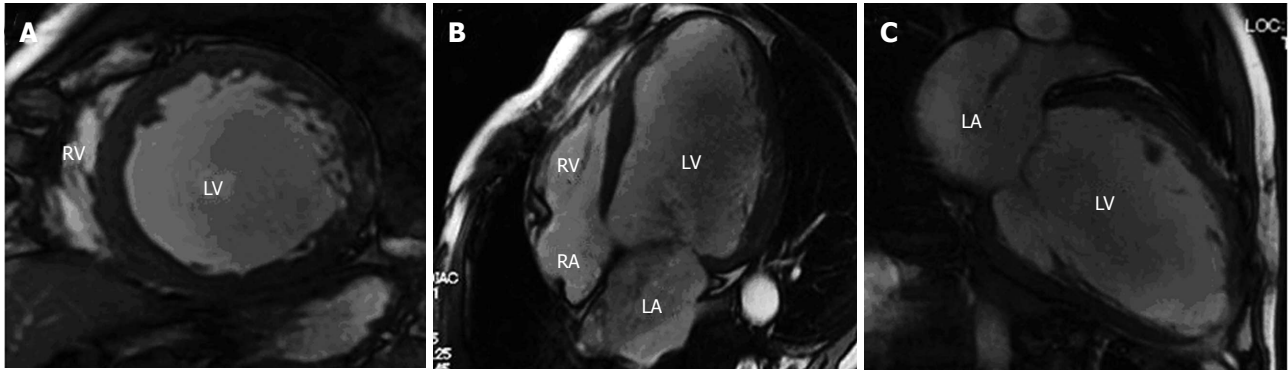
The diagnosis of patients with NICM originates with the differentiation of ICM. In general, coronary angiography is routinely performed for the differentiation, and when patients have no obstructive coronary arteries or coronary risk factors, the diagnosis of NICM is usually made. However, it has to be kept in mind that no obstructive coronary artery on angiography is inadequate to exclude ICM<sup>[16]</sup>. The spontaneous recanalization after coronary occlusion caused by a rupture of minimally stenotic but unstable plaque, embolization or spasm may mask the occurrence of coronary events. Conversely, it is also a common situation that patients with DCM have coronary arterial disease during their natural courses. An autopsy study in some patients diagnosed with DCM has described subendocardial and transmural fibrosis indistinguishable from myocardial infarction<sup>[22]</sup>.

CMR technique is now recognized as a useful tool to determine whether the LV dysfunction is caused by ischemic coronary events. Cine-CMR with excellent spatial and temporal resolution can detect segmental wall motion abnormalities or wall thinning in a particular coronary arterial territory. LGE-CMR can also define the subendocardial or transmural LGE as fibrosis caused by coronary events because the ischemic wave front starts from subendocardium.

On the other hand, LGE in NICM generally does not correspond to any particular coronary artery distribution and is often located in the mid-wall<sup>[23]</sup>. A previous study detected striated or patchy pattern of LGE in a certain part of patients diagnosed with DCM<sup>[16]</sup>.

The differential diagnosis of ICM and NICM is also crucial for management of patients with cardiac dysfunction. Treatment with  $\beta$ -adrenoceptor blockers and renin-angiotensin-aldosterone inhibitors are recommended for both ICM and NICM. Patients with ICM have worse outcome but may benefit from revascularization and/or aneurysmectomy and from secondary prevention with aspirin and statins. Furthermore, LV remodeling after





**Figure 1** Representative cine-cardiac magnetic resonance images in a 62-year-old male patient with dilated cardiomyopathy. The images show mid-ventricular short axis (A), horizontal axis (4-chambers) (B) and vertical long axis views (C). The images reveal dilatation of left ventricular (LV) cavity and diffuse wall thinning (relatively homogenous). The LV end-diastolic volume, LV end-systolic volume, LV ejection fraction (EF) and LV mass are 329.1 mL, 252.5 mL, and 23.3%, 153.2 g, respectively. LV and RV: Left and right ventricles; LA and RA: Left and right atria; LV: Left ventricular.

myocardial infarction often occurs with non-extensive infarction but the absence of suitable preventive therapy. Conversely, in patients with NICM, the early diagnosis may recommend genetic studies to indentify inherited abnormalities and help to start early aggressive study with intensified medical and device therapies<sup>[2,16]</sup>.

## DCM

### General

DCM is the most common isoform of NICM, and is characterized by dilatation of LV chamber and systolic dysfunction, which leads to progressive heart failure, high risk for fatal arrhythmias and high mortality rate<sup>[3]</sup>.

Although over the half of cases are idiopathic, DCM is not a single tree of disease spectrum but may include several undetermined etiologies, such as chronic myocarditis, tachycardia-induced cardiomyopathy, undiagnosed sarcoidosis, and end-stage HCM<sup>[16,24]</sup>.

### CMR features

In cine-CMR, all cardiac chambers are enlarged and a decrease in LV ejection fraction (EF) is evident. The LV wall thickness is normal or decreased, but relatively homogenous. Figure 1 shows representative cine-CMR images of different views in a patient with DCM.

In LGE-CMR, DCM has been shown to demonstrate mostly a lack of LGE or the presence of mid-wall enhancement, and a fewer part of cases shows patchy or diffuse striated LGE. The distribution of LGE is unrelated to a particular coronary arterial territory, and corresponds to focal fibrosis at autopsy<sup>[1,9,25]</sup>. Our recent study showed various patterns of LGE as described in Figure 2<sup>[13]</sup>. However, the prevalence of LGE varies among reports between 12% and 67%, which may be caused by different etiologies, disease states and duration, or by a limitation of LGE-CMR technique. The mechanisms of myocardial fibrosis in DCM are complex and include inflammation, genetic predisposition, micro-vascular ischemia, and neurohumoral changes<sup>[9]</sup>. LGE-CMR technique may miss a diffuse type of fibrosis, and hence a certain

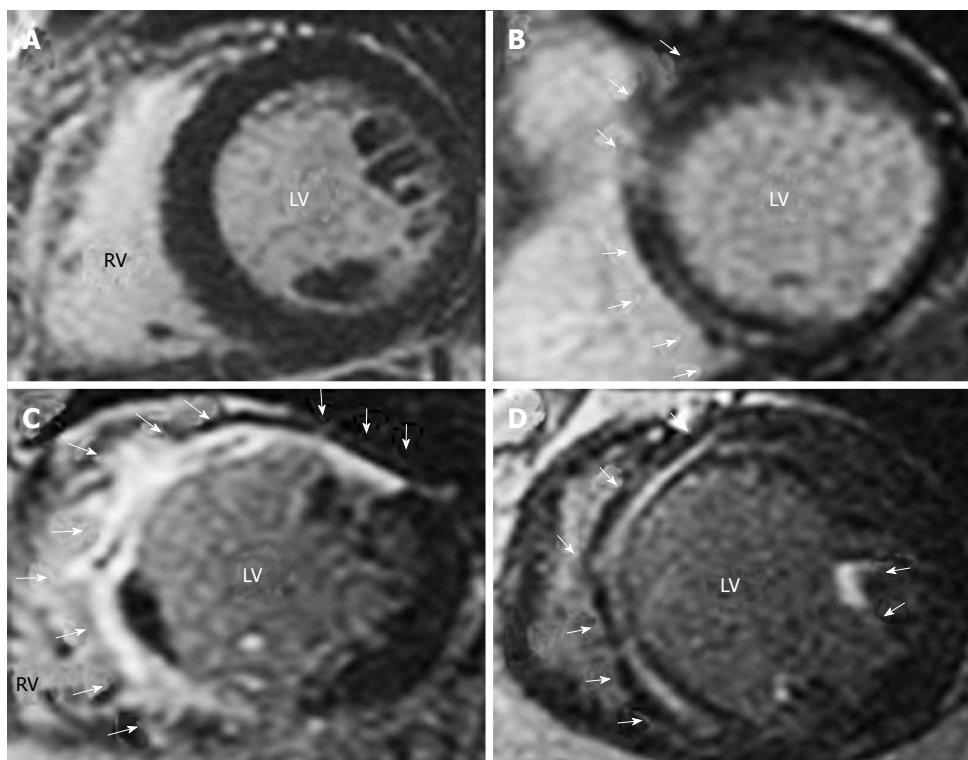
part of DCM patients may have no LGE<sup>[16,17]</sup>. Different thresholds used to detect LGE may also affect the variation in the prevalence of LGE. A recent development of T1 mapping technique is expected to estimate such a diffuse type of fibrosis<sup>[17,18]</sup>.

### Clinical implications

Several previous studies showed the lack of relationship between the presence of LGE or LGE volume, and LV volume and function<sup>[9,12,26]</sup>. We and other investigators found that LGE volume did not correlate with LV end-diastolic volume, global left ventricular ejection fraction (LVEF) or segmental LV contraction, but the washout rate of 99m-technecium-sestamibi (<sup>99m</sup>Tc-MIBI) did<sup>[12,27]</sup>. Since the increase in washout rate of <sup>99m</sup>Tc-MIBI reflects mitochondrial dysfunction in cardiomyocytes<sup>[28]</sup>, the increased LV volume and impairment of LV function in DCM may be ascribed to the dysfunction of individual myocytes rather than segmental fibrosis. However, recent studies have shown the resistance of patients with mid-wall LGE to reverse remodeling by  $\beta$ -adrenergic blockers and/or cardiac re-synchronization therapy<sup>[9,29]</sup>. We also showed that reverse remodeling occurred after treatment in patients with no LGE and with LGE localized in inter-ventricular septum, but did not in patients with extensively distributed LGE<sup>[13]</sup>. Since LV segments with a lower amount of LGE are expected to have more viable but functionally disturbed cardiomyocytes and reversible matrix fibrosis, they are more likely to benefit from therapies<sup>[12,27]</sup>.

The mid-wall LGE in DCM correlates with intra-ventricular conduction disturbance, and is independently predictive of sudden cardiac death (SCD) or ventricular tachycardias (VTs)<sup>[13,30,31]</sup>. Thus, LGE-CMR can help to identify the arrhythmogenic substrate and plan an appropriate mapping and ablation strategy.

In DCM, a series of factors is associated with adverse prognosis, such as age, gender, LVEF, QRS duration and cardiac biomarkers<sup>[13]</sup>. Although the larger LGE volume is associated with poor prognosis in patients with ICM<sup>[13,32]</sup>, the prognostic implication of LGE in DCM remains controversial. However, the severity of irrevers-



**Figure 2** Representative short axis late gadolinium enhancement-cardiac magnetic resonance images in patients with dilated cardiomyopathy. A: No LGE; B: localized LGE. Mid-wall LGE distributed only into anterior and inferior septum; C: Extensive LGE. LGE distributed at anterior and inferior septum, anterior, antero-lateral and inferior LV segments; D: Extensive LGE. Mid wall LGE distributed at anterior and inferior septum, and at anterior papillary muscle. Arrows indicate LGE in LV wall segments. All the images are taken from Machii *et al.*<sup>[13]</sup> with permission. LGE: Late gadolinium enhancement; LV: Left ventricular.

ible fibrosis is related to the impairment of cardiac function, the propensity to ventricular arrhythmias and the resistance to reverse remodeling, and recent studies have shown that LGE volume is well concordant with high probabilities of cardiac mortality and morbidity<sup>[30,33,34]</sup>. We also exhibited the lowest event-free survival rate in patients with extensively distributed LGE<sup>[13]</sup>. Therefore, the analysis of LGE volume or distribution, not only the presence of LGE, may be valuable to predict prognosis and identify high-risk patients in DCM.

## HCM

### General

HCM is a relatively common genetic disorder of the cardiac sarcomere, characterized by an idiopathic LV hypertrophy. Typically, this disorder demonstrates asymmetric septal hypertrophy, but can also present atypical patterns of hypertrophy involving the mid-ventricle and apex. Hence, HCM has a wide variety of morphological, functional, and clinical features.

### CMR features

Because of various phenotypic expressions of HCM and other mimicking diseases which show LV hypertrophy, cardiac imaging has a central role in establishing the final diagnosis. Although transthoracic echocardiography has been the standard tool for the diagnosis of HCM, it has limitations for precise visualization of whole ventricles

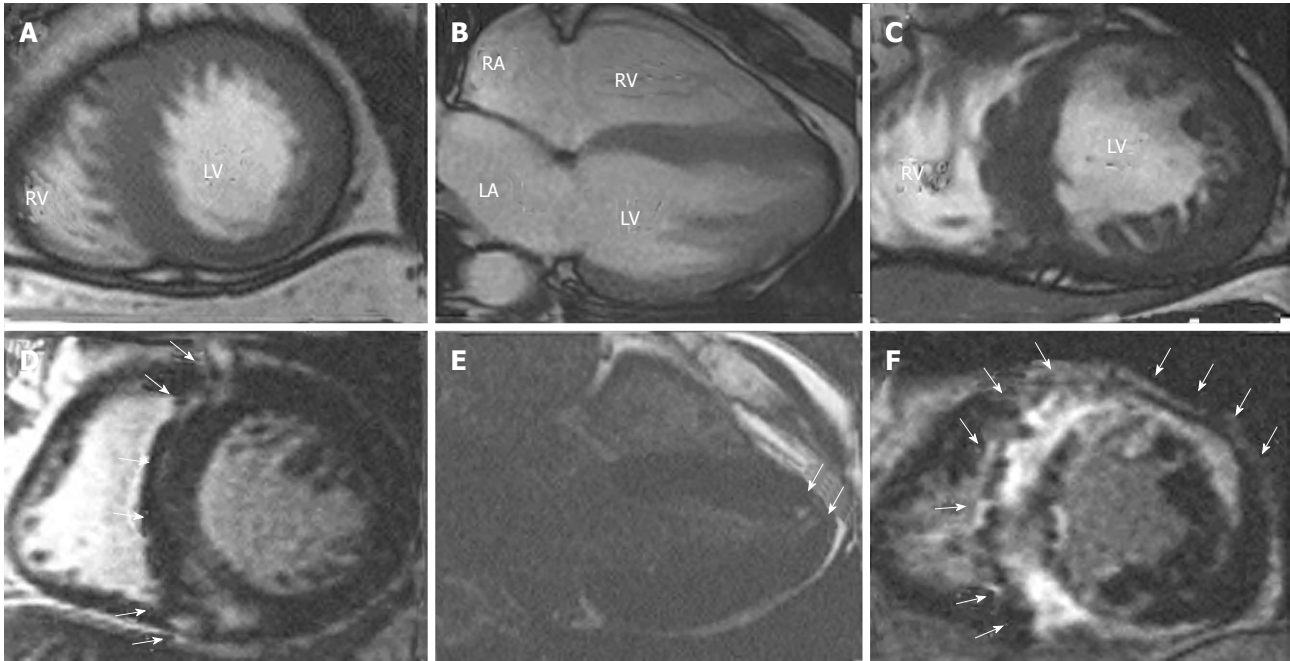
and quantification of hypertrophy. CMR is capable of identifying regions of LV hypertrophy not readily recognized by echocardiography<sup>[6-8]</sup>, especially for apical hypertrophy and apical aneurysm<sup>[11,35,36]</sup>.

The myocardial LGE is a common feature of HCM, and can be focal or spread diffusely into any areas of LV<sup>[11,37,38]</sup>. A previous study showed that more than 55% of HCM patients have some LGE, most commonly at the anterior and posterior RV insertion points. Gene-positive patients are more likely to have LGE and may even precede hypertrophy<sup>[39,40]</sup>. LGE in HCM usually represents areas of increased interstitial fibrosis but may also indicate myocardial disarray, necrosis, and scarring<sup>[41]</sup>. Figure 3 shows representative cine-CMR and LGE-CMR images in various types of HCM.

In other MRI sequences, a previous study showed focal T2 abnormalities in the areas of LGE with severe LV hypertrophy<sup>[42]</sup>. In addition, stress CMR can demonstrate reduced vasodilator response in subendocardium particularly in the area of severe hypertrophy<sup>[14]</sup>.

### Clinical implications

In contrast to DCM, the presence of LGE and LGE volume have been well associated with New York Heart Association (NYHA) functional classes, LV systolic and diastolic function, and left atrial volume<sup>[9,11,43]</sup>. Since 15% to 20% of HCM patients have progressive heart failure<sup>[44]</sup>, determining the prognostic implications of LGE in HCM patients is crucial in order to identify high-risk



**Figure 3** Representative cine-cardiac magnetic resonance (A-C) and late gadolinium enhancement-cardiac magnetic resonance (D-F) images in patients with various phenotypes of hypertrophic cardiomyopathy. A, D: ASH (short axis views); B, E: APH (horizontal views); C, F: End-stage HCM (short axis views). LGE was mainly localized in the ventricular septum and right ventricular insertion points in ASH and in the apex in APH (arrows). Note the inhomogeneous LV wall thickness and diffusely spread LGE in end-stage HCM. All the images are taken from Sato *et al.*<sup>[11]</sup>. ASH: Asymmetrical septal hypertrophy; APH: Apical hypertrophy; LGE: Late gadolinium enhancement; LV: Left ventricular; HCM: Hypertrophic cardiomyopathy.

patients who are most likely to benefit from early aggressive therapies.

Since myocardial fibrosis may provide an arrhythmogenic underlying substrate, previous studies examined the correlation between LGE and ECG abnormalities or ventricular arrhythmias in HCM. The disturbance of conduction system, exhibited as prolonged QRS duration and/or QRS axis deviation was correlated with LGE volume and LGE distribution into inter-ventricular septum<sup>[11,45]</sup>. Although the contribution of LGE to abnormal Q waves still remains controversial, the segmental and transmural extent rather than the mere presence of LGE may be the underlying mechanism of abnormal Q waves<sup>[11,45,46]</sup>. The apical hypertrophy (APH) is a common type of HCM especially in Japan<sup>[47]</sup>. The giant negative T waves are one of the characteristics of APH, and the depth of negative T waves was related to the asymmetric distal hypertrophy<sup>[11]</sup>. We and others also reported the progression of apical myocardial damage expressed as LGE reduced the QRS voltage, the depth of negative T waves, and caused fragmentation of QRS waves<sup>[48]</sup>. Recent studies have also shown that HCM patients with LGE are more likely to have episodes of non-sustained VTs, higher frequency of ventricular extrasystoles as well as VT inducibility in the electrophysiological study<sup>[9,13,49]</sup>.

Risk stratification in HCM is difficult because of the heterogeneity in the clinical and phenotypic expression and the low event rate<sup>[44,49]</sup>. However, HCM is one of the most common disorders causing SCD. There are five clinically accepted high-risk factors for SCD, including a family history of sudden death, extreme LV hypertro-

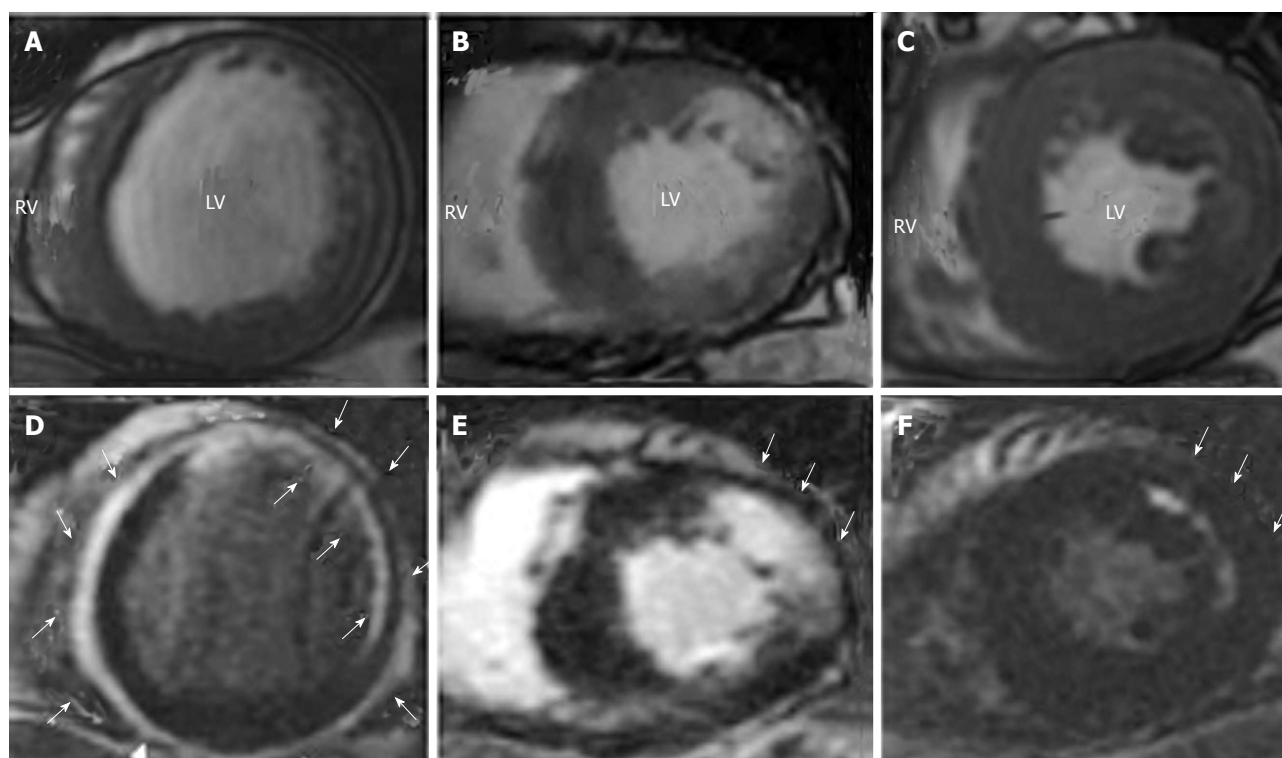
phy ( $> 30$  mm), unexplained syncope, a documentation of non-sustained VTs, and an abnormal blood pressure response during upright exercise<sup>[50]</sup>. A recent review has shown a close relationship between LGE and cardiovascular mortality, heart failure death, and all-cause mortality in HCM<sup>[51]</sup>. Additionally, stress perfusion CMR could be used to further stratify the risk for SCD, since inducible myocardial ischemia is another risk in HCM, which was proven by a study on single-photon emission computed tomography (SPECT)<sup>[52]</sup>.

### End-stage (dilated phase) HCM

End-stage HCM, which is characterized by LV systolic dysfunction and enlargement of LV cavity, is recognized as a part of HCM disease spectrum<sup>[53]</sup>. Since the clinical condition in end-stage HCM resembles that in DCM, the differential diagnosis of them becomes difficult, if the hypertrophy was undiagnosed or underestimated during the natural course of the disease. Patients with end-stage HCM frequently exhibit severe heart failure and lethal ventricular arrhythmias, thus resulting in higher mortality rates than the overall HCM or DCM population<sup>[13,53]</sup>. Therefore, the early and correct recognition of those patients is necessary to start aggressive medical and device therapies.

Cine-CMR exhibits that LV wall thickness in end-stage HCM is normal or relatively larger and is inhomogeneous among LV segments compared with that in DCM (Figure 3)<sup>[13]</sup>. LGE-CMR also shows that LGE in end-stage HCM distributes more diffusely into all the LV segments, whereas that in DCM is localized mainly in the





**Figure 4** Representative cine-cardiac magnetic resonance (A-C) and late gadolinium enhancement-cardiac magnetic resonance (D-F) images in patients with cardiac sarcoidosis. A, D: A patient with LV dilatation, reduced LVEF (22%) and circumferential subepicardial and subendocardial LGE with spared mid-myocardium; B, E: A patient with reduced LVEF (38%) and nodular LGE in antero-lateral wall; C, F: A patient with preserved LVEF (58%) with mid-wall striated LGE in antero-lateral wall. White arrows indicate LGE areas. A part of the images is taken from Matoh *et al.*<sup>[10]</sup> with permission. LGE: Late gadolinium enhancement; LV: Left ventricular; LVEF: Left ventricular ejection fraction

inter-ventricular septum<sup>[6,9,11,38]</sup>. Detailed analyses of both cine-CMR and LGE-CMR can help differentiation of end-stage HCM from DCM and other secondary cardiomyopathies that exhibit LV dysfunction with hypertrophy (*e.g.*, cardiac amyloidosis and Anderson-Fabry disease).

## CARDIAC SARCOIDOSIS

### General

Sarcoidosis is a multi-system disorder of unknown etiology. Clinical cardiac involvement is found in only 5% to 7% of patients with sarcoidosis, whereas postmortem studies have identified myocardial lesions in 20% to 60%<sup>[54]</sup>. Autopsy studies showed that cardiac sarcoid lesions were mainly non-transmural and located in the basal LV and subepicardial myocardium<sup>[55-57]</sup>.

The diagnosis of cardiac sarcoidosis has been made with endomyocardial biopsy, and the guideline of Japanese Ministry of health and welfare (JMH) is also based on histological diagnosis<sup>[58]</sup>. However, biopsy results are sometimes false negative because of discrete distribution of sarcoid lesions. Hence, patients with systemic sarcoidosis and those with impaired LV function who are suspected cardiac involvement of sarcoidosis are not always positive according to the guideline. Therefore, some patients have been misdiagnosed with normal or DCM, and do not benefit from immunosuppressive therapies. Since patients with cardiac sarcoidosis have a poor prognosis, and a treatment with corticosteroid can improve long-

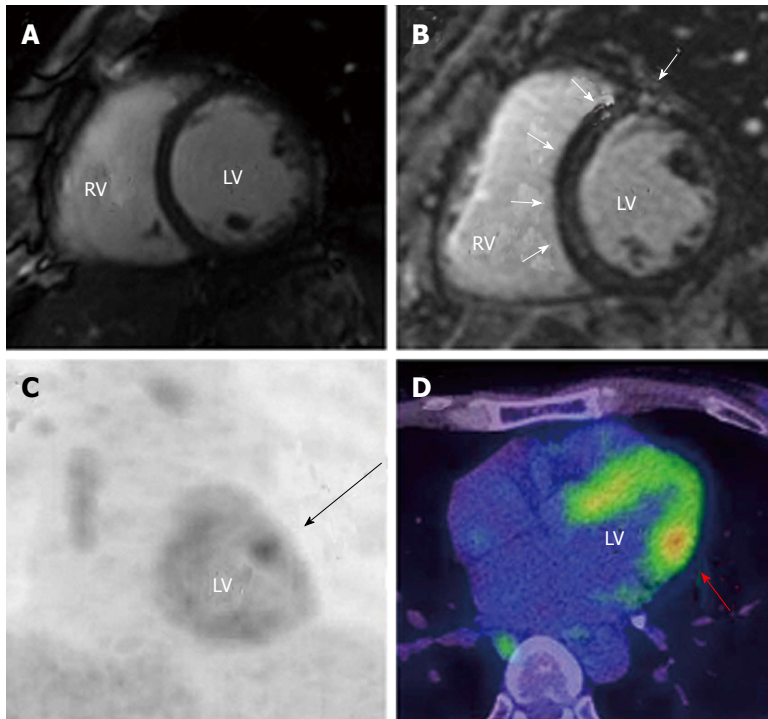
term prognosis, an earlier diagnosis of cardiac involvement of sarcoidosis with non-invasive imaging modalities is crucial.

### CMR features

In cardiac sarcoidosis, cine-CMR can image segmental wall motion abnormalities, wall thinning, and aneurysm formation. LGE-CMR identifies LGE in the LV wall<sup>[10,55-58]</sup>. The mechanism of LGE in cardiac sarcoidosis is considered to be heterogeneous, and may contain not only fibrotic scar but also an increased interstitial space due to the formation of non-caseating epithelioid cell granuloma<sup>[59]</sup>. LGE in cardiac sarcoidosis may reflect irreversible myocardial damage, since we and others could not demonstrate a reduction in LGE volume during various follow-up periods<sup>[10,58]</sup>.

Previous studies compared findings between LGE-CMR and SPECT or <sup>18</sup>F-fluorodeoxyglucose-positron emission computed tomography (FDG-PET) in the diagnosis and assessment of cardiac sarcoidosis. A previous paper noted that the transmural extent of LGE was well associated with defect scores in <sup>201</sup>Tl-SPECT<sup>[56]</sup>. We found that LGE distributed mostly into the basal and mid inter-ventricular septum, but also spread into all the LV segments. Additionally, we and other investigators found that nodular, circumferential, and subepicardial and subendocardial types of LGE distribution exhibited high specificity for differential diagnosis from DCM (97%-100%, Figure 4)<sup>[57,58]</sup>. Although the new JMH guideline includes





**Figure 5** Representative short axis cine-cardiac magnetic resonance (A), late gadolinium enhancement-cardiac magnetic resonance (B),  $^{18}\text{F}$ -fluorodeoxyglucose-positron emission computed tomography (C), and positron emission computed tomography (D) images in a 57-year-old male patient with systemic sarcoidosis. The diagnosis of sarcoidosis was made with liver biopsy. Cine-CMR images shows normal LV size and contraction (LVEDV: 119 mL, LVEF: 73%), but LGE-CMR reveals patchy and striated LGE in anterior wall and inter-ventricular septum (white arrows). The patient was negative for cardiac involvement of sarcoidosis according to the guideline of Japanese Ministry of Health and Welfare. However, FDG-PET and PET-CT images demonstrate hot spot in postero-lateral wall of LV, indicative of active inflammatory change (black and red arrows). FDG-PET:  $^{18}\text{F}$ -fluorodeoxyglucose-positron emission computed tomography; LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance.

the presence of LGE as a minor criterion for cardiac sarcoidosis<sup>[60]</sup>, the characteristic patterns of LGE distribution may help more precise diagnosis.

T2-weighted CMR sometimes shows punctuated or patchy signals in the acute lesions of cardiac sarcoidosis with myocardial edema<sup>[61]</sup>.

### Clinical implications

In sarcoidosis, patients with LGE in myocardium show heart failure symptoms, and a higher prevalence of ECG abnormalities and VTs<sup>[58]</sup>. The correlations between LGE volume, and LV volume and function are also described. Hence, the cardiac outcome in patients with LGE is significantly lower than that without LGE<sup>[57,58]</sup>.

While LGE and defects in  $^{201}\text{Tl}$ -SPECT represent irreversible fibro-granulomatous replacement, the hot spots in  $^{67}\text{Ga}$ -SPECT or FDG-PET indicate active inflammatory change, which can also be used for assessing the effect of corticosteroid therapy<sup>[62,63]</sup>. Since FDG-PET can provide better sensitivity compared with SPECT, the combination of CMR and FDG-PET may improve overall sensitivity for diagnosis and help therapeutic strategies (Figure 5)<sup>[63,64]</sup>.

## STRESS (TAKOTSUBO) CARDIOMYOPATHY

### General

Stress cardiomyopathy (SC), initially reported in Japan as Takotsubo cardiomyopathy, is characterized by an acute, severe but reversible LV dysfunction without significant coronary artery disease<sup>[65,66]</sup>. The majority of patients have a clinical presentation similar to that of acute coronary syndrome (ACS)<sup>[66]</sup>. The precise incidence of SC is unknown, but recent studies have revealed a prevalence

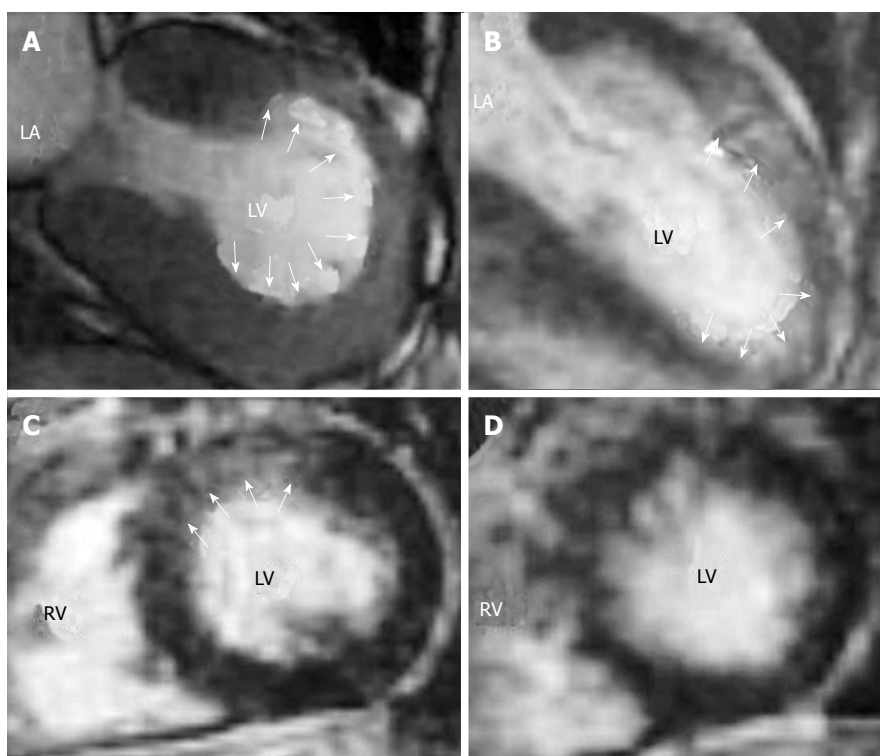
of approximately 2% of patients presenting ACS in the United States and Europe<sup>[66,67]</sup>. There is a high predominance in elderly women, and several instances are possibly triggered by physical or emotional stress<sup>[68,69]</sup>. Despite severe presentation in acute phase, complications are rare and the prognosis of patients with SC is generally considered favorable<sup>[67,70]</sup>.

Although the mechanism of SC has not yet been fully clarified, considerable evidence suggests that enhanced sympathetic activity might play a pathogenic role in the transient myocardial dysfunction observed in SC<sup>[71]</sup>. At the tissue level, myocardial edema as a sign of acute but reversible injury and diffuse inflammation in the absence of significant necrosis/fibrosis are characteristics of SC. However, other histological analyses of the heart in SC showed sparse foci of myocardial necrosis with contraction bands in the akinetic area<sup>[71,72]</sup>.

### CMR features

CMR at acute phase (approximately 5 d after onset) is mostly suited for the evaluation of patients with SC. Since CMR imaging can provide markers for reversible and irreversible injury, it may be particularly important to diagnose SC from ACS and myocarditis<sup>[66,70,73]</sup>.

A previous study suggested diagnostic criteria with CMR: (1) severe LV dysfunction in a non-coronary regional distribution pattern; (2) myocardial edema collocated with the regional wall motion abnormality; (3) absence of high-signal areas in LGE images; and (4) increased early myocardial gadolinium uptake<sup>[66]</sup>. The LV dysfunction in cine-CMR is typically apical ballooning shape with akinesis of apical and mid-ventricular LV segments (so-called Takotsubo-like). However, fewer patients presented a mid-ventricular variant with apical sparing or with isolated basal ballooning<sup>[66,69]</sup>. Mean LVEF was 39%



**Figure 6** Representative cine-cardiac magnetic resonance (A) and late gadolinium enhancement-cardiac magnetic resonance (B-D) images in a case of stress (Takotsubo) cardiomyopathy. The images show vertical long axis (A, B) and mid-ventricular short axis (C, D) views. The cine-CMR image during systole (A) shows mid-anterior dyskinesia (white arrows). LGE-CMR images on the sub-acute phase (B, C) show that the area of LGE was well matched with the area of wall motion abnormality (white arrows). On the follow-up phase, LV systolic function recovered, and the LGE-CMR image (D) could not detect significant LGE in the LGE area observed on the sub-acute phase. All the images are taken from Naruse *et al*<sup>[69]</sup> with permission. LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance.

in the acute setting and 65% in the recovery phase. Cine-CMR also clarified right ventricular (RV) dysfunction in 38.5% of patients, and apical thrombus in 5.1%<sup>[73]</sup>. T2-weighted images can also show myocardial edema co-located with the regional wall motion abnormality<sup>[66]</sup>. The absence of LGE has been described in many case studies and is a common diagnostic criterion<sup>[66,70]</sup>. However, a recent meta-analysis has demonstrated LGE in a certain part of cases with SC<sup>[73]</sup>. A previous study showed evidence for the immune-histological basis of the LGE phenomenon in patients with SC<sup>[74]</sup>.

We found LGE in 8 of 20 patients with SC<sup>[69]</sup>. The signal intensity was lower than that usually documented in cases of myocardial infarction or myocarditis (Figure 6). Another study also showed that focal and patchy LGE was detected in a certain part of patients when using a threshold of 3 standard deviation (SD) instead of 5 SD above the mean of remote myocardium to define significant enhancement<sup>[66]</sup>. Possible speculations are that severe stress-induced stunning of the apical segments leads to a patchy pattern of myocardial contraction-band necrosis possibly accompanied by a certain amount of transient focal/patchy edema or deposition of extracellular matrix resulting in LGE with low signal intensity. We also detected LGE at the recovery phase in fewer patients.

### Clinical implications

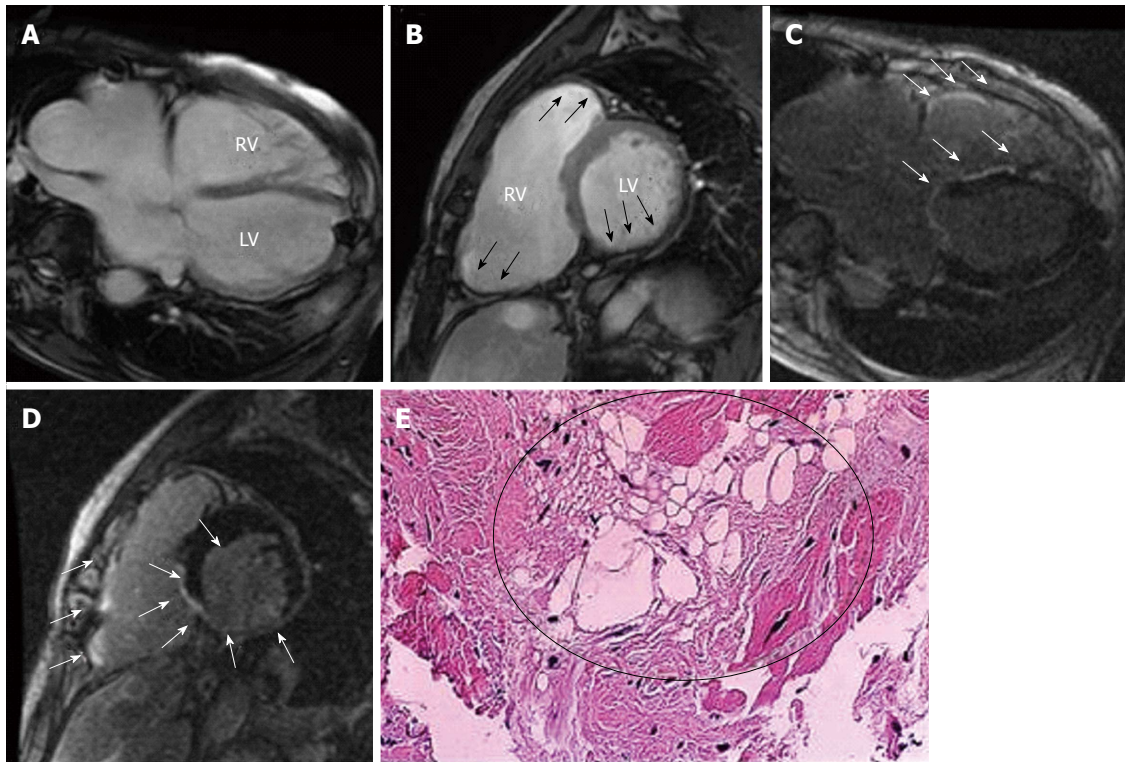
Although the LV dysfunction in SC is mostly reversible,

an involvement of RV is associated with longer hospitalization, heart failure, and older age. Cine-CMR can clarify the exact incidence of bi-ventricular ballooning<sup>[66,75]</sup>. We also showed that patients with LGE experienced cardiogenic shock more frequently and had a longer duration to ECG normalization and recovery of wall motion than did those without LGE<sup>[69]</sup>. Contrary, another study exhibited that the presence of less rigorously defined LGE during the acute phase had no persisting effect on global LV function, and there was no evidence of LGE at CMR follow-up<sup>[66]</sup>. Thus, the clinical implications of such type of LGE remain still elusive. In both studies, however, the absence of significant LGE was consistent with the complete normalization of LV function in patients with SC.

## OTHER CARDIOMYOPATHIES

### Arrhythmogenic right ventricular cardiomyopathy/dysplasia

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a disease of heart muscle characterized by structural and functional abnormalities of RV wall due to replacement of the myocardium by fatty and fibrous tissue. This disorder is relatively uncommon but life-threatening cardiomyopathy with progressive RV failure, ventricular arrhythmias and SCD. Although RV is the predominantly diseased chamber, LV can also be the affected chamber in some cases<sup>[76]</sup>.



**Figure 7** Representative cine- cardiac magnetic resonance (A, B) and late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 55- year-old male patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia. The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal dilatation of both RV and LV chamber. Focal dilatation of RV and wall thinning in inferior LV wall are also apparent (black arrows). LGE-CMR images show diffuse LGE in RV wall and in inferior LV wall (white arrows). A sub-endocardial biopsy demonstrates fatty infiltration in RV myocardium (Circle, H-E stain, 100×). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

The diagnosis of ARVC/D is challenging due to heterogeneous clinical presentation and non-specific ECG findings<sup>[77,78]</sup>. The diagnosis is currently made on the presence of major and minor Task Force criteria that include structural, functional, histological, electrocardiographic, arrhythmic, and genetic factors<sup>[79]</sup>. Endomyocardial biopsy is considerably unreliable for the diagnosis of ARVC/D, because the patchy distribution of the fibro-fatty change may cause sampling error.

CMR can visualize RV wall better than echocardiography. Functional abnormalities in cine-CMR include regional wall motion defects, focal aneurysms, global RV dilation and dysfunction<sup>[1,21]</sup>. In addition, the diagnosis could be supported by the presence of fatty infiltration of RV free wall that can be suppressed in fat suppression sequences<sup>[2,21]</sup>. LGE imaging has been shown to provide additional evidence of fibrosis which often co-exists in the fat-infiltrated RV myocardium (Figure 7).

Despite the limitations of thin RV wall and small volume of affected myocardium, CMR frequently identifies individuals with early disease, in whom Task Force criteria are relatively insensitive<sup>[21]</sup>. The presence of LGE can also predict inducible VTs on electrophysiological studies<sup>[80]</sup>.

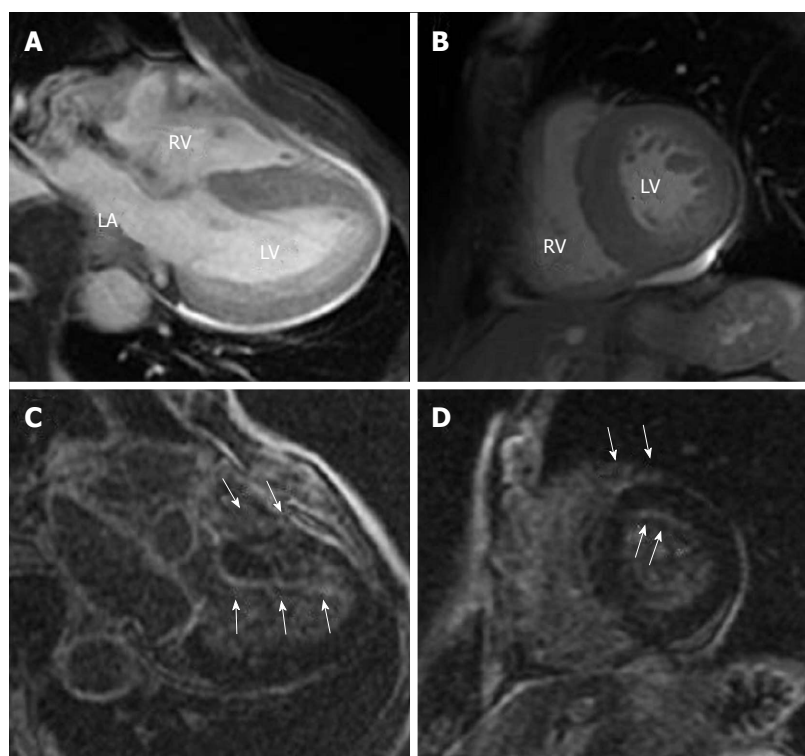
### Cardiac amyloidosis

Cardiac involvement has been described in most forms

of amyloidosis, but is most common and clinically significant in type AL amyloidosis (primary amyloidosis)<sup>[81]</sup>. Cardiac amyloidosis is a common cause of restrictive cardiomyopathy, and reduced ventricular wall compliance leads to impairment of diastolic filling and diastolic heart failure even when systolic function was preserved. At a histological level, amyloidosis is evident by extra-cellular deposition of insoluble fibrillar proteinaceous material (amyloid fibrils) in various cardiac tissues including valve leaflets and coronary vessels.

On cine-CMR, diffuse myocardial hypertrophy including both ventricles and atria is seen with thickened valve leaflets and pericardial effusion. The accumulation of amyloid fibrils in the myocardial interstitium also results in unique LGE appearances. In the early disease stage, a characteristic subendocardial enhancement of LV and RV, sparing the mid-wall of the inter-ventricular septum has been reported. However, as the accumulation of amyloid fibrils expands interstitial space, the volume of distribution of gadolinium increases. Therefore, there is usually a homogeneous pattern of enhancement, such that the signal from the myocardium cannot be adequately suppressed and differentiated from the adjacent blood pool. Actually, previous studies showed an atypically dark appearance of the blood pool, which reflects the similar myocardial and blood T1 values attributable to high myocardial uptake and fast blood pool washout (Figure 8)<sup>[1,2,82]</sup>.





**Figure 8** Representative cine-cardiac magnetic resonance (A, B) and Late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 76-year-old male patient with AL amyloidosis (IgA type multiple myeloma, Bence-Jones protein positive). The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal diffuse hypertrophy in LV and RV wall. LGE-CMR images show a characteristic subendocardial enhancement of the LV and RV with an atypically dark appearance of the blood pool (white arrows). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

A recent study has also demonstrated a potential of remarkable prolongation of non-contrast (native) T1 in AL amyloidosis<sup>[83]</sup>.

A positive CMR finding, that is biventricular hypertrophy, characteristic LGE distribution, and pericardial effusion, is associated with poor outcomes (heart failure and death) in patients with AL amyloidosis<sup>[82,84]</sup>.

### Myocarditis

Myocarditis is most commonly caused by a viral infection resulting in myocardial inflammation and immune-mediated damage in cardiomyocytes. Acute myocarditis causes chest pain, ST-T changes and elevated cardiac enzymes, which are sometimes difficult to be differentiated from ACS, and is occasionally complicated by fulminant heart failure and SCD<sup>[85]</sup>. Chronic myocarditis is one of the common causes of NICM, and sometimes misdiagnosed as DCM<sup>[1]</sup>.

The most characteristic features in CMR are the presence of myocardial edema, diffuse wall motion abnormalities, subepicardial patchy myocardial LGE, and the concomitant involvement of the pericardium<sup>[86,87]</sup>. Edema imaging using T2 black blood sequences plays an important role in the evaluation of patients with suspected myocarditis<sup>[20,61]</sup>. Edema should be verified by a quantitative signal intensity analysis, best by calculating the ratio between myocardium and skeletal muscle. Early gadolinium enhancement and prolonged native T1 are also indicative of myocardial edema<sup>[20,88]</sup>. On LGE-CMR, the subepicardial layer especially in postero-lateral wall has LGE, and in severe cases, LGE may be more diffuse and circumferential<sup>[89]</sup>.

### Anderson-fabry disease

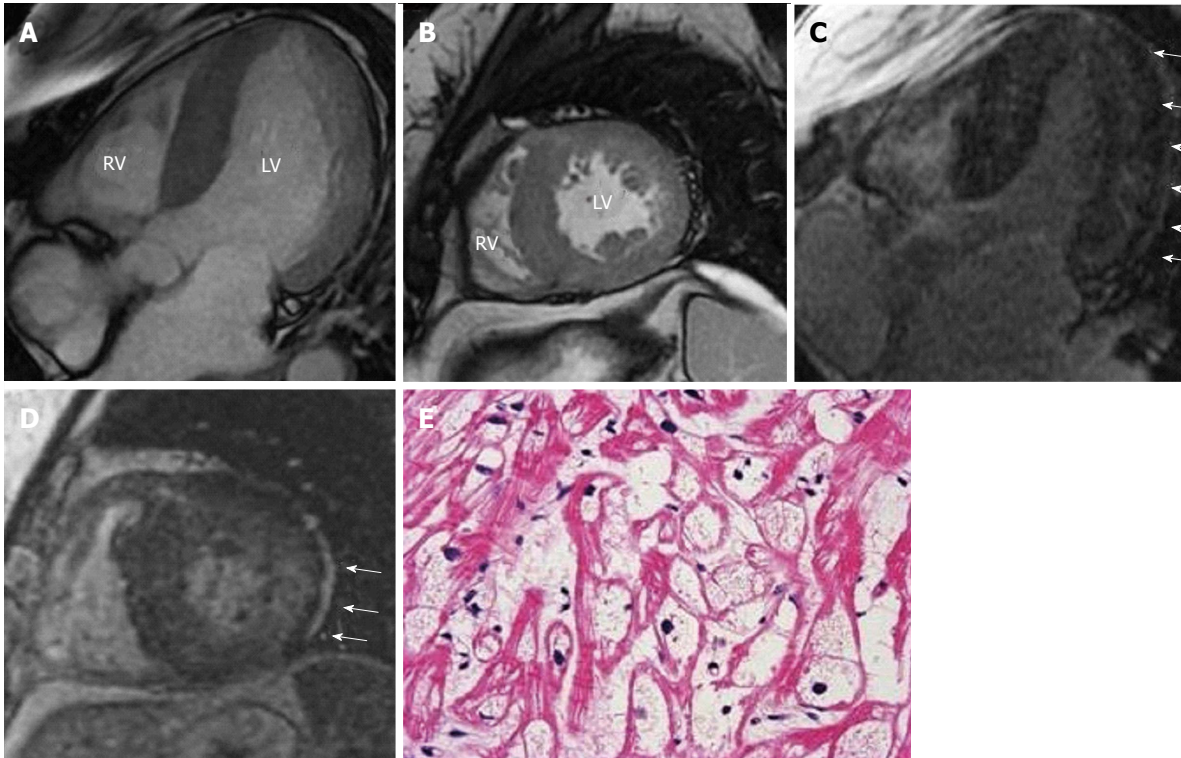
Anderson-fabry disease (AFD) is an X-linked lysosomal

storage disorder caused by the partial or complete deficiency of  $\alpha$ -galactosidase A. The enzymatic deficit results in progressive intracellular accumulation of excess cellular glycosphingolipid substrate in multiple organs<sup>[90]</sup>. Cardiac involvement in AFD is frequent, and the myocardial accumulation of glycosphingolipids acts as a trigger leading to myocardial cell hypertrophy and interstitial fibrosis. Hence, most patients present LV hypertrophy, and often exhibit conduction defects, supra-ventricular and ventricular arrhythmias, and heart failure symptoms associated with progressive LV dysfunction<sup>[91,92]</sup>. The presence and extent of cardiac damage increase progressively with age. Enzyme replacement therapy with recombinant  $\alpha$ -galactosidase A clears microvascular deposits of glycosphingolipids, and several recent studies have shown a reduction in LV hypertrophy and improvement in systolic function after treatment<sup>[93,94]</sup>.

Therefore, differentiating AFD from other causes of LV hypertrophy is critical but is usually difficult on common imaging modalities including echocardiography. A binary endocardial appearance, initially expected as a highly sensitive and specific finding in AFD, was later ascertained to be insufficient for a screening tool<sup>[95,96]</sup>.

Instead, CMR has become a promising tool to diagnose cardiac involvement of AFD. Cine-CMR can exhibit a symmetrical and non-obstructive LV hypertrophy, and LGE-CMR can demonstrate a particular LGE distribution to the infero-lateral wall of mid to basal LV and to mid-myocardial layer (Figure 9)<sup>[92,97]</sup>. Furthermore, a recent non-contrast T1 mapping technique has potential to detect early cardiac involvement of AFD by showing T1 shortening<sup>[98]</sup>. Thus, AFD should always be considered if unexplained LV hypertrophy is seen, particularly in a young patient with family history.





**Figure 9** Representative cine- cardiac magnetic resonance (A, B) and late gadolinium enhancement-cardiac magnetic resonance (C, D) images in a 46-year-old female patient with Anderson-fabry disease. The images show horizontal axis (4-chambers) (A, C) and mid-ventricular short axis (B, D) views. Cine-CMR images reveal diffuse hypertrophy of LV wall. LGE-CMR images show a particular LGE distribution pattern to the infero-lateral mid to basal segments and to mid-myocardial layer (white arrows). E: A sub-endocardial biopsy from RV wall demonstrates interstitial fibrosis and cardiomyocyte hypertrophy with cytoplasmic vacuolization (H-E stain, 40×). LGE: Late gadolinium enhancement; LV: Left ventricular; LGE-CMR: Late gadolinium enhancement-cardiac magnetic resonance; RV: Right ventricular.

### Endomyocardial fibrosis

Endomyocardial fibrosis (EMF) is the most frequent restrictive cardiomyopathy especially affecting poor children and young adults in the tropical zone. The characteristic features are fibrotic tissue deposition in the endocardium of the inflow tract and apex of one or both ventricles. The pathogenesis of EMF is poorly understood, but early hyper eosinophilia may play a role<sup>[99]</sup>.

Cine-CMR can clearly demonstrate distorted ventricles with normal or reduced volume and enlarged atria. LGE-CMR can also show areas of LGE in the endocardium where the histopathological examination revealed extensive fibrous thickening, proliferation of small vessels and scarce inflammatory infiltrate. The LGE pattern may have a “V sign” at the ventricular apex, characterized by a 3-layer appearance of myocardium, thickened fibrotic endocardium, and overlying thrombus<sup>[100]</sup>. The relationships between increased LGE burden and worse NYHA functional classes, and increased probability of surgery and mortality rate are reported<sup>[100]</sup>.

Since the reports of EMF have been increasing in areas where the disease had not been previously recognized, the role of CMR may increase for the early diagnosis of EMF<sup>[101]</sup>.

### Systemic sclerosis

Systemic sclerosis (SSc) is characterized by vascular changes and fibrosis of the skin and internal organs.

Among many autoimmune disorders, SSc has been considered to have a high prevalence of cardiac involvement. The prevalence is clinically 1.4% to 5.4% for systolic or 18% to 30% for diastolic dysfunction<sup>[102,103]</sup>. While in autopsy, myocardial fibrosis was identified in 50% to 80%<sup>[104]</sup>. Cardiac involvement in SSc is assumed to be derived from impairment of the microcirculation and primary myocardial fibrosis, and from ischemic damage due to coronary atherosclerosis<sup>[105,106]</sup>. Patients with cardiac involvement have a poor prognosis because of congestive heart failure and fatal arrhythmias associated with conduction disturbance<sup>[107]</sup>. Unfortunately, most patients with cardiac involvement are asymptomatic and difficult to be detected in subclinical stage.

Recently, the values of CMR are suggested for the early detection of cardiac involvement in SSc. Actually, previous reports revealed LGE in 21% to 66% of patients with SSc<sup>[108-110]</sup>. LGE distributed mainly into the basal to mid inter-ventricular septum and RV insertion points, and spread into all the myocardial layers, reflecting various mechanisms for myocardial fibrosis.

We showed the correlations between LGE and enlargement of LV/RV volume, impaired LV/RV function and pulmonary arterial hypertension. The ability of LGE-CMR to detect cardiac fibrosis in the subclinical stage may help identification of high risk patients and early initiation of therapeutic interventions, although the relevance in long term prognosis remains to be elucidated.

**Table 1** Distribution and patterns of late gadolinium enhancement and other cardiac magnetic resonance findings in various types of cardiomyopathies

Cardiomyopathies		LGE distribution		LGE patterns	Other CMR features
		Intra-cardiac	Intra-myocardium		
Ischemic		LV regions corresponding to coronary distribution	Subendocardium to transmural	Striated, transmural	LV wall motion abnormality, wall thinning, aneurysm
Non-ischemic	Dilated cardiomyopathy	Inter-ventricular septum	Mid-wall	Striated	Diffuse LV wall thinning, shortened post-contrast T1
	Hypertrophic cardiomyopathy	Regions with hypertrophy	Any	Patchy, striated	Asymmetrical or symmetrical LV hypertrophy
	End-stage HCM	Diffuse	Any	Patchy, striated, transmural	LV dilatation with Inhomogenous LV wall thickening
	Cardiac sarcoidosis	Any	Any	Patchy, striated, transmural	LV aneurysm, myocardial edema in T2 black blood sequences
	Stress cardiomyopathy	Regions with ballooning	Any	Patchy, transient	Myocardial ballooning, RV motion abnormality
	Arrhythmogenic right ventricular cardio-myopathy/dysplasia	RV (sometimes with LV)	Any	Striated	Focal or global RV dilatation, fatty infiltration in fat suppression sequence
	Cardiac amyloidosis	Any	Subendocardial, transmural	Diffuse	Diffuse hypertrophy, thickened valve leaflets, prolonged native T1
	Myocarditis	Any	Subepicardial	Diffuse	Myocardial edema in T2 black blood sequences, early GE, prolonged native T1
	Anderson-Fabry disease	Postero-lateral LV	Mid-wall	Striated	Concentric/eccentric but non-obstructive LV hypertrophy, shortened native T1
	Endomyocardial fibrosis	Inflow tract to apex	Subendocardial	Diffuse	Distorted ventricles with normal or reduced volume and enlarged atria
	Cardiac involvement in SSc	Any	Any	Any	
	LV non-compaction	NA	NA	NA	High non-compacted/compacted myocardial ratio
	Iron-overload cardio-myopathy (cardiac hemochromatosis)	NA	NA	NA	Shortened T2-star

LV: Left ventricular; LGE: Late gadolinium enhancement; CMR: Cardiac magnetic resonance; RV: Right ventricular; NA: No available information; ICM: Ischemic; NICM: Non-ischemic; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy.

Table 1 summarizes the typical distribution and patterns of LGE, and other characteristic CMR features in ICM and NICM. In addition to above mentioned NICM, cine-CMR can show clearer images in terms of the presence of apical trabeculations, deep inter-trabecular recesses and high non-compacted/compacted myocardial ratio in patients with LV non-compaction<sup>[11]</sup>. In addition, a T2\* technique allows to estimate iron deposition in myocardium, and to correlate it with cardiac function and the effect of chelation in iron overload cardiomyopathy (cardiac hemochromatosis)<sup>[19]</sup>.

## LIMITATION OF CMR

Despite the benefits with much evidence, CMR is not necessarily available in all institutes and patients, and has a problem of cost. Claustrophobia is a frequent reason to cancel MRI. Patients with decompensated heart failure cannot be tolerant to long data acquisition time of MRI. MRI is still contraindicated in patients who have had device implantation (*e.g.*, permanent pacemaker, implantable cardioverter defibrillator or cardiac resynchronization therapy with and without defibrillation). Furthermore, gadolinium contrast agents cannot be administered to

patients with chronic renal failure because of the risk of nephrogenic systemic fibrosis. The determination of threshold and quantification of LGE are also limitations in NICM.

## CONCLUSION

Currently, CMR has become one of the most important methods to diagnose and follow-up patients with ICM and NICM. This review showed that the analysis of LGE distribution in myocardium is particularly valuable for differential diagnosis and risk stratification. However, the differential diagnosis of cardiomyopathies should be made generally on the basis of combination of various CMR sequences and with other imaging modalities and endomyocardial biopsy.

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## Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment

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**Key words:** Cardiomyopathy; Catecholamine; Heart failure; Myocardial Infarction; Stress

**Core tip:** Takotsubo cardiomyopathy (TCM) is an important disease entity that differs from acute myocardial infarction. It occurs more often in postmenopausal elderly women, is characterized by a transient hypokinesis of the left ventricular (LV) apex, and is associated with emotional or physical stress. Wall motion abnormality of the LV apex is generally transient and resolves within a few days to several weeks. The prognosis of TCM is generally good. It has been suggested that coronary spasm, coronary microvascular dysfunction, catecholamine toxicity and myocarditis might contribute to the pathogenesis of TCM. However, its pathophysiology is not clearly understood.

### Abstract

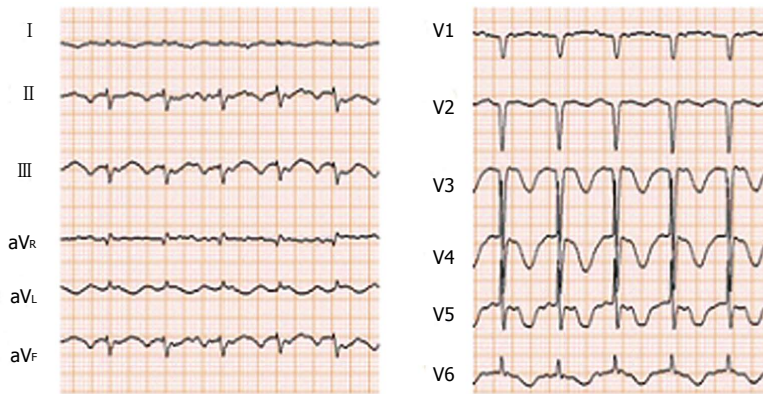
In 1990, takotsubo cardiomyopathy (TCM) was first discovered and reported by a Japanese cardiovascular specialist. Since then, this heart disease has gained worldwide acceptance as an independent disease entity. TCM is an important entity that differs from acute myocardial infarction. It occurs more often in postmenopausal elderly women, is characterized by a transient hypokinesis of the left ventricular (LV) apex, and is associated with emotional or physical stress. Wall motion abnormality of the LV apex is generally transient and resolves within a few days to several weeks. Its prognosis is generally good. However, there are some reports of serious TCM complications, including hypotension, heart failure, ventricular rupture, thrombosis involving the LV apex, and torsade de pointes. It has been suggested that coronary spasm, coronary microvascular dysfunction, catecholamine toxicity and myocarditis might contribute to the pathogenesis of TCM. However, its pathophysiology is not clearly understood.

Komamura K, Fukui M, Iwasaku T, Hirotani S, Masuyama T. Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment. *World J Cardiol* 2014; 6(7): 602-609 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/602.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.602>

### INTRODUCTION

Takotsubo cardiomyopathy (TCM) is a transient wall motion abnormality of the left ventricular (LV) apex accompanied with emotional or physical stress that usually resolves completely. Takotsubo is a Japanese word meaning a pot with a narrow neck and a round bottom used to catch octopuses. Left ventriculography during systole of patients with TCM demonstrates such a shape. Although TCM is a novel concept, the number of cases reported is increasing rapidly. Other words have been used to refer this cardiomyopathy, including stress-related cardiomyopathy<sup>[1]</sup>, transient LV apical ballooning syndrome<sup>[2,3]</sup>, broken heart (heartbreak) syndrome, and ampulla cardiomyopathy<sup>[4]</sup>. In





**Figure 1** Inverted T waves are found in the limb and precordial leads, which is a common characteristic of takotsubo cardiomyopathy with apex balloon-like dilation.

2006, the American Heart Association incorporated this disease under the class of acquired cardiomyopathies<sup>[5]</sup>. This article aimed to review this newly recognized cardiomyopathy, paying particular attention to clinical characteristics, pathophysiology, diagnosis, and treatment.

## EPIDEMIOLOGY

TCM symptoms were considered extremely rare until the past 20 years. The increasing number of medical reports on these symptoms has highlighted the higher incidence of TCM than that previously reported. Currently, 1000 or more studies reporting cases of TCM have been published. According to a retrospective review, patients with TCM accounted for approximately 2% of all the patients with suspected acute coronary syndrome<sup>[6,7]</sup>. Further, 90% of these patients were postmenopausal women<sup>[8,9]</sup>. A few reports indicated that the average age of TCM patients was 68 years, although children or young adults may also be affected<sup>[10,11]</sup>. Another report indicated that most men with TCM were inpatients, which suggests that physical stresses might play a role for the progress of the disease<sup>[12]</sup>. In a recent study, demographic and clinical course data in patients with TCM were compared between the United States and Japan. Few Japanese patients with TCM had a history of overt coronary disease (CAD) and family history of early-onset CAD. However, there was no significant difference in long-term prognosis and the recurrence rate between the United States and Japanese patients with TCM<sup>[13]</sup>.

## DIAGNOSIS

The diagnosis of TCM remains controversial. The diagnostic criteria most widely accepted were published by the Mayo Clinic<sup>[14]</sup> in 2004. In 2008, a new criterion was added to them: a normal epicardial coronary artery (Table 1)<sup>[15]</sup>. Kawai *et al.*<sup>[16]</sup> classified this disease as a syndrome of unknown etiology that was characterized by acute balloon-like dilation in the LV apex (Table 2). As shown by these two diagnostic criteria, the patients with TCM have nonspecific or normal findings on physical examination; however, the clinical course resembles that of acute coronary syndrome or acute decompensated heart fail-

ure<sup>[14-16]</sup>. The most common presenting symptoms listed in the diagnostic criteria are chest pain and dyspnea. In rare cases, patients developed palpitations, nausea, vomiting, syncope, or cardiogenic shock<sup>[14-16]</sup>.

The following six symptoms are especially indicative of TCM: (1) acute onset and stressful inducement: One of the unique features of TCM is its relation with stressful emotional or physical events. This characteristic was described in nearly two-thirds of the patients who developed TCM<sup>[17]</sup>. Unlike acute coronary syndrome, with an onset peak early in the morning, TCM presents in the afternoon in most cases when stressful inducible events are likely to occur; (2) electrocardiographic characteristics: Although the initial electrocardiogram (ECG) of patients with TCM is nonspecific, an ST segment elevation can be found mainly in the precordial leads in 50% of patients at onset<sup>[18,19]</sup>. In addition, reciprocal ST-segment depression in the inferior wall leads is unlikely<sup>[20]</sup>. In comparison with patients with base deformity, inverted T waves are more frequently observed in patients with apex balloon-like dilation<sup>[21]</sup> and they resolve spontaneously within a few weeks to several months (Figure 1). Furthermore, patients with TCM usually present abnormal Q waves in precordial leads. These Q waves are transient in most patients and generally resolve within a few days to several weeks<sup>[22]</sup>; (3) cardiac enzymes: In most patients with TCM, there is slight elevation in the cardiac enzyme level on admission<sup>[6,20]</sup>. The enzyme levels decrease rapidly and do not seem to have prognostic significance<sup>[22]</sup>; (4) absence of coronary lesion: It is characteristic that no specific coronary lesions are detected in TCM<sup>[23,24]</sup>. Generally, patients with TCM have chest pain, changes in ECG, elevation of cardiac enzyme levels, and wall motion abnormalities. Therefore, coronary angiography has to be conducted to rule out acute coronary syndrome; (5) balloon-like dilation of the ventricle: In contrast with acute myocardial infarction, LV wall motion abnormalities are found beyond a single coronary artery perfusion area in patients with TCM. Most patients with TCM show loss of motion or hypokinesia at the apex and an apical balloon-like dilation pattern associated with preservation of the base (Figure 2). However, cases of a TCM subtype without abnormalities of the apex were reported recently<sup>[25,26]</sup>. TCM is essentially characterized by LV failure,

**Table 1** Diagnostic criteria of the Mayo Clinic

Suspicion of AMI based on precordial pain and ST elevation observed on the acute-phase ECG
Transient hypokinesia or akinesia of the middle and apical regions of the LV and functional hyperkinesia of the basal region, observed on ventriculography or echocardiography
Normal coronary arteries confirmed by arteriography (luminal narrowing of less than 50% in all the coronary arteries) in the first 24 h after the onset of symptoms
Absence of recent significant head injury, intracranial hemorrhage, suspicion of pheochromocytoma, myocarditis, or hypertrophic cardiomyopathy

AMI: Acute myocardial infarction; ECG: Electrocardiogram; LV: Left ventricular.

although, approximately, one-third of patients also have abnormalities in the right ventricle<sup>[27]</sup>. Cardiac magnetic resonance imaging (MRI) is a suitable method to establish the diagnosis of TCM because this modality allows the accurate identification of reversible myocardium damage by visualization of wall motion abnormalities in each area, quantification of ventricular function, and assessment of inflammation and fibrosis. This modality brings new insight into the pathophysiology of TCM. It could enable early treatment of acute symptoms, raise awareness, and improve clinical outcomes. Cardiac MRI is appropriate to evaluate wall motion abnormalities and LV ejection fraction, and to confirm the absence of delayed gadolinium enhancement in patients with TCM. This allows differentiation of TCM from myocardial infarction and myocarditis, both pathologies associated with delayed gadolinium enhancement<sup>[17]</sup>. Although coronary computed tomography angiography is not applicable to the first diagnosis of patients with TCM, there are many reports on its use for clinical course evaluation after TCM onset; (6) recovery of cardiac function: One of the characteristics of TCM is that thorough recovery of cardiac function is achieved. In contrast to other serious wall motion abnormalities at onset, recovery of ventricular function is proven in follow-up evaluations. Most patients with TCM show significant improvement of systolic function within a week and achieve complete recovery by the end of third or fourth week after onset. Generally, another diagnosis should be considered in patients with suspected TCM whose systolic function is not normalized within 12 wk after onset.

The differential diagnosis of TCM includes the following: esophageal spasm, gastroesophageal reflux disease, myocardial infarction, myocardial ischemia, unstable angina, acute coronary syndrome, angina, aortic dissection, myocarditis, acute pericarditis, pneumothorax, cardiogenic pulmonary edema, pulmonary embolism, Boerhaave syndrome (spontaneous esophageal rupture), cardiac tamponade, cardiogenic shock, cocaine-induced cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, and coronary artery spasm.

## PATHOPHYSIOLOGY

The exact pathogenesis of TCM is unknown, but various

**Table 2** Diagnostic criteria of Kawai *et al.*<sup>[16]</sup>

Exclusion criteria
Significant organic stenosis or spasm of a coronary artery. In particular, AMI due to a lesion of the anterior descending artery of the left coronary artery, which irrigates a large territory including the apex of the LV (urgent coronary angiography is desirable in order to view the image in the acute phase; during the chronic phase, coronary angiography is necessary to confirm the presence or absence of significant stenotic lesions or abnormal lesions that could explain the ventricular contraction)
Cerebrovascular disturbances
Pheochromocytoma
Viral or idiopathic myocarditis
(Note: Coronary angiography is required for the exclusion of coronary artery lesions. Takotsubo-like myocardial dysfunction can occur in conditions such as cerebrovascular disorders or pheochromocytoma)
Diagnostic references
Symptoms: Precordial pain and dyspnea similar to the findings in the acute coronary syndrome. TCM can also occur without symptoms
Triggers: Emotional or physical stress, although it can also occur without any obvious trigger
Age and gender: There is a recognized tendency to a higher frequency in elderly individuals, principally women
Ventricular morphology: Apical ballooning with rapid recovery on ventriculography and echocardiography
ECG: ST elevation may be observed immediately after the event. T waves progressively become negative in various leads and the QT interval progressively lengthens. These changes gradually improve, but the T waves may remain negative for months. Pathological Q waves and alterations of the QRS voltage may be observed in the acute phase
Cardiac biomarkers: There is only a slight rise in the cardiac enzymes and troponin
Nuclear medicine scan of the heart: Abnormalities may be detected on myocardial gamma scan in some cases
Prognosis: Recovery is rapid in most cases, but some patients develop acute pulmonary edema and other sequel, even death

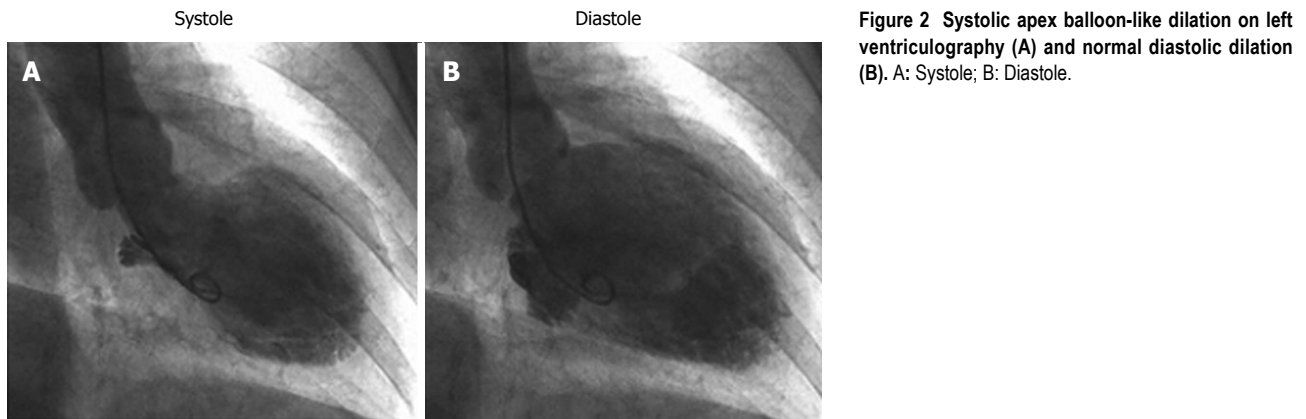
AMI: Acute myocardial infarction; ECG: Electrocardiogram; LV: Left ventricular; TCM: Takotsubo cardiomyopathy.

hypotheses have been suggested and discussed, including coronary microvascular dysfunction, coronary artery spasm, catecholamine-induced myocardial stunning, reperfusion injury following acute coronary syndrome, myocardial microinfarction and abnormalities in cardiac fatty acid metabolism. Currently, catecholamine-induced cardiotoxicity and microvasculature dysfunction are the most supported theories.

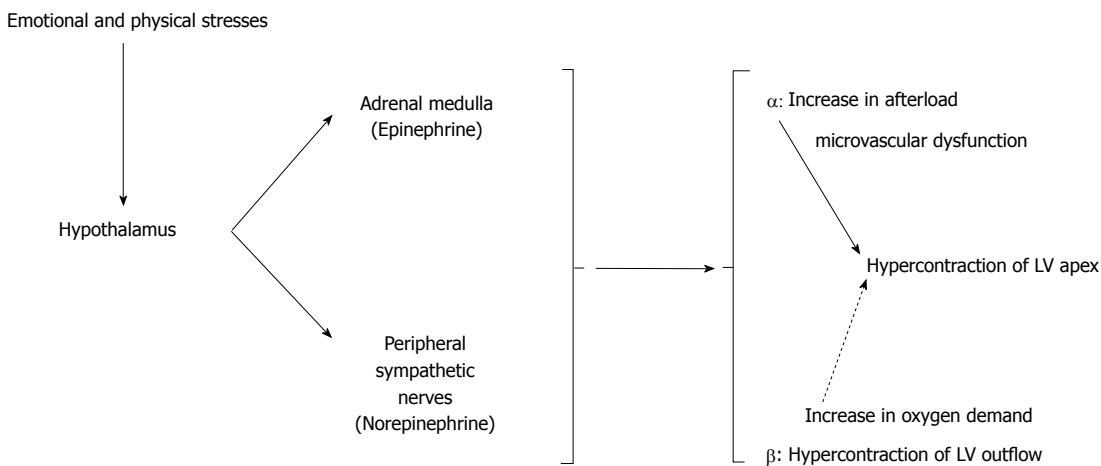
### Catecholamine theory (Figure 3)

Wittstein *et al.*<sup>[22]</sup> found that the serum catecholamine concentration was two to three times greater in patients with TCM than that in patients with myocardial infarction, and described that serious emotional stress is a precipitating factor. It has been reported that exogenously administered catecholamines and pheochromocytoma cause typical characteristics of TCM, which supports this theory further<sup>[28,29]</sup>.

Lyon *et al.*<sup>[30]</sup> advocated a theory called “stimulus trafficking” that could explain the decline of myocyte contractile function in patients with TCM. Supraphysiological levels of catecholamines induce  $\beta_2$ -coupling from Gs to Gi. Therefore, the decline of myocyte contractile func-



**Figure 2** Systolic apex balloon-like dilation on left ventriculography (A) and normal diastolic dilation (B). A: Systole; B: Diastole.



**Figure 3** The catecholamine theory of takotsubo cardiomyopathy. LV: Left ventricular.

tion is evidenced by hypokinesia in ECG. Involvement of the apex can be attributed to higher adrenoceptor density in the apex than in the base<sup>[31]</sup>. The rationale of stimulus trafficking is that a switch to Gi occurs to protect the myocytes from the strong stimulation of Gs, which causes apoptosis. Slow increases in serum troponin level explain early minimal necrosis of the myocardial tissue. Nef *et al*<sup>[32]</sup> showed increased activity of the phosphatidylinositol 3-kinase-protein kinase B (PI3K/AKT) signaling pathway, which has important anti-apoptosis functions and plays a role in the rapid recovery of myocytes. Thus, the transient LV dysfunction can be attributed to the PI3K/AKT pathway and inversely switching from Gi to Gs, associated with the homogeneous, prompt and clinically thorough recovery of systolic function observed in TCM.

Patients with TCM consistently present microvasculature dysfunction findings<sup>[33]</sup>. The characteristics of microvasculature dysfunction after acute psychological stress in patients with TCM include abnormality of endothelium-dependent vasodilation, excessive vasoconstriction, and impairment of myocardial perfusion<sup>[34]</sup>. Uchida *et al*<sup>[35]</sup> reported that extensive endothelial cell apoptosis was observed by myocardial biopsy. According to another report, increased susceptibility to ergonovine or acetylcholine followed by large vessel spasm, similar to vasospastic angina, may contribute to transient LV dysfunction<sup>[36]</sup>.

However, because only 30% of patients showed the characteristics of vasospasm in a challenge test, this theory was ruled out<sup>[37,38]</sup>. Afonso *et al*<sup>[39]</sup> demonstrated that circulatory disturbance, indicating coronary microvascular dysfunction was found on a myocardial contrast echocardiography and the epicardial coronary arteries were normal.

Myocardial biopsy of patients with TCM showed regions with contraction band necrosis, inflammatory cell infiltration, and localized fibrosis<sup>[40]</sup>. These changes were caused by direct catecholamine toxicity on cardiac muscle cells<sup>[41]</sup>. Morel *et al*<sup>[42]</sup> found that C-reactive protein levels and white blood cell counts increased with the increase in norepinephrine levels in patients with TCM and inferred that catecholamines produced more systemic inflammation *via* the induction of proinflammatory cytokines such as tumor necrosis factor- $\alpha$  and interleukin-6. Several studies have pointed out that the remarkable myocardial edema, observed on cardiac MRI, occurs despite normal perfusion, which provides further evidence to support the inflammation theory<sup>[43,44]</sup>. Ueyama *et al*<sup>[45]</sup> examined restraint stress in rats with TCM and reported that heme oxygenase 1 (HO-1) levels, a marker of oxidative stress that has cardioprotective properties, was increased significantly. Macrophages play an important main role in oxidative stress induction and expression of  $\beta$ - and  $\alpha$ -adrenergic receptors. As a result of pretreatment with

$\beta$ - and  $\alpha$ -antagonists, HO-1 expression and its altering gene expression, decreased.

## RISK FACTORS

### Lack of estrogen

More than 90% of patients with TCM are postmenopausal women. In fact, in a study to investigate if hormone replacement therapy had an effect on TCM, the authors concluded that none of the 31 patients with TCM received estrogen replacement therapy<sup>[46]</sup>. Moreover, Ueyama *et al.*<sup>[47]</sup> demonstrated that the decrease in LV function was greater in ovariectomized rats subjected to restraint stress than in rats receiving estradiol supplementation. The myocytes are known to express estrogen receptor- $\alpha$  and estrogen receptor- $\beta$ . According to Ueyama *et al.*<sup>[47]</sup>, estrogen enhanced transcription of cardioprotective factors such as heat shock protein and atrial natriuretic peptide, and in turn, protected against the toxic effects of catecholamines, calcium overload and reduced oxidative stress<sup>[48]</sup>.

### Emotional or physical stress inducers

A study reported on the prevalence of mood disorders and use of antidepressants in patients with TCM<sup>[28]</sup>. When patients with depressive disorders experienced a stressful event, vagus nerve tension was decreased and response to adrenal medullary hormone was increased, which may be relevant to the cause of the disease<sup>[49]</sup>. Further, some patients with depression showed very high noradrenaline extravasation<sup>[50]</sup>.

### Genetic factors

Certain polymorphisms of  $\alpha$ - and  $\beta$ -adrenergic receptors are associated with neurogenic stunned myocardium that occurs as symptom of subarachnoid hemorrhage and has overlapping pathophysiology with TCM<sup>[51]</sup>. Although adrenoceptor polymorphisms have not yet been identified in patients with TCM, patients with this disease showed L41Q polymorphism of G protein coupled receptor kinase (GRK5) more frequently compared with the control group<sup>[52]</sup>. L41Q polymorphism of GRK5 responds to catecholamine stimulation and attenuates the response of  $\beta$ -adrenergic receptors. Under catecholamine stimulation, balloon dilation of the ventricle may occur either by negative inotropic effect by  $\beta$ -receptor decoupling or ischemia because of an imbalance between  $\alpha$ 1-adrenergic coronary artery vasoconstriction and  $\beta$ -adrenergic vasodilation. These reports suggest the very interesting possibility that the susceptibility to TCM in individuals may be partially related to genetic factors.

## TREATMENT

Treatment of TCM during the acute phase is mainly symptomatic treatment. Intra-aortic balloon pump equipment is required for hemodynamically unstable patients in addition to cardiopulmonary circulatory support and continuous veno-venous hemofiltration<sup>[53-55]</sup>. There is

controversy on the use of cardiac stimulants because of increased circulating catecholamines<sup>[56]</sup>. However, cardiac stimulants are used in 20%-40% of patients with TCM<sup>[2,57]</sup>. Levosimendan may be beneficial because of its inotropic action and vasodilator effect<sup>[30,58]</sup>. Usage of anticoagulants may be considered at least until systolic function is recovered.

For patients with severe LV outflow tract obstruction with hemodynamic compromise, treatment with a  $\beta$ -blocker or  $\alpha$ -adrenoceptor agonist such as phenylephrine and volume expansion should be considered. Calcium channel blockers can be used to decrease LV outflow tract pressure gradient. It is of utmost importance to avoid treatment with nitrites or inotropic drugs in these cases<sup>[59-63]</sup>. For patients with suspected vasospasm, the use of calcium channel blockers such as verapamil or diltiazem is suggested<sup>[64]</sup>.

Hemodynamically stable patients are often treated with diuretics, angiotensin-converting enzyme (ACE) inhibitors and  $\beta$ -blockers. To reduce the risk of thromboembolism, patients with loss of motion of the LV apex should be treated with anticoagulant therapy until the contractility of the apex is improved unless there is a definite contraindication.

There is no consensus regarding long-term management of TCM, although it is reasonable to treat patients with  $\beta$ -blockers and ACE inhibitors during the ventricular recovery period. However, no data support the continuous use of these drugs for the prevention of TCM recurrence or improvement of survival rate. After LV function normalizes, physicians may consider discontinuation of these drugs.

## PROGNOSIS AND RECURRENCE

Patients with TCM usually have a good prognosis, and almost perfect recovery is observed in 96% of the cases<sup>[65]</sup>. Mortality rate in hospital vary at one to two percent<sup>[18,66]</sup>. TCM was formerly thought to follow a relatively benign course. However, Sharkey *et al.*<sup>[18]</sup> described that approximately 5% of TCM patients experienced cardiac arrest. While their long-term survival rate is the same as that in healthy subjects, patients with TCM have a greater risk of death at the time of initial onset<sup>[65]</sup>. Elesber *et al.*<sup>[65]</sup> reported that the most frequent chief complaint was chest pain (30%) and that recurrence of the symptom occurred in 11% of patients with TCM after a 4-year follow-up. Some studies have been conducted to assess prognostic indicators such as ECG findings, signs of thrombolysis in myocardial infarction, grade of myocardial perfusion, and N-terminal pro-brain natriuretic peptide level. However, a definite outcome marker has not been established<sup>[66-68]</sup>.

## CONCLUSION

A lot of attention has been focused on TCM recently and this entity has been characterized as a transient LV dysfunction with rapid recovery generally induced by a stressful emotional or physical event. The number of



TCM cases continues to increase. Because of close resemblance of its presentation and clinical course to acute myocardial infarction, we believe that TCM should be included in one of the differential diagnosis for acute myocardial infarction. Although the cause of this disease has not been completely understood to date, some promising hypotheses have been suggested. The occurrence of this disease is attributed to the large-scale production of catecholamines that causes myocardial hypokinesia *via* direct cardiomyocyte toxicity and induction of coronary microvascular dysfunction. Further, the high prevalence of TCM in postmenopausal women suggests an important role of estrogen for myocardial protection. Another hypothesis includes oxidative/inflammatory stress-induced myocardial dysfunction. Although the treatment of TCM remains controversial, adrenergic blockade is suggested as a reasonable therapy based on the presumptive pathophysiology of TCM.

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## WJC 6<sup>th</sup> Anniversary Special Issues (5): Myocardial infarction

# Stem cell mechanisms during left ventricular remodeling post-myocardial infarction: Repair and regeneration

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**Core tip:** Stem cell (SC)-based therapies hold promise to improve damaged myocardium repair and regeneration and thereby restore normal tissue function post-MI. In addition to the potential of SCs to regenerate myocardium, intrinsic properties of SCs such as their ability to home to areas of tissue damage make them an attractive tool for drug delivery. SCs, specifically mesenchymal stem cells, secrete multiple factors that can act in an autocrine and paracrine manner to regulate cell activation, recruitment, and survival during myocardium repair and regeneration.

## Abstract

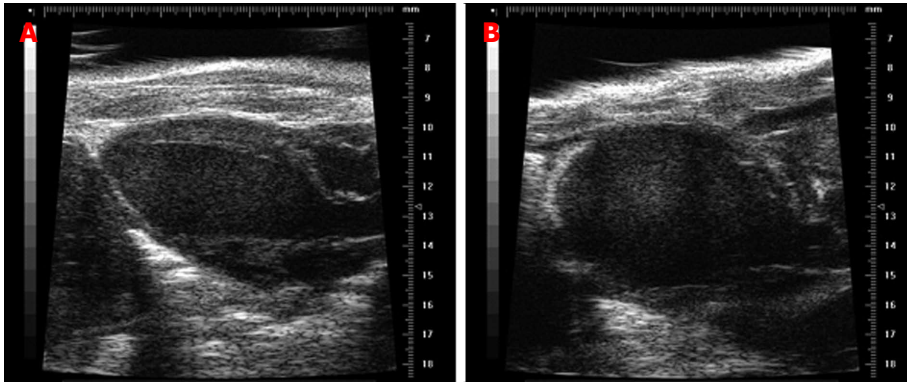
Post-myocardial infarction (MI), the left ventricle (LV) undergoes a series of events collectively referred to as remodeling. As a result, damaged myocardium is replaced with fibrotic tissue consequently leading to contractile dysfunction and ultimately heart failure. LV remodeling post-MI includes inflammatory, fibrotic, and neovascularization responses that involve regulated cell recruitment and function. Stem cells (SCs) have been transplanted post-MI for treatment of LV remodeling and shown to improve LV function by reduction in scar tissue formation in humans and animal models of MI. The promising results obtained from the application of SCs post-MI have sparked a massive effort to identify the optimal SC for regeneration of cardiomyocytes and the paradigm for clinical applications. Although SC transplantations are generally associated with new tissue formation, SCs also secrete cytokines, chemokines and growth factors that robustly regulate cell behavior in a paracrine fashion during the remodeling process. In this review, the different types of SCs used for cardiomyogenesis, markers of differentiation, paracrine factor secretion, and strategies for cell recruitment and

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## INTRODUCTION

In the United States alone, it is estimated that a myocardial infarction (MI) occurs every 35 s and approximately 20% of patients that experience a first-MI develop heart failure (HF) within 5 years<sup>[1]</sup>. An MI is consensually defined as the death of cardiomyocytes after a prolonged period of ischemia causing a progressive decline in cardiac function that ultimately results in HF<sup>[2]</sup>. Although the mortality associated with acute MI continues to decline as a result of revascularization, the morbidity and mortality





**Figure 1** During the course of left ventricular remodeling, (A) the normal elliptical shape of the left ventricular changes to spherical (B) as illustrated by the echocardiograms of the mouse permanent ligation myocardial infarction model. Image A was recorded at baseline and image B was recorded at day 7 post-myocardial infarction.

caused by HF is on the rise<sup>[3,4]</sup>.

Current post-MI pharmacological therapies such as ACE inhibitors and beta-blockers improve cardiac repair and slow down the progression to HF. However, the growing interest in stem cell (SC) therapies which not only promote repair but also hold promise to regenerate damaged myocardium has sparked a tremendous effort aimed at the development of an effective paradigm for ventricular remodeling post-MI. The possibility that SC therapies can restore cardiac function post-MI and increased evidence that the heart contains resident SCs niches has also contributed to this growing interest<sup>[5-7]</sup>.

Post-MI, the LV undergoes a remodeling process that results in the replacement of damaged myocardium with a collagen scar<sup>[8-10]</sup>. During the remodeling process, the normal elliptical shape of the LV (Figure 1A) changes to spherical (Figure 1B) as illustrated by the echocardiogram of the murine heart following MI induced by permanent ligation of the left anterior descending coronary artery. Along with the architectural and structural changes, LV contractile function declines<sup>[10]</sup>.

The magnitude of LV contractile dysfunction is dependent on the extent of the infarct and the wound healing response that follows which includes cardiomyocyte death, inflammatory response, granulation tissue synthesis and granulation tissue maturation and remodeling. Historically, the use of stem cells has automatically been associated with direct replacement of dead cardiomyocytes; however, more recent research has indicated that stem cells possess intricate properties that can regulate other aspects of myocardium repair post-MI. In this review we will focus on the application of stem cells as a therapeutic tool for treatment of myocardial damage post-acute MI and discuss the role of stem cells during cardiac repair and regeneration.

## OVERVIEW OF STEM CELL ROLES IN REPAIR AND REGENERATION

SCs are sophisticated cells with multifunctional properties that can orchestrate the wound healing process post-MI

leading to restoration of normal tissue function (Figure 2). One of these properties is the ability to home to areas of injury which has led to the investigation of stem cells for targeted drug delivery<sup>[11-13]</sup>. Post-MI, SC transplantations have been shown to rescue apoptotic cardiomyocytes and give rise to mature cardiomyocytes through cell fusion<sup>[14,15]</sup>. In addition, multiple SC types have the capability of differentiating into functional cardiomyocytes which suggest that SCs can be used to replace necrotic or apoptotic cells post-MI. Further, SC transplantations have been shown to regulate the inflammatory response, reduce scarring, and promote angiogenesis through the paracrine effects, all of which lead to improved cardiac function in humans and animal models post-MI.

### Cell fusion

A major mechanism of action of SC transplantation post-MI that contributes to cardiac repair and regeneration is achieved through cell fusion. Using a combination of *in vitro* cell culture models and *in vivo* animal models of MI, fusion rates of SCs with injured cardiomyocytes were shown to significantly increase<sup>[14,15]</sup>. As a result, there was a decrease in cardiomyocyte apoptosis and an increase in the generation of mature cardiomyocytes<sup>[14-16]</sup>. Interestingly, inhibition of apoptosis was also achieved through paracrine effects using *in vitro* co-culture models through activation of the anti-apoptotic AKT/PKB pathway<sup>[15,16]</sup>.

### Replacement of dead cardiomyocytes

One of the primary goals of SC therapies post-MI is the replacement of dead cardiomyocytes. The current challenge in this regard is to identify the optimal SC for cardiomyocyte replacement. SCs are broadly classified based on their tissue of origin including embryonic *vs* adult, hematopoietic *vs* non-hematopoietic, and are further subcategorized by their differentiation potential. Stem cell differentiation potential is their ability to differentiate into specialized cells. By definition, a SC is not committed to one specific lineage and must therefore be given the appropriate differentiation signals if the paradigm calls for a cardiac progenitor or cardiomyocyte-differentiated cell.



**Figure 2 Stem cells possess multifunctional properties to promote damaged myocardium repair and regeneration post-myocardial infarction.** As illustrated by this model, stem cells have a tremendous ability to home to sites of injury, fuse with injured cells, inhibit cardiomyocyte apoptosis, replace dead cardiomyocytes, as well as secrete paracrine factors to regulate the inflammatory response, fibrosis, and neovascularization post-myocardial infarction. LV: Left ventricle; SC: Stem cell; MI: Myocardial infarction.

In Table 1, SCs that have been differentiated into a cardiogenic lineage and the methods of differentiation are listed.

Embryonic stem cells (ESCs) have been differentiated into cardiomyocytes *in vitro* and *in vivo*. Expression of transcription factors GATA-4, myocyte-specific enhancer factors (MEF) 1 and 2C, and Nkx2.5 are commonly used for assessment of cardiomyocyte differentiation. Other factors such as atrial natriuretic factor, myosin light chain (MLC)-2v, myosin heavy chain (MHC), and phospholamban have also been used<sup>[16-18]</sup>.

Human induced pluripotent stem (iPS) cells from various sources, including reprogrammed cardiac fibroblasts, have been differentiated into functional cardiomyocytes<sup>[19-23]</sup>. Early cardiac lineage differentiation markers include GATA-4, GATA6, Nkx2-5, the T-box 5 (Tbx5), insulin gene enhancer protein-1 (Isl1), and LIM homeodomain transcription factor<sup>[20]</sup>.

To date, the most commonly used SCs for cardiac tissue regeneration have been derived from adult bone marrow. In 2001, Orlic *et al.*<sup>[24]</sup> demonstrated that c-kit positive cells derived from bone marrow were able to generate de novo myocardium indicating that these cells might be ideal for treatment post-MI. Expansion of this study has demonstrated that bone marrow hematopoietic SCs give rise to cardiomyocytes through cell fusion rather than differentiation. Expression of  $\alpha$ -actin, cardiac troponin T, and connexin-43 has been used for cardiac lineage differentiation<sup>[25]</sup>. In addition to c-kit positive cells, the bone marrow contains fibroblast-like, mesenchymal stromal cells (MSCs) also known as mesenchymal stem cells<sup>[26,27]</sup>.

Studies using bone marrow MSCs have demonstrated that transplanted MSCs mobilize from the bone marrow into the ischemic myocardium post-MI. Consequently, these cells differentiate into cardiomyocytes suggesting that these cells play important roles in repair and regeneration post-MI<sup>[27-29]</sup>. Expression of  $\alpha$ -actin, cardiac titin, cardiac troponin T, desmin, MHC, MEF 2A and 2D, and phospholamban have been used as markers for MSC cardiomyocyte differentiation<sup>[27,30,31]</sup>.

Human-derived adipose MSCs, amniotic fluid SCs, umbilical cord blood hematopoietic cells and MSCs, and Wharton's Jelly MSCs have also been differentiated to cardiomyocytes. Expression of  $\alpha$ -actin, cardiac troponin I, GATA4, MHC, N-cadherin, Nkx2.5, and Tbx5 have been used for cardiac lineage differentiation characterization<sup>[32-38]</sup>.

Interestingly, cardiac tissue homeostasis and regenerative potential has been shown to involve resident cardiac SCs and progenitor cells which have been isolated and expanded from adult human and mouse heart tissue biopsies<sup>[39-41]</sup>. At least four different types of resident cardiac SCs have been isolated and shown to differentiate into cardiomyocytes<sup>[41-47]</sup>. Interestingly, three of the four types of resident cardiac SCs identified so far have the ability to form cardiospheres (CS)<sup>[41,47]</sup>.  $\alpha$ -actin and MEF2C are expressed by cardiomyocyte progenitors and developing cardiomyocytes. In addition to cardiosphere-derived cells, cardiac side population cells isolated from neonatal rat hearts have also been differentiated into beating cardiomyocytes by treatment with oxytocin or trichostatin A. *In vivo*, cardiac side specific cells demonstrated a superb

**Table 1 Stem cells differentiated into cardiomyocytes**

Cell type	Method of differentiation	Ref.
ESCs	EB-mediated differentiation	[17,18]
iPS	Transdifferentiation of iPS cell factor-based reprogrammed cardiac fibroblasts using EB-based method + transwell CM co-culture system	[19]
	Direct reprogramming of cardiac fibroblasts <i>in vivo</i> by local delivery of GMT	[21]
	Suspension EB-mediated differentiation of reprogrammed adult fibroblasts	[22,23]
Bone marrow MSC	<i>In vitro</i> differentiation induced by treatment with 5-azacytidine	[27,28]
	<i>In vivo</i> differentiation of stem cells transplanted and mobilized to damaged myocardium	[29]
	<i>In vivo</i> differentiation of stem cells engrafted into the myocardium	[30]
	Differentiation using a cardiomyogenic differentiation medium containing insulin, DMSO, and ascorbic acid	[31]
Adipose-derived MSC	Co-culture in direct contact with contracting cardiomyocytes	[37]
	DMSO at 0.1% for 48 h	[38]
Amniotic fluid SCs	<i>In vivo</i> differentiation of cells transplanted into myocardium	[33]
	<i>In vitro</i> differentiation through EB formation	[35]
Umbilical cord blood SCs	Co-culture with primary rat neonatal ventricular myocytes	[32]
	Co-culture with mouse neonatal cardiomyocytes	[34]
Wharton's Jelly MSCs	<i>In vitro</i> differentiation induced by treatment with 5-azacytidine or by culture in cardiomyocyte CM	[36]
CS	Co-culture with neonatal rat cardiomyocytes	[41]
CSP	Treatment with oxytocin or trichostatin A	[48]

ESC: Embryonic stem cell; EB: Embryoid body; iPS: Induced pluripotent stem cells; SCs: Stem cells; CM: Conditioned medium; GMT: Gata4, Mef2c, and Tbx5; MSC: Mesenchymal stem cell; DMSO: Dimethylsulfoxide; CS: Cardiospheres; CSP: Cardiac side population.

ability to home to injured heart and differentiate into cardiomyocytes. Expression of cardiac transcription factors GATA-4, Nkx2.5 and MEF 2C as well as contractile proteins MHC and MLC-2v have been used for SP cell cardiomyocyte differentiation<sup>[48]</sup>.

### Regulation of the inflammation

In addition to the ability of SCs to potentially replace dead cardiomyocytes, SCs provide a rich source of cytokines and growth factors that can act in an autocrine, paracrine, or endocrine fashion to regulate cell behavior during the inflammatory reaction post-MI<sup>[49]</sup>.

The inflammatory response that follows an MI is necessary and plays a crucial role in proper healing and ventricular remodeling. Post-MI, myocardial necrosis initiates an inflammatory response that includes a cascade of cytokines and chemokines followed by recruitment of neutrophils and macrophages<sup>[50,51]</sup>. As summarized by Frangogiannis *et al.*<sup>[51]</sup>, the inflammatory reaction clears

the damaged myocardium of cellular and matrix debris and activates the reparative process<sup>[51]</sup>. A prolonged inflammatory reaction leads to adverse remodeling and ventricular dysfunction due to untimely resolution of the acute inflammatory response, increased cardiomyocyte loss and resultant negative downstream effects to extracellular matrix (ECM) metabolism and neovascularization<sup>[50]</sup>.

The most commonly used SC for transplantations post-MI are bone marrow-derived MSCs. The paracrine effects of MSCs have received far more recognition than their ability to replace dead cardiomyocytes. One of the therapeutic goals post-MI is to minimize cardiomyocyte loss. Transplantation of bone marrow MSCs has been shown to reduce cardiomyocyte loss through activation of the cell survival gene Akt<sup>[52]</sup>. Further, other anti-apoptotic effects of MSCs are postulated to include inhibition of nuclear factor  $\kappa$ B (NF- $\kappa$ B) activity, reduced production of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6) as well as increased expression of IL-10<sup>[53-55]</sup>.

As part of their involvement in the inflammatory response post-MI, polymorphonuclear granulocytes (PMNs; neutrophils) leave the circulation, infiltrate into the injured myocardium, secrete proteolytic enzymes and reactive oxygen species, and clear cellular and ECM debris<sup>[56,57]</sup>. Increased production of IL-6 by MSCs has been shown to prevent apoptosis by activated neutrophils thereby increasing the lifespan of neutrophils through STAT3 transcription factors<sup>[58-60]</sup>. In addition, the increased production of IL-6 regulates neutrophil activation by attenuation of the respiratory burst<sup>[59,60]</sup>.

Macrophages in the injured myocardium undergo a biphasic activation that begins with a pro-inflammatory phase (also known as M1 or classically activated) that is followed by an overlapping anti-inflammatory phase (also known as M2 or alternatively activated)<sup>[61,62]</sup>. The macrophage polarization switch from M1 to M2 is a key event in myocardium repair<sup>[51,63]</sup>. MSC transplantations post-MI increase the number of M2 macrophages<sup>[64]</sup>. While the mechanism is still unclear, it is likely mediated through paracrine effects that include CCL2, galectin-1, interferon- $\gamma$ , IL-1 $\beta$ , indoleamine-2,3-dioxygenase, IL-4, IL-6, IL-10, IL-13, prostaglandin-E2, TNF- $\alpha$ , NF- $\kappa$ B, nitric oxide, heme oxygenase-1, hepatocyte growth factor, transforming growth factor-b1, and Human Leukocyte Antigen-G5<sup>[53,64,65]</sup>.

MSC paracrine factors have also been shown to suppress T cell, natural killer cell, and B cell proliferation and attenuate the maturation of dendritic cells through paracrine factors as listed in Table 2<sup>[60,66-68]</sup>.

### Regulation of fibrosis

Post-MI, necrotic cardiomyocytes are replaced with a fibrous scar. The extent of damaged tissue degradation and subsequent production of a provisional ECM affects scar thickness which in turn influences contractility of the surrounding myocardium. An increased degradation of ECM results in wall thinning and the development of aneurysms and LV rupture while an increased production



**Table 2 Stem cell trophic factors**

Factor	Outcome	Ref.
↑Akt	Reduction cardiomyocyte loss	[52]
↓NF-κβ	Anti-apoptotic effects	[53-55]
↓TNF-α	Anti-apoptotic effects	[53-55]
↓IL-6	Anti-apoptotic effects	[53-55]
↑IL-10	Anti-apoptotic effects	[53-55]
↑IL-6	Prevention activated neutrophil apoptosis <i>via</i> Stat3; regulation of neutrophil activation	[56-60]
↑IL-10, ↑TNF-α, and ↑IL-6	Macrophage M2 polarization	[53,61-65]
↓Collagen I and III, ↓TIMP-1 and ↓TGF-β	Reduction in fibrosis and scar size	[55,69-76]
↑VEGF	Promote angiogenesis; improved contractile function	[77-86]
↑IL-6	DC maturation inhibition	[60,66-68]
↑IDO and ↑PGE2	Reduced T cell activation	[60,66-68]
↑IDO and ↑PGE2	Decreased NK proliferation	[60]
Factor to be identified	B-Cell arrest	[60]

Akt: Serine/threonine kinase; NF-κβ: Nuclear factor κβ; TNF-α: Tumor necrosis factor α; IL-6: Interleukin 6; IL-10: Interleukin 10; TIMP-1: Tissue inhibitor of metalloproteinase 1; TGF-β: Transforming growth factor β; VEGF: Vascular endothelial growth factor; IDO: Indoleamine 2,3-dioxygenase; DC: Dendritic cell; NK: Natural killer cell; PGE2: Prostaglandin E2.

of ECM results in fibrosis and can predispose the LV to HF<sup>[69]</sup>. Interestingly, SC transplantations post-MI have been shown to regulate scar formation post-MI and improve ventricular function.

Transplantation of beating cardiomyocytes produced *in vitro* from ESCs has been shown to attenuate scar thinning and increase fractional shortening post-MI<sup>[70]</sup>. iPS cell therapy in the mouse permanent ligation model has also been shown to reduce wall thinning post-MI<sup>[71]</sup>. Additionally, MSC transplantations have been shown to reduce fibrosis and scar size<sup>[55,72-74]</sup>. Studies by Xu and colleagues demonstrated that MSC transplantations in rats post-MI regulate LV remodeling by decreasing mRNA expression and protein levels of TGF-β, type I and type III collagens, and tissue inhibitor of metalloproteinase (TIMP)-1<sup>[75]</sup>. Interestingly, in sheep, MSC progenitor cell-injections into the border zone altered collagen dynamics in a cell concentration-dependent manner as a result of spatial changes in matrix metalloproteinases (MMPs) and TIMPs. MMPs -1, -2, -3, -7, -9, -13, MT1-MMP, and TIMPs -1, -2, -4 were differentially altered in the remote, border zone, and infarct zones post-injection<sup>[76]</sup>.

### Regulation of angiogenesis

Angiogenesis is essential for myocardium repair and scar formation post-MI, and paracrine factors released following SC transplantations promote angiogenesis<sup>[77,78]</sup>. MSCs that engraft after transplantation post MI have been shown to express endothelial cell markers<sup>[79,80]</sup>. Consistent with these findings, MSCs have also been shown to secrete significantly elevated levels of vascular endothelial growth factor (VEGF). Concomitantly, capillary density increases in the infarct region contributing to improved regional and contractile function<sup>[81-83]</sup>. It is important to

note that MSCs, preconditioned under hypoxic conditions, have an enhanced capacity to stimulate vascularization compared to MSCs cultured under normoxic conditions due to increased expression of VEGF, angiopoietin-1, and survival post-transplantation<sup>[84-86]</sup>.

### Stem cell recruitment and delivery strategies

Several strategies have been used for SC therapeutic applications post-MI. These include cell infusion intravenously, intramyocardial injections, intracoronary applications, endocardial applications, and engineered delivery methods such as cardiac patches<sup>[87,88]</sup>. For SC recruitment, identification of chemoattractants that are responsible for SCs homing to damaged myocardium has shown an improvement in repair and ventricular function post-MI. Overexpression of stromal cell-derived factor-1 by transfected fibroblasts injected into the peri-infarct zone increased hematopoietic SC homing and improved fractional shortening in the rat MI model<sup>[89]</sup>. Monocyte chemotactic protein-3 also delivered in a similar fashion *via* transfected fibroblasts was shown to increase MSC engraftment. Although no significant regeneration of cardiomyocytes was observed, fractional shortening increased and LV end diastolic dimensions decreased<sup>[90]</sup>. In the porcine MI model, the combination of insulin growth factor-1 and hepatocyte growth factor activated endogenous cardiac SCs resulting in regeneration of cardiomyocytes and angiogenesis as well as improved cardiac function<sup>[91]</sup>. Interestingly, thymosin β4 has also been shown to play important roles in epicardial progenitor cell mobilization in the mouse heart for neovascularization<sup>[92,93]</sup>.

For delivery, biological and synthetic scaffolds used as vehicles for SC transplantations have shown improvement in cell survival, engraftment and cardiomyogenesis. In the rat MI model, transplanted cardiac SCs using nano-topographical hydrogel patches that mimicked the native cardiac ECM improved cell integration, retention and myocardium regeneration<sup>[94]</sup>. Similarly, cardiac patches containing adipose stromal vascular cells increased coronary blood flow and significantly improved ejection fraction post-MI<sup>[95,96]</sup>. The combination of a hydrogel patch with encapsulation of MSCs, as designed by Levit and colleagues, improves cell survival and takes full advantage of MSC paracrine factors. In addition to significantly reduced scar size, delivery of encapsulated MSCs increased peri-infarct microvasculature and improved ejection fraction in the rat MI model<sup>[97]</sup>.

## LIMITATIONS ASSOCIATED WITH SC TRANSPLANTATIONS POST-MI

Although numerous studies in humans and animal models have demonstrated that SC transplantations post-MI are safe and can improve cardiac healing and function, several common limitations associated with SC transplantations have been reported. The most common issues with SC transplantations for ventricular remodeling post-MI include reduced cell survival and engraftment which



**Table 3** Recently published clinical trials of stem cell therapies for acute treatment post-myocardial infarction

	Clinical trial	Outcome	Ref.
2010	Influence of bone marrow stem cells on left ventricular perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: Randomized clinical trial	Slight improvement of myocardial perfusion	[109]
2011	HEBE trial	No significant improvement on regional or global function	[110]
2011	Late TIME trial	No improvement on global or regional function at 6 mo	[111]
2012	Stem cell treatment for acute myocardial infarction	Reduced LVESV, LVEDV, and infarct size	[109]
2012	CADUCEUS trial	Reduced scar mass, increased myocardium viability, regional contractility and wall thickness	[112]
2012	Enhanced mobilization of the bone marrow-derived circulating progenitor cells by intracoronary freshly isolated bone marrow cells transplantation in patients with acute myocardial infarction	Feasibility, safety, and improvement on recovery of LV contractility	[113]
2013	The C-CURE trial	Feasibility, safety and improved LV ejection fraction	[114]

LV: Left ventricular; LVESV: Left ventricular end systolic volume; LVEDV: Left ventricular end diastolic volumes; CADUCEUS: CARDiosphere-derived autologous stem cells to reverse ventricular dysfunction; C-Cure: Cardiopoietic stem cell therapy in heart failure.

ultimately result in diminished cardiac regeneration and limited functional benefits. In human clinical trials, 3.2% of bone marrow SCs remained 24 h post-infusion, and in agreement with this outcome, other studies report less than 10% SC retention in human and animal studies<sup>[97-102]</sup>. Further, SCs that do engraft may differentiate into other lineages such as endothelial cells and fibroblasts rather than cardiomyocytes<sup>[103-105]</sup>. With regard to delivery methods, intravenous infusions may have decreased efficacy due to entrapment of cells in non-target tissues and organs such as bone marrow, lungs, liver and spleen<sup>[106,107]</sup>. Similarly, intracoronary and intramyocardial delivered cell retention is also limited and may reduce the efficacy of the transplanted cells due to the hostile milieu of the damaged heart<sup>[108,109]</sup>. Other reported issues with SC delivery methods include the potential for microembolism formation (intravenously and intracoronary), and the potential to induce arrhythmias (intracoronary and intramyocardial)<sup>[87,88]</sup>.

### Translation from bench to bedside

The ultimate goal of SC applications is the translation of what has been learned in the laboratory to the production of safe and effective therapies for attenuation of adverse LV remodeling. In Table 3, the results from the most recently published clinical trials of SC therapies in treatment of myocardial damage post-acute MI are listed. In addition to the feasibility of cell delivery, the safety associated with SC transplantations continues to evolve in clinical trials. Conversely, common issues such as standardization of methodology (including cell dosing, cell product formulation, and timing of transplantation) and the innate heterogeneity of study populations which include other clinical factors such as advanced aging and diabetes hinder interpretation of trial outcomes resulting in the need for a larger-scale study<sup>[100-114]</sup>. On this front, it is very encouraging to see a significant increase in the number of clinical trials being performed across the globe. The Alliance for Regenerative Medicine annual report for 2012-2013 indicates there were 326 industry-sponsored cell therapy trials ongoing in early 2013. The report fur-

ther indicates that the number of early to mid-stage cell therapy trials in cardiovascular-related diseases ranks second only to cell therapy studies involving cancer<sup>[115]</sup>.

A more specific review of acute MI clinical trials reveals that there were 36 open studies registered under “acute myocardial infarction and stem cells”. For congestive heart disease clinical trials the search revealed that there were 48 open studies registered under “congestive heart failure and stem cells” as of the writing of this review. Of these studies, there were 16 listed in phase 1 trials, 25 phase 2 trials, and 9 phase 3 trials (note that some studies are listed in overlapping phases). The majority of these studies are being conducted in the European Union and the United States with 15 and 12 registered studies, respectively<sup>[116]</sup>.

## CONCLUSION

The results from post-MI SC transplantations in animal models and humans have provided promising results in reducing scar formation and improved LV function which are achieved primarily through paracrine effects. While a great deal of information has been obtained in the past two decades on the roles SCs play in the post-MI setting, additional studies are needed to improve the efficacy of stem cell transplantations post-MI. Further, a consensus on the best time to initiate treatment, dosage, and delivery method is needed.

In summary, we have reviewed the current literature on the roles SCs play during LV remodeling post-MI. This evaluation includes different types of SCs with cardiomyogenic potential, markers of differentiation, trophic effects for the inflammatory, fibrotic and vascularization responses as well as strategies for cell homing and delivery post-MI.

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## Chronic total occlusion: To treat or not to treat

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this review, we will consider the available information supporting percutaneous treatment for chronic occlusions, as well as the areas of uncertainty where more research projects are required.

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**Key words:** Chronic total occlusion; Percutaneous coronary intervention

**Core tip:** This is a critical review about the available information supporting percutaneous treatment for chronic occlusions, as well as the areas of uncertainty where more research projects are required.

### Abstract

Over the last two decades, there has been increasing interest in new techniques for the percutaneous treatment of coronary chronic total occlusions (CTO), which have a success rate that is much higher than that of a few years ago. The rise in percutaneous treatment for these lesions is due to its ability to improve the symptoms and prognosis of patients in the chronic and stable phase of coronary disease. Current data suggest that successful percutaneous coronary intervention for CTO is associated with improvement in patient symptoms, quality of life, left ventricular function, and survival, compared with those with unsuccessful CTO PCI. However, all the scientific evidence supporting this treatment comes from observational studies, and no randomized study comparing percutaneous treatment with medical treatment has yet been published. A major limitation of these studies is their observational design, with limited information with regard to potential baseline differences between the successful vs unsuccessful cohorts. Pending randomized studies, patients should be selected very carefully, especially if they are asymptomatic or very few symptoms, and the benefits obtained in terms of complications during the procedure, the quality of life obtained and further ischemic events avoided should be evaluated systematically. In

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### INTRODUCTION

Chronic total occlusions (CTO) are considered to be 100% coronary lesions, of more than 3 mo evolution<sup>[1]</sup>. They are therefore always found in stable chronic patients, with varying levels of symptoms. After the culprit artery has been treated, patients with acute coronary syndrome may occasionally also have a chronic occlusion in another artery that was not responsible for the acute event, and is therefore considered a CTO.

### DEFINITION AND INCIDENCE

The prevalence of CTO in patients undergoing coronary angiography varies, ranging between 18% and 52% depending on the clinical profile of the patient being examined<sup>[2-7]</sup> (Table 1). Although revascularization surgery is the most frequent treatment, clinicians and invasive cardi-

**Table 1** Chronic total occlusion prevalence, location and treatment applied in different studies *n* (%)

Ref.	Type of study	Population	CTO prevalence	CTO location			Medical treatment	PCI	CABG
				RCA	LAD	LCA			
Kahn <i>et al</i> <sup>[2]</sup> , 1993	Retrospective	333	101 (35)	58%	18%	24%	-	-	-
Christofferson <i>et al</i> <sup>[3]</sup> , 2005	Retrospective	Coronary disease (stenoses $\geq$ 50%)	1612 (25)	49.4%	22%	28.60%	49%	11%	40%
		Underwent coronarography because of suspected CD							
		3087	1612 (52)						
Srinivas <i>et al</i> <sup>[4]</sup> , 2002	Retrospective	Coronary disease (stenoses $\geq$ 70%)	1761					14.50%	-
		Multivessel disease							
Yamamoto <i>et al</i> <sup>[5]</sup> , 2013	Prospective	15263	2491 (19)	44.9%	41.10%	28.50%	-	61.18%	-
Fefer <i>et al</i> <sup>[6]</sup> , 2012	Prospective	First revascularization procedure	14439						
		Underwent coronariography because of suspected CD	2630 (18.2)	46.9%	19.86%	15.43%	64%	10%	26%
Jeroudi <i>et al</i> <sup>[7]</sup> , 2013	Prospective	1015	319 (31.34)	-	-	-	19% (61)	50% (161)	30% (97)
		Coronary disease (stenoses $\geq$ 50%)							

CTO: Chronic total occlusion; RCA: Right coronary artery; LAD: Left anterior descending; LCA: Left circumflex artery; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass graft.

ologists often consider the need and feasibility of percutaneous treatment for these lesions, based on symptoms and prognostic factors. However, as it is a common problem in all Cath Labs, the extensive variability between different centres is striking. For example, in North American centres<sup>[4]</sup>, with an incidence rate of CTO of between 29% and 33% in all the catheterizations performed, only between 6% and 9% of patients were treated percutaneously. However, in Japanese centres, with an incidence of 19% of CTO in all the catheterizations performed, 61.2% of all cases were treated percutaneously<sup>[5]</sup>. There are also significant differences in the treatment of CTO within the same geographical area or healthcare system. For example, in the Canadian CTO registry<sup>[6]</sup>, some hospitals percutaneously treat 16% of their patients, while others only do so for 1%. These differences are very striking, and can only be justified by some generally ill-defined indications, as well as the technical difficulty that means that not all invasive cardiologists can or should deal with complex lesions of this type. However, there is another factor that also needs to be mentioned. Patients with CTO probably have a clinical profile that is different to that of patients with chronic coronary ischemic disease in general. There not only are differences in terms of greater severity of coronary disease, but also in terms of increased non-coronary comorbidity, such as a higher rates of prevalence of diabetes, peripheral arterial disease, heart failure and a history of strokes<sup>[8]</sup>. The indications for percutaneous treatment of CTO are not well defined in the European guidelines for revascularization<sup>[9]</sup>, or in the guidelines for patients with stable chronic coronary disease<sup>[11]</sup> (Table 2). The American guidelines on revascularization<sup>[10,11]</sup> and chronic stable ischemic heart disease<sup>[12]</sup> are also unclear as regards the indications for treatment of CTO. Only the American guidelines for the appropriate use of percutaneous coronary treatment<sup>[13]</sup> contain a clear position on treatment that is appropriate, uncertain

or not indicated in CTO lesions (Table 3).

## CTO TREATMENT IN PATIENTS WITH ANGINA

There should be no doubt that a treatment of a CTO affecting an ischemic myocardial area that causes symptoms such as angina should improve patients' symptoms, by providing a greater perfusion flow than that provided by collateral circulation, as a consequence of opening the occluded artery<sup>[14]</sup>. However, this has been poorly studied and quantified in the medical literature. Very few studies have specifically evaluated the changes in the ischemic threshold and quality of life scales of symptomatic and asymptomatic patients with percutaneously treated CTO. In the FACTOR Trial (FlowCardia Approach to CTO Recanalization), 125 patients completed the Seattle Angina Questionnaire at baseline and one month after percutaneous coronary intervention<sup>[15]</sup>. Successful treatment was associated in overall terms with an improvement in the frequency of angina, physical capacity and quality of life. However, this improvement was only observed in previously symptomatic patients but not in asymptomatic patients. In fact, this symptomatic improvement is similar to that obtained with percutaneous coronary intervention in the treatment of lesions without chronic total occlusion<sup>[16]</sup>.

## TREATMENT OF CTO IN ISCHEMIC PATIENTS

Often no distinction is made between patients with angina and patients with myocardial ischemia when percutaneous treatment of CTO is indicated<sup>[17]</sup>. However, these two concepts are different in our opinion, and should be clarified. In patients with angina (and therefore with



**Table 2** Specific recommendations on the treatment of chronic total occlusion in the American and European Practice Guidelines

Society	Guideline	Specific recommendation on the treatment of CTO
EUROPEAN	2010 Guidelines of myocardial revascularization <sup>[9]</sup>	"Revascularization of CTO may be considered in the presence of angina or ischemia related to the corresponding territory"
	2013 ESC guidelines on the management of stable coronary artery disease <sup>[1]</sup>	"Revascularization needs to be discussed in patients with symptoms of occlusion or large ischemic areas"
AMERICAN	2011 ACCF/AHA Guideline for Coronary Artery Bypass Graft Surgery <sup>[10]</sup>	Not mentioned
	2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention <sup>[11]</sup>	Recommendation IIa. Evidence level B. PCI of a CTO in patients with appropriate clinical indications and suitable anatomy is reasonable when performed by operators with appropriate expertise "The decision to try PCI for a CTO ( <i>vs</i> continued medical therapy or surgical revascularization) requires an individualized risk-benefit analysis encompassing clinical, angiographic, and technical considerations"
	2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the Diagnosis and Management of Patients With Stable Ischemic Heart Disease <sup>[12]</sup>	Not mentioned

CTO: Chronic total occlusion; ACCF: American College of Cardiology Foundation; AHA: American Heart Association; SCAI: Society for Cardiovascular Angiography and Interventions; ACP: American College of Physicians; AATS: American Association for Thoracic Surgery; PCNA: Preventive Cardiovascular Nurses Association; STS: Society of Thoracic Surgeons; PCI: Percutaneous Coronary Intervention.

**Table 3** Specific recommendations on the treatment of chronic total occlusion in the 2012 ACCF/SCAI/STS/AATS/ASNC/HFSA/SCCT Appropriate Use Criteria for Coronary Revascularization Focused Update<sup>[14]</sup>

		ANGINA							
		Asymptomatic	I	II	III	IV			
Risk in the ischemia test	High	Uncertain	Appropriate	Appropriate	Appropriate	Appropriate	Max	Treatment level	
		Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Med		
		Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Min		
	Medium	Uncertain	Uncertain	Uncertain	Appropriate	Appropriate	Max		
		Inappropriate	Uncertain	Uncertain	Uncertain	Uncertain	Med		
		Inappropriate	Uncertain	Uncertain	Uncertain	Uncertain	Min		
	Low	Inappropriate	Inappropriate	Inappropriate	Uncertain	Uncertain	Max		
		Inappropriate	Inappropriate	Inappropriate	Inappropriate	Inappropriate	Med		
		Inappropriate	Inappropriate	Inappropriate	Inappropriate	Inappropriate	Min		

It shows the 45 possible scenarios depending on the risk of mortality based on the findings on ischemia tests, symptoms and level of treatment.

ischemia), the benefit of CTO treatment is for the symptoms and possibly the prognosis. However, as mentioned above, in patients with ischemia but without angina, the benefit is not symptomatic and can only be evaluated in prognostic terms. It is therefore important to determine whether patients with myocardial ischemia but who are asymptomatic benefit from percutaneous treatment of a CTO. The rationale for this approach is based on relatively early studies in which the improvement of ischemia provided by the revascularization obtained by an angioplasty, in both symptomatic patients<sup>[18]</sup> and asymptomatic patients<sup>[19]</sup>, was associated with an improved prognosis. In the SWISSI II Trial<sup>[19]</sup> in the late 1990s, on asymptomatic patients after myocardial infarction, with coronary disease in 1 or 2 vessels and inducible myocardial ischemia in an imaging stress test, coronary angioplasty significantly reduced coronary events during a long-term follow-up period. However, in this study, both the medical treatment, which was very limited, and the percutaneous treatment (the use of bare metal stents) were obviously different to those currently in use. More recent studies of

symptomatic patients with chronic coronary disease, frequently presenting a positive test for ischemia, have failed to show that percutaneous revascularization improves prognosis<sup>[20]</sup>, even in diabetic patients<sup>[21]</sup>. In the COURAGE trial, the small benefit in terms of improved quality of life in percutaneously treated patients compared to those receiving medical therapy without revascularization disappeared after 36 mo follow-up<sup>[22]</sup>. The data from the COURAGE trial substudy, with quantification of ischemia by a stress test with nuclear imaging, show that in patients with stable chronic ischemic heart disease, angioplasty provides a greater improvement in the ischemic area than medical treatment<sup>[23]</sup>. However, this improvement in the ischemic area had no effect on the medium-term prognosis<sup>[24]</sup>. A recent meta-analysis including all the randomized studies in patients with stable chronic ischaemic cardiopathy and proven myocardial ischemia concluded that percutaneous treatment does not affect rates of mortality, myocardial infarction, unplanned revascularization or angina compared to medical treatment alone<sup>[25]</sup>. At present, the hypothesis that moderate to severe

**Table 4 Findings on left ventricular ejection fraction and regional wall motion variations after percutaneous coronary intervention treatment of chronic total occlusion**

	Type of study	Population	LVEF estimation	Follow up	Results				
					LVEF	Regional wall motion	Symptoms	Collateral function	Ventricular remodeling
1994-1995 Sirnes <i>et al</i> <sup>[30]</sup>	Prospective	95 CTOs treated with PCI	Ventriculography	Angiography 6 mo	LVEF increase (from 0.62 ± 0.13 to 0.67 ± 0.12) <i>P</i> < 0.001	Increase in regional radial shortening (from 0.279 ± 0.106 to 0.319 ± 0.107) <i>P</i> < 0.001	Improvement in angina class	Not mentioned	Not mentioned
1999-2003 Werner <i>et al</i> <sup>[31]</sup>	Prospective	126 CTOs treated with PCI	Ventriculography	Angiography	LVEF increase (from 0.60 ± 0.19 to 0.67 ± 0.16) <i>P</i> < 0.001	Increase in wall motion severity index (from -1.92 ± 1.32 to -1.30 ± 1.28) <i>P</i> < 0.001	Not mentioned	No changes in collateral function	Not mentioned
2008 Kirschbaum <i>et al</i> <sup>[32]</sup>	Prospective	21 CTOs treated with PCI	NMR	NMR 5 mo and 3 yr	LVEF increase (from 60% ± 9% to 63% ± 11%) <i>P</i> = 0.11	Increase in segmental wall thickening. From 19% ± 21% to 31% ± 30% at 5 mo ( <i>P</i> < 0.001) and 47% ± 46% at 3 yr ( <i>P</i> = 0.04)	Not mentioned	Not mentioned	Less ventricular remodeling in NMR at 3 yr

LVEF: Left ventricular ejection fraction; CTO: Chronic total occlusion; PCI: Percutaneous coronary intervention; NMR: Nuclear magnetic resonance.

myocardial ischemia should be revascularized in order to improve the prognosis must therefore be reviewed<sup>[26]</sup>. In the context of patients with chronic coronary artery disease, the ISCHEMIA clinical trial will attempt to demonstrate whether the strategy of cardiac catheterization for revascularization is better than strategy of medical treatment in patients with moderate to severe ischemia detected in a stress test with imaging<sup>[27]</sup>. In this trial, there will presumably be few patients with CTO, meaning that it is possible that its findings cannot be fully extrapolated to this specific population. As regards patients specifically with CTO, there are two ongoing clinical trials that are randomizing patients with angina or ischemia in an imaging test for medical treatment or angioplasty. The EURO-CTO clinical trial, being run at a European level, has the primary objective of evaluating quality of life at 12 mo, as well as assessing major coronary events after 3 years<sup>[28]</sup>. The DECISION-CTO clinical trial, conducted in Asian countries, has a composite primary endpoint (cardiac death, myocardial infarction, stroke or further revascularization) evaluated after 3 years<sup>[29]</sup>.

## CTO TREATMENT IN PATIENTS WITH VENTRICULAR DYSFUNCTION

Chronic hypoperfusion due to the presence of a CTO on a viable myocardium can cause ventricular dysfunction, and may lead to symptoms such as exercise intolerance and heart failure resulting from this dysfunction. It therefore seems logical that the opening of an occluded artery which irrigates a viable but dysfunctional myocardium could reverse this dysfunction and improve these

patients' symptoms and prognosis. There are few studies, all of which are observational, that have specifically addressed this issue (Table 4). Most available data suggest a very modest improvement in ventricular function as a result of opening an occluded artery. For example, Sirnes assessed the changes in ventricular function by ventriculography and was only able to demonstrate a 2% increase in ejection fraction, although the regional radial shortening increased by 16% in the revascularized areas<sup>[30]</sup>. This slight improvement in regional ventricular function does not appear to depend on the presence of pre-existing collaterals, but probably on preserved microvascular integrity<sup>[31]</sup>. The use of more accurate methods for quantifying ventricular function, such as cardiac magnetic resonance imaging, also confirms that the improvement in ventricular function as a result of opening an occluded artery is very modest<sup>[32]</sup>. The improvement in the prognosis of patients with ventricular dysfunction due to revascularization is currently a topic of heated debate, following the results of randomized STICH study<sup>[33]</sup>. In this clinical trial, patients with multivessel disease and ventricular dysfunction did not improve their prognosis as a result of revascularization surgery, in comparison with the medically treated group. Surprisingly, even the specifically studied patients with myocardial viability did not benefit from revascularization<sup>[34]</sup>. The STICH study did not include patients with CTO, but the concept and the comments are relevant, because the percutaneous treatment of CTO is often justified simply on the basis of viability.

Meanwhile, the treatment of a CTO as a cause of deterioration in the ejection fraction due to complications

Table 5 Baseline characteristics of clinical and angiographic variables in studies included on Joyal meta-analysis<sup>[42]</sup>

Ref.	Age (yr)		Male sex (%)		Multivessel disease (%)		Diabetes (%)		LVEF (%)		NYHA class 3-4 (%)		Renal dysfunction (%)		Occlusion length (mm)		Calcified vessel (%)		Ischemic burden	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure	Success	Failure
Finci <i>et al.</i> <sup>[42]</sup> , 1990	55 ± 11	55 ± 12	93	88	24	23	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Warren <i>et al.</i> <sup>[43]</sup> , 1990	54	55	53	47	48	52	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Ivanhoe <i>et al.</i> <sup>[44]</sup> , 1992	55 ± 10	56 ± 11	81	82	30	54 (0.0001)	10	15	55 ± 10	56 ± 11	3	3	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Angiot <sup>[45]</sup> , 2000	55 ± 10	56 ± 11	52	88	37	45	10	11	59 ± 14	59 ± 14	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Noguchi <i>et al.</i> <sup>[46]</sup> , 2000	61 ± 9	61 ± 11	78	80	47	67 (0.01)	26	32	56 ± 12	54 ± 9	n/d	n/d	n/d	11.3 ± 8.3	14.1 ± 8.1	37	56 (< 0.01)	n/d	n/d	n/d
Suero <i>et al.</i> <sup>[47]</sup> , 2001	60 ± 11	61 ± 12	78	80	73	82 (0.001)	21	20	51 ± 14	52 ± 14	n/d	n/d	8.2	7.1	n/d	n/d	n/d	n/d	n/d	n/d
Olivari <i>et al.</i> <sup>[48]</sup> , 2003	58 ± 10	59 ± 11	86	85	45	60 (0.014)	17	20	56 ± 10	56 ± 10	9	7	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Hoye <i>et al.</i> <sup>[49]</sup> , 2005	60 ± 11	61 ± 10	74	72	54	67 (0.03)	12	9.1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Drozdz <i>et al.</i> <sup>[50]</sup> , 2006	57 ± 10	58 ± 10	81	80	46	53	11	11	n/d	n/d	14.4	18 (NYHA ≥ 2)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Aziz <i>et al.</i> <sup>[51]</sup> , 2007	59	59	76	81	50	40 (0.006)	14	9	53	53	12.2	15.7	0.3	1.8	n/d	n/d	n/d	n/d	n/d	n/d
Prasad <i>et al.</i> <sup>[52]</sup> , 2007	63 ± 11	64 ± 11	76	75	70	74	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Valenti <i>et al.</i> <sup>[53]</sup> , 2008	67 ± 11	70 ± 11	81	83	85	87	24	21	42 ± 13	41 ± 14	n/d	n/d	n/d	25 (15-52.5)	28 (21-47.5)	n/d	n/d	n/d	n/d	n/d
de Labriolle <i>et al.</i> <sup>[54]</sup> , 2008	61 ± 12	64 ± 10	72	87	45	66 (0.002)	19	40.5 (0.005)	50 ± 12	48 ± 15	n/d	n/d	9.1	6.3	n/d	n/d	n/d	n/d	n/d	n/d

LVEF: Left ventricular ejection fraction; n/d: No data; NYHA: New York Heart Association.

during the procedure should not be ruled out. In recent years, major breakthroughs have been described in the material used for the percutaneous treatment of CTO, which has led to a significant reduction in complications<sup>[35]</sup>. However, the statement that today's complication rates are similar to those occurring in the treatment of less complex lesions could not be further from the truth. The Multinational CTO Registry mentions a rate of residual coronary dissection and perforation of 4.3% and 1.7% in successfully patients treated. However, among patients treated without success, these rates are 9.4% and 7.4%, respectively<sup>[35]</sup>. In the series from a large Japanese centre, the overall rate of perforation in all percutaneous coronary intervention procedures is 1.2%, but 44% of these occur in patients with CTO<sup>[36]</sup>. In another large Japanese centre, the rates of coronary dissection, perforation, distal embolization are 14.7%, 8.2% and 3.7% respectively, when an antegrade approach is used, and 10.1%, 13% and 1.4% respectively, when the procedure is performed *via* the retrograde route<sup>[37]</sup>. Some authors have postulated that this high rate of complications in unsuccessfully treated patients partially explains their worse prognosis compared with those who are successfully treated<sup>[38,39]</sup>.

## CTO TREATMENT TO IMPROVE PROGNOSIS

Some registries have reported that patients with complete revascularization have a better prognosis than those with incomplete revascularization, including the presence of

an untreated CTO<sup>[40]</sup>. On this basis, the main argument which normally supports the treatment of CTO is that successfully treated patients have a better prognosis than those treated without success. This is apparent in the Joyal meta-analysis, in which successful treatment was associated with a significant improvement in mortality compared to unsuccessfully treated patients<sup>[41]</sup>. This meta-analysis, conducted on studies with mainly retrospective data, seems to suggest that the baseline characteristics of successfully treated patients are similar to those treated without success, and that the unsuccessfully treated patients act as a medically treated control group. However, the studies performed with retrospective data<sup>[42-54]</sup> often lack information on some of the baseline characteristics of patients which have a clear effect on prognosis (Table 5). When studies with prospectively collected data are analyzed, it becomes apparent that the baseline characteristics of patients treated without success are clearly different from those who are successfully treated. For example, the Canadian prospective registry contains many variables of poor prognosis among the unsuccessfully treated patients, such as having a longer history of prior infarction, prior multivessel disease, a longer CTO, higher rate of residual dissection and perforation during the procedure, which undoubtedly influences the these patients' poor prognosis<sup>[36]</sup>. Furthermore, when the collection of variables is prospective and they are included in the predictive statistical model<sup>[55]</sup>, the benefit of successful treatment of CTO is cancelled out as these patients have baseline characteristics with a better prognosis than those treated without success. This hypothesis is corroborated by the recent publication of the long-term results of patients in the CREDO-Kyoto Registry<sup>[5]</sup>. In this large series, the clinical evolution of 1192 successfully treated patients was compared with 332 unsuccessfully treated patients. Hospital mortality tended to be lower among the successfully treated patients than among those who were unsuccessfully treated (1.4% *vs* 3%,  $P = 0.053$ ). During a three-year follow-up period, all-cause mortality did not differ between the two groups (9% *vs* 13.1%,  $P = 0.18$ ), while the incidence of cardiac-related death was significantly lower in the successfully treated group (4.5% *vs* 8.4%,  $P = 0.03$ ). However, after adjustment for confounding variables, successful treatment was not associated with either reduced total mortality (hazard ratio 0.93, 95%CI: 0.64 to 1.37,  $P = 0.69$ ) or cardiac mortality (HR = 0.71, 95%CI: 0.44-1.16,  $P = 0.16$ ). The only benefit associated with success in the treatment of CTO was a lower rate of surgical revascularization.

One group of patients deserves special consideration. These are patients with acute coronary syndrome in which the culprit artery is treated initially, but who have another chronically occluded artery which is considered for recanalization in a second procedure. This argument is based on the fact that these patients have a worse prognosis than patients with acute coronary syndrome with no CTO<sup>[56]</sup>. The EXPLORE clinical trial, which randomizes patients with CTO with no culprit artery after an acute coronary artery syndrome on revas-

cularization treatment within 7 d of the ischemic event *vs* medical treatment<sup>[57]</sup> attempts to clarify this important issue, which is currently performed frequently without any scientific evidence.

## CONCLUSION

Treatment of CTO has emerged in recent years as a result of a revolution in medical equipment that enables these patients to be managed with success rates well above those of a few years ago. However, there is an urgent need for randomized studies to support this therapy as it is not risk-free, and is very expensive. Pending randomized studies, patients should be selected very carefully, especially if they are asymptomatic or very few symptoms, and the benefits obtained in terms of complications during the procedure, the quality of life obtained and further ischemic events avoided should be evaluated systematically.

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## Significance of lead aVR in acute coronary syndrome

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### Abstract

The 12-lead electrocardiogram (ECG) is a crucial tool in the diagnosis and risk stratification of acute coronary syndrome (ACS). Unlike other 11 leads, lead aVR has been long neglected until recent years. However, recent investigations have shown that an analysis of ST-segment shift in lead aVR provides useful information on the coronary angiographic anatomy and risk stratification in ACS. ST-segment elevation in lead aVR can be caused by (1) transmural ischemia in the basal part of the interventricular septum caused by impaired coronary blood flow of the first major branch originating from the left anterior descending coronary artery; (2) transmural ischemia in the right ventricular outflow tract caused by impaired coronary blood flow of the large conal branch originating from the right coronary artery; and (3) reciprocal changes opposite to ischemic or non-ischemic ST-segment depression in the lateral limb and precordial leads. On the other hand, ST-segment depression in lead aVR can be caused by transmural ischemia in the inferolateral and apical regions. It has been recently shown that an analysis of T wave in lead aVR also provides useful prognostic information in the general population and patients with prior myocardial infarction. Cardiologists should pay more attention to the tracing of lead aVR when interpreting the 12-lead ECG in clinical practice.

served.

**Key words:** Electrocardiography; Lead aVR; ST-segment; T wave; Acute coronary syndrome

**Core tip:** In this article, I will review current evidence on lead aVR in the field of acute coronary syndrome.

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### INTRODUCTION

Lead aVR, an augmented and unipolar limb lead, was constructed to obtain specific information from the right upper portion of the heart, including the outflow tract of the right ventricle and the basal portion of the interventricular septum. However, lead aVR has been long neglected until recent years. This is thought to be because most cardiologists have considered that the tracing of lead aVR merely reflects reciprocal information from the lateral limb and precordial leads<sup>[1]</sup>. However, in the last decade, evidence indicating the importance of lead aVR in the field of acute coronary syndrome (ACS) has been accumulating. In this article, the author will review current evidence on lead aVR in the field of ACS.

### ST-SEGMENT SHIFT IN LEAD AVR

#### *Prediction of acute left main trunk occlusion*

Because the left coronary artery mostly supplies approximately 75% of the left ventricular (LV) myocardial mass, acute occlusion of the left main trunk (LMT) causes life-threatening hemodynamic deterioration and malignant arrhythmias, resulting in an adverse outcome. Therefore, a rapid diagnosis and subsequent urgent revascularization with percutaneous coronary intervention (PCI) or



coronary bypass surgery is very important in acute LMT occlusion. The 12-lead electrocardiogram (ECG) is a crucial tool in the diagnosis of ACS. Yamaji *et al*<sup>[2]</sup> compared electrocardiographic findings among 16 patients with acute LMT occlusion, 46 patients with acute left anterior descending coronary artery (LAD) occlusion, and 24 patients with acute right coronary artery (RCA) occlusion and found that ST-segment elevation  $> 0.05$  mV in lead aVR was more common in acute LMT occlusion (88%) compared to acute LAD occlusion (43%) and acute RCA occlusion (8%). Furthermore, the magnitude of ST-segment elevation in lead aVR greater than or equal to that of ST-segment elevation in lead V<sub>1</sub> was found to have 81% sensitivity and 80% specificity for differentiating acute LMT occlusion from acute LAD occlusion. They considered that ST-segment in lead aVR observed in acute LMT occlusion is caused by transmural ischemia in the basal part of the interventricular septum through impaired coronary blood flow of the first major septal branch arising from the LAD and that smaller ST-segment elevation in lead V<sub>1</sub> is due to the counterbalance of injury currents produced by transmural ischemia in both the anterior and posterior walls. The Yamaji's criterion requires validation by further studies with a large sample size.

In acute LMT occlusion, ST-segment elevation in lead aVR can also occur as a mirror image of ST-segment depression in the lateral limb and precordial leads. For example, global subendomyocardial ischemia caused by acute LMT occlusion can produce widespread ST-segment depression, especially in the lateral precordial leads, resulting in ST-segment elevation in lead aVR. In a review article, Nikus *et al*<sup>[3]</sup> classify the electrocardiographic findings of acute LMT occlusion into the following patterns: (1) widespread ST-segment depression with maximal changes in lead V<sub>4-6</sub> with inverted T waves; (2) ST-segment elevation in lead aVR; and (3) anterior (anterolateral) ST-segment elevation. Ischemia-induced conduction disturbances, including right bundle branch block, left anterior fascicular block, and intraventricular conduction disturbance, are also frequently observed in acute LMT occlusion<sup>[3]</sup>. The lack of one single uniform electrocardiographic pattern of acute LMT occlusion is thought to be greatly due to the heterogeneity of the amount and localization of the ischemic jeopardized myocardium.

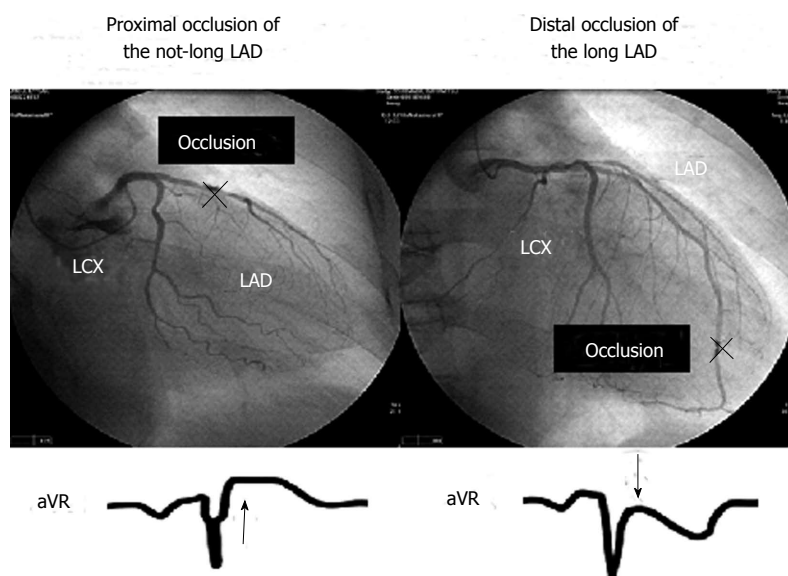
In summary, the electrocardiographic findings of acute LMT occlusion do not show one single uniform electrocardiographic pattern. The classification proposed by Nikus and Eskola requires validation. Whether there is a specific electrocardiographic finding to predict a poor outcome in patients with acute LMT occlusion needs to be investigated.

### ST-segment elevation in lead aVR in non-ST-segment-elevation ACS

Several studies have examined the significance of ST-segment elevation in lead aVR on the admission ECG in non ST-segment elevation ACS (NSTEMI-ACS)<sup>[4-8]</sup>. Barrabés *et al*<sup>[4]</sup> examined the association between ST-segment

shift in lead aVR and in-hospital mortality in 775 patients with a first non ST-segment elevation myocardial infarction (NSTEMI) and found that the rates of in-hospital mortality were 1.3% in 525 patients without ST-segment elevation in lead aVR, 8.6% in 116 patients with 0.05 mV to 0.1 mV of ST-segment elevation in lead aVR, and 19.4% in 134 patients with ST-segment elevation  $\geq 0.1$  mV in lead aVR. After adjusting for clinical variables, the odds ratios (ORs) for in-hospital mortality in the last 2 groups were 4.2 (95%CI: 1.5-12.2) and 6.6 (95%CI: 2.5-17.6), respectively. In 437 patients who underwent coronary arteriography within 6 mo of the onset of symptoms, the prevalence of LMT or 3-vessel disease among the 3 groups was 22.0%, 42.6%, and 66.3%, respectively. They concluded that in NSTEMI, ST-segment elevation in lead aVR is independently associated with increased in-hospital mortality probably because of severe coronary artery disease. In a GRACE substudy, including 5064 patients with NSTEMI-ACS, Yan *et al*<sup>[5]</sup> showed that neither minor (0.05-0.1 mV) nor major ( $> 0.1$  mV) ST-segment elevation in lead aVR was an independent predictor of in-hospital and 6-mo mortality after adjusting for other validated prognosticators in the GRACE risk model. The results are inconsistent with those of Barrabés *et al*<sup>[4]</sup>. In the study of Yan *et al*<sup>[5]</sup>, the prevalence of ST-segment elevation  $> 0.1$  mV in lead aVR was only 1.5% ( $n = 76$ ), which was much lower compared to the study by Barrabés *et al*<sup>[4]</sup>. A small number of patients with ST-segment elevation  $> 0.1$  mV in lead aVR might have led to the negative result. In addition, entering ST-segment deviation in other leads and ST-segment elevation in lead aVR simultaneously into the multivariate analysis might have led to the negative result because all patients with ST-segment elevation  $> 0.1$  mV in lead aVR had ST-segment deviation in other leads. Taglieri *et al*<sup>[6]</sup> showed that ST-segment depression  $\geq 0.05$  mV in any lead plus ST-segment elevation  $\geq 0.1$  mV in lead aVR was independently associated with culprit LMT disease and increased in-hospital and 1-year cardiovascular deaths in 1042 patients with NSTEMI-ACS. In these three studies<sup>[4-6]</sup>, coronary arteriography was not performed in all patients.

There are a few studies<sup>[7-9]</sup> to examine the significance of ST-segment elevation in lead aVR in NSTEMI-ACS patients undergoing emergent coronary arteriography. Kosuge *et al*<sup>[7]</sup> analyzed ECGs of 310 patients with NSTEMI-ACS undergoing coronary arteriography and found that ST-segment elevation  $\geq 0.05$  mV in lead aVR was the strongest predictor of LMT or 3-vessel disease, with 78% sensitivity and 86% specificity. In another study, Kosuge *et al*<sup>[8]</sup> examined the prognostic value of ST-segment elevation  $\geq 0.05$  mV in lead aVR in 333 patients with NSTEMI-ACS undergoing coronary arteriography and showed that ST-segment elevation  $\geq 0.05$  mV in lead aVR as well as serum troponin T level  $\geq 0.1$  ng/mL were independent predictors of 90-d adverse outcomes, including death, myocardial infarction (MI), or urgent revascularization. When the patients were divided into 4 groups based on ST-segment shift in lead aVR and serum troponin T levels, patients with ST-segment elevation  $\geq 0.05$  mV in lead aVR combined with



**Figure 1 Association between ST-segment shift in aVR and coronary angiographic anatomy in a first anterior wall ST-segment elevation myocardial infarction.** LAD: Left anterior descending coronary artery; LCX: left circumflex coronary artery.

an increased serum troponin T level had the highest rates of LMT or 3-vessel disease (62%) and 90-d adverse outcomes (47%). In another study, Kosuge *et al*<sup>[9]</sup> examined 572 patients with NSTEMI-ACS undergoing coronary arteriography and showed that ST-segment elevation  $\geq 0.1$  mV in lead aVR identified severe LMT or 3-vessel disease ( $\geq 75\%$  stenosis of LMT and/or 3-vessel disease with  $\geq 90\%$  stenosis in  $\geq 2$  proximal lesions of the LAD and other major epicardial arteries), with 80% sensitivity and 93% specificity.

The current evidence suggests that in patients with NSTEMI-ACS, ST-segment elevation in lead aVR is associated with LMT or 3-vessel disease and increased adverse events. Considering the location of lead aVR, global subendomyocardial ischemia can produce ST-segment elevation in lead aVR. Therefore, it is reasonable that ST-segment elevation in lead aVR is associated with LMT or 3-vessel disease in NSTEMI-ACS.

#### **ST-segment shift in lead aVR in anterior wall STEMI caused by LAD occlusion**

A few studies<sup>[10-12]</sup> have examined the significance of ST-segment shift in lead aVR on the admission ECG in first anterior wall STEMI caused by LAD occlusion. Kosuge *et al*<sup>[10]</sup> analyzed ECGs of 105 patients with a first anterior wall STEMI undergoing successful reperfusion and found that 35 patients with ST-segment depression  $\geq 0.05$  mV in lead aVR had a larger infarct size, as estimated by peak creatine kinase levels, and a lower LV ejection fraction at predischage compared to 23 patients with ST-segment elevation  $\geq 0.05$  mV in lead aVR and 47 patients without ST-segment deviation in lead aVR. They speculated that ST-segment depression in lead aVR may reflect transmural ischemia extending to the apical and inferolateral walls, thereby resulting in a large MI. However, they did not evaluate the precise coronary angiographic anatomy. Accordingly, we<sup>[11]</sup> examined the association between ST-segment shift in lead aVR and emergent coronary angiographic anatomy in 261 patients

with a first anterior wall STEMI and found that ST-segment depression  $\geq 0.05$  mV in lead aVR was associated with distal LAD occlusion (defined as occlusion of the LAD distal to the origin of the first septal branch) and a long LAD (defined as an LAD perfusing  $\geq 25\%$  of the inferior wall) and that ST-segment elevation  $\geq 0.05$  mV in lead aVR was associated with proximal LAD occlusion (defined as occlusion of the LAD proximal to the origin of the first septal branch) and a not-long LAD. Interestingly, patients with proximal occlusion of the long LAD, who would suffer from a large MI, had a relatively lesser degree of ST-segment shift in lead aVR. We considered that this is due to the counterbalance of injury currents produced by transmural ischemia in both the basal part of the interventricular septum and the inferolateral and apical walls. In another study<sup>[12]</sup>, we examined the association between ST-segment shift in lead aVR and left ventriculography findings at predischage in 237 patients with a first anterior wall STEMI and found that LV ejection fraction at predischage did not differ significantly among 85 patients with ST-segment elevation  $\geq 0.05$  mV in lead aVR, 106 patients without ST-segment deviation, and 46 patients with ST-segment depression  $\geq 0.05$  mV in lead aVR. We concluded that both ST-segment elevation and depression in lead aVR may not be associated with a large infarct size in first anterior wall STEMI.

On the basis of the results of our 2 studies<sup>[11,12]</sup>, the association between ST-segment shift in lead aVR and emergent coronary angiographic anatomy in first anterior wall STEMI can be summarized as follows (Figure 1): (1) ST-segment elevation in lead aVR is more common in proximal occlusion of the not-long LAD; and (2) ST-segment depression in lead aVR is more common in distal occlusion of the long LAD. Acute LAD occlusion proximal to the origin of the first septal branch can produce ST-segment elevation in lead aVR through transmural ischemia in the basal portion of the interventricular septum, and acute occlusion of the long LAD can produce ST-segment depression in lead aVR though transmural

ischemia in the inferolateral and apical regions. However, it should be noted that the following conditions that can cause ST-segment elevation in lead aVR may disturb the theory: concomitant ischemia in the non-LAD region caused by multivessel disease, LV hypertrophy with strain pattern, and some types of conduction disturbances.

The current evidence suggests that in anterior wall STEMI caused by LAD occlusion, the length of the LAD and the site of occlusion of the LAD can affect ST-segment in lead aVR. The prognostic significance of ST-segment shift in lead aVR in such STEMI needs to be clarified.

### **Infarct-related coronary artery and ST-segment depression in lead aVR in inferior wall STEMI**

Inferior wall STEMI can be caused by RCA or left circumflex coronary artery (LCX) occlusion, although the RCA is much more likely to be the infarct-related vessel. A few studies<sup>[13-15]</sup> have examined whether ST-segment shift in lead aVR on the admission ECG can differentiate inferior wall STEMI caused by LCX occlusion from that caused by RCA occlusion. Nair *et al*<sup>[13]</sup> analyzed admission ECGs in 30 patients with inferior wall STEMI and found that ST-segment depression  $\geq 0.1$  mV in lead aVR had 80% sensitivity and 96% specificity to identify LCX occlusion. Sun *et al*<sup>[14]</sup> analyzed admission ECGs of 90 patients with inferior wall STEMI and showed that ST-segment depression  $\geq 0.1$  mV in lead aVR had 70.0% sensitivity and 94.3% specificity to identify LCX occlusion. In contrast, Kanei *et al*<sup>[15]</sup> showed that ST-segment depression  $\geq 0.1$  mV in lead aVR had a high specificity (86%) but a low sensitivity (53%) to identify LCX occlusion in 106 patients with inferior wall STEMI. Thus, the diagnostic value of ST-segment depression in lead aVR to identify LCX occlusion in inferior wall STEMI is not yet established.

The current evidence suggests that in inferior wall STEMI, ST-segment depression in lead aVR is more common in LCX occlusion than in RCA occlusion. Large population studies are needed to determine the diagnostic value of ST-segment depression in lead aVR to identify LCX occlusion in inferior wall STEMI.

### **Significance of ST-segment depression in lead aVR in inferior wall STEMI**

A few studies<sup>[15-17]</sup> have examined the association between ST-segment depression in lead aVR on the admission ECG and infarct size in inferior wall STEMI. Menown *et al*<sup>[16]</sup> examined 173 patients with ST-segment elevation  $\geq 0.1$  mV in inferior or lateral (I, aVL, V<sub>5</sub>, and V<sub>6</sub>) leads and found that ST-segment elevation  $\geq 0.1$  mV in inverted lead aVR (lead -aVR) was associated with a larger infarct size, as estimated by peak creatine kinase levels. Kosuge *et al*<sup>[17]</sup> examined 225 patients with a first inferior wall STEMI and found that the degree of ST-segment depression in lead aVR was an independent predictor of impaired myocardial reperfusion defined as myocardial blush grade of 0 or 1. They considered that in inferior wall STEMI, ST-segment depression in lead aVR reflects transmural ischemia extending to the inferolateral and apical walls

and that it therefore relates to a larger infarct size and impaired myocardial reperfusion. Kanei *et al*<sup>[15]</sup> reported that ST-segment depression  $\geq 0.1$  mV in lead aVR was associated with a large infarct size, as estimated by peak creatine kinase levels, in 86 patients with inferior wall STEMI caused by RCA occlusion but not in 19 patients with inferior wall STEMI caused by LCX occlusion. In 86 patients with RCA occlusion, the prevalence of a large posterolateral branch was higher in 12 patients with ST-segment depression  $\geq 0.1$  mV in lead aVR than in 74 patients without it (67% *vs* 16%,  $P = 0.0006$ ). They considered that acute occlusion of the RCA with a large posterolateral branch occlusion can cause transmural ischemia extending to the inferolateral and apical walls, resulting in ST-segment depression in lead aVR and that it therefore relates to a larger infarct size. Since their study included only 19 patients with LCX occlusion, the association between ST-segment depression in lead aVR and infarct size in inferior wall STEMI caused by LCX occlusion needs to be further investigated.

The current evidence suggests that in inferior wall STEMI caused by RCA occlusion, ST-segment depression in lead aVR is associated with the RCA with a large posterolateral branch, which would result in a large MI. The prognostic significance of ST-segment depression in lead aVR in inferior wall STEMI needs to be determined by further studies with a large sample size.

### **Large population studies on the prognostic significance of ST-segment shift in lead aVR in STEMI**

There are two large-population studies<sup>[18,19]</sup> to examine the prognostic significance of ST-segment shift in lead aVR on the admission ECG in STEMI. In a HERO-2 substudy, including 15315 patients with STEMI, Wong *et al*<sup>[18]</sup> found a U-shaped relationship between ST-segment shift in lead aVR and 30-d mortality in anterior wall STEMI. In inferior wall STEMI, only ST-segment elevation  $\geq 0.1$  mV in lead aVR was independently associated with increased 30-d mortality. However, the underlying mechanisms for the observations are unclear, because that study did not evaluate the coronary angiographic anatomy. In an APEX-AMI substudy<sup>[19]</sup>, including 5683 patients with STEMI treated by PCI, ST-segment elevation  $\geq 0.1$  mV in lead aVR was independently associated with increased 90-d mortality (HR = 5.87, 95%CI: 2.09-16.5) in inferior wall STEMI, whereas ST-segment depression  $\geq 0.1$  mV in lead aVR was independently associated with increased 90-d mortality (HR = 1.53, 95%CI: 1.06-2.22) in non-inferior wall STEMI. However, the results have to be interpreted with some cautions. First, the precise mechanisms responsible for the observations are unclear, because that study did not evaluate the detailed coronary angiographic anatomy (the site of occlusion and the length of the coronary arteries). Second, both the inferior wall STEMI group and the non-inferior wall STEMI group included patients with STEMI caused by LMT, LAD, RCA, LCX, or graft occlusion, among whom the outcome would be different. Therefore, the heterogeneity of each group might have affected the results.



The current evidence suggests that the prognostic significance of ST-segment shift in lead aVR may differ according to the site of STEMI. The exact prognostic significance of ST-segment shift in lead aVR in anterior wall STEMI and inferior wall STEMI remains to be determined.

## T-WAVE ABNORMALITY IN LEAD AVR

Although numerous studies have examined the association between T-wave abnormalities with or without ST-segment changes and cardiovascular events, the significance of T-wave abnormality in lead aVR has not been investigated until recent years. Tan *et al*<sup>[20]</sup> firstly examined the association between T-wave amplitude in lead aVR and cardiovascular mortality during a mean follow-up period of 4 years in 24270 male veterans whose electrocardiograms were obtained for any clinical reasons. In that study, an upright ( $> 0$  mV) T wave in lead aVR was found to be associated with increased cardiovascular mortality after adjusting for age and heart rate (HR = 2.8, 95%CI: 2.3-3.3). Anttila *et al*<sup>[21]</sup> examined the prognostic impact of a positive T wave ( $\geq 0$  mV) in lead aVR in 6254 subjects aged  $\geq 30$  years who participated in the field healthy examination. In that study, the positive T wave in lead aVR was observed in 2.2% of the subjects, and the relative risk for cardiovascular mortality for the positive T wave in lead aVR was 2.94 (95%CI: 1.47-2.49) after adjusting for other risk factors. In a NHANES sub-study, including 7928 participants aged  $> 40$  years, Badheka *et al*<sup>[22]</sup> showed that a positive ( $> 0$  mV) T wave in lead aVR was the strongest multivariate predictor of cardiovascular mortality (OR = 3.37, 95%CI: 2.11-5.36) and that the addition of T-wave amplitude in lead aVR to the Framingham risk score improved model discrimination and calibration with better reclassification of intermediate-risk subjects. However, in these three studies<sup>[20-22]</sup>, the underlying mechanisms for these observations are not identified.

A few studies<sup>[23,24]</sup> have investigated the significance of T-wave positivity in lead aVR in prior MI. We<sup>[23]</sup> examined 122 patients with anterior wall prior MI and found that 20 patients with a T wave ( $\geq 0.1$  mV) in lead aVR had higher pulmonary arterial, pulmonary capillary wedge, and LV end-diastolic pressures, a lower cardiac index, and a lower LV ejection fraction than 102 patients without such a T wave in lead aVR. The prevalence of a long LAD was significantly higher in the former group than in the latter group (60% *vs* 30.4%,  $P = 0.01$ ), and none of the former group had an LAD that did not reach the apex. We concluded that in anterior wall prior MI, the positive T wave in lead aVR is associated with severely reduced cardiac function, with the long LAD. In another study, we<sup>[24]</sup> examined the prognostic significance of an upright ( $> 0$  mV) T wave in lead aVR in 167 patients with a prior MI and found that the upright T wave in lead aVR was independently associated with increased cardiac death or hospitalization for heart failure for a follow-up period of  $6.5 \pm 2.8$  years (HR = 3.10, 95%CI: 1.23-7.82).

However, because of a relatively small sample size, we could not evaluate the prognostic significance of the upright T wave in lead aVR in each of anterior wall MI and non-anterior wall MI.

The current evidence suggests that the positive T wave in lead aVR is associated with cardiovascular mortality in the general population and patients with prior MI. Further studies are needed to clarify the underlying mechanisms for increased cardiovascular mortality in subjects with a positive T wave in lead aVR in the general population and determine the prognostic significance of the positive T wave in lead aVR in anterior wall MI and non-anterior wall MI.

## Q WAVE IN LEAD -AVR

In normal subjects, QRS configuration in lead aVR indicates QS pattern. We have noticed that a Q wave in lead -aVR (R wave in lead aVR) is sometimes observed in patients with anterior wall MI. Accordingly, we examined the association between a prominent Q wave (duration  $\geq 20$  ms) in lead -aVR and LV wall motion at predischARGE in 87 patients with a first anterior wall STEMI<sup>[25]</sup>. In that study, 17 patients with a prominent Q wave in lead -aVR on the predischARGE ECG was found to have a lower LV ejection fraction and more reduced regional wall motion in the apical and inferior regions than 70 patients without a Q wave in lead -aVR. Furthermore, the former had a higher prevalence of a long LAD compared to the latter (70.6% *vs* 32.9%,  $P = 0.01$ ), and none of the former group had an LAD that did not reach the apex. We concluded that in anterior wall STEMI, the prominent Q wave in lead -aVR is associated with severe regional wall motion abnormality in the apical and inferior regions, with the long LAD. Further studies are needed to clarify the clinical and prognostic significance of the prominent Q wave in lead -aVR in anterior wall MI and non-anterior wall MI.

## ORDERLY DISPLAY OF THE LIMB LEADS

The conventional display of the 6 precordial leads provides an anatomically contiguous view of the electrical activity progressing horizontally from the right anterior ( $V_1$ ) to left lateral ( $V_6$ ). In contrast, the conventional display of the 6 limb leads provides only a suboptimal representation of the electrical activity on the frontal plane. The 6 limb leads are anatomically better to be displayed by the following sequence: aVL, I, -aVR, II, aVF, and III (Figure 2). This orderly display of the 6 limb leads (known as the Cabrera format or sequence) provides a 150° view of the heart at regular 30° intervals. When using the orderly display, we can globally visualize the electrical activity on the frontal plane and easily understand the localization of the transmurally ischemic myocardium on the frontal plane in the setting of STEMI. The orderly display of the 6 limb leads has been routinely used in Sweden since the late 1970s. In 2009, the AHA/ACC/HRS recommends that ECG machines should be equipped with switching systems that will allow the limb leads to be displayed and



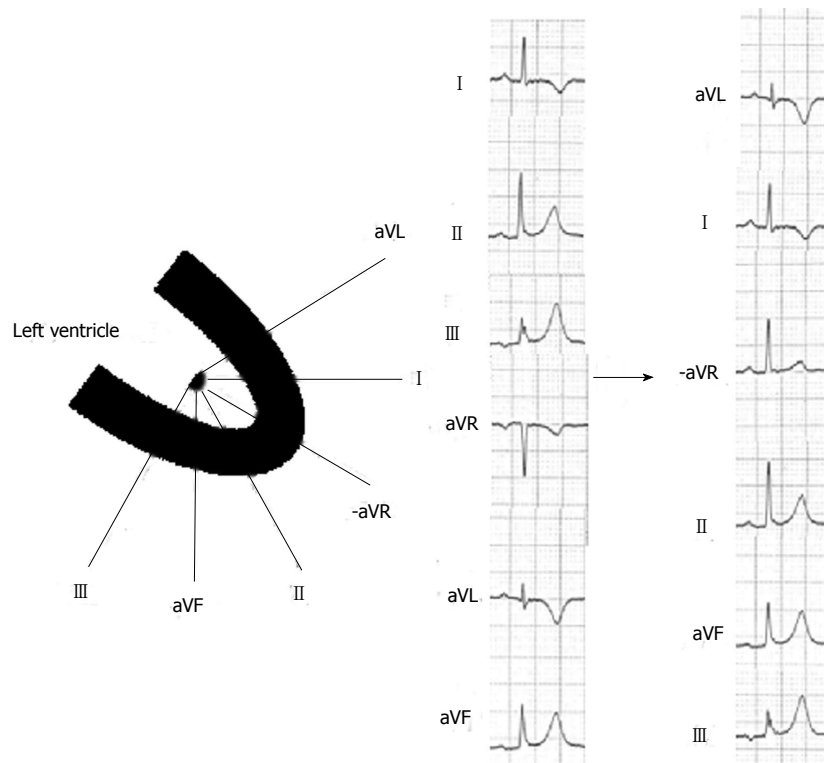


Figure 2 Orderly display of the 6 limb leads.

**Table 1 Possible mechanisms of ST-segment elevation or depression in lead aVR and coronary angiographic anatomy in acute coronary syndrome**

Lead aVR	Possible mechanisms
ST-segment elevation	Global subendomyocardial ischemia caused by LMT or 3-vessel disease Transmural ischemia in the basal portion of the interventricular septum caused by proximal LAD (especially, not-long LAD) occlusion Transmural ischemia in the right ventricular outflow tract caused by proximal occlusion of the RCA with a large conal artery Reciprocal changes opposite to ischemic or non-ischemic ST-segment depression in the lateral limb and precordial leads
ST-segment depression	Transmural ischemia in the inferolateral and apical regions caused by occlusion of the long LAD (especially, distal occlusion) Transmural ischemia in the inferolateral and apical regions caused by occlusion of the RCA with a large posterolateral branch Transmural ischemia in the inferolateral and apical regions caused by occlusion of the LCX (especially, with impaired coronary blood flow of the obtuse marginal or posterolateral branch that perfuses the inferolateral and apical regions)

LMT: Left main trunk; LAD: Left anterior descending coronary artery; RCA: Right coronary artery; LCX: Left circumflex coronary artery.

**Table 2 Current evidence concerning the prognostic significance of ST-segment elevation or depression in lead aVR in acute coronary syndrome**

Type of ACS	Findings of previous studies
NSTE-ACS	ST-segment elevation in lead aVR was independently associated with increased in-hospital mortality <sup>[4]</sup> Neither minor (0.05-0.1 mV) nor major (> 0.1 mV) ST-segment elevation in lead aVR was an independent predictor of in-hospital or 6-mo mortality <sup>[5]</sup> ST-segment depression $\geq 0.05$ mV in any lead plus ST-segment elevation $\geq 0.1$ mV in lead aVR was independently associated with increased in-hospital and 1-year cardiovascular deaths <sup>[6]</sup> ST-segment elevation $\geq 0.05$ mV in lead aVR was an independent predictor of 90-d adverse outcomes, including death, myocardial infarction, or urgent revascularization <sup>[8]</sup>
Anterior wall STEMI	U-shaped relationship between ST-segment shift in lead aVR and 30-d mortality was observed <sup>[18]</sup>
Non-inferior wall STEMI	ST-segment depression $\geq 0.1$ mV in lead aVR was independently associated with increased 90-d mortality <sup>[19]</sup>
Inferior wall STEMI	ST-segment elevation $\geq 0.1$ mV in lead aVR was independently associated with increased 30-d mortality <sup>[18]</sup> ST-segment elevation $\geq 0.1$ mV in lead aVR was independently associated with increased 90-d mortality <sup>[19]</sup>

ACS: Acute coronary syndrome; NSTE: Non ST-segment elevation; STEMI: ST-segment elevation myocardial infarction.

labeled appropriately in their anatomically contiguous sequences<sup>[26]</sup>. This useful display of the 6 limb leads should

be routinely used in everyday clinical practice.

## CAUTIONS WHEN INTERPRETING PREVIOUS DATA ON LEAD AVR

It should be noted that the point at which the magnitude of ST-segment elevation or depression in lead aVR was measured varies among previous studies on ST-segment shift in lead aVR. In “Third universal definition of MI”<sup>[27]</sup>, abnormal ST-segment elevation or depression measured at the J point is defined. Therefore, the clinical and prognostic significance of ST-segment shift in lead aVR measured at the J point has to be determined in various conditions of ACS.

## CONCLUSION

Accumulating evidence indicates that the analysis of ST-segment shift in lead aVR provides useful information on the coronary angiographic anatomy and risk stratification in various conditions of ACS. The possible mechanisms of ST-segment elevation or depression in lead aVR in ACS and the current evidence concerning the prognostic significance of ST-segment elevation or depression in lead aVR in ACS are summarized in Tables 1 and 2, respectively. It has been also shown that the analysis of T wave in lead aVR provides useful prognostic information in the general population and patients with prior MI. Cardiologists should pay more attention to ST-segment shift and T-wave positivity in lead aVR in everyday clinical practice.

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## Calpain system and its involvement in myocardial ischemia and reperfusion injury

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### Abstract

Calpains are ubiquitous non-lysosomal  $\text{Ca}^{2+}$ -dependent cysteine proteases also present in myocardial cytosol and mitochondria. Numerous experimental studies reveal an essential role of the calpain system in myocardial injury during ischemia, reperfusion and postischemic structural remodelling. The increasing  $\text{Ca}^{2+}$ -content and  $\text{Ca}^{2+}$ -overload in myocardial cytosol and mitochondria during ischemia and reperfusion causes an activation of calpains. Upon activation they are able to injure the contractile apparatus and impair the energy production by cleaving structural and functional proteins of myocytes and mitochondria. Besides their causal involvement in acute myocardial dysfunction they are also involved in structural remodelling after myocardial infarction by the generation and release of proapoptotic factors from mitochondria. Calpain inhibition can prevent or attenuate myocardial injury during ischemia, reperfusion, and in later stages of myocardial infarction.

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**Key words:** Calpain; Calpain inhibition; Calcium overload; Myocardial injury; Ischemia; Reperfusion; Myocardial infarction; Remodelling

**Core tip:** Calpains, calcium-dependant cytosolic cysteine proteases, are essentially involved in the pathophysiology of myocardial infarction. Their inhibition has shown in animal experiments an enhanced tolerance towards ischemia, a reduction of myocardial infarction and reperfusion injury, and an improvement of the process of remodelling. The availability of specific calpain inhibitors offers new prophylactic and therapeutic possibilities for patients with myocardial infarction, revascularisation and coronary surgery.

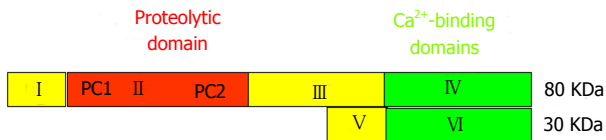
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### INTRODUCTION

Calpains are calcium-dependent, cytosolic cysteine proteases and are expressed as two “ubiquitous” isoenzymes ( $\mu$ - and m-calpains) and several “tissue specific” isoforms (n-calpains). Their primary structure contains as well calmodulin-like calcium-binding proteins as well as papain protease-like components, reflected by the term calpain<sup>[1]</sup>. A non-lysosomal  $\text{Ca}^{2+}$ -activated cysteine protease was isolated for the first time by Guroff<sup>[2]</sup> 1964 from rat brain. Calpains are meanwhile found in all cells of vertebrates that have been examined<sup>[2-5]</sup>, in cells of invertebrates<sup>[6,7]</sup> and fungi<sup>[8]</sup>, but not in bacteria and plants.

Besides their physiological functions they are also implicated in pathophysiological processes<sup>[4,9-12]</sup>, especially with disturbed calcium homeostasis<sup>[4,13,14]</sup>. Thus, calpains were found to be involved in myocardial tissue damage resulting from ischemia and reperfusion<sup>[15,16]</sup>. Calpain inhibition on the other hand ameliorates, respectively, prevents these lesions in animal experiments with potential prophylactic and therapeutic implications even in clinical





**Figure 1** Domain structure of the catalytic 80-kDa and the regulatory 30-kDa subunits of the  $\mu$ - and m-calpain dimers.

situations.

The following review will give an overview of the physiological and pathophysiological basis of the calpain system and finally focus on its role in myocardial ischemia, infarction and reperfusion and the effectiveness of calpain inhibition based on experimental studies.

## BASICS OF THE CALPAIN SYSTEM

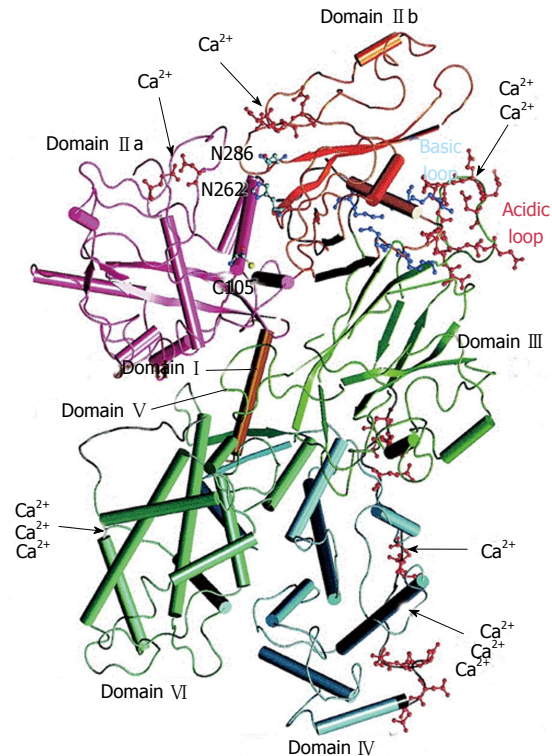
### Nomenclature

The terms  $\mu$ -calpain and m-calpain were first used by Cong *et al.*<sup>[17]</sup> in 1989. They indicate the micromolar ( $\mu$ -calpain) respectively millimolar (m-calpain)  $\text{Ca}^{2+}$ -concentrations required for their activation. Thus,  $\mu$ -calpain is activated in the presence of 3-50  $\mu\text{mol/L}$   $\text{Ca}^{2+}$  and m-calpain in the presence of 400-800  $\mu\text{mol/L}$   $\text{Ca}^{2+}$ <sup>[17,18]</sup>. Meanwhile, more than 25 proteins with structural similarities were identified as calpains or calpain-like molecules. The genes assigned to 15 of these proteins are numerically named as CAPN1 up to CAPN15 and their coded molecules are named as calpain1 up to calpain15, correspondingly. Calpain1 as well as Calpain2 are biologically active as proteases not as monomers but only as dimers with an identical 30-kDa subunit each. Both biologically active calpains are usually called  $\mu$ -calpain (calpain 1 + 30-kDa subunit) and m-calpain (calpain 2 + 30-kDa subunit), respectively<sup>[4,12]</sup>.

According to Suzuki *et al.*<sup>[19]</sup> calpains are subdivided into two main categories: (1) "typical" calpains with a calmodulin-like domain IV at their COOH-terminus; and (2) "atypical" calpains without this component. Typical calpains are  $\mu$ -calpain, m-calpain and the calpains 5, 7, 10, 13 and 15 which are also named "ubiquitous" calpains as they are present in almost all cells of vertebrates. In contrast to the "ubiquitous" calpains the "tissue-specific" calpains are exclusively expressed in special cells and tissues, such as calpain 3 in skeletal muscle<sup>[20]</sup>, calpain 6 in placenta and embryonic muscles<sup>[21]</sup>, calpain 8 and 9 in the gastrointestinal tract<sup>[22]</sup>, calpain 11 in the testis<sup>[23]</sup>, and calpain 12 in hair follicles<sup>[24]</sup>.

### Domain structure of $\mu$ - and m-calpain

Both proteases  $\mu$ - and m-calpain exist as dimers with two subunits of 80-kDa and 30-kDa each (Figure 1)<sup>[25,26]</sup>. The larger 80-kDa catalytic subunits of  $\mu$ -calpain and m-calpain are coded in humans by different genes on chromosome 11 respectively chromosome 1<sup>[27]</sup>. On the base of their amino acid sequences they are composed of four regions/domains: (1) a N-terminal domain; (2) a catalytic CysPc protease domain consisting of two protease core regions PC1 and PC2; and (3) a C2-like  $\text{Ca}^{2+}$ -regulated



**Figure 2** Crystallographic structure of human m-calpain by Suzuki *et al.*<sup>[33]</sup>.

phospholipid-binding domain, and IV a  $\text{Ca}^{2+}$ -binding penta-EF-hand domain<sup>[28-31]</sup>.

Domain I contains an amphipathic alpha-helix in the N-terminus of  $\mu$ -calpain which was shown to be important in targeting and migrating of  $\mu$ -calpain into the intermembrane space of mitochondria. Domain I of m-calpain, however, does not contain a similar N-terminal component<sup>[32]</sup>.

Domain II represents the catalytic CysPc protease domain. It consists of two separate protease core domains PC1 with a cysteine (Cys) residue and PC2 with a histidine (His) residue and an asparagine (Asn) residue. These residues form a catalytic triade as known from cysteine proteases such as papain or cathepsin (Figure 2). Both core domains PC1 and PC2 have  $\text{Ca}^{2+}$ -binding sites for a single  $\text{Ca}^{2+}$  by each<sup>[33,34]</sup>.

Domain III is structurally related to C2 domains and can bind phospholipids in a  $\text{Ca}^{2+}$ -dependent manner. It links the  $\text{Ca}^{2+}$ -binding domains with the catalytic domain II and is supposed to be involved in the adjustment of the calpain activity *via* electrostatic interactions<sup>[35]</sup>.

Domain IV shows a slight sequence homology to calmodulin (51%-54%) and has five  $\text{Ca}^{2+}$ -binding COOH-terminal EF-hand motifs. The fifth motif binds to the corresponding EF-hand sequences of domain VI of the smaller 30 kDa subunit and, thus, contributes to the dimer formation of both calpain subunits<sup>[4,31,33,36]</sup>.

The smaller regulatory 30 kDa subunit, responsible for the stability of the larger catalytic subunit, consists of the N-terminal Gly-rich domain V and the  $\text{Ca}^{2+}$ -binding calmodulin-like penta-EF-hand domain VI. The long stretches of Gly residues and an unordered structure of

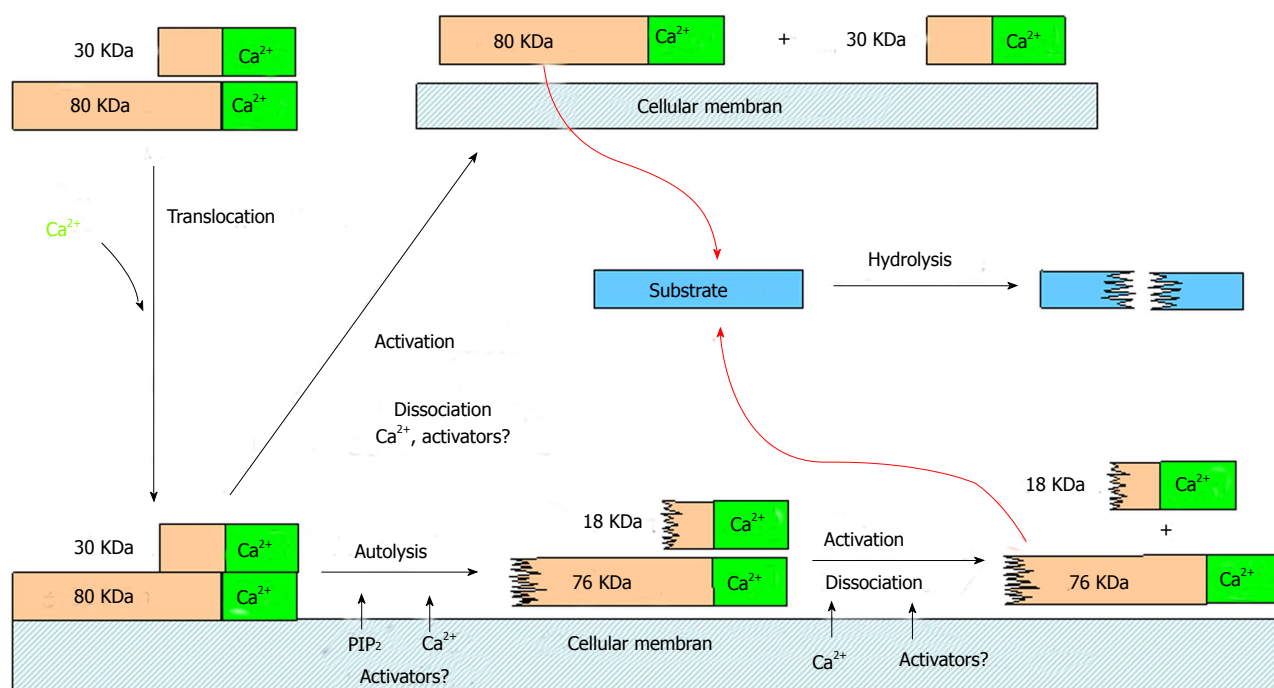


Figure 3 Mechanisms and consequences of calpain activation at biological membranes. Modified from Suzuki *et al.*<sup>[40]</sup>.

the amino acid sequence in domain V are supposed to bind to other molecules and structures.

The “calmodulin-like” domain VI is involved in  $\text{Ca}^{2+}$ -binding and dimerization by their penta-EF-hand motifs, as also known from domain V of the 80-kDa subunit<sup>[4,31,37,38]</sup>.

### Activation of $\mu$ - and m-calpain

Increase of the intracellular  $\text{Ca}^{2+}$ -concentration is the decisive trigger for calpain activation. The  $\text{Ca}^{2+}$ -binding core domains PC1 and PC2 of domain II and the terminal EF-hand motifs of domain V and VI cause electrostatic conformational changes in these domains. By this electrostatic switch mechanism the PC1 and PC2 core domains approaches each other. Thus the distance of the Cys-residue from the  $\alpha$ His- and Asn-residues of the initially inactive catalytic triade shrinks from 10 to approximately 3.7 Å to form the proteolytic active centre<sup>[30,39]</sup>. Simultaneously, the change of conformation intensifies the affinity of calpain to membrane phospholipids and thus induces its translocation to the cell membranes (Figure 3)<sup>[40,41]</sup>.

Immediately with the binding of  $\text{Ca}^{2+}$  the autolysis of both subunits of the calpain dimers happens by splitting off the NH2-terminal amino acids. The 80-kDa subunits of  $\mu$ - and m-calpain are reduced by this process to active fragments of 76-kDa and 78-kDa, respectively, and both 30-kDa subunits are reduced to fragments of 18-kDa each<sup>[42-44]</sup>. The autolysis facilitates the dissociation and re-association of the calpain dimers, but is not necessary for their activation, as the dissociated 80-kDa subunits are enzymatically full active<sup>[45]</sup>.

Confusion still exists with regard to the  $\text{Ca}^{2+}$ -concentration required for calpain activation. The *in vitro* con-

centrations for  $\mu$ -calpain (3-50  $\mu\text{mol/L}$ ) and m-calpain (200-1000  $\mu\text{mol/L}$ ) to cause a half-maximal calpain activity are far above the physiological concentrations of 100-300 nmol/L necessary in living cells<sup>[46-48]</sup>. Additional mechanisms and factors are therefore supposed to contribute to the activation and activity in a physiological environment. Autolysis is known to increase the  $\text{Ca}^{2+}$ -sensitivity of  $\mu$ - and m-calpain for activation<sup>[19,49]</sup>, however, the problem remains, that far higher  $\text{Ca}^{2+}$ -concentrations are required to initiate autolysis as they occur in a physiological environment<sup>[50]</sup>. Autolysis normally happens in contact with biological membranes in presence of phospholipids such as  $\text{PIP}_2$  which considerably reduces the  $\text{Ca}^{2+}$ -concentration necessary for autolysis<sup>[10,51]</sup>. Thus, in presence of  $\text{PIP}_2$  autolysis of  $\mu$ -calpain already happens with  $10^{-5}$ - $10^{-7}$  mol  $\text{Ca}^{2+}$ .

In addition, activator proteins from rat brain lower the  $\text{Ca}^{2+}$ -concentrations necessary for autolysis of  $\mu$ -calpain to a tenth<sup>[52]</sup> and from rat skeletal muscle for autolysis of m-calpain from 400  $\mu\text{mol/L}$  to 15  $\mu\text{mol/L}$ <sup>[53]</sup>. Both activators are  $\text{Ca}^{2+}$ -binding proteins combining with calpains and becoming effective upon contact with cell membranes. Further activator proteins are known which increase the catalytic activity of calpains against particular substrates twice<sup>[54]</sup>, ten times<sup>[55]</sup> or twenty-five times<sup>[56]</sup> without influencing the required  $\text{Ca}^{2+}$ -concentration.

### Regulation of calpain activity

Calpastatin is the only known specific endogenous inhibitor and regulator of  $\mu$ - and m-calpain. In addition also H-kininogen and  $\alpha$ 2-macroglobulin are inhibiting calpain besides other proteases<sup>[57]</sup>. Human calpastatin is encoded by a single gene on chromosome 5<sup>[58]</sup> and expressed in several isoforms from 17.5 to 107 kDa<sup>[59-61]</sup>.

It consists of four inhibitory domains I, II, III and IV, and one N-terminal domain L without inhibitory capability<sup>[62, 63]</sup>. Each inhibitory unit inhibits one calpain molecule competitively by blocking the substrate access to the catalytic centre<sup>[64, 65]</sup>. Calpastatin inhibits exclusively calpain and not other proteases<sup>[57]</sup>. Binding of calpastatin to calpain and its inhibition is  $\text{Ca}^{2+}$ -dependent. The  $\text{Ca}^{2+}$ -concentrations for this are lower as needed for the half-maximal proteolytic activity of  $\mu$ - and m-calpain<sup>[66]</sup>. Calpains and calpastatin are found in physical proximity within the cells<sup>[67, 68]</sup>. Therefore, mechanisms are necessary to enable calpain to perform its biological purpose, since calpastatin already binds to calpain with increasing  $\text{Ca}^{2+}$ -concentrations. Thus, the translocation of calpain to the membranes could cause a spatial distance to calpastatin. Furthermore, special mechanisms/factors could lower the threshold for  $\text{Ca}^{2+}$  to activate calpain without influencing the binding of calpastatin<sup>[3]</sup>. With regard to activation and deactivation of calpain many questions are still open concerning a regulating, respectively, modifying role of substrate phosphorylation.

#### Localization of $\mu$ - and m-calpain in cell and tissue

In all examined cells of vertebrates  $\mu$ -calpain, m-calpain and calpastatin are found at least as the only constituents of the calpain system or they exist in various combinations with great varying patterns of distribution. Thus, human erythrocytes and platelets only contain  $\mu$ -calpain, and smooth muscles of vessels and stomach predominantly contain m-calpain, whereas, in skeletal muscles and kidneys of the most representatives of vertebrates nearly equal amounts of  $\mu$ - and m-calpain are found<sup>[67, 69, 70]</sup>. Both calpains as well as calpastatin are exclusively localized intracellular and apparently associated with subcellular structures. Thus, 93% of the  $\mu$ -calpain are found in human red blood cells within the cytosol and 7% membrane associated<sup>[71]</sup>. Most of the  $\mu$ -calpain, m-calpain and calpastatin is localized close to the Z-disc in the myofibrils of skeletal and cardiac muscle, smaller amounts are found in the I- and A-bands. In mitochondria and nuclei only a tenth, respectively, a fifth of calpains and calpastatin was identified compared to their concentration in the Z-disc region<sup>[67, 72, 73]</sup>. Calpain and calpastatin are normally localized with a close spatial proximity.

#### Substrates for calpain

Normally, calpains have only access to intracellular substrates, whereby their cleavage decisively depends on the local activity of calpain and its inhibitor calpastatin. Many proteins are cleaved by calpains *in vitro*, but there is no conclusive evidence that they cannot also be splitted by calpain *in vivo*.

Calpain cleaves the cytoskeleton and membrane-associated proteins: adducin<sup>[74]</sup>, ankyrin<sup>[75]</sup>, caldesmon<sup>[9]</sup>, cadherin<sup>[76, 77]</sup>, C-protein<sup>[78]</sup>, desmin<sup>[79]</sup>, dystrophin<sup>[80]</sup>, the filamin/actin-binding proteins MAP1 and MAP2<sup>[81]</sup>, myosin<sup>[82]</sup>, the neurofilament-proteins NFH, NFM and NFL<sup>[83]</sup>, NR2-subunit<sup>[84]</sup>, the anchoring protein PSD-95

of NMDA-receptors<sup>[85]</sup>,  $\alpha$ II-spectrin<sup>[16]</sup>,  $\beta$ -spectrin<sup>[86]</sup>, talin<sup>[87, 88]</sup>, titin<sup>[89]</sup>, tropomyosin and troponin I<sup>[78]</sup>, troponin T<sup>[90]</sup>, vimentin<sup>[79, 91]</sup>, and vinculin<sup>[92]</sup>.

Furthermore, kinases, phosphatases and transcription factors are cleaved, such as: EGF-receptor-kinase<sup>[93]</sup>, myosin light-chain kinase<sup>[94]</sup>, protein-kinase C<sup>[95]</sup>, calcineurin<sup>[96]</sup>, inositol-polyphosphat-4-phosphatase<sup>[97]</sup>, protein-tyrosin-phosphatase-1B<sup>[98]</sup>, the transcription factors c-Jun, c-Fos<sup>[99, 100]</sup>, and p53<sup>[101, 102]</sup>.

## PHYSIOLOGICAL FUNCTIONS AND PATHOPHYSIOLOGICAL IMPLICATIONS OF THE CALPAIN SYSTEM

### Physiological function of $\mu$ - and m-calpain

Calpains are not seen to play an essential role in the intracellular protein digestion. In contrast to lysosomal proteases and the proteasome calpains split proteins by a limited proteolysis into large fragments with potential regulatory and signalling functions<sup>[4]</sup>. Many studies including experiments with transgenic mice indicate, that calpains are involved in the embryonic development and cell function<sup>[103-105]</sup>, cytoskeletal/membrane attachments/cell motility<sup>[79, 81, 86-88, 106]</sup>, intracellular signal transduction<sup>[95, 107-109]</sup>, cell cycle<sup>[110, 111]</sup>, regulation of gene expression<sup>[99, 101]</sup>, apoptosis<sup>[112-115]</sup>, and in the long-term potentiation of synaptic transmission<sup>[84, 85, 116]</sup>.

### Involvement of calpains in inherited and acquired diseases

A lacking synthesis of calpains or the dysregulation of the calpain activity disturbing the proteolysis of structural and regulatory proteins is found in a series of genetic and acquired diseases, such as: limb girdle muscular dystrophy (LGMD2A)<sup>[117, 118]</sup>, muscular dystrophy (type Duchenne and Becker)<sup>[119]</sup>, diabetes mellitus (type 2)<sup>[120]</sup>, gastric cancer<sup>[121]</sup>, Alzheimer's disease<sup>[122-125]</sup>, multiple sclerosis<sup>[126, 127]</sup>, and cataract formation<sup>[127]</sup>.

## THE KEY ROLE OF CALCIUM HOMEOSTASIS WITHIN THE CALPAIN SYSTEM

### Regulation of $\text{Ca}^{2+}$ -homeostasis

Many vital cell functions are regulated by the concentration of intracellular available  $\text{Ca}^{2+}$ , such as muscle contraction, neurotransmitter release, glandular secretion, and intercellular communication<sup>[128, 129]</sup>. And last but not least, calpains are  $\text{Ca}^{2+}$ -activated proteases. Because of its key role, normally the  $\text{Ca}^{2+}$  concentration is controlled at different cellular levels *via* mitochondria, plasmalemma/sarcolemma and endoplasmatic reticulum. The transmembrane transport of ions is regulated actively, selectively and directionally-oriented by voltage gated ion channels, by ATP-consuming ion pumps ( $\text{Na}^+$ - $\text{K}^+$ -ATPases,  $\text{Ca}^{2+}$ -ATPases, proton-ATPases) and by the concentration gradient due to carrier proteins ( $\text{Na}^+$ / $\text{H}^+$ -exchanger,



$\text{Na}^+/\text{HCO}_3^-$ -symporter,  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger)<sup>[130-133]</sup>. Failing of this control mechanisms may result in an excessive intracellular accumulation of  $\text{Ca}^{2+}$  ( $\text{Ca}^{2+}$ -overload) with severe cellular dysfunction up to cell death<sup>[14,134,135]</sup>.

### Events with increasing myocardial $\text{Ca}^{2+}$ concentration

Studies with isolated perfused mammalian hearts have shown an increasing cytosolic  $\text{Ca}^{2+}$  concentration during hypoxia in hearts of rabbits<sup>[136]</sup> and ferrets<sup>[137]</sup>, during ischemia in hearts of rabbits<sup>[138]</sup> and rats<sup>[139]</sup>, and during post-ischemic reperfusion in hearts of rats<sup>[140]</sup> and ferrets<sup>[141]</sup>. Severe burn trauma also augments the  $\text{Ca}^{2+}$  content in myocytes<sup>[142,143]</sup> and mitochondria<sup>[144]</sup> of rat hearts. The same effect can be observed upon exposure of isolated perfused rabbit hearts<sup>[145]</sup> and isolated rat cardiomyocytes<sup>[146,147]</sup> to hydroxyl free radicals. In analogy to the heart, a  $\text{Ca}^{2+}$ -overload was also observed in rat brains<sup>[148,149]</sup> during hypoxia/ischemia and in the spinal cord<sup>[150]</sup> after traumatization.

### Disturbance of $\text{Ca}^{2+}$ homeostasis in the heart:

#### Pathomechanisms and consequences

The underlying mechanisms and consequences of an imbalance in  $\text{Ca}^{2+}$  homeostasis are documented the most extensively in heart during hypoxia, ischemia and post-ischemic reperfusion. They are initiated by the decreasing ATP generation and developing acidosis resulting from oxygen deficiency. The activation of the  $\text{Na}^+/\text{H}^+$ -exchanger (NHE-1)<sup>[152,151,152]</sup>, which causes the influx of  $\text{Na}^+$  into the cell for exchange with  $\text{H}^+$  in order to regulate pH, and the simultaneous inhibition of the  $\text{Na}^+/\text{K}^+$ -ATPase<sup>[153]</sup>, due to lack of ATP, plays a key role in the intracellular  $\text{Ca}^{2+}$ -overload. Thus,  $\text{Na}^+$  accumulates intracellular and lowers the transmembranous  $\text{Na}^+$  gradient, which is the driving force behind the  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger by transporting  $\text{Ca}^{2+}$  out off the cell, resulting in  $\text{Ca}^{2+}$ -accumulation. The  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger which represents a bidirectional transport system is also able to transport  $\text{Ca}^{2+}$  in exchange with  $\text{Na}^+$  in a reverse mode into the cell<sup>[152,154,155]</sup>. Driving forces for this are the increasing intracellular  $\text{Na}^+$  concentration and depolarisation of the sarcolemma.

Today, disturbance of  $\text{Ca}^{2+}$ -homeostasis is seen as the main triggering factor of cardiac dysfunction and myocardial injury during ischemia and reperfusion, such as the myocardial stunning, a long-lasting reversible reduction of heart contraction after ischemia<sup>[156-158]</sup>, or like the  $\text{Ca}^{2+}$ -overload induced hypercontracture during reperfusion/reoxygenation<sup>[14,159-161]</sup>, or the incidence of arrhythmias during reperfusion<sup>[162]</sup>. Other factors, such as reactive oxygen species or inflammation seem to play a minor role in these situations<sup>[163]</sup>.

Many studies demonstrate as a consequence of an increasing intracellular  $\text{Ca}^{2+}$ -concentration the activation of calpains, which cleave numerous functional and structural proteins, and thereby decisively contribute to ischemic and postischemic injury. Thus, the activation of the calpain system during hypoxia or ischemia is well documented in the myocardium of rats<sup>[164-167]</sup> and humans<sup>[168]</sup>,

as well as in the brain of rats<sup>[169-171]</sup>. In rat renal proximal tubules hypoxia induces the increase of  $\mu$ -calpain activity<sup>[172]</sup>, whereas calpain inhibition reduces the renal functional and structural damage following ischemia and reperfusion<sup>[173]</sup>. Hypoxia was also found to up-regulate the activity and gene expression of calpains in endothelial cells of the pulmonary artery<sup>[174]</sup>.

## ROLE OF CALPAINS IN MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

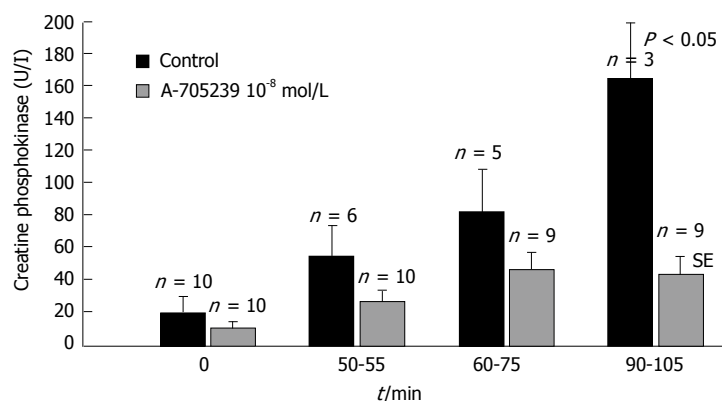
### Global ischemia

Most studies on the implication of calpains for myocardial dysfunction and failure are based on experiments in isolated perfused mammalian hearts, in which the duration of perfusion stop (global ischemia) is restricted to enable at least a recovery with reperfusion.

Global ischemia in isolated perfused rat hearts was found to induce a time-dependent translocation of m-calpain to the membrane initially not associated with calpain activation which occurred only during reperfusion and intracellular pH normalization<sup>[175]</sup>. Under comparable conditions, a loss of myofibrillar desmin,  $\alpha$ -actinin, and spectrin was observed in guinea pig hearts, which was reduced by calpain inhibitor I<sup>[176]</sup>. Immunohistochemical studies revealed the proteolysis of caldesmon and  $\alpha$ -fodrin at the intercalated discs and the sarcolemma after post-ischemic reperfusion in rat hearts. Degradation of both proteins could be suppressed and myocardial function improved by calpain inhibitor I<sup>[16,177]</sup>. The inhibition of  $\alpha$ -fodrin degradation associated with the attenuation of myocardial dysfunction could also be observed after cardioplegic cardiac arrest in rat hearts in the presence of calpain inhibitor SNJ-1945<sup>[178]</sup>. As a result of calpain activation, the essential  $\text{Ca}^{2+}$ -handling proteins  $\text{Ca}^{2+}$ -ATPase (SERCA2a) and the SERCA regulatory protein PLB were degraded upon global ischemia and reperfusion in a working rat heart preparation. Their degradation, the depression of cardiac performance and the release of lactate dehydrogenase, indicating the myocardial damage, could be significantly attenuated by calpain inhibition with calpain inhibitor III (MDL28170)<sup>[179]</sup>. As an indicator of myocardial tissue damage creatine phosphokinase and lactate dehydrogenase are released from myocytes into the perfusion fluid during reperfusion in concentrations dependent on the duration of ischemia (Figure 4). Calpains seem to be responsible or to contribute to these effects, as calpain inhibition with A-705239 significantly reduces the enzyme release<sup>[180]</sup>.

Cardiac muscle contraction is initiated by  $\text{Ca}^{2+}$  via troponin/tropomyosin which are known as substrates of calpain. Therefore, their cleavage is supposed to be jointly responsible for myocardial dysfunction in ischemia/reperfusion injury. With regard to this, degradation of troponin T (TnT) was observed during ischemia/reperfusion of isolated perfused rat hearts and was reduced by calpain inhibition with PD150606 and PD151746<sup>[181]</sup>. In addition, "overexpression of calpastatin by gene trans-





**Figure 4** Release of creatine phosphokinase into the perfusion fluid of isolated rabbit hearts subjected to ischemia and reperfusion<sup>[180]</sup>. Control experiments without inhibitor are represented by black-coloured columns and inhibitor (A-705239 10<sup>-8</sup> mol/L) treated hearts by grey-coloured columns. Data are expressed as means  $\pm$  SE of  $n = 10$  experiments each. Both groups differ significantly ( $P < 0.05$ ) at the end of reperfusion.

fer prevents troponin I (TnI) degradation and ameliorates contractile dysfunction in rat hearts subjected to global ischemia followed by reperfusion<sup>[182]</sup>.

### Mitochondrial function impairment

Damage of mitochondria plays a central role in the pathophysiology of reperfusion injury *via* the impairment of oxidative metabolism, respectively, energy production and the generation and accumulation of metabolic products toxic to the myocytes. Cardiac mitochondria are located subsarcolemmal beneath the plasma membrane and interfibrillar between the myofibrils<sup>[183-185]</sup>. In animal and human hearts  $\mu$ -calpain, m-calpain and calpain 10 are present in cytosol and in the intermembrane space of mitochondria<sup>[67,186-189]</sup>. Cytosolic calcium content is found to increase in hearts of rats and rabbits during myocardial ischemia and reperfusion and is made responsible for the subsequent activation of calpains<sup>[190,191]</sup>. The damage of Ca<sup>2+</sup>-handling proteins by direct cleaving or detaching the Na<sup>+</sup>/K<sup>+</sup>-ATPase and the Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger from their binding ankyrin<sup>[174,192]</sup>, and by proteolysis of sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA)<sup>[179,193]</sup> and Ryanodine receptor RyR<sup>[194]</sup>, sustains Ca<sup>2+</sup>-influx and calpain activation and aggravates myocardial injury. Thus, SERCA2a and the SERCA regulatory protein PLB were found to be degraded upon global ischemia and reperfusion in a working rat heart preparation. Their degradation, the depression of cardiac performance and the release of lactate dehydrogenase, indicating the myocardial damage, could be significantly attenuated by calpain inhibition with calpain inhibitor III (MDL28170)<sup>[179]</sup>.

One of the most serious consequences of mitochondrial damage by calpains is the impairment of oxidative phosphorylation with loss of ATP generation. Damage to mitochondrial oxidative metabolism can be caused on various levels of the electron transport chain (ETC). In isolated renal cortical mitochondria from rats and rabbits calpain 10 was shown to cleave complex I subunits of the ETC, which could be prevented by pretreatment with calpeptin<sup>[195]</sup>. The impairment of mitochondrial respiration is documented in isolated perfused rabbit hearts<sup>[180,196]</sup>. State 3 respiration decreased significantly during 45 min of global ischemia and further decreased during 60 min of reperfusion, and this reaction could be

significantly attenuated by addition of calpain inhibitor A-705239 to the perfusion fluid (Table 1).

Reduced state 3 respiration reflects the impairment of the electron transport chain (ETC), above all complex I, which is an early target of myocardial ischemia<sup>[197]</sup>.

Calpain inhibitor A-705239 administered before ischemia and reperfusion also attenuated the increase in permeability of the inner mitochondrial membrane (mitochondrial permeability transition), as reflected by the reduced state 4 respiration and leak-respiration<sup>[180]</sup>.

Besides their deleterious effect on mitochondrial oxidative metabolism, calpains are also recognized to cause the generation and release of substances toxic to myocytes.

During reperfusion, mitochondria generate reactive oxygen species that lead to additional mitochondrial and myocyte injury<sup>[197-200]</sup>.

Dependent on the degree of oxidative damage in concert with mitochondrial calcium overload and calpain activation, mitochondrial permeability transition can occur by formation of inner membrane pores<sup>[201,202]</sup>. Mitochondrial permeability transition can result in disruption of the outer mitochondrial membrane and the release of cytochrome c, a key step inducing apoptosis<sup>[203]</sup>. Cytochrome c is detectable in the cytosol of rabbit myocardium at 30 min of ischemia<sup>[204]</sup>, whereas cytochrome c content decreases in subsarcolemmal mitochondria<sup>[205]</sup>. Mitochondrial calpain plays an important role in programmed cell death by generation or release of apoptotic factors in mitochondria during ischemia and reperfusion. Thus, the cleavage of Bid, a pro-apoptotic BH3-only Bcl-2 family member, is documented in isolated perfused adult rabbit hearts during ischemia/reperfusion, and in secondary *in vitro* studies recombinant Bid was cleaved by calpain to an active fragment that was able to mediate cytochrome c release<sup>[206]</sup>. It was also shown, that activated mitochondrial  $\mu$ -calpain, mostly located in the intermembrane space, cleaves and releases apoptosis inducing factor (AIF) from isolated mouse heart mitochondria. Besides, mitochondrial  $\mu$ -calpain activity increased in buffer perfused mouse hearts during ischemia/reperfusion whereas the mitochondrial AIF content decreased. Inhibition of mitochondrial  $\mu$ -calpain using MDL-28170 preserved the AIF content within the mitochondria and

**Table 1** Effect of calpain inhibitor A-705239 on impairment of mitochondrial function following myocardial ischemia and reperfusion<sup>[180]</sup>

	<i>n</i>	State 3 respiration (nmol O <sub>2</sub> /min per milligram)	State 4 respiration (nmol O <sub>2</sub> /min per milligram)	RCI (state3 rate): (state 4 rate)	Leak respiration(nmol O <sub>2</sub> /min per milligram)	Stimulation by cytochrome c %
Control						
Before ischemia	4	6.4 ± 1.1	0.5 ± 0.1	12.5 ± 2.7	0.15 ± 0.07	6.0 ± 10.0
Ischemia 45 min	8	3.5 ± 1.4 <sup>abc</sup>	0.9 ± 0.3 <sup>a</sup>	4.4 ± 2.5 <sup>a</sup>	0.32 ± 0.14 <sup>a</sup>	10.0 ± 6.0
Reperfusion 60 min	4	2.6 ± 1.3 <sup>abc</sup>	0.9 ± 0.3 <sup>a</sup>	3.2 ± 2.1 <sup>a</sup>	0.43 ± 0.29	28.0 ± 16.0
A-705239 treated hearts						
Before ischemia	4	6.8 ± 1.3	0.6 ± 0.1	12.4 ± 1.1	0.12 ± 0.06	16.0 ± 9.0
Ischemia 45 min	9	5.0 ± 0.8 <sup>abc</sup>	0.6 ± 0.2	8.2 ± 2.3 <sup>abc</sup>	0.20 ± 0.14 <sup>a</sup>	15.0 ± 13.0
Reperfusion 60 min	5	4.2 ± 1.2 <sup>abc</sup>	0.7 ± 0.2	6.4 ± 2.7 <sup>a</sup>	0.26 ± 0.24	

Data are presented as means of 4 to 9 experiments mean ± SD measured as duplicates or triplicates. A significant difference from baseline before ischemia is represented by <sup>a</sup>*P* < 0.05, and between both groups by <sup>c</sup>*P* < 0.05.

reduced cardiac injury<sup>[186]</sup>.

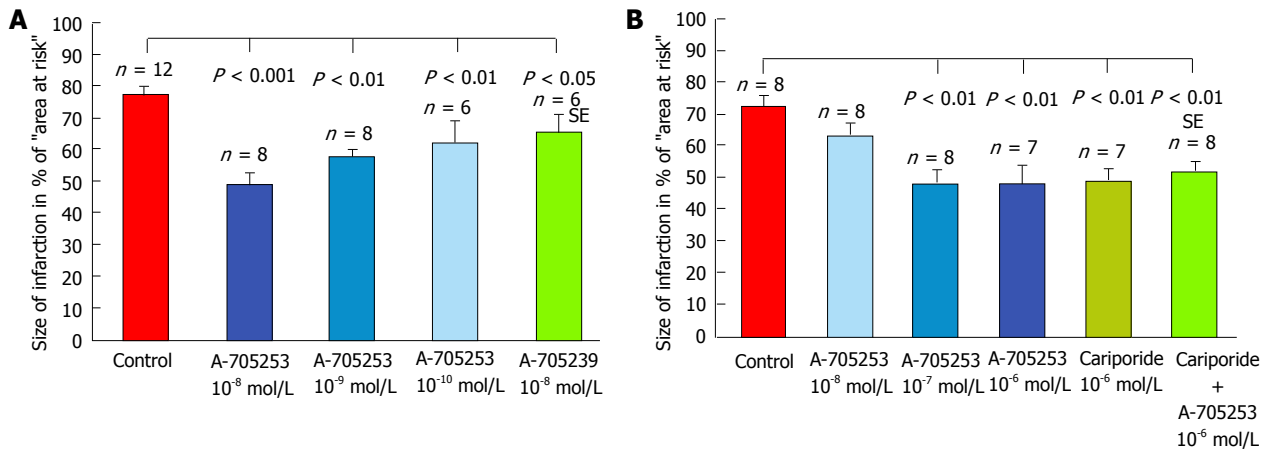
### Partial ischemia and myocardial infarction

In contrast to models of global ischemia, in the experimental setting of partial ischemia by temporary occlusion of coronary arteries the duration of ischemia can be extended in time to enable irreversible myocardial damage to a restricted area with myocardial infarction without the risk of early global heart failure with reperfusion. In isolated perfused rat hearts it was shown, that during a 30 min occlusion of the left anterior descending coronary artery calpain translocates to the cell membranes without being activated initially. Calpain activation, as indicated by the hydrolysis of  $\alpha$ -fodrin, only started with the onset of reperfusion and could be prevented by calpain inhibition with MDL-28170, just as the infarct size could be reduced by 32%<sup>[175]</sup>.

Inhibition of  $\alpha$ -fodrin degradation and improvement of left ventricular function by calpain inhibitor SNJ-1945, administered 30 min before a gradual and partial coronary occlusion, was also found after mild ischemic-reperfusion in another study in rat hearts<sup>[207]</sup>. Protecting effects of calpain inhibition on myocardial injury could also be demonstrated by own experiments with inhibitor administration both before and during reperfusion. "Two novel calpain inhibitors (A-705239 and A-705253) were studied in isolated perfused rabbit hearts subjected to a 60 min occlusion of the ramus interventricularis of the left coronary artery (below the origin of the first diagonal branch), followed by 120 min of reperfusion<sup>[208,209]</sup>. The inhibitors were added to the perfusion fluid in various final concentrations from the beginning of the experiments before the coronary artery was blocked. The infarct size was significantly reduced in presence of both calpain inhibitors. The best effect was achieved with 10<sup>-8</sup> mol/L A-705253 which reduced the infarcted area by 61.8 % (Figure 5A). In a second study in isolated perfused rabbit hearts subjected to a 60 min occlusion of the ramus interventricularis of the left coronary artery followed by 120 min of reperfusion calpain inhibitor A-705253 and/or the Na<sup>+</sup>/H<sup>+</sup>-exchange inhibitor cariporide<sup>®</sup> were added to the perfusion fluid at the beginning of reperfusion solely or in combination<sup>[210]</sup>. The infarct size was signifi-

cantly reduced dose-dependently in presence of both inhibitors (Figure 5B). The best effect was achieved with 10<sup>-6</sup> mol/L A-705253, which reduced the infarcted area by 33.6%. Cariporide<sup>®</sup> (10<sup>-6</sup> mol/L) reduced the infarct size in the same extent. The combination of both inhibitors, however, didn't further improve cardioprotection. Thus, the protective effect can be attributed exclusively to its influence on the calpain system, since the combination of both inhibitors didn't augment the protective effect of sole calpain inhibition. The calpain inhibitor A-705253 is known to directly block the catalytic centre of activated calpains, whereas the Na<sup>+</sup>/H<sup>+</sup>-exchange inhibitor cariporide<sup>®</sup> prevents or reduces the ischemic intracellular Ca<sup>2+</sup>-overload and thus prevents or reduces the following calpain activation". This is shown in postischemic perfused rat and rabbit hearts where reduced calpain activation<sup>[211]</sup> and calcium overload<sup>[212]</sup> were observed upon inhibition of Na<sup>+</sup>/H<sup>+</sup>-exchange. Even in patients undergoing coronary bypass surgery pretreatment with cariporide<sup>®</sup> reduced mortality and the risk of myocardial infarction<sup>[213]</sup>, however, cerebrovascular events increased<sup>[214]</sup>. In accordance with the findings in rabbit hearts, also in pigs undergoing occlusion of the left anterior descending coronary artery for 45 min followed by 6 h of reperfusion infarct size was reduced by 35% and hemodynamic alterations attenuated using calpain inhibitor A-705253<sup>[215]</sup>. In experiments with isolated mouse hearts undergoing ischemia and reperfusion infarct size was decreased and ventricular function improved in calpain-1 knockout mice, whereas myocardial injury was greatly increased in transgenic mice hearts with calpain-1 overexpression<sup>[216]</sup>.

No sufficient information is available to what extent polymorphonuclear leukocytes (PMN) contribute to ischemic/reperfusion injury. In one study in isolated rat hearts perfused with PMNs, exposed to 20 min of ischemia and followed by 45 min of reperfusion, calpain inhibition with Z-Leu-Leu-CHO reduced the adherence of PMNs to the vascular endothelium and improved ventricular function, however, controls without PMNs are missing<sup>[217]</sup>. Thus, with regard to the numerous experiments discussed in this review, which were all performed without PMNs in the perfusion fluid, polymorphonuclear leukocytes appear not to be essential for reperfusion



**Figure 5** Development of myocardial infarction in isolated perfused rabbit hearts after occlusion of ramus interventricularis of left coronary artery for 60 min, followed by 120 min of reperfusion<sup>[209]</sup>. A: The inhibitors were added to the perfusion fluid before ischemia; B: With reperfusion. Infarct size is expressed in percentage of the area at risk (the transiently not perfused myocardium). Control experiments without inhibitor are represented by a red-coloured column and inhibitor treated hearts by blue-coloured columns. Data are presented as means  $\pm$  SE. Infarct size is significantly reduced by calpain inhibition in all treated hearts compared to untreated controls.

injury.

### Remodelling after myocardial infarction

Myocardial infarction is followed by a progressive structural remodelling of the heart, replacing and reconstructing the irreversibly damaged myocardium<sup>[186,203,206]</sup>. After the early phase of ischemia-induced myocyte necrosis a longer lasting myocyte death by apoptosis can be observed. Proapoptotic factors are generated and released from myocardial mitochondria already during ischemia and reperfusion which are considered to be essentially involved in remodelling after myocardial infarction<sup>[186,203,206]</sup>. Characteristics of apoptosis, DNA fragmentation and chromatin condensation, could be detected in isolated perfused rabbit hearts subjected to 30 min ischemia and 4 h reperfusion<sup>[220]</sup>. In ischemic/reperfused rat hearts undergoing 30 min coronary occlusion followed by 6 h reperfusion the administration of calpain inhibitor I (CAI) 10 min before reperfusion significantly reduced DNA fragmentation and infarct size<sup>[221]</sup>. Comparable results were achieved in mouse hearts with persistent coronary artery ligation for 4 d. Calpain inhibition with calpeptin was started 15 min before artery occlusion and continued during the observation time. Calpeptin administration reduced apoptotic cell death, as detected by TUNEL staining, and reduced infarct size and myocardial dysfunction<sup>[222]</sup>. The important contribution of calpains to the process of myocardial remodelling is also documented by a transgenic mouse model with cardiomyocyte-specific deletion of gene *Capn4* (*Capn4-ko*) which is indispensable for  $\mu$ - and m-calpain stability and activity. Mice were subjected to persistent left coronary artery ligation and followed up for 30 d. Deletion of *Capn4* reduced infarct expansion, apoptosis, myocardial remodelling and dysfunction<sup>[223]</sup>.

## CONCLUSION

Numerous studies have shown an essential contribution

of calpains in myocardial injury following ischemia and reperfusion. Proven prevention or attenuation of post-ischemic heart damage by calpain inhibition with various tested inhibitors could offer a novel prophylactic or therapeutic approach for patients with myocardial infarction, revascularisation and coronary surgery.

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## Neuregulin-1/erbB activities with focus on the susceptibility of the heart to anthracyclines

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### Abstract

Neuregulin-1 (NRG1) signaling through the tyrosine kinase receptors erbB2 and erbB4 is required for cardiac morphogenesis, and it plays an essential role in maintaining the myocardial architecture during adulthood. The tyrosine kinase receptor erbB2 was first linked to the amplification and overexpression of *erbB2* gene in a subtype of breast tumor cells, which is indicative of highly proliferative cells and likely a poor prognosis following conventional chemotherapy. The development of targeted therapies to block the survival of erbB2-positive cancer cells revealed that impaired NRG1 signaling through erbB2/erbB4 heterodimers combined with anthracycline chemotherapy may lead to dilated cardiomyopathy in a subpopulation of treated patients. The ventricular-specific deletion of either *erbB2* or *erbB4* manifested dilated cardiomyopathy, which is aggravated by the administration of doxorubicin. Based on the exacerbated toxicity displayed by the combined treatment, it is expected that the relevant pathways would be affected in a synergistic manner. This review examines the NRG1 activities that were monitored in

different model systems, focusing on the emerging pathways and molecular targets, which may aid in understanding the acquired dilated cardiomyopathy that occurs under the conditions of NRG1-deficient signaling.

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**Key words:** Ventricular dilation; Cardiotoxicity; ErbB2; ErbB4; Neuregulin; Trastuzumab; Doxorubicin

**Core tip:** We have reviewed the cardiac requirement of neuregulin-1 (NRG1) signaling through the receptor tyrosine kinase erbB2/erbB4. The evidence indicates that the NRG1/erbB signaling pathway displays a panel of activities implicated in maintaining the myocardial architecture during remodeling, which may explain why the combined treatment with antibodies against erbB2 and anthracycline chemotherapy may evolve into a severe dilated cardiomyopathy. We have further examined the potential molecular targets, which have been either inferred from impaired NRG1 signaling or directly assessed by the administration of NRG1. The current working hypotheses have been delineated towards a prospective molecular understanding of NRG1 signaling in heart.

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### INTRODUCTION

Dilated cardiomyopathy (DCM) results from the abnormal remodeling of the myocardium with the eccentric growth of cardiomyocytes in response to valve defects, toxic and metabolic causes or gene defects<sup>[1,2]</sup>. An ac-

quired form of DCM is manifested in a subpopulation of breast cancer patients treated with anthracycline chemotherapy combined with humanized antibodies against erbB2. The amplification and over-expression of erbB2 occurs in 25% of all breast cancer types, inducing a highly invasive tumor that has a poor prognosis when treated using conventional therapies. Targeted therapies with antibodies against erbB2 were shown to be clinically effective for erbB2-positive breast cancer patients through an objective tumor regression analysis, with lower rates of both recurrence and mortality<sup>[3]</sup>. However, the iatrogenic effect of the combined immunotherapy and chemotherapy results in increased incidence of dilated cardiomyopathy, initially affecting a subpopulation of 27% of treated patients<sup>[4]</sup>.

We reviewed the panel of activities of the NRG1 pathway that affect cardiomyocyte survival, proliferation, differentiation and specification to further focus on the synergistically deregulated molecular pathways under the experimental conditions of impaired NRG1 signaling and doxorubicin therapy.

## THERAPEUTIC CONSIDERATIONS

Evidence from long-term retrospective analyses of treatment with the humanized antibodies against erbB2 (trastuzumab) have suggested that deficient NRG1 signaling sensitizes the heart to anthracycline cardiotoxicity<sup>[5,6]</sup>. These studies prompted the sequential administration of immunotherapy after chemotherapy in patients with no signs of cardiotoxicity to reduce the incidence of cardiomyopathy. The continuous development of immunotherapeutic drugs aimed at improving efficacy in tumor cell death have recently provided a novel humanized monoclonal antibody against erbB called pertuzumab, which prevents erbB receptor dimerization and thus blocks the activity of both erbB2 and erbB4. Currently, there are ongoing clinical trials about the safety and efficacy of these immunotherapeutics at escalating doses, when used to treat a diverse group of patients with epithelial-derived cancers. Thus far, the results from the CLEOPATRA study group indicate the beneficial effect of the combined action of pertuzumab and trastuzumab with docetaxel, which leads to the significantly progression-free and prolonged survival of patients with breast cancer while having a comparable level of cardiotoxicity both to previous formulations and to placebo with trastuzumab and docetaxel<sup>[7]</sup>. These results prompted the FDA priority review of pertuzumab for its approval and release into the market in June 2012.

In the light of the cardiac adverse effects of the therapeutics used to block the survival of cancer cells, researchers have indicated that drugs, specifically those that target signaling pathways or kinase receptors and have a broad range of effects on cancer cells, should also be studied for cardiac safety<sup>[8]</sup>. In this regard, the multidisciplinary workshop of the Association of the European Society of Cardiology aimed for a consensus in the management of treatments while prioritizing the awareness

of anthracycline cardiotoxicity and the development of new targeted therapeutics<sup>[9]</sup>.

Interestingly, the design of an observational trial on cardiotoxicity in cancer therapies has included an additional evaluation of cardiac risk incidence by analyzing the Single Nucleotide Polymorphism/haplotype variations in the NRG1/Erbb signaling gene components (NCT01173341). In addition to its impact on disease management, the results from this trial may contribute to the knowledge of genetic modifiers by identifying polymorphisms on the genes of the NRG1/erbB pathway that are associated with disease.

## THE COMPONENTS OF THE NRG1 PATHWAY

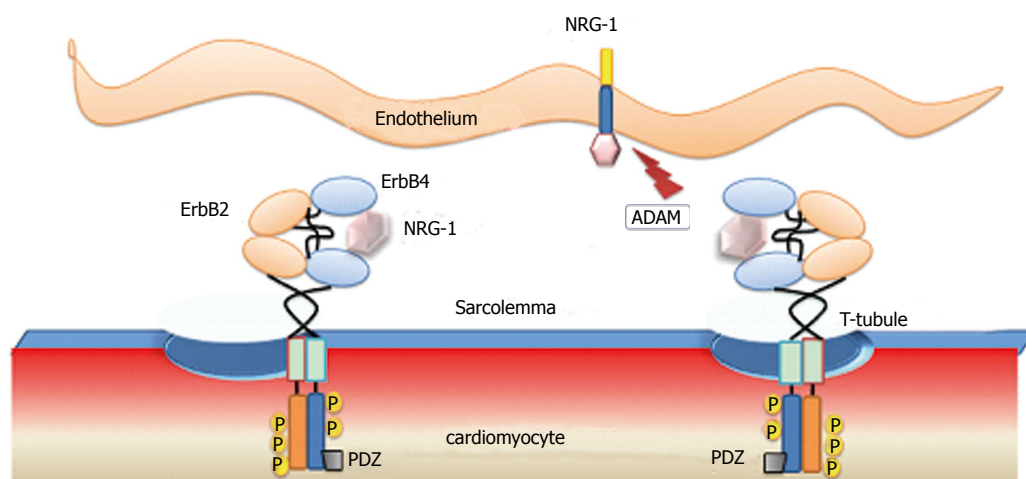
Neuregulins are transmembrane proteins of four isotypes (NRG1-4). Neuregulin-1 is classified into at least three subgroups (type I - III) and has approximately 30 isoforms as a result of its synthesis from different promoters and splicing variants<sup>[10]</sup>. Neuregulins: of types I and II are processed at the membrane by metalloproteinase, ADAM17, 19 and are cleaved by  $\alpha$ -secretase activity<sup>[11]</sup>. The release rate of the amino-terminal active domain is modulated by protein kinase C (PKC)-delta<sup>[12]</sup>. The active peptide of the NRG is related to the epidermal growth factor (EGF), which contains a cysteine-rich domain that binds to and activates the tyrosine kinase receptors erbB4 and erbB3, which belong to the EGF receptor (erbB1) family. The active forms of erbB2 and erbB3 receptors are considered heterodimers because they lack either an opened ligand binding domain or tyrosine kinase activity, respectively, as opposed to the potential function of erbB4 receptor homodimers<sup>[13]</sup>.

In the heart, the active domain of NRG1 secreted from endothelial cells binds and activates the erbB2/erbB4 heterodimers expressed in cardiomyocytes (Figure 1). The NRG1 pathway, which was initially characterized as inducing cardiomyocyte differentiation and specification, has been identified as inducing a broader panel of activities according to the experimental model system and the induced heart condition (Table 1).

## THE NRG1 INTRACELLULAR SIGNALING CASCADE

The erbB-dependent intracellular cascades have been extensively studied because of their important role in cancer cells, thereby providing a basis for analyses of signaling mechanisms in other cell types. The NRG activation of erbB receptors mediates the auto- and trans-phosphorylation of tyrosine residues at the receptor intracellular domain. A subgroup of phosphotyrosine residues bind specific adaptor molecules (*e.g.*, Grb, Shc, Src, SH3 domain)<sup>[14]</sup>, ultimately inducing intracellular pathways, *e.g.*, MAP kinase and PI 3'-kinase cascades, PLC  $\gamma$ , the regulation of the  $\text{Ca}^{2+}$ -dependent PKC and Nuclear Factor of Activated T cells activity (Figure 2)<sup>[13,15]</sup>.





**Figure 1 Endothelium-Cardiac muscle interactions through paracrine neuregulin-1 signaling.** Secreted neuregulin-1 from endothelial cells binds to erbB4 inducing auto- and trans-phosphorylation of ErbB2/ErbB4 heterodimers, expressed in cardiomyocytes. NRG-1: Neuregulin-1; PDZ: Postsynaptic density 95, disc large and zona occludens-1 homologous protein domain; ADAM: A desintegrin and metalloproteinase; P: Phosphorylated tyrosine.

**Table 1 Biological effects of neuregulin-1/erbB in embryonic and postnatal cardiomyocytes**

Experimental system	Monitored response	Intracellular signal	Ref.
Cultured cells			
NRG1 administration in neonatal cardiomyocytes	Sarcomeric F-actin polymerization	PI 3'-kinase	[16]
	Myofibrillogenesis - Growth	Ras-Mek-erk1/2	[17]
	Proliferation	PI 3'-kinase	[17]
	NOS activation		[42]
	Muscarinic activation		[43]
	Karyo- cytokinesis		[44]
NRG1 administration in human ESC	Specification of cardiomyocytes of the conduction lineage		[45]
Animal models			
NRG1b injection to <i>ex vivo</i> developing E12.5 dpc mouse	Differentiation of trabeculae		[17]
	DNA synthesis, Proliferation	PI 3'-kinase	[17]
NRG1 administration in mouse with heart failure	Improved cardiac performance		[40]
Hypomorphic NRG1 deficiency in E8.5dpc mouse	Destabilization of gene regulatory network in the left ventricle	Erk1/2	[39]
Mouse ventricular-erbB2-KO	Overt dilation and reduced survival in erbB2 F/- postnatal mouse		[31]
Mouse ventricular-erbB2-KO	Dilation with cellular apoptosis		[32]
Mouse ventricular-erbB4-KO	Overt ventricular dilation at 3 mo and reduced survival		[33]
Lapatinib inhibition of erbB1/erbB2 in Mouse	erbB2-physiologic hypertrophy in pregnancy	MEK1/erk1/2	[34]
Adult ventricular-erbB4-KO	Cell division in myocardial infarction		[44]

Summary of cardiac activities of neuregulin-1 (NRG1)-erbB2/erbB4 signaling inferred from *in vitro* system and animal model studies. The NRG1 activities, -proliferation, myofibrillogenesis, ventricular remodeling, and repair-, were assessed based on the outcomes of the exogenous administration of NRG1 or by the complete or partial loss of erbB2/erbB4 signaling. NOS: Nitric oxide synthase; MEK1: Mitogen activated kinase erk kinase 1; ESC: Embryonic stem cells.

A link between NRG1 and focal adhesion kinase (FAK) has been observed in proliferative and migrating cells.

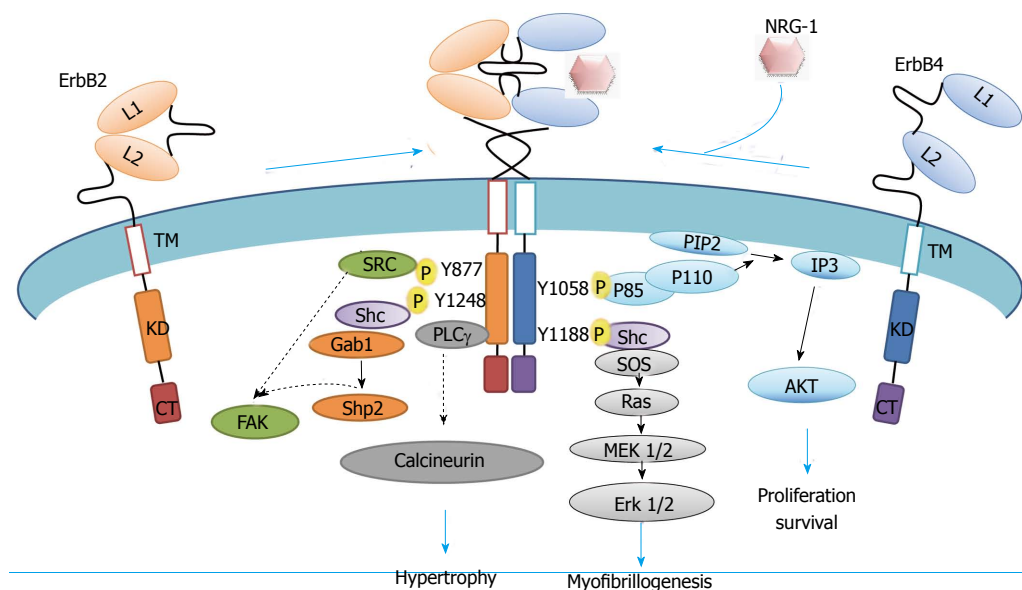
The Ras/MAPK/erk1/2 pathway was required for the NRG1-driven myofibrillogenesis in cultured cardiomyocytes. This activity was mimicked by a constitutively active form of Ras and inhibited by both its dominant negative form and the MEK1 inhibitor PD98059<sup>[16,17]</sup>. The NRG1-induced ability of cardiomyocytes to proliferate was manifested by its combined administration with Insulin-like growth factor I (IGF-I). The NRG1/IGF-I induction of cardiomyocyte DNA synthesis in both *ex vivo* embryonic development and cultured neonatal cardiomyocytes was prevented by wortmannin, an inhibitor of PI3'-Kinase. Cellular transfection with an adenovirus harboring a constitutively active Akt mimicked the proliferative and protective activities, which were inhibited

by a dominant negative form of Akt in the presence of NRG1/IGF-I (Figure 2)<sup>[17]</sup>.

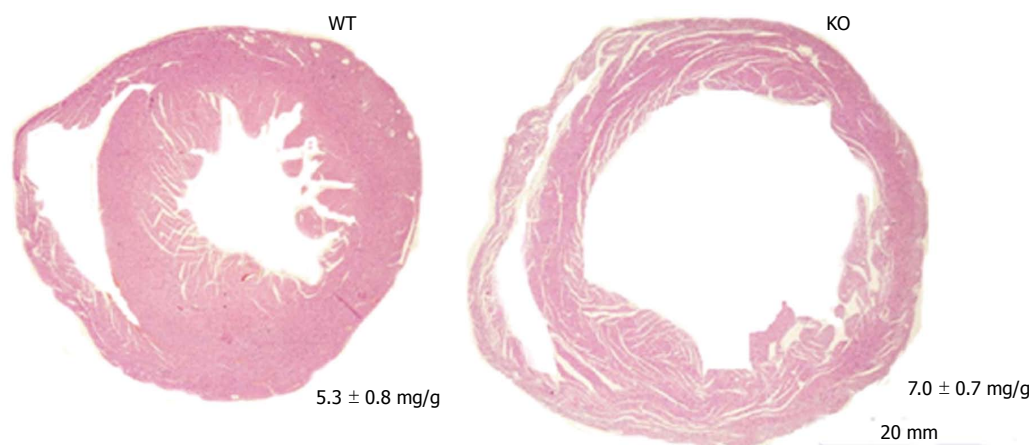
Alternative pathways may be activated by the cross-communication of erbB2 and G protein coupled receptors. Pro-hypertrophic GPCR agonists (*e.g.*, angiotensin II, endothelin I and isoproterenol) have been implicated in the transactivation of EGFR and erbB2, thereby inducing hypertrophic and survival stimuli in cardiac cells<sup>[18,19]</sup>.

### **ErbB non-phosphorylated interactions**

Intracellular signaling also depends on the binding ability of specific non-phosphorylated residues of erbB receptors to PDZ (postsynaptic density 95, discs large and Zonula occludens-1) domain-containing proteins. This interaction with PDZ domain proteins is relevant for the



**Figure 2 Representation of neuregulin-1-erbB2/erbB4 intracellular signaling cascade.** Schematic representation of active ErbB2/ErbB4 heterodimers through phosphorylation, which phosphosites are docking sites for intracellular molecules involved in pathways that modulate myocyte biology. Specific non-phosphorylated residues interact to PDZ domain proteins. CT: Cytoplasmic tail; KD: Kinase domain; L: Ligand binding site; TM: Transmembrane domain; NRG-1: Neuregulin-1; PIP2: Phosphoinositol-2-phosphate; SOS: Son of sevenless; IP3: Inositol triphosphate; AKT: Thymoma viral oncogene homolog 1, a serine/threonine protein kinase; MEK: Mitogen activated kinase erk kinase; Shc: Src homology domain containing transforming protein; Shp: Protein tyrosine phosphatase; FAK: Focal adhesion kinase; Gab: Binding protein of growth factor bound protein Grb2; P: Phosphorylated tyrosine residues.

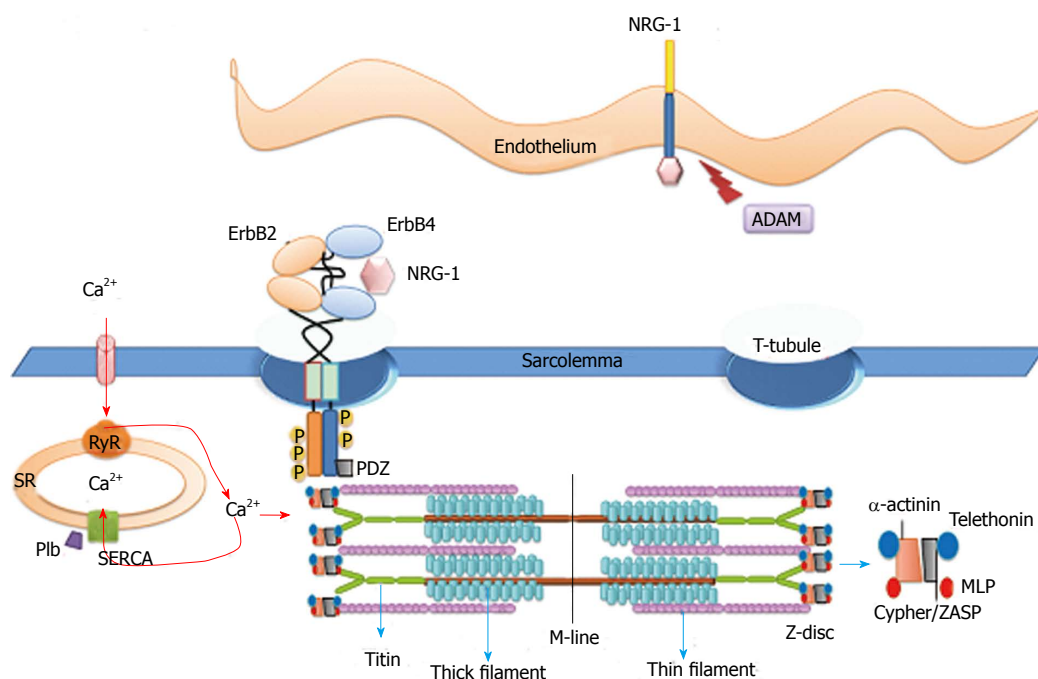


**Figure 3 Ventricular specific erbB4-knockout leads to adult dilated cardiomyopathy.** Representative image of transverse ventricular sections stained with hematoxylin-eosin. Camber dilation is overt in mouse erbB4-KO hearts in the adulthood. WT: Wild type; KO: Knock-out.

specific location of erbB proteins in particular membrane compartments and for the modulation of the receptor stability and activity<sup>[20]</sup>. Despite the significance of PDZ domain proteins in the heart (*e.g.*, MAGUK, actinin binding proteins), there is not yet evidence for the specific PDZ-erbB-interacting proteins in cardiomyocytes. The erbB4 receptors, which are endocytosis-impaired, are also regulated through proteolysis, which is mediated by the proteasome system and the alternative transcriptional activity of the cleavable juxtamembrane isoform JMa<sup>[21,22]</sup>. These mechanisms either drive the erbB4 protein degradation or induce the nuclear translocation of the JMa intracellular domain. As occurs for the release of the NRG1 active peptides, the release of the erbB4 JMa C-terminal domain is modulated by the activation

of PKC and cleaved by the activity of the tumor necrosis factor- $\alpha$  converting enzyme and of  $\gamma$ -secretase at the plasma membrane<sup>[23]</sup>. In the heart, the identified erbB4 protein in cardiomyocytes is the JMb non-cleavable splice variant<sup>[24]</sup>, which may be proteolytically modulated by the proteasome system. Three PPXY motifs couple erbB4 with WW domain proteins, such as Wwox and ubiquitin ligases, thereby either modulating the transcriptional activity of the c-terminal domain when translocated into the nucleus or promoting the isoform degradation<sup>[25]</sup>. Of the two cytoplasmic splice variants CYT1 and CYT2, CYT1 mediates specific interactions with SH2 and WW binding domain proteins (*e.g.*, PI 3'-kinase, ubiquitin ligases)<sup>[26]</sup>.

Interestingly, *erbB4* polymorphisms and splicing vari-



**Figure 4 Functional interaction of molecules placed at the T-tubules.** The ErbB2 and ErbB4 proteins are localized to the T-tubules. This compartment of the sarcolemma provides specific sites for functional interactions with molecules at the sarcoplasmic reticulum and at the myofibril Z-band. Molecular interactions at the T-tubules and at the intercalated discs provide the electric-contraction coupling of the myocardium. Scheme of the sarcomeric units of thin and thick filaments are anchored by titin and actin at the Z-band via  $\alpha$ -actinin scaffold protein complex. Muscle LIM domain protein (MLP), telethonin (T-cap) and PDZ and LIM domain protein (ZASP). PDZ: Postsynaptic density 95, disc large and zonula occludens-1; ADAM: A desintegrin and metalloproteinase; SERCA: Sarcoplasmic reticulum calcium ATPase; Plb: Phospholamban; RyR: Ryanodine receptor; SR: Sarcoplasmic reticulum.

ants of the *erbB4* gene and, more recently, of *Nrg-1* and *erbB2* have been linked to the pathogenesis of non-neoplastic disorders<sup>[27-30]</sup>. However, further experimentation is still needed to address the regulation and specific functions of isoforms in cardiovascular, psychiatric, and other diseases.

## ERBB REQUIREMENT IN POSTNATAL AND ADULT HEART

The clinical implication of the NRG1 signaling in cardiology results from the increased incidence of dilated cardiomyopathies in a subpopulation of breast tumor patients undergoing the combined administration of anthracycline chemotherapy and humanized antibodies against erbB2 protein<sup>[4]</sup>. The cardiac effect of the antibodies (*i.e.*, trastuzumab, pertuzumab), which are species specific and do not cross-react with the mouse protein, were experimentally assessed through the ventricular cardiomyocyte-specific deletion of either the *ErbB2* or the *ErbB4* gene in mice. A ventricular-specific mutation in either of these genes caused dilated cardiomyopathy during adulthood (Figure 3)<sup>[31-33]</sup>. These murine models were useful for demonstrating the cardiomyocyte-autonomous requirement of erbB2/erbB4 during the postnatal remodeling of the myocardium. In agreement with the role of the NRG1-erbB2/erbB4 pathway to prevent ventricular dilation during remodeling, the lapatinib-mediated inhibition of erbB2 phosphorylation in mice resulted in a pathological pregnancy-related dilation of the ventricular

chambers that occurred without apparent apoptotic cell death (Table 1)<sup>[34]</sup>.

The association of changes in the NRG1/erbB pathway with disease was also suggested by the downregulation of both erbB2 and erbB4 expression during the pathologic remodeling of the myocardium in rodents under pressure overload and in humans with a failing myocardium<sup>[35]</sup>. However, it is plausible that a different isoform than the normally expressed JMb could be expressed in the failing myocardium. In this regard, the human erbB4 JMa CYT1 isoform manifested a similar activity to the endogenous erbB4 JMb isoform in cardiac morphogenesis in transgenic mice<sup>[36]</sup>. A different role than the classical activity of transmembrane tyrosine kinase receptor is displayed by the cleavable erbB4 isoform, which acts as a transcriptional co-activator or co-repressor<sup>[37]</sup>. The nuclear localization of the full-length erbB4 protein has recently been observed in cultured adult rat cardiomyocytes under the stress conditions of cell isolation, and the protein was suggested to participate in DNA damaging processes<sup>[38]</sup>.

### Subcellular localization of erbB2/erbB4

Clues about a local cardiomyocyte effect of NRG1 on the maintenance of the myocardial architecture may arise from the subcellular localization of the receptors. The erbB2/erbB4 proteins accumulated within the T-tubule membrane system of cardiomyocytes and the intercalated discs (ID) (Figure 4)<sup>[33]</sup>.

The relevance of the receptor localization is that the

T-tubules place membrane molecules in close apposition with the myofibril Z-disc, thereby providing a specific context for the functional interactions among molecules at the plasma membrane, sarcoplasmic reticulum (SR) and the myofibril Z-disc. The ID are highly specialized Z-disc structures at the cell-cell contact that provide the anchorage of the sarcomeres to the membrane and place the connexin-43 gap junctions, assuring electric transmission and rhythmic contraction of cardiomyocytes.

Concerning the erbB subcellular context, it is speculated that NRG1 may act on cues at the cytoskeletal pathways that are required for myocardial remodeling. A connection to the cytoskeleton may be required for the feedback modulation of NRG1 signaling with wall stress and contractile parameters during cardiac chamber morphogenesis<sup>[39]</sup>.

## CARDIOPROTECTIVE AND REGENERATIVE ACTIVITIES

The *in vivo* administration of NRG1, under different conditions of mouse cardiac pathology, contributed to the amelioration of ventricular contraction (*e.g.*, reduced left ventricular end systolic dimensions, increased ejection fraction). In these experiments, the NRG1-mediated functional performance of the ventricular chambers was correlated with the increased phosphorylation of the myosin regulatory light chain (RLC) (Table 1)<sup>[40]</sup>. However, the administration of NRG1 in knockout mice for the myosin light chain kinase (*Mlk*) gene improves cardiac function without increasing the phosphorylation level of RLC<sup>[41]</sup>, thus implicating additional mechanisms for contractile improvement.

In cultured cardiomyocytes, NRG1 exerts a negative inotropic effect through either NOS activity or the activation of the muscarinic response (Table 1)<sup>[42,43]</sup>. Both nitric oxide and active muscarinic receptors modulate the inotropic response to beta-adrenergic stimulation, which may result in an improved fractional shortening. Indeed, there is a general lack of evidence for either a direct NRG1-mediated inotropic effect or an induced change in calcium handling or in myofilament calcium sensitivity that may provide a molecular explanation for the NRG1-mediated enhancement of cardiac systolic function.

Additional repair activities have been suggested through the induced proliferation of cardiomyocytes in a murine model of cardiac infarction. In this setting, NRG-1/erbB4 was shown to induce mononucleated cardiomyocytes to proliferate, thereby contributing to the cardiac repair mechanisms in one-week-old myocardial infarction without affecting the level of apoptotic cell death (Table 1)<sup>[44]</sup>. This activity is particularly interesting for the renewed research of the cardiomyocytes' re-entry into the cell cycle and the use of stem cells to re-populate the injured myocardium<sup>[45-47]</sup>. In this regard, pluripotent cells may also repair damage myocardial areas through the secretion of relevant growth factors<sup>[48]</sup>.

The evidence for the essential role of NRG1-erbB2/

erbB4 prompted an evaluation of the safety and efficacy of NRG1 administration in patients with chronic heart failure and focal ischemia (Phase I NCT01258387, Phase III NCT01439893, NCT01541202 trials)<sup>[49,50]</sup>. The panel of activities displayed upon NRG1 administration has been well reviewed<sup>[51]</sup> and indicates a pleiotropic effect on cardiac muscle and vascular cells<sup>[51]</sup>. The ultimate contribution of each identified NRG1-mediated activity on cardiac performance remains to be defined. Moreover, the employment of NRG1 for the treatment of cardiac dysfunction still requires a mechanistic understanding of how this signal exerts its effect as well as which critical molecular targets of this pathway affect cardiac remodeling.

## MECHANISMS OF CARDIOTOXICITY: THE ROLE OF NRG1/ERBB SIGNALING

The evidence of the critical activity of NRG1 towards anthracycline cardiotoxicity led to improvements in the clinical management of administering chemotherapy and antibodies against erbB2. Further investigation is required to understand the molecular link of a NRG1 deficiency and exacerbated cardiotoxicity. The individual therapies with either antibodies against erbB2 or anthracyclines exert a cardiotoxic effect at a lower incidence rate compared to the combined treatment. Chemotherapy with anthracycline derivatives may result in both immediate and delayed cardiomyocyte toxic events. The induction of the oxidative stress response that underlies anthracycline cardiotoxicity has been related to cellular apoptosis and necrosis as the mechanism of toxicity. However, employing antioxidants in radiation- or chemotherapy-treated patients resulted in an unclear improvement in cardiac function. These results led to the conclusion that oxidative stress may be viewed as a two-way process by which radical oxygen species (ROS) mediate tumor cell death and promote cell survival through the degradation of anthracyclines<sup>[52]</sup>. In addition, clinical studies aimed to reduce the oxidative and inflammatory process during ischemic dilated cardiomyopathy and chronic heart failure have not yet provided a therapeutic strategy because of dissimilar explanations among the trial results<sup>[53]</sup>. It is therefore likely that ROS-independent mechanisms may play a more important role in the doxorubicin-induced myocardial damage than previously evaluated<sup>[54]</sup>.

Direct evidence for the role of NRG1 against anthracycline cardiotoxicity was provided by the protective activity of NRG1 against doxorubicin-mediated myofibril disarray and in preventing the toxic degradation of troponins in cultured cardiomyocytes (Table 2)<sup>[55,56]</sup>. The cardioprotective activity of NRG1 was also inferred *in vivo* by the doxorubicin-aggravated contractile dysfunction in heterozygous NRG1 mutant mice and by the exacerbated cardiac chamber dilation in the ventricular-specific erbB4-knockout mice (Table 2)<sup>[57,58]</sup>.

The serum level of both the cardiac troponins (*e.g.*, cTnI) and brain natriuretic peptide (BNP) are relevant markers for the follow-up of acquired cardiac pathology



**Table 2 Biological effects of combined neuregulin-1/erbB signaling and anthracyclines**

Experimental system	Monitored response	Ref.
Cultured cardiomyocytes		
NRG-1 administration	Prevented myofibril disarray Attenuated troponin degradation	[55] [56]
Animal models		
NRG1-deficiency/doxorubicin (NRG1 heterozygous mouse)	Induced heart failure	[57]
NRG1-deficiency/doxorubicin (Ventricular specific-erbB4-KO)	Deregulated Protein Homeostasis Autophagic vacuolization	[58]

Summary of effects mediated by anthracycline-induced toxicity and by neuregulin-1 (NRG1)-erbB2/erbB4 signaling in *in vitro* system and animal models. The NRG-1 activities on cardiomyocyte survival, myofibril organization and ventricular homeostasis were assessed based on the exogenous administration of NRG1 or by the outcome of an impaired signaling as discussed throughout the article.

**Table 3 Cardiac phenotypic modifications**

Morphology	WT	KO	WTD	KOD
Young adult (1 mo)				
Heart/body weight (mg/g)	5.2 ± 0.4	5.3 ± 0.6	5.2 ± 0.5	6.2 ± 0.8 <sup>a</sup>
Body weight (g)	17.4 ± 2.0	17.3 ± 2.2	17.3 ± 2.0	17.2 ± 1.5
Adult (3 mo)				
Heart/body weight (mg/g)	5.6 ± 0.6	7.0 ± 0.8 <sup>d</sup>	5.4 ± 0.7	6.7 ± 0.8 <sup>d</sup>
Body weight (g)	30.1 ± 2.5	30.3 ± 2.2	29.4 ± 2.2	28.4 ± 2.2
Cardiotoxic gene groups				
Dilation (ratio)	-	3	1.8	2.8
Hypertrophy (ratio)	-	9.9	3.3	6.5
Damage (ratio)	-	3.7	3.7	3.6
Cell death/necrosis (Ratio)	-	3.0	4.3	3.1
Measured activity				
Caspase 3 (arbitrary units)	0.25 ± 0.17	0.44 ± 0.17	0.84 ± 0.09 <sup>a</sup>	0.35 ± 0.07
Autophagic vacuolization (n°)	0.07 ± 0.1	0.35 ± 0.3	0.2 ± 0.2	2.4 ± 2.1 <sup>a</sup>
Serum cTnI (mg/mL)	0.15 ± 0.1	3.6 ± 1.5	0.7 ± 0.2	14.5 ± 4.2 <sup>a</sup>
LVDP (mmHg)	109.2 ± 7.1	42 ± 8.2 <sup>a</sup>	94.5 ± 5.5	31.5 ± 5.4 <sup>a</sup>
Tau ½	37.4 ± 1.9	38.1 ± 4.9	36.1 ± 3.1	34.6 ± 2.9

Morphological and biochemical modifications studied in the aggravated cardiotoxic condition of the doxorubicin-treated-ventricular specific erbB4-KO mouse model. Summary of hypertrophic-associated morphological changes determined in mouse at the age of 1 and 3 mo, group of differentially expressed genes clustered into a wide-range of cardiotoxic conditions, biochemical activities associated to cell death and of physiological systolic and diastolic parameters are represented as discussed in the text.

<sup>a</sup>*P* < 0.05 *vs* WT, <sup>d</sup>*P* < 0.01 *vs* WT and WTD. WTD: Doxorubicin-treated wildtype; KOD: Doxorubicin-treated erbB4-KO; WT: Treated wildtype.

in trastuzumab- and anthracycline-treated patients<sup>[59,60]</sup>. Indeed, the detection of the cTnI and BNP serum levels was useful for validating the exacerbated cardiotoxicity displayed by an injection of doxorubicin in the ventricular-specific erbB4-knockout mouse. The hypertrophic hallmark, *i.e.*, the natriuretic peptide of the atrial type, was expressed at a relatively low level in doxorubicin-treated wildtype (WTD) animals and at intermediate levels in the doxorubicin-treated erbB4-KO (KOD), compared to the robust expression in erbB4-KO<sup>[33,58]</sup>. The expression of BNP was at a similar level in the erbB4-KO and

in both WTD and KOD mice compared to the wildtype. A contrasting finding between WTD and treated KOD mice was the differential expression of both a group of genes related to oxidative stress and molecules of the apoptotic-death pathway that underlined the WTD (Table 3)<sup>[58]</sup>. The administration of doxorubicin to erbB4-KO mice led to the synergistic deregulation of the ubiquitin-proteasome system. The observed downregulation of the IGF-I/PI3<sup>3</sup>-Kinase axis, which may respond to lower levels of PPAR<sup>[61]</sup> or to a deficient activity of CREB under the control of NRG1<sup>[62]</sup>, may represent a potential mechanism acting on the deregulation of the ubiquitin-proteasome system in erbB4-KOD hearts.

The deregulated group of genes of the ubiquitin-proteasome system was characterized by the upregulation of ubiquitin-ligase, which induced large protein aggregates in cardiomyocytes within the doxorubicin-treated erbB4-KO. Autophagic vacuolization, which is the recommended term for the cellular appearance of large ubiquitin-positive protein aggregates<sup>[63]</sup>, resulted in a 7-fold increase of affected cardiomyocytes relative to the non-treated erbB4-KO (Table 3). The perturbation of the ubiquitin-proteasome system induced by a genetic modification in mice led to cardiomyocyte necrosis and cardiac chamber dilation<sup>[64]</sup>. Moreover, the level of protein ubiquitination was documented as a useful predictor of myocardial deterioration in patients to follow cardiac transplantation<sup>[65]</sup>. The monitoring of necrotic cardiomyocytes, by the determination of the cTnI serum level, is a highly sensitive cardiotoxic marker that is employed in the follow-up of breast tumor patients undergoing trastuzumab and chemotherapy<sup>[59,60]</sup>.

A search for biomarkers in the early detection of doxorubicin cardiotoxicity in both heart and peripheral blood mononuclear cells through the determination of differential gene expression indicated that the most significant group of genes was represented by changes in the canonical NRF2-oxidative stress response pathway, protein ubiquitination and the PI3<sup>3</sup>K/AKT signaling pathway<sup>[66]</sup>. Collectively, these results extend our current knowledge by demonstrating that the impaired NRG1 response in erbB4-KO hearts to doxorubicin toxicity has a net result of the induced autophagic vacuolization of cardiomyocytes, which is consistent with the association of abnormal protein homeostasis with a severe cardiac

disorder.

## CONCLUSION

The NRG1/erbB pathway is critical for the maintenance of the myocardial structure in the adult heart, and moreover, impaired NRG1 signaling exacerbates anthracycline-mediated cardiotoxicity. The accumulated evidence indicates that NRG1 displays a panel of protective and repair activities in the heart during the lifespan of an individual. In this context, an impaired NRG1 signaling sensitizes the heart to the toxicity of anthracycline. There is an ongoing search for drugs and immunotherapies that can inhibit the erbB receptors implicated in tumorigenesis, which may also display an iatrogenic effect in the heart. The individual treatment with either anthracycline derivatives or the induced deficiency in the NRG1 pathway displayed different gene expression profiles in experimental murine models. The doxorubicin-treated hearts were characterized by an oxidative stress response, which may induce cardiomyocyte apoptosis. The sensitization of the NRG1-deficient heart to the anthracycline toxicity resulted in a potentiated deregulation of the ubiquitin-proteasome system, with a net result of the autophagic vacuolization of cardiomyocytes.

Altogether, the NRG1 activities that affect the myocardial architecture and homeostasis await a mechanistic understanding of how NRG1 modulates remodeling and thereby prevents ventricular dilation. Continuous research in this area will provide critical molecules and targets that may help in the design of diagnostic tools and therapeutic.

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## Assessment of coronary artery disease using coronary computed tomography angiography and biochemical markers

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### Abstract

Chronic inflammatory mechanisms in the arterial wall lead to atherosclerosis, and include endothelial cell damage, inflammation, apoptosis, lipoprotein deposition, calcification and fibrosis. Cardiac computed tomography angiography (CCTA) has been shown to be a promising tool for non-invasive assessment of these specific compositional and structural changes in coronary arteries. This review focuses on the technical background of CCTA-based quantitative plaque characterization. Furthermore, we discuss the available evidence for CCTA-based plaque characterization and the potential role of CCTA for risk stratification of patients with coronary artery disease.

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**Key words:** Atherosclerotic plaque composition; Quantification analysis; Multi-slice cardiac computed tomography; Biomarkers

**Core tip:** This review gives an overview of the current status of noninvasive assessment of coronary artery disease (CAD) and the ability of cardiac computed tomography angiography (CCTA) and cardiac biomarkers

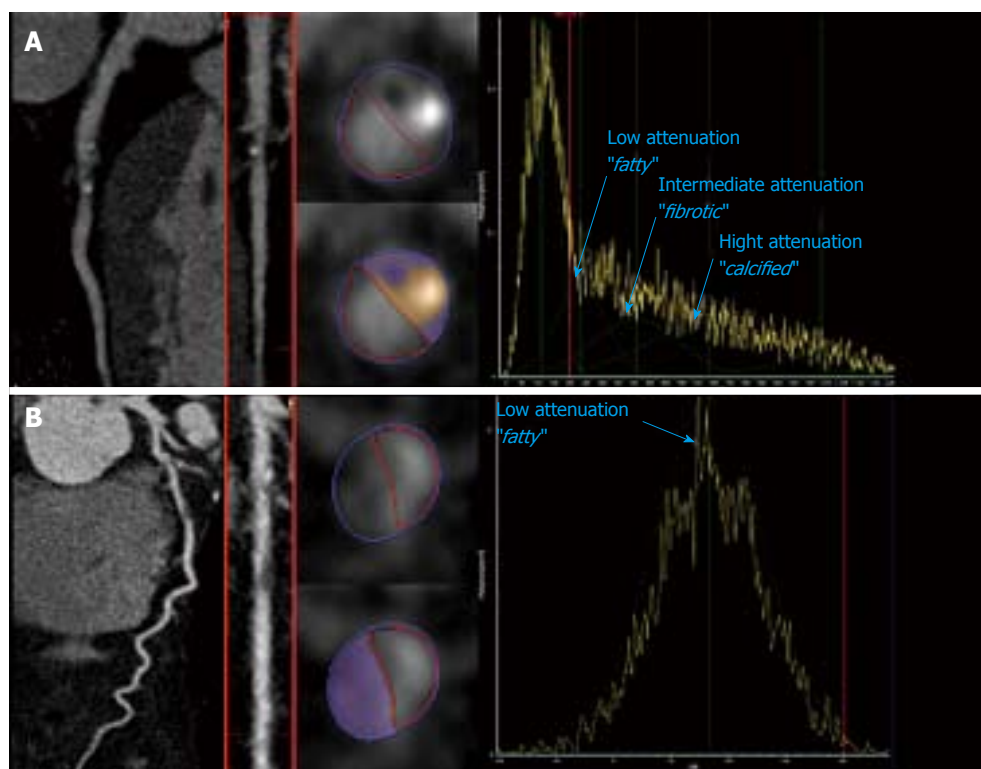
for the diagnostic classification and risk stratification of patients with suspected and known CAD. Since all techniques described herein are available in the clinical routine and are associated with an acceptable time spent the translation to the clinical realm appears promising. Focusing on CCTA-based quantitative plaque characterization we herein present the (1) available evidence; (2) comparison with other techniques of plaque characterization; and (3) the value of "bio-imaging" for the risk stratification of patients with CAD.

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### INTRODUCTION

Sudden vessel occlusion as a consequence of atherosclerotic plaque rupture with subsequent coronary artery thrombosis is the most common cause of acute myocardial infarction (AMI) and sudden cardiac death in the industrialized world<sup>[1]</sup>. Conventional X-ray coronary angiography still remains the gold standard for detection of coronary artery disease (CAD). However, this technique is invasive and provides limited information on the composition of atherosclerotic plaque<sup>[2]</sup>. Coronary computed tomography angiography (CCTA) on the other hand, is a very fast evolving and in the meanwhile well-established non-invasive technique for the visualization of both coronary artery lumen narrowing and coronary calcification<sup>[3]</sup>. In addition, CCTA with the help of commercially available software tools provides objective and quantitative assessment of atherosclerotic plaque composition<sup>[4-6]</sup>.

Based on recent developments with CCTA hardware



**Figure 1** Representative example of (A) a partially calcified and (B) of a non-calcified atherosclerotic coronary plaque with the corresponding Gaussian curves, respectively for different plaque components (lipid-rich, fibrotic and calcified).

and software technologies, including iterative reconstruction algorithms, a substantial reduction in radiation exposure and improvement of image quality could be achieved<sup>[7-11]</sup>. In addition, dedicated post-processing tools constituted major steps towards the reliable and quantitative assessment of atherosclerotic plaque composition<sup>[12-17]</sup>.

The growing body of evidence for the prognostic value of CCTA-based plaque characterization underscores its potential for implementation in the clinical realm. In this regard, features indicating plaque vulnerability include a large necrotic core, thin fibrous cap and positive vessel remodeling<sup>[6,18-22]</sup>. The early and non-invasive detection of such vulnerable rupture-prone atherosclerotic lesions remains a major challenge in patient care.

## DATA ON THE FEASIBILITY OF CCTA-BASED CORONARY PLAQUE CHARACTERIZATION

First generation CCTA scanners offered limited ability for the reliable detection of coronary lesions due to technical limitations, including limited spatial and temporal resolution, and partial volume effects caused by coronary calcifications. With the development of 256- or even 320-slice multi-slice CT-scanners however, faster gantry rotation speed, Z-direction focal-spot sampling and spherical detector design could overcome these limitations, offering high isotropic spatial resolution of

approximately 400-600  $\mu\text{m}$  and a temporal resolution of approximately 83-175 ms<sup>[7,9,23-26]</sup>.

Current SCCT guidelines introduced a scheme for the qualitative characterization of different plaque types for clinical reporting<sup>[27]</sup>. In general, the percentage of calcium content is < 20% in non-calcified plaque, between 20% and 80% in mixed plaque and > 80% in calcified plaque. The reproducibility of this qualitative assessment (calcified, non-calcified, mixed plaques) has been shown to be good for both intra- and inter-observer agreements with more than 88%<sup>[28,29]</sup>. The accuracy of this qualitative plaque characterization approach has been validated by virtual histology-intravascular ultrasound (VH-IVUS) for different plaque types<sup>[30]</sup>.

Others and we showed the feasibility and practicability of semi-automated and automated post-processing software tools for the quantitative assessment of atherosclerotic coronary plaque size and composition in patients undergoing CCTA for clinical reasons<sup>[17,31-33]</sup>. This volumetric approach allows for assessment of (1) total plaque volume, (2) plaque composition (distribution of (non-) calcified content) and (3) maximum, mean and minimum plaque intensities in hounsfield units (HU). Hoffman *et al.*<sup>[33]</sup> showed that limits of agreement are approximately 60% for small volumes (10 mm<sup>3</sup>) and 28% for larger volumes (100 mm<sup>3</sup>). According to the tissue specific attenuation properties, three different plaque components can potentially be distinguished, including: (1) lipid-rich (14-70 HU); (2) fibrotic (71-150 HU); and (3) calcified components (> 150-200 HU)<sup>[14]</sup>. Lipid and fi-

**Table 1** Table summarizing the current key studies on comprehensive “bio-imaging” with coronary computed tomography and biomarkers in presumably stable coronary artery disease patients

Ref.	Biochemical markers	CT scanner	Number of patients	Results
Laufer <i>et al</i> <sup>[59]</sup>	hsTnT	64-sl. MDCT	615	Even mild CAD is associated with hsTnT levels in symptomatic patients
Korosoglou <i>et al</i> <sup>[58]</sup>	hsTnT	≥ 64-sl. MDCT	124	hsTnT is associated with the extend of positive remodeled NCP. Only weak association was detected for hsCRP
Blaha <i>et al</i> <sup>[68]</sup>	hsCRP	4-sl. MDCT	6762	hsCRP was not associated with coronary artery calcification
Duivenvoorden <i>et al</i> <sup>[70]</sup>	hsCRP, MPO, and others	<sup>18</sup> FDG-PET/CT	130	MPO levels are associated with carotid plaque inflammation
Andrassy <i>et al</i> <sup>[62]</sup>	HMBG-1	256-sl. MDCT	152	HMBG1 is associated with the composition and extend of atherosclerotic plaques
Nakazato <i>et al</i> <sup>[64]</sup>	LDL, HDL, TC	≥ 64-sl. MDCT	4575	Presence and extend of NCP are associated with high non-HDL level
Voros <i>et al</i> <sup>[63]</sup>	ApoB, HDL, LDL	64-sl. MDCT IVUS/VH	60	ApoB and small HDL particles are associated with larger plaque burden and more NCP plaque. Larger HDL and pre-b2-HDL particles are associated with plaque burden and less NCP

CAD: Coronary artery disease; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ApoB: Apolipoprotein B; TC: Total cholesterol; hsTnT: High-sensitive Troponin T; hsCRP: High-sensitive C-reactive protein; MPO: Myeloperoxidase; MDCT: Multi-slice computer tomography.

brotic plaque components are often summarized as “non-calcified”. However, there is still a lack of a uniform attenuation cut-off values defining these tissue qualities due to overlapping attenuation intervals. Figure 1 shows representative examples of a (A) non-calcified and (B) of a partially calcified atherosclerotic coronary plaques with the corresponding Gaussian curves, respectively for different plaque components.

Previous *ex vivo* studies compared CCTA-based plaque characteristics with histopathology<sup>[34-36]</sup>. In this regard, 16- and 64-slice CCTA provided precise detection of calcified lesion, while its accuracy for the differentiation between lipid-rich and fibrotic components was lower<sup>[37-39]</sup>. Further experimental studies are now warranted to reevaluate the potential of 256- and 320-slice scanners in this context.

## VIRTUAL HISTOLOGY-INTRAVASCULAR ULTRASOUND

VH-IVUS with radiofrequency backscatter analysis is the clinical gold standard technique for the visualization of coronary vessel wall morphology<sup>[40,41]</sup>. In *ex vivo* studies of coronary arteries, IVUS has been shown to successfully identify plaque features as regional calcification, lipid-rich necrotic cores and fibro-fatty plaques with high accuracy<sup>[42-44]</sup>. From a clinical point of view, the PROSPECTIVE trial could show the prognostic impact of IVUS-based plaque characterization in patients with acute coronary syndromes<sup>[21]</sup>. In contrast to CCTA, VH-IVUS enables for detailed measurement of fibrous cap thickness and for the detection of thin-cap fibroatheromas (TCFA)<sup>[38,45]</sup>. Pundziute *et al*<sup>[40]</sup> showed that 32% of partially calcified plaques in CCTA were characterized as TCFA by VH-IVUS.

However, there are still some limitations both during IVUS data acquisition and in the post-processing raw data handling<sup>[46]</sup>. In addition, the assessment of the entire coronary tree requires a 3-vessel catheter-based interrogation, which may involve additional risks for the

patients<sup>[21]</sup>. In this regard, CCTA would be a valuable non-invasive alternative to IVUS, especially in light of the good correlation of the 2 techniques in terms of plaque composition assessment<sup>[14,32,38,47-49]</sup>.

## OPTICAL COHERENCE TOMOGRAPHY AND NEAR INFRARED SPECTROSCOPY

Other intravascular imaging techniques like optical coherence tomography (OCT) and near infrared spectroscopy (NIS) have also been applied for the assessment of coronary plaque composition. OCT which is the light analogue of IVUS enables for a resolution of 10-20 μm, which is about 10 times higher than that provided by IVUS. OCT detects erosions and can also differentiate between red and white thrombus<sup>[50]</sup>. However, OCT cannot visualize vessel wall structures under the condition of blood flow, has limited penetration depths of 1-2 mm, and is therefore not appropriate for deeper imaging of blood vessels<sup>[51]</sup>. Despite continuing improvements in the performance of both IVUS and OCT, their use has been mostly limited to structural imaging so far. On the other hand, near infrared spectroscopy (NIS) belongs to a different class of imaging methods which measures absorption spectra from blood vessels in order to assess lipid content<sup>[51,52]</sup>. However, additional experimental and clinical data are required to assess the methodological reliability and to define precise clinical applications with this technique. Finally, the detection of lipid subtypes, such as oxidized low-density lipoprotein (ox LDL) is still limited using NIS.

## RISK STRATIFICATION USING CCTA AND BIOCHEMICAL MARKERS

The primary adverse outcome of CAD is acute myocardial infarction (AMI) and sudden cardiac death. Therefore, there is a great need for robust diagnostic algorithms, which may include cardiac biomarkers and non-invasive imaging techniques, for the risk stratification

of patients with subclinical or presumably stable CAD. In this regard, the detection of rupture-prone coronary plaques or of elevated cardiac troponins may help the classification of patients with presumably low risk *vs* those with high-risk, aiding in the guidance of pharmacologic and interventional treatment strategies. Non-invasive assessment of functional wall motion analysis by dobutamine stress cardiac magnetic resonance imaging (MRI) or stress echocardiography has also been shown to identify patients at high risk for future cardiac events<sup>[53,54]</sup>. However, in contrast to CCTA these imaging modalities provide no information on coronary artery pathologies and plaque composition.

Several cardiovascular biomarkers are well established in clinical routine to complement clinical assessment and 12-lead ECG in the diagnosis, risk stratification, triage, and management of patients with suspected acute coronary syndrome (ACS). Especially cardiac troponins were shown to aid the diagnostic classification and risk stratification of patients with ACS<sup>[55-57]</sup>. Recently others and we could show an association between CTA atherosclerotic plaque characteristics and small blood level troponin increases in patients with stable CAD<sup>[58,59]</sup>, which could be explained by chronic clinically silent rupture of non-calcified plaque with subsequent microembolisation. In an experimental setting, high mobility group box 1 (HMBG1) protein was found to be a critical mediator of acute ischemic injury, predicting adverse outcomes after myocardial infarction<sup>[60,61]</sup>. In addition, we could show that HMBG1 serum levels are associated with coronary calcification and with non-calcified plaque composition in patients with suspected or known stable CAD<sup>[62]</sup>.

Incorporation of ox-LDL transforms macrophages into foam cells, which built the core of atherosclerotic plaques. In this regard, the presence and extent of non-calcified plaques are associated with high non-HDL, which suggest a relationship between lipid profile and plaque composition<sup>[63,64]</sup>.

CRP was initially supposed to be a causal player for atherosclerotic plaque development and inflammation<sup>[65]</sup>. However, further basic science research has questioned a direct atherogenic mechanism<sup>[66,67]</sup>. Others and we could show that serum levels of hsCRP are only weakly correlated with plaque composition and coronary artery calcification and largely determined by the presence of risk factors<sup>[58,68,69]</sup>. More specific markers of inflammation could provide a stronger association with plaque formation and atherosclerotic inflammation. In this regard, the dal-PLAQUE study recently showed that myeloperoxidase levels are associated with carotid plaque inflammation, which was assessed using 18F-fluorodeoxyglucose positron emission tomography/computed tomography<sup>[70]</sup>. An overview of the most interesting studies in the area of comprehensive “bio-imaging” using cardiac computed tomography and biomarkers are presented in Table 1.

Several CCTA outcome studies on the other hand, have assessed the prognostic value of plaque burden and plaque morphology in both symptomatic and asymp-

tomatic cohorts<sup>[18,71-74]</sup>. The value of risk assessment in patients with CAD using a CCTA-based semi-automated plaque assessment has been recently shown<sup>[6]</sup>. Ongoing studies now investigate the potential complementary value of high-sensitive Troponin T (hsTnT) and quantitatively assessed coronary plaque burden for the risk stratification of patients with intermediate likelihood for CAD.

## CONCLUSION

Imaging of coronary artery disease using CCTA is a feasible and robust approach for non-invasive plaque characterization. Growing body of evidence exists for the ability of CCTA based quantitative plaque characterization for the prediction of clinical outcome in patients with suspected or known coronary artery disease.

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## Lipid profile in children with coronary artery disease in Sindh, Pakistan

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**CONCLUSION:** CAD risk factors are significant regarding abnormal lipid levels. Genetic tendency seems to be important in the development of CAD in children.

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**Key words:** Serum; Lipid profile; Coronary artery disease; Children; Sindh

**Core tip:** It is well known that cholesterol accumulates in the coronary wall and conditions of blood pressure are recurrently connected with coronary artery disease in early adult life.

Baloch S, Devrajani BR, Baloch MA, Pir MA. Lipid profile in children with coronary artery disease in Sindh, Pakistan. *World J Cardiol* 2014; 6(7): 671-674 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/671.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.671>

### Abstract

**AIM:** To evaluate lipid profile in children with coronary artery disease (CAD) in Hyderabad, Sindh, Pakistan.

**METHODS:** The study included 100 children (6-15 years), of which 43 were children of young parents (one or both) with recognized CAD, while the other 57 were children with no evidence of CAD (controls). All were evaluated for fasting blood lipid profile. Blood samples were collected from patients with CAD and healthy controls and analysis of the levels of lipid profile were carried out using a kit method on Microlab 300.

**RESULTS:** Children with CAD had significantly higher levels of total serum cholesterol and triglycerides and decreased levels of high density lipoprotein and low density lipoprotein compared to children in the control group. Systolic and diastolic blood pressures were significantly higher, without any significant difference.

### INTRODUCTION

Coronary artery disease (CAD) is one of the main causes of mortality and morbidity in Pakistan. It is assessed that in the future these diseases will constitute major public health problems. Propensity CAD risk factors stimulate the progression of main and conditional CAD risk factors that cause CAD. Numerous lifestyle aspects with diet, environmental factors and genetic predisposition affect the outcome and development of atherosclerosis and thrombosis<sup>[1]</sup>. The progression of risk factors and their association with the manifestation of CAD has been developed from worldwide prospective epidemiological studies. These studies have revealed a constant correlation among characteristics examined in healthy individuals with the consequent prevalence of CAD<sup>[2]</sup>. The results have drawn attention to the status of risk factors in formative outcomes<sup>[3]</sup> and heterogeneity of CAD

**Table 1 Lipid Profile in children with coronary artery disease and control group**

Variables	Children with CAD	Control group
Total cholesterol	62.1 ± 41.1	44.6 ± 15
Triglycerides	45.3 ± 21.2	29.4 ± 17
HDL	15.1 ± 13	19.2 ± 22
LDL	12.3 ± 0.2	17.2 ± 12

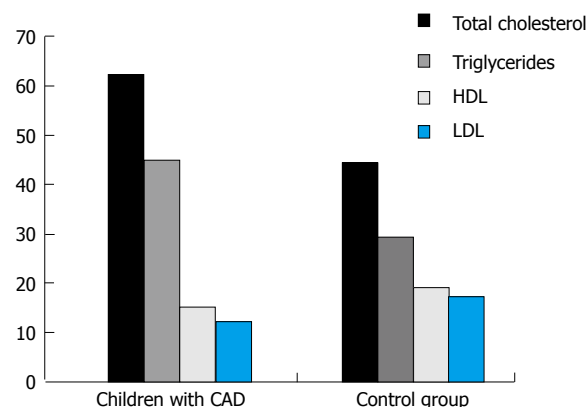
CAD: Coronary artery disease; HDL: High density lipoprotein; LDL: Low density lipoprotein.

patients. Family history of CAD is highly associated with disease occurrence<sup>[4]</sup>. Hypertension<sup>[5]</sup> frequently has a correlation with CAD. Increased serum cholesterol levels<sup>[6]</sup> are associated with the risk of CAD and decreased levels of low density lipoprotein (LDL) and high density lipoprotein (HDL) are important in the progression<sup>[7]</sup> of CAD. Hypertriglyceridemia is known<sup>[8]</sup> in the progression of CAD.

It is well known that cholesterol accumulates in the coronary wall and conditions of blood pressure are recurrently connected by CAD in early adult life. Fatty streaks can be noticed in infants by 2-3 mo of age and increase in size and number throughout the first two decades of life<sup>[9-13]</sup>. Dyslipoproteinemia with high levels of total cholesterol and LDL and low levels of HDL and family history of early CAD have been demonstrated to be predisposing factors of early CAD<sup>[9-14]</sup>. Recently, more emphasis has been laid on the role of lipoproteins than cholesterol alone<sup>[15,16]</sup>. The aim of this study is to analyze main lipid and lipoprotein cholesterol spectrum in children with respect to the CAD history of their parents with or without hypercholesterolemia. Although cardiovascular diseases do not manifest until maturity, dyslipidemia risk factors are present in children and remain into old age<sup>[17]</sup>. It was suggested that lipid profile levels should be screened in children, providing a procedure to recognize and treat those who are at risk for the progression of CAD<sup>[18,19]</sup>. Cardiovascular disease is one of the major problems in Asia but very few studies have been documented about the lipid profile and incidence of dyslipidemia in children, which prove irregular lipid profiles<sup>[20]</sup>. These studies also reported the high levels of lipid disorders in children. Therefore, the current study was carried out to detect serum lipid profiles and the prevalence CAD among children.

## MATERIALS AND METHODS

The study included 100 children (6-15 years), of which 43 were children with recognized coronary artery disease (CAD) and 57 children with no confirmation of CAD (healthy controls). The case group was selected from patients admitted or visiting the pediatric unit at LUMHS City and Jamshoro Hospital for angiography or medical treatment. The inclusion criterion for the case group was structural CAD diagnosed by echocardiography or angiography, and the inclusion criteria for both groups were



**Figure 1 Total cholesterol, triglyceride high density lipoprotein and low density lipoprotein levels in children with coronary artery disease compared to the control group.** HDL: High density lipoprotein; LDL: Low density lipoprotein; CAD: Coronary artery disease.

not having chronic liver or kidney disease which may disturb lipid profile levels. The children and their parents received complete justifications about the study (including procedural details and sampling) and informed consent was obtained from the parents before the beginning of the study. The echocardiographic studies comprised using an echocardiographic machine. All the measurements were performed by one pediatric cardiologist. Ten mL blood samples from coronary artery disease patients and healthy control subjects were collected and serum was separated and immediately levels of the lipid profile were analyzed using a kit method on Microlab 300. Excel and SPSS.15 were used for data analysis.

## RESULTS

Table 1 and Figure 1 show mean serum levels of lipid profile in the CAD and control groups. The results showed a significant increased level of total cholesterol and triglycerides and a decreased level of HDL and LDL compared to the controls, with  $P < 0.001$ .

## DISCUSSION

The genetic factor is supposed to be the leading factor when CAD presents early in life. Several studies have documented the association between cholesterol levels and prevalence of CAD<sup>[21,22]</sup>. The association between CAD and levels of cholesterol is complex to estimate in children because clinically significant CAD does not happen. In the current study, the children of parents with CAD have a significant occurrence of hyperlipidemia and there is an association between lipid profile levels of children<sup>[23-26]</sup>. It was reported<sup>[27]</sup> that 72 children whose ancestors had myocardial infarction had increased levels of cholesterol; however, there was no significant difference in levels of triglyceride. It was reported that there was an association among lipid profile levels of parents and their children with total cholesterol levels. This study is similar to other studies<sup>[27]</sup>.

Increased levels of serum cholesterol, triglycerides and LDL are several of the significant factors in these patients. It was reported that hypercholesterolemia is common in children of parents with recognized hypercholesterolemia and symptomatic coronary artery disease. An increased total cholesterol along with HDL ratio influences primary coronary artery disease<sup>[28]</sup>. This ratio in the current study of high risk children was significantly more increased than the ratio given by earlier workers<sup>[29]</sup>. It has been revealed that the entire risk factor separately enhances the risk of coronary artery disease by 5 to 10 times compared with having no risk factors.

The present study observed the lipid profile in children with CAD compared to the control group. High levels of cholesterol and triglyceride and low levels of HDL and LDL in children with CAD were found. Our results conclude that it is useful to monitor the lipid profile of children of parents with coronary artery disease. Children of parents with CAD and hyperlipidemia are at high risk of progression to premature atherosclerosis and need lipid profile assessment monitoring.

The lipid profile of children diagnosed with intermittent major risks can be taken to reduce these risks. Further studies with greater sample numbers are necessary to confirm these findings.

## COMMENTS

### Background

Coronary artery disease (CAD) patients are at risk for poor nutritional status. The entire measures lead to life intimidating problems and are predictive factors.

### Research frontiers

Lipid profile disturbances between the patients were compared with healthy subjects. This study is designed with the objective of investigating the similarities and differences in patients with CAD, clinically and metabolically.

### Innovations and breakthroughs

Children with CAD had significantly higher levels of total serum cholesterol and triglycerides and decreased levels of high density lipoprotein and low density lipoprotein compared to the control group. Systolic and diastolic blood pressures were significantly higher, without any significant difference.

### Applications

By understanding the lipid profile in CAD patients and controls for the progression of future remedial guidelines, this study may need to be sustained in a further extensive manner at different nursing homes.

### Peer review

The authors made a good effort to analyze the lipid profile in children whose parents are known to have coronary artery disease.

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## Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis?

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### Abstract

**AIM:** To evaluate the referrals with suspected arrhythmogenic right ventricular cardiomyopathy (ARVC) and compare cardiac MR (cMR) findings against clinical diagnosis.

**METHODS:** A retrospective analysis of 114 (age range 16 to 83, males 55% and females 45%) patients referred for cMR with a suspected diagnosis of ARVC between May 2006 and February 2010 was performed after obtaining institutional approval for service evaluation. Reasons for referral including clinical symptoms and family history of sudden death, electrocardiogram and echo abnormalities, cMR findings, final clinical diagnosis and information about clinical management were obtained. The results of cMR were classified as major, minor, non-specific or negative depending on both functional and tissue characterisation and the cMR results

were compared against the final clinical diagnosis.

**RESULTS:** The most common reasons for referral included arrhythmias (30%) and a family history of sudden death (20%). Of the total cohort of 114 patients: 4 patients (4%) had major cMR findings for ARVC, 13 patients (11%) had minor cMR findings, 2 patients had non-specific cMR findings relating to the right ventricle and 95 patients had a negative cMR. Of the 4 patients who had major cMR findings, 3 (75%) had a positive clinical diagnosis. In contrast, of the 13 patients who had minor cMR findings, only 2 (15%) had a positive clinical diagnosis. Out of the 95 negative patients, clinical details were available for 81 patients and none of them had ARVC. Excluding the 14 patients with no clinical data and final diagnosis, the sensitivity of the test was 100%, specificity 87%, positive predictive value 29% and the negative predictive value 100%.

**CONCLUSION:** CMR is a useful tool for ARVC evaluation because of the high negative predictive value as the outcome has a significant impact on the clinical decision-making.

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**Key words:** Arrhythmogenic right ventricular cardiomyopathy; Cardiomyopathy; Right ventricular; Arrhythmias; Magnetic resonance imaging; Diagnosis; Implantable cardiac defibrillator

**Core tip:** This study was designed to evaluate the referrals with suspected Arrhythmogenic right ventricular cardiomyopathy (ARVC) and compare the findings of cardiac magnetic resonance imaging (cMR) against clinical diagnosis. Currently the diagnosis depends upon a combination of variety of factors including imaging findings. We evaluated all the referrals in our institution over a 4-year period and found a high sensitivity and specificity of cMR for ARVC diagnosis. We have concluded that cMR is a very useful tool for ARVC evalua-

tion because of the very high negative predictive value as the outcome has a significant impact on the clinical decision-making.

Chellamuthu S, Smith AM, Thomas SM, Hill C, Brown PWG, Al-Mohammad A. Is cardiac MRI an effective test for arrhythmogenic right ventricular cardiomyopathy diagnosis? *World J Cardiol* 2014; 6(7): 675-681 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/675.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.675>

## INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetic form of cardiomyopathy characterized by fibro-fatty replacement primarily of right ventricular muscle. ARVC is inherited predominantly as an autosomal dominant pattern. There are also recessive forms described caused by mutations in the plakoglobin and desmoplakin (*e.g.*, Naxos Disease, Carvajal Syndrome) which are associated with a cutaneous phenotype<sup>[1-3]</sup>. Although the name describes a “right” ventricular process, it is now recognized that left ventricular involvement is much more common and acknowledged at an earlier stage than before<sup>[4-6]</sup>. In the early stage of the disease, structural changes may be absent or subtle and are due to myocardial injury, inflammation and repair<sup>[7]</sup> and are confined to a localized region of the right ventricle (RV), typically the RV outflow tract, pulmonary infundibulum or the RV apex, which form a “triangle of dysplasia”<sup>[8]</sup> (Figure 1, see arrows). These areas are eventually replaced by fibrous and fatty tissue<sup>[7,9]</sup> resulting in aneurysm formation, which is commonly seen in the basal inferior wall below the tricuspid valve<sup>[10]</sup>. Ventricular aneurysms at these sites can be considered pathognomonic of ARVC<sup>[7]</sup> (Figure 2, see arrows). These changes contribute to the electrical instability, which triggers ventricular tachycardia and sudden cardiac death (SCD)<sup>[11,12]</sup>.

Clinical manifestations of ARVC are variable. The most common symptoms are palpitations and syncope, due to the occurrence of ventricular tachycardia. Occasionally, SCD may be the first event. ARVC is a relatively common cause of unexpected SCD in the young, especially in athletes<sup>[13,14]</sup>. Progressive structural involvement results in failure of the right or left ventricle depending on which one is predominantly affected and eventually to biventricular heart failure<sup>[15-17]</sup>. Following the diagnosis of ARVC or the occurrence of SCD due to suspected ARVC; evaluation of family members is frequently initiated.

Histological confirmation is required for the definitive diagnosis of ARVC; however myocardial biopsy may not necessarily be sensitive due to the segmental nature of the disease<sup>[18]</sup>. An International Task Force (Table 1) was developed in 1994, which proposed major and minor criteria for the diagnosis of ARVC<sup>[19]</sup>. The diagnostic criteria depends on a variety of factors including functional

**Table 1 Task force criteria for the diagnosis of arrhythmogenic right ventricular cardiomyopathy<sup>[19]</sup>**

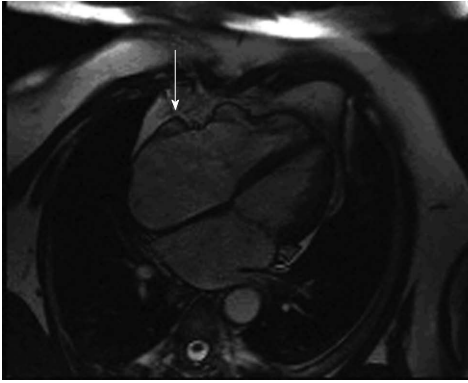
1 Global and/or regional dysfunction and structural alterations (detected by echocardiography, angiography, magnetic resonance imaging, or radionuclide scintigraphy)
Major: Severe dilatation and reduction of right ventricular ejection fraction with no (or only mild) left ventricular impairment. Localized right ventricular aneurysms (akinetic or dyskinetic areas with diastolic bulging). Severe segmental dilatation of the right ventricle
Minor: Mild global right ventricular dilatation and/or ejection fraction reduction with normal left ventricle Mild segmental dilatation of the right ventricle Regional right ventricular hypokinesia
2 Tissue characterization of wall
Major: Fibro-fatty replacement of myocardium on endomyocardial biopsy
3 Repolarisation Abnormalities
Minor: Inverted T waves in right precordial leads (V2 and V3) in people aged > 12 yr, in absence of right bundle branch block
4 Depolarization/conduction abnormalities
Major: Epsilon waves or localized prolongation (> 110 ms) of the QRS complex in right precordial leads (V1-V3)
Minor: Late potentials (signal-averaged ECG)
5 Arrhythmias
Minor: Left bundle branch block type ventricular tachycardia (sustained and non-sustained) by ECG, Holter or exercise testing. Frequent ventricular extra-systoles (> 1000/24 h) by Holter
6 Family history
Major: Familial disease confirmed at necropsy or surgery
Minor: Family history of premature sudden death (< 35 yr) due to suspected right ventricular dysplasia. Familial history (clinical diagnosis based on present criteria)

The diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) would be fulfilled in the presence of 2 major criteria or 1 major plus 2 minor or 4 minor criteria from the different groups. ECG: Electrocardiogram.

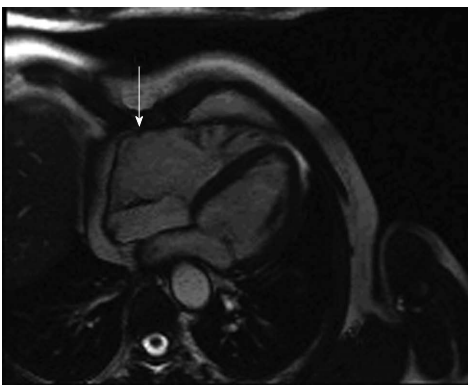
and structural abnormalities on imaging such as cMR or echocardiography, tissue characterisation (*i.e.*, endomyocardial biopsy), repolarization and depolarisation abnormalities in the electrocardiogram (ECG), arrhythmias, family history and genetic analysis<sup>[20]</sup>. These criteria although were highly specific, lacked the sensitivity for early disease<sup>[21,22]</sup>. In 2010, revised criteria were proposed to include quantitative parameters particularly for the imaging studies (Table 2) to improve diagnostic sensitivity whilst maintaining specificity<sup>[5]</sup>.

cMR has an important role in the diagnosis of ARVC as it allows three-dimensional visualisation of the ventricles and is very useful in the assessment of functional and structural abnormalities<sup>[23]</sup>. Previous studies have demonstrated that cMR has high sensitivity and specificity for ARVC diagnosis<sup>[24,25]</sup> and also play an important role in the evaluation of ARVC even in patients who do not meet the Task Force Criteria<sup>[26]</sup>. Due to the high sensitivity and specificity, cMR has also been suggested as a routine method of examination if ARVC is clinically suspected<sup>[25]</sup>. Apart from excluding ARVC, cMR is also sometimes useful in finding ARVC mimics or other clinically significant findings<sup>[26,27]</sup>.

The requests to rule out ARVC constitute a significant proportion of the total cMR referrals in our institution from the cardiologists. Although the previous studies



**Figure 1** Cardiac magnetic resonance 4 chamber image from cine imaging demonstrating dyskinesia of the right ventricle free wall.



**Figure 2** Cardiac magnetic resonance 4 chamber image from cine imaging demonstrating aneurysm of the basal segment free right ventricle wall.

have demonstrated the critical role of cMR in the diagnosis of ARVC, the impact of cMR outcome in an unselected population is not widely analysed. So this study was undertaken to find out what percentage of patients with a suspected diagnosis of ARVC referred for cMR had positive clinical results and whether the outcome helped in deciding further patients' care.

## MATERIALS AND METHODS

### Study population

All the patients who were referred for cMR with a suspected diagnosis of ARVC or with a family history of suspected or confirmed ARVC from May 2006 to February 2010 in our institution were included in this retrospective analysis (Table 3). A total of 121 patients were referred in this period. Seven patients were not scanned due to claustrophobia. Therefore, the study included 114 patients. Out of the 114 patients who underwent cMR, 63 patients were male (55%) and 51 were female (45%). The age range was from 16 to 83 years, however 82% of the patients were between 20 and 60 years of age. The majority of these patients (84%) were referred from the cardiologists in our teaching institution, and the rest came from the cardiologists from the five district general hospitals and one private hospital. The three most common reasons for referral were arrhythmias (30%), fam-

**Table 2** Revised task force criteria for imaging<sup>[5]</sup>

#### Major

##### By 2D echo:

Regional RV akinesia, dyskinesia or aneurysm and one of the following (end diastole):

- 1 Parasternal long axis view RVOT (PLAX)  $\geq 32$  mm (corrected for body size (PLAX/BSA)  $\geq 19$  mm/m<sup>2</sup>)
- 2 Parasternal short axis view RVOT (PSAX)  $\geq 36$  mm (corrected for body size (PSAX/BSA)  $\geq 21$  mm/m<sup>2</sup>)
- 3 Or fractional area change (FAC)  $\leq 33\%$

##### By MRI:

Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following:

- 1 Right ventricular end diastolic volume (RVEDV/BSA)  $\geq 110$  mL/m<sup>2</sup> (male) or  $\geq 100$  mL/m<sup>2</sup> (female)
- 2 Or RVEF  $\leq 40\%$

##### By RV angiography:

Regional RV akinesia, dyskinesia or aneurysm

#### Minor

##### By 2D echo

Regional RV akinesia or dyskinesia and one of the following (end diastole):

- 1 Parasternal long axis view RVOT (PLAX)  $\geq 29$  -  $< 32$  mm (corrected for body size (PLAX/BSA)  $\geq 16$  -  $< 19$  mm/m<sup>2</sup>)
- 2 Parasternal short axis view RVOT (PSAX)  $\geq 32$  -  $< 36$  mm (corrected for body size (PSAX/BSA)  $\geq 18$  -  $< 21$  mm/m<sup>2</sup>)
- 3 Or FAC  $> 33\%$  -  $\leq 40\%$

##### By MRI

Regional RV akinesia or dyskinesia or dyssynchronous RV contraction and one of the following:

- 1 Right ventricular end diastolic volume/BSA  $\geq 100$  -  $< 110$  mL/m<sup>2</sup> (male) or  $\geq 90$  -  $< 100$  mL/m<sup>2</sup> (female)
- 2 RVEF  $> 40\%$  -  $\leq 45\%$

RVEF: Right ventricular ejection fraction; RVOT: Right ventricular outflow tract; FAC: Fractional area change.

ily history of sudden death (20%) and abnormal RV on echocardiography or on a previous cMR (19%). The other reasons for referral included history of palpitations and syncope, Supra-ventricular tachycardia, suspected Brugada syndrome, abnormal left ventricle (LV) and dilated cardiomyopathy on echo, frequent ectopics and cardiac arrest.

The institutional review board approved this retrospective study. Since this was a service evaluation, formal ethical approval was not required. However, the patients' confidentiality was respected.

### Acquisition protocol

All the cardiac magnetic resonance imaging (MRI) scans were performed on a Siemens Avanto 1.5 Tesla magnetic resonance scanner using a body coil. The ARVC protocol included: (1) scout images; (2) dark blood Half-Fourier Acquisition Single-Shot Turbo Spin-Echo imaging in 3 planes-axial, coronal and sagittal; and (3) a series of balanced, steady state free precession (25 phase) cine images, with standard views in the following planes; Vertical long-axis (VLA) or 2-chamber; 4-chamber (FCH); Left ventricular outflow tract (LVOT) or 3 chamber; Right ventricular outflow tract; short axis stack at 10 mm intervals to allow quantification of ventricular volumes and function; Axial stack to assess for RV free wall regional wall motion abnormality; (4) gadolinium was adminis-

**Table 3 Study population**

Time period	May 2006-Feb 2010
Total No. of patients referred	121
Total No. of patients scanned	114
Age range	16 to 83
16-20	7 (6%)
21-30	19 (17%)
31-40	25 (22%)
41-50	28 (24%)
51-60	21 (18%)
61-70	10 (9%)
71-80	3 (3%)
81-90	1 (1%)
Males	63 (55%)
Females	51 (45%)
Referrals	
Teaching Hospitals	96 (84%)
District General Hospitals	18 (16%)
Reasons for referral	
Arrhythmias	34 (30%)
Family history of sudden death	23 (20%)
Others	57 (50%)
Others	
Abnormal RV in echo	22 (19%)
Brugada syndrome	8 (7%)
Syncope	8 (7%)
Palpitations	6 (5%)
Dilated cardiomyopathy in echo	4 (3.5%)
Frequent ectopics	4 (3.5%)
SVT	3 (3%)
Abnormal LV	1 (1%)
Cardiac arrest	1 (1%)

RV: Right ventricle; LV: Left ventricle.

tered at a rate of 0.1 mmol/kg and followed by early gadolinium inversion recovery images in the LVOT, FCH, VLA planes, with an inversion time (TI) of 440 ms; and then (5) late gadolinium inversion recovery images were obtained of the LVOT, FCH and VLA planes and as a short axis stack; repeated with a phase swap, with the TI set to produce optimal myocardial nulling. Right ventricular volumes were analysed from the short axis stack and compared with indexed normal values.

### Data analysis

Patient details including age, sex, ECG and echo abnormalities, family history, MRI findings and information about clinical management were obtained. We analyzed the data for cMR findings, which fitted with the major and minor criteria for ARVC. The major MRI criteria for ARVC included severe global/segmental dilatation of the RV and global systolic dysfunction. The minor criteria included mild global/segmental dilatation of the RV, regional contraction abnormalities and global diastolic dysfunction according to the Task Force Criteria. The gadolinium enhancement if seen was also recorded. At least 2 radiologists and 1 cardiologist jointly reported the scans. The MRI findings were correlated with clinical outcome.

### Statistical analysis

The overall sensitivity and specificity of cMR to diagnose

**Table 4 Results**

RV abnormalities related to ARVC	19 (17%)
Major	4 (4%)
Minor	13 (11%)
Non specific	2 (2%)
Other diagnoses	95 (83%)
Normal	63 (55%)
Dilated cardiomyopathy	8 (7%)
Left to right shunt	1 (1%)
RV infarction	1 (1%)
LV infarction	1 (1%)
Other mild abnormalities	21 (18%)
Clinically proven ARVC	5 (4%)
cMR major	3
cMR minor	2
Non specific (out of 2)	0
Others (out of 95) <sup>1</sup>	0
Family history of sudden death	23
Clinically proven ARVC	1
Minor and non specific criteria for ARVC (not clinically proven)	3
Normal	15
LV hypertrophy	1
LV dyssynchrony	1
LV infarct	1
Dilated cardiomyopathy	1

<sup>1</sup>Clinical data was available for only 81 patients. RV: Right ventricle; ARVC: Arrhythmogenic right ventricular cardiomyopathy; LV: Left ventricular.

ARVC and the positive and negative predictive values in three different groups (arrhythmias, family history and others) were calculated. Values were expressed in percentages.

## RESULTS

CMR findings with abnormalities related to the right ventricle were classified as major, minor and non-specific according to whether they met major or minor cMR diagnostic criteria of ARVC. There were also some non-specific findings related to the RV such as mild dyskinesia, thinning of RV wall and mildly impaired RV function.

Of the 114 patients, 19 patients had RV related abnormalities (Table 4). Of these, 4 patients had major cMR criteria, 13 had minor criteria and 2 patients had non-specific features. Out of the 4 who had major criteria for ARVC, 3 were clinically proven to have ARVC of whom 2 had an implantable cardioverter defibrillator (ICD) fitted. The third patient, who also had family history of ARVC, died soon after the diagnosis. Only this patient (1 out of 4) showed extensive RV free wall late gadolinium enhancement. The fourth patient did not meet the full diagnostic criteria, but still had an ICD fitted for recurrent episodes of ventricular tachycardia (VT). Out of the 13 patients with minor criteria, 2 were clinically proven to have ARVC. None of these patients had late gadolinium enhancement. Eight patients with either minor or non-specific criteria had repeat MR scan, which suggested either no significant or mild change compared to the earlier scans. Out of the 95 patients who had nega-



**Table 5** Positive predictive value in different groups

Clinical history - No of patients	Major criteria present	Clinically proven ARVC	Positive predictive value	Minor criteria present	Clinically proven ARVC	Positive predictive value
Arrhythmia (30%)	2	1	50%	3	2	67%
Family history of SCD (20%)	1	1	100%	1	0	0%
Others (50%)	1	1	100%	9	0	0%

ARVC: Arrhythmogenic right ventricular cardiomyopathy; SCD: Sudden cardiac death.

tive cMR for ARVC, clinical data were available for 81 patients and none of them had a positive clinical diagnosis of ARVC. Of these patients, 63 patients had normal scans and 8 had dilated cardiomyopathy. Other significant diagnoses included left to right shunt (1), LV infarction (1) and RV infarction (1). The remaining patients had mild chamber or aortic root abnormality.

There were 23 patients referred with a family history of sudden death. One had major cMR criteria, one had minor cMR criteria and 2 had non-specific findings related to the RV on cMR. Apart from the one patient who had major criteria, there was no other clinically proven ARVC in patients with family history. Out of the remaining 19 patients, 15 patients had normal scans, 1 had LV hypertrophy, 1 had LV dyssynchrony, 1 had an LV infarct with RV impairment and one had dilated cardiomyopathy.

In summary, of the total cohort of 114 patients, 17 % had scans showing abnormalities related to RV and 83% had scans not suggestive of ARVC. Four percent of the study population had clinical proven ARVC and in all of them, MR was positive for either major or minor criteria. Excluding the 14 patients with no clinical data and final diagnosis, the overall sensitivity of the test was 100%, specificity 87%, positive predictive value 29% and the negative predictive value 100%. If split by criteria, the positive predictive value for major criteria was 75% and minor criteria 15%. The positive predictive values for different patient groups (arrhythmias (30%), family history (20%), and others (50%)) are given in Table 5. The negative cMR diagnoses were reassuring in terms of clinical management especially for the patients with family history of sudden death.

## DISCUSSION

This study has shown that in this cohort of patients 15% fulfill imaging criteria for ARVC, with a subsequent 75% of cases fulfilling Task Force criteria for the diagnosis of ARVC if they had a major CMR criterion. Conversely a negative CMR scan for ARVC on imaging criteria translated into no subsequent diagnosis of ARVC.

Referrals for cMR in our centre come from a variety of sources, including the Inherited Cardiac Conditions service and the Arrhythmia service, which are based regionally at our institution. In addition, referrals are received from general cardiology clinics in our institution and from the district general hospitals in our region. The scans are reported jointly by radiologists and cardiologists at multidisciplinary meetings to maximize the clinical rel-

evance of the reports.

In our study 15% of the patients fulfilled imaging criteria for ARVC. Major criteria were found in 4% of the cases and minor criteria were found in 11%. Data from other centers report a detection rate of 3%-10%<sup>[26,27]</sup>, for unselected cMR referrals for possible ARVC. Therefore our detection rate does not differ significantly from these. These detection rates may fall following implementation of the modified Task Force Criteria<sup>[5]</sup>, with one study showing a significant drop in the number of positive scans<sup>[28]</sup>.

Our study showed that none of the patients with CMR scans that were negative for ARVC on imaging criteria were subsequently diagnosed with ARVC. This is an important finding and is reassuring to the clinicians involved. This negative predictive value of 100% compares to previous studies which have also shown the diagnostic accuracy of CMR in the diagnosis of ARVC<sup>[28]</sup>. While it may be perceived by some that many studies proved to be negative, we claim that these studies are particularly helpful to the clinician and the patient alike in excluding important pathology such as ARVC, particularly in patients presenting with arrhythmias or those with a family history of either ARVC or of sudden cardiac death. Interestingly we also found evidence of other pathologies in 11% of patients with scans negative for ARVC on imaging criteria, the majority of these were diagnosed as dilated cardiomyopathy. This is in keeping with other studies, which report an incidence of significant other etiologies being diagnosed in 4%-8% of the cases<sup>[26,27]</sup>.

We have also shown that scans positive for ARVC on imaging criteria translate into a high percentage of patients formally fulfilling Task Force criteria for ARVC (75% of cases with a major imaging criteria, 15% with a minor criteria) and that no scans negative for ARVC on imaging criteria were subsequently diagnosed with ARVC. These reflect a reassuring performance by our clinically effective service where scans are reviewed in a joint cardiology and radiology multidisciplinary team meeting. In conclusion, CMR is a useful tool for excluding ARVC, because of a high negative predictive value and is especially helpful in patients with family history of sudden death. A positive scan correlates well with clinical findings. The technique is cost effective as the positive or negative outcomes have significant impact on the clinical decision-making.

Limitations of this paper are that the number of patients studied were small, with no control group and this precluded more detailed statistical analysis, such as hazard

ratio's. Further work in this area should include a larger study population.

## COMMENTS

### Background

Arrhythmogenic right ventricular cardiomyopathy (ARVC), an inherited disorder is a relatively common cause of sudden death especially in young athletes. The diagnostic criteria is however not simple and depends on fulfilling modified Task Force Criteria which involves a lot of diagnostic work up including multimodality imaging. Cardiac magnetic resonance imaging (cMR) is one such useful tool and also the one frequently requested to evaluate for ARVC. Although the previous studies have demonstrated the critical role of cMR in the diagnosis of ARVC, the impact of cMR outcome in an unselected population is not widely analysed.

### Research frontiers

The definitive diagnosis requires endomyocardial biopsy, which is an invasive procedure, however even the biopsy may still not be sensitive due to the patchy nature of the disease. Cardiac MR has emerged over the years as a very useful non-invasive modality of choice and the research hotspot is to find out whether it can serve as a one-stop shop for the evaluation of ARVC.

### Innovations and breakthroughs

Data from other centers report a detection rate of 3%-10%, for unselected CMR referrals for possible ARVC. This study results show 4% of the referrals had positive clinical diagnosis and none of the patients with negative CMR scans were subsequently diagnosed with ARVC. This is an important finding and is reassuring to the clinicians involved. Although significant proportion of studies proved to be negative, the authors claim that these studies are particularly helpful to the clinician and the patient alike in excluding important pathology such as ARVC, particularly in patients presenting with arrhythmias or those with a family history of either ARVC or of sudden cardiac death.

### Applications

The study results show that cardiac MR is a useful tool for excluding ARVC, because of a high negative predictive value and the positive scan also correlates well with clinical findings, which makes the study cost effective as both the outcomes have significant impact on the clinical decision-making.

### Terminology

"Arrhythmogenic cardiomyopathy" - "cardiomyopathy" refers to disease of the heart muscle which when affected by inflammation with subsequent fibrosis and fat infiltration can cause "arrhythmogenic" potential which triggers the heart muscle to produce very high heart rates such as ventricular tachycardia and fibrillation due to electrical instability which can result in sudden death.

### Peer review

The manuscript is well written and highly organized. The readability is excellent. In this retrospective analysis of 114 patients referred for CMR because of arrhythmias or family history of sudden death, the results of CMR were classified depending on both functional and tissue characterisation and the clinical information were used. The assessment and judgment of the images of CMR was performed jointly by radiologist and cardiologist. This study shows that CMR has an important role in the diagnosis of ARVC as it allows 3-D visualization of the ventricles and CMR is sometimes useful in finding other disorders for patient's symptoms.

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## Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon

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**Key words:** Coronary artery fistula; Coronary artery anomalies in adult; Coronary artery disease

**Core tip:** This report highlights the presence of an extremely rare coronary anomaly in adult, in the form of a fistula between left anterior descending coronary artery and left superior pulmonary vein with steal phenomenon causing angina that resolved by medical treatment.

Barsoum EA, Saiful FB, Asti D, Morcus R, Khoeiry G, Lafferty J, McCord DA. Rare case of coronary to pulmonary vein fistula with coronary steal phenomenon. *World J Cardiol* 2014; 6(7): 682-684 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/682.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.682>

### Abstract

Coronary artery fistulas are abnormal connections between coronary artery territories and cardiac chambers or major vessels, most of them are congenital. Patients with coronary artery fistula can be asymptomatic or present with different symptoms like angina. Cardiac computed tomography (CT) is one of the best modalities for diagnosis. We present an elderly patient that presented with angina symptoms, non invasive stress test was positive for ischemic heart disease, coronary angiogram could not reveal any obstructive lesions, but an abnormal branch of the left descending coronary artery (LAD), cardiac CT showed fistula that connect left anterior descending coronary artery to left superior pulmonary vein. Our case is extremely rare as most of the reported cases were fistulas between LAD and pulmonary artery, but in our case the fistula between LAD and left superior pulmonary vein. In addition, our patients' symptoms resolved with anti-ischemic medical treatment without any surgical intervention.

### INTRODUCTION

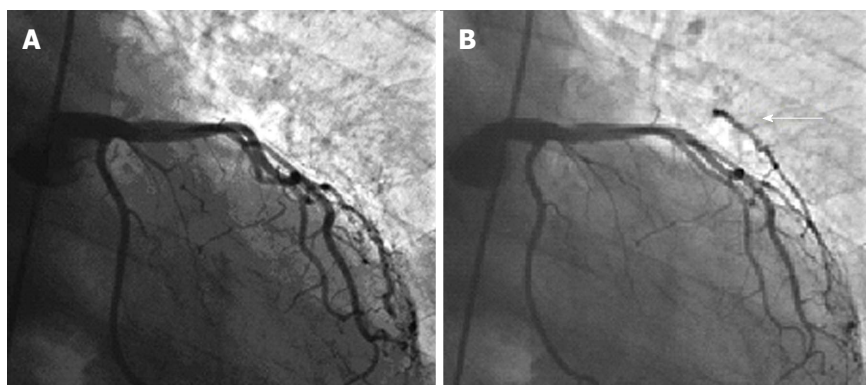
Coronary artery anomalies are found in 1% of coronary angiograms<sup>[1]</sup>. Some of these anomalies are clinically insignificant but, many others are associated with serious morbidity and potential mortality. Coronary artery anomalies can be detected by a variety of means including echocardiography, coronary artery angiography and multidetector-row computed tomography<sup>[2,3]</sup>.

The following case report describes an elderly patient presenting with angina, whose coronary angiography and cardiac computed tomography (CT) revealed an abnormal communication between the left anterior descending (LAD) and the left superior pulmonary vein.

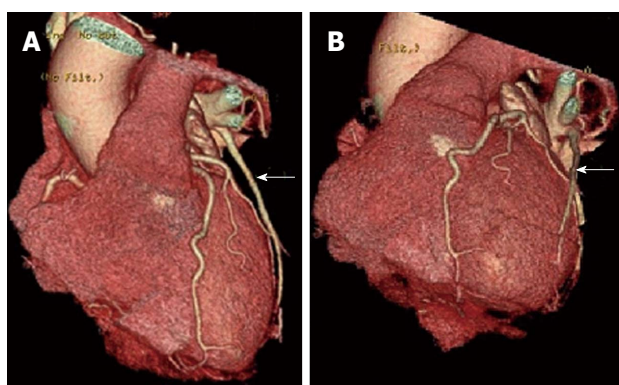
### CASE REPORT

A 67-year-old man presented to the office with exertional chest pain of six weeks. He had a past medical history significant for hypercholesterolemia and gastro esopha-





**Figure 1** Coronary angiogram. A: Showing coronaries without significant atherosclerotic lesion; B: Showing fistula arises from left anterior descending.



**Figure 2** Multislice computed tomographic angiography. A: Showing fistula between left anterior descending (LAD) and superior pulmonary vein; B: Showing fistula between LAD and superior pulmonary vein.

geal reflux disease. He quit smoking 30 years ago. A thallium stress test revealed a moderate sized, completely reversible, anterior and anterolateral wall defect suggestive of LAD territory ischemia. Coronary angiography was performed, which failed to reveal any obstructive disease in the LAD as well any other coronary vessel. However, in the distal segment of the LAD there was a small aneurysmal dilatation and a communication with an extracardiac vessel that was well opacified with antegrade injection of contrast (Figure 1). We concluded that it was likely to be a fistula between the LAD and a segment of the left pulmonary artery system. We decided to get a CT angiogram of the chest to better delineate the nature of the fistula as an outpatient. The patient was discharged home on a regimen including a beta-blocker, statin, ACE inhibitor and a long acting nitrate. Soon after, a multislice computed tomographic angiography of the thorax and coronaries was performed. A fistula arising from the distal LAD and connecting to the left superior pulmonary vein was elucidated (Figure 2). The patient has been followed up at one and three month intervals. He continues to do very well with optimal medical therapy and remains free of exertion and rest angina.

## DISCUSSION

Coronary artery fistula (CAF) is an abnormal connection between coronary artery territories and cardiac chambers

or major vessels, CAF represent 17% of angiographic diagnosed anomalies<sup>[1]</sup>. Majority of them are congenital, but can be acquired secondary to increasing application of intravascular diagnostic instrumentations and therapeutic procedures or even secondary to blunt or penetrating trauma<sup>[4-11]</sup>.

Patient with coronary artery fistulas usually asymptomatic that the fistula accidentally detected by echocardiography and coronary angiography, but patients may have varied symptoms such as angina pectoris, palpitations, syncope, congestive heart failure, and may even present with sudden cardiac death. In some cases, physical examination may or reveal a murmur if the flow is significant<sup>[12]</sup>.

The majority of reported LAD fistulas have been between the LAD and pulmonary artery, but in our case the anomaly is between the LAD and the left superior pulmonary vein. Also, it is interesting that we have concomitant coronary steal phenomenon by the pulmonary venous system. This is evident due to the presence of angina symptoms and a reversible defect on nuclear imaging that is not explained by coronary artery disease. It is plausible that a small fistulas increase in size with advancing age secondary to changes in vessels compliance and pressure and as it reaches a certain flow threshold, begins to exhibit steal phenomenon.

The main treatment of symptomatic coronary fistulas is surgical and a variety of operative techniques have been described in the literature including internal closure of the fistula from within the distal communication, distal ligation alone, proximal and distal ligations and closure from within the aneurysmal coronary artery<sup>[13,14]</sup>. In addition, transcatheter retrograde coil embolization became a safe and effective alternative to standard surgical closure<sup>[15,16]</sup>. However, our patient's symptoms are resolved with optimal anti ischemic medical therapy.

In a conclusion, Coronary artery fistula to pulmonary vein is extremely rare, medical treatment is effective to resolve patient's symptoms, long term follow up is highly recommended.

## COMMENTS

### Case characteristics

A 67-year-old man with a history of hypercholesterolemia and gastro esopha-

geal reflux disease presented with exertional chest pain.

### Clinical diagnosis

Normal physical exam.

### Differential diagnosis

Ischemic heart disease, non cardiac causes of chest pain.

### Laboratory diagnosis

Cardiac enzymes were within normal limits.

### Imaging diagnosis

Coronary angiography and cardiac computed tomography revealed an abnormal communication between the left anterior descending (LAD) and the left superior pulmonary vein.

### Treatment

The patient was treated with beta-blocker, statin, ACE inhibitor and a long acting nitrate.

### Related reports

A fistula between left anterior descending coronary artery and left superior pulmonary vein is extremely rare. Most of the reported cases are between a coronary artery and the pulmonary artery.

### Term explanation

Coronary artery anomalies are rare in adults, but they can induce angina symptoms.

### Experiences and lessons

This case report represents rare fistula between LAD and left superior pulmonary vein, cardiac computed tomography scan was a sensitive modality in the detection of the fistula and the patient improved on medical treatment without surgical intervention.

### Peer review

This article presents an extremely rare fistula between LAD and left superior pulmonary vein in adult.

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## Worsening of coronary spasm during the perioperative period: A case report

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### Abstract

We present the case of a 65-year-old male with vasospastic angina (VSA) whose condition worsened during the perioperative period. He had been diagnosed with VSA 10 years prior. He was treated with two types of vasodilators and had not experienced any chest symptoms for 5 years. At this juncture, he underwent surgery for relapsed maxillary sublingual carcinoma. He had taken two vasodilators one day prior to surgery. Intravenous infusion of nitroglycerin (NTG) was initiated immediately before the surgery and continued the following day. Instead of stopping NTG, a dermal isosorbide dinitrate tape was applied on post-operative day 1. Two days later, a complete atrioventricular block with pulseless electrical activity appeared. After cardiopulmonary resuscitation, emergent coronary angiography showed severe coronary spasm in both the left and right coronary arteries. Intracoronary infusion of nitroglycerin and epinephrine with percutaneous cardiopulmonary support relieved the coronary spasm. During the perioperative period, several factors can trigger coronary vasospasm, including the discontinua-

tion of vasodilators. Thus, surgeons, anesthetists, and cardiologists should watch for coronary vasospasm during this period and for worsening coronary spasm when discontinuing vasodilators in patients at risk for VSA.

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**Key words:** Coronary vasospasm; Perioperative period; Discontinuation of vasodilator

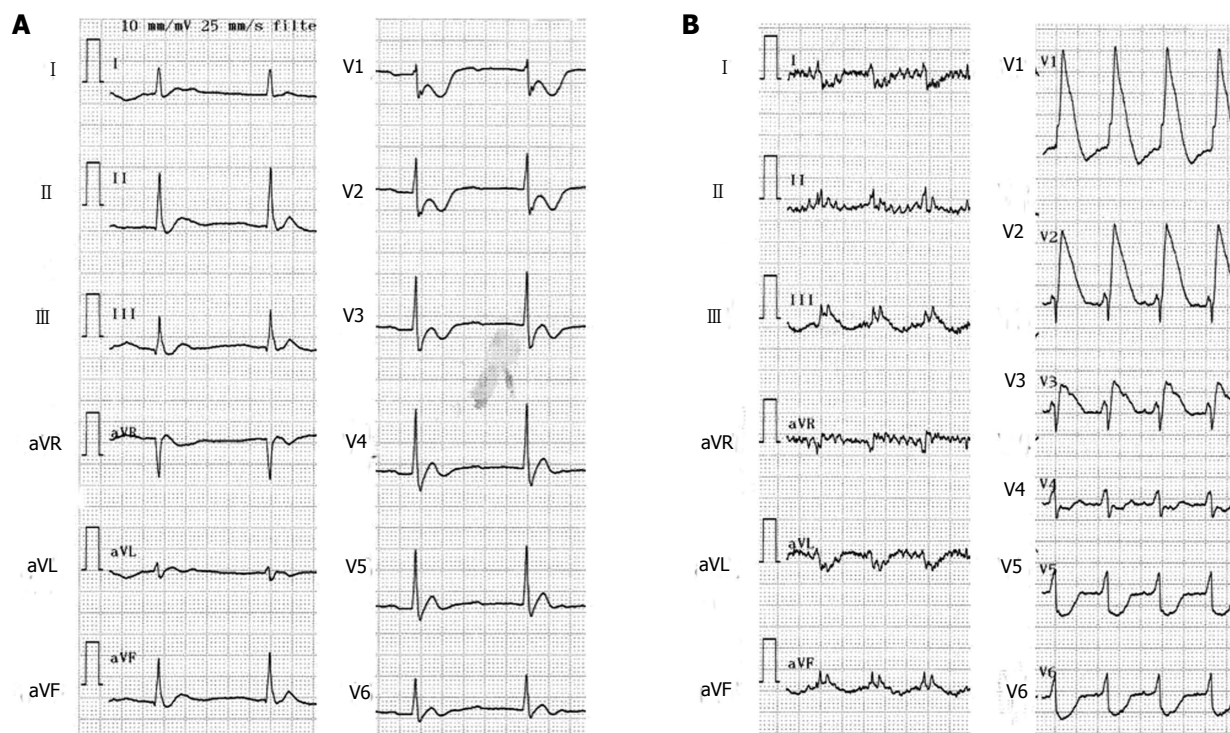
**Core tip:** Coronary spasm during the perioperative period often emerges severely as either cardiogenic shock or ventricular fibrillation. Although there are several surgery-related factors that influence the activity of coronary spasm, discontinuing vasodilators during the perioperative period is an important problem in patients with vasospastic angina (VSA). We encountered an out-patient with VSA whose condition had been stabilized using two types of vasodilators but subsequently worsened, leading to cardiogenic shock during the perioperative period. In light of this event, physicians should carefully evaluate their patients regarding the possibility of a coronary spasm during the perioperative period.

Teragawa H, Nishioka K, Fujii Y, Idei N, Hata T, Kurushima S, Shokawa T, Kihara Y. Worsening of coronary spasm during the perioperative period: A case report. *World J Cardiol* 2014; 6(7): 685-688 Available from: URL: <http://www.wjgnet.com/1949-8462/full/v6/i7/685.htm> DOI: <http://dx.doi.org/10.4330/wjc.v6.i7.685>

### INTRODUCTION

The abrupt cessation of vasodilators causes rebound coronary spasm in patients with vasospastic angina (VSA)<sup>[1-6]</sup>. During the perioperative period, physicians sometimes have to discontinue oral vasodilators, even in patients with VSA. Furthermore, other factors that worsen VSA





**Figure 1** Electrocardiograms showed complete atrioventricular block when blood pressure decreased (A) and ST elevations were noted in leads II, III, aVF, and V1-3 immediately after cardiopulmonary resuscitation (B).

occur during the perioperative period. Thus, VSA activity may be accelerated during this interval. However, little is known about VSA management during this period<sup>[7,8]</sup>. In this study, we present a case in which coronary spasm worsened during the perioperative period.

## CASE REPORT

A 65-year-old male, who was diagnosed with VSA 10 years prior and treated with two types of vasodilators (benidipine hydrochloride, 8 mg/d, and nicorandil, 5 mg/d) was admitted to the Department of Otolaryngology at our institution to undergo surgery for relapsed maxillary sublingual carcinoma. He had a coronary risk factor due to smoking (30/d  $\times$  37 years). With regard to the results of the interview and medical records, the patient experienced spontaneous and/or imminent episodes of VSA several times per year for the first 5 years, but he had experienced no chest symptoms for the past 5 years. On admission, his vitals were stable. Blood examination, electrocardiogram (ECG), and echocardiography showed no specific findings.

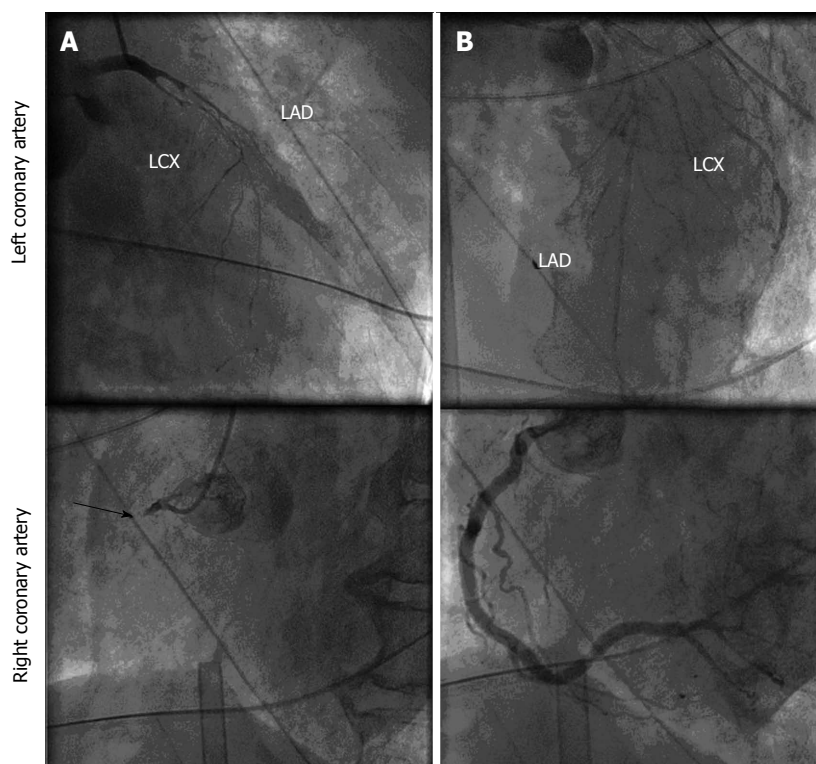
On pre-operative day 1, the patient took the same 2 vasodilators; this oral medication was stopped on the day of surgery. Instead, from the morning of the surgery, an intravenous infusion of nitroglycerin (NTG) at 2 mL/h was started. A large left maxillary resection and submaxillectomy were performed. On post-operative day 1, the patient was sedated with midazolam. Intravenous NTG was stopped on post-operative day 1, and a dermal isosorbide dinitrate tape was applied. In the evening of post-operative day 2, his blood pressure decreased, and ECG

showed a complete atrioventricular block (Figure 1A). Pulseless electrical activity (PEA) was subsequently noticed, and cardiopulmonary resuscitation was initiated with repeated infusions of epinephrine. Ventricular fibrillation and PEA were repeated and percutaneous cardiopulmonary support (PCPS) was started 1 h later. ECG at that time showed ST elevations in leads II, III, aVF, and V1-3 (Figure 1B). Emergent coronary angiography revealed an occlusion at the proximal segment of the right coronary artery, and severe and diffuse narrowing due to coronary spasms of the left coronary artery (Figure 2A). Intracoronary infusions of nitroglycerin and epinephrine (20-100  $\mu$ g) were repeated, which relieved the bilateral coronary spasms (Figure 2B). Intravenous NTG and dopamine were continued, and PCPS was removed 4 d later. From post-operative day 19, oral benidipine hydrochloride at 8 mg/d was started, in addition to intravenous NTG infusion. From post-operative day 21, NTG infusion was discontinued, and only oral benidipine hydrochloride was prescribed. Thereafter, the patient had no chest symptoms; however, he died on post-operative day 74 from hydrocephalus due to the original disease.

## DISCUSSION

We describe a case with VSA, where the condition was stable for 5 years but worsened during the perioperative period. The cessation or reduction of vasodilators worsens coronary spasm in patients with VSA<sup>[1-6]</sup>. In fact, we have seen several VSA cases in which noncompliance to vasodilators increased angina attacks in the clinical setting. In the perioperative period, vasodilators sometimes





**Figure 2** Emergent coronary angiography showed severe narrowing of the left anterior descending coronary artery and the left circumflex coronary artery and occlusion at the proximal segment of the right coronary artery (indicated by an arrow) in initial shots (A), and the trunks of the left anterior descending coronary artery, left circumflex coronary artery, and right coronary artery were found to be dilated despite remaining coronary spasm at the branches after intracoronary infusions of nitroglycerin and epinephrine (B). LAD: Left anterior descending coronary artery; LCX: Left circumflex coronary artery.

have to be stopped or reduced, even in patients with VSA. Thus, VSA management during the perioperative period is of pivotal importance, based on the knowledge gained from the present case.

Although the occurrence or exacerbation of VSA during the perioperative period is not frequent<sup>[8]</sup>, the problem certainly exists. At our institution, the incidence from 1999 to 2008 was 0.042% (17 cases/40466 operations, unpublished data). Koshiba *et al*<sup>[7]</sup> reviewed the clinical characteristics of perioperative coronary spasm and raised several important points, including possible contributing factors and the surgical site. Contributing factors for perioperative coronary spasm may include inadequate anesthesia, use of vasopressors, vagal nerve stimulation, drugs other than vasopressors, epidural block, hypotension, mechanical stimulation of the heart, allergic reaction, and/or mental stress. Regarding the surgical site, abdominal surgery was performed in 49% of cases. At our institution, abdominal surgeries comprised 76% of all perioperative VSA cases (13 of 17), and upper abdominal surgery was the most frequent type with 65% (11 of 17 cases, unpublished data). An increase in vagal nerve stimulation, a possible contributing factor, may be the result of abdominal surgery, which was the most frequently performed procedure. Nagayoshi *et al*<sup>[8]</sup> reported a case of perioperative coronary spasm at their institution and showed that the surgical risks are fairly low in patients who developed coronary spasm in the perioperative period. Preoperative consultations with a cardiologist were reported in only 2 of 18 cases with perioperative coronary spasm. Therefore, a cardiologist should be consulted before surgery in patients with known VSA or coronary artery disease (CAD). In the clinical setting,

even though the presence of VSA or CAD has not been indicated, we sometimes encounter patients who have taken vasodilators and experienced no chest symptoms. A preoperative cardiology consultation should be recommended, even for such patients. Cardiologists should check all patient information, including interview notes, medical records, and preoperative cardiovascular examinations, and consider the possibility of a coronary spasm. Discontinuing vasodilators, including calcium-channel blockers, during the perioperative period is another important factor<sup>[1,9]</sup>. In principle, vasodilators should be taken during the perioperative period; however, the surgery, which normally requires the discontinuation of these medications, must proceed as planned. In the present case, where a large left maxillary resection and submaxillectomy were performed, vasodilators were discontinued during the surgery. Under such circumstances, two alternatives exist: intravenous vasodilators and dermal nitrate tape/patch. We previously conducted a survey regarding the perioperative management of VSA patients by 31 cardiologists from the Hiroshima Prefecture (unpublished data). Based on the results of the questionnaires, intravenous vasodilators was not routine but frequently given, depending on the patient's VSA status, in 93% of cases. The patient's VSA status includes VSA episodes while taking oral medications, spasm-provocation test results (*e.g.*, organic stenosis, multivessel spasm, severely provoked spasm), and the number of vasodilators (more than or equal to 2). Intravenous vasodilators generally include NTG, nicorandil, and diltiazem, and determining when to stop the intravenous vasodilators may be a problem. They can be instantly stopped in VSA patients who can take oral vasodilators, particularly those with low

VSA activity. However, in patients who cannot take vasodilators orally, are compelled to use a dermal nitrate tape/patch, or have a high VSA activity, the use of intravenous vasodilators and oral medications (or dermal nitrate tape/patch) simultaneously may be necessary for 1 or 2 d. In the present case, because the patient used a dermal NTG tape after terminating intravenous NTG, the vasodilating effect may have decreased, leading to severe VSA. In contrast, a dermal nitrate tape/patch is usually prescribed for VSA patients with less active disease. In our survey, 69% of all effective answers indicated that the dermal nitrate tape/patch was used in patients who had taken vasodilators for an extensive period, in spite of a low probability of having VSA. Long-term use of nitrates, particularly a dermal nitrate tape/patch, increases the possibility of nitrate tolerance<sup>[9-11]</sup>; therefore, physicians should be extremely judicious in prescribing these drugs.

Learning from the present case, we recommend the following management of coronary spasm during the perioperative period: (1) not only the cardiologist but also the surgeon and anesthetist have to rule out the presence of VSA according to the patient's medical records, interview, and results from the preoperative cardiovascular examinations; (2) if VSA is established, vasodilators must be included in the patient's treatment plan. If there is a suspicion of VSA, *i.e.*, the patient has been taking vasodilators for a long time, the regimen should be continued during the perioperative period; and (3) the use of intravenous vasodilators should be determined on the basis of the patient's VSA activity. During the transition from IV to oral administration, no gap should be allowed. The simultaneous use of two drugs may be required for 1 or 2 d.

In conclusion, perioperative coronary spasm has been proven to exist, despite its low frequency. In evaluating a patient's medical history and presenting symptoms, physicians should consider and investigate the possibility of perioperative coronary spasm. Intravenous infusion of vasodilators during the perioperative period is sometimes required, but a discontinuation may pose a risk of coronary spasm for the patient.

## COMMENTS

### Case characteristics

A 65-year-old male with vasospastic angina (VSA) whose condition worsened during the perioperative period.

### Differential diagnosis

Authors' present a case in which coronary spasm worsened during the perioperative period.

### Treatment

The patient had been diagnosed with VSA 10 years prior.

### Peer review

Teragawa *et al* present an interesting case report about worsening of coronary spasm during the perioperative period in a patients with previous diagnosis of vasospastic angina.

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## 3D-echo in preoperative assessment of aortic cusps effective height

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**Key words:** Aortic valve; Aortic repair; Aortic prolapse; Echocardiography

**Core tip:** 3D transesophageal echocardiography and multiple plane reconstruction lets visualize all the three coaptation planes between the aortic valve (AV) cusps and overcomes the limits of 2-D echocardiography which allows to see only two of three AV coaptation planes and this may lead to misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair. It is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root.

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### Abstract

Effective height, which represents the height difference between the central free margins and the aortic insertion lines can be easily determined by 2-D echocardiography and allows for identification of prolapse in the native cusps and assessment of prolapse correction after valve repair. Nonetheless, it allows to see only two of three aortic valve (AV) coaptation planes and this may lead to misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair. In contrast, 3D transoesophageal echocardiography and multiple plane reconstruction lets visualize all the three coaptation planes between the AV cusps and it represents an invaluable tool in the assessment of aortic valve geometry. It is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root.

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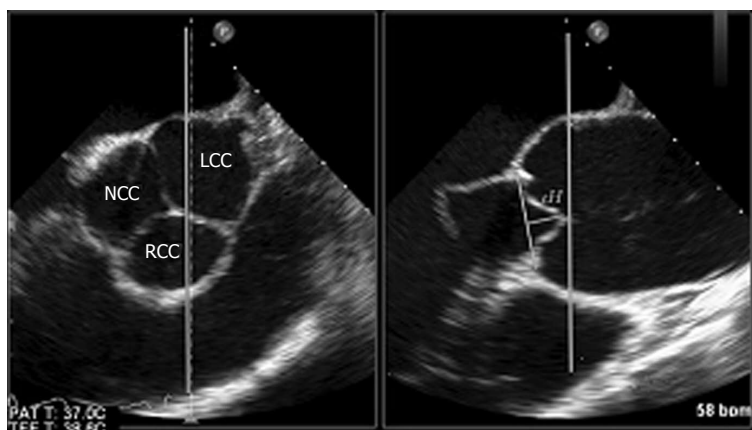
### TO THE EDITOR

In the recent years aortic valve (AV) repair has gained increasing interest in the treatment of aortic root pathology<sup>[1]</sup> as a feasible alternative to aortic valve replacement<sup>[2]</sup>.

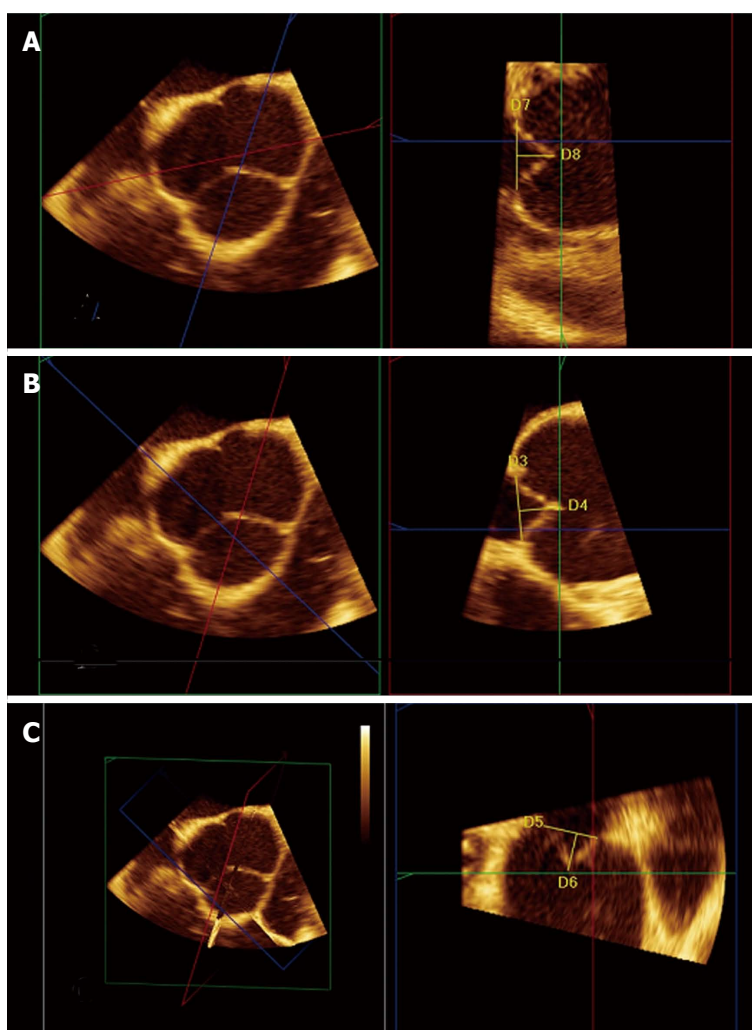
Good results have been achieved with valve-preserving aortic replacement for patients in whom aortic regurgitation is solely caused by aortic root dilatation with morphologically preserved valve leaflets<sup>[3]</sup>. In contrast, cusp repair still remains a surgical challenge when prolapse of cusp tissue impairs coaptation<sup>[4]</sup>.

The most prominent echocardiographic phenomenon indicating cusps prolapse is a decreased effective height (eH) which represents the height difference between the central free margins and the aortic insertion lines<sup>[4]</sup>. This measurement, which depends on the complex re-





**Figure 1** Established method of measuring the effective height in the 2-D short axis (left) and long axis image of the proximal aorta (right). As shown, only the coaptation between the right coronary cusp anteriorly and the left coronary cusp posteriorly can be measured. RCC: Right coronary cusp; LCC: Left coronary cusp; NCC: Noncoronary cusp.



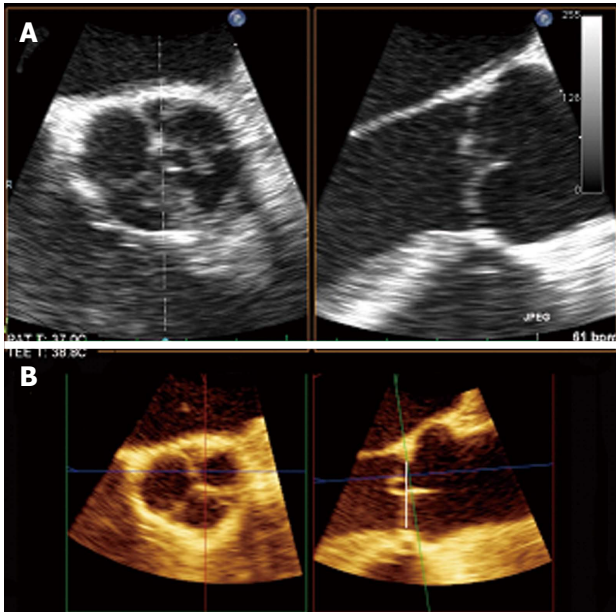
**Figure 2** Images of a normal aortic valve from multiple plane reconstruction of the aortic valve with 3D transesophageal echocardiography. A: The red plane intersects the coaptation surface of the noncoronary cusp (NCC) and left coronary cusps (LCC) The yellow line labelled D8 (12.7 mm) represents the LCC effective height; B: The red plane intersects the coaptation surface of the LCC and right coronary cusps (RCC) The yellow line labelled D4 (13.9 mm) represents the effective height; C: The red plane intersects the coaptation surface of the NCC and RCC. The yellow line labelled D6 (12.7 mm) represents the effective height.

relationship of root and cusp, can be easily determined by 2-D echocardiography and allows for identification of prolapse in the native cusps and assessment of prolapse correction after valve repair. Nonetheless, with 2-D transesophageal echocardiography (2D-TEE) only two of three AV coaptation planes can be seen and the eH, a unidimensional value, can be measured only between the right coronary cusp anteriorly and either the non- or left coronary cusp (depending on probe rotation) posteriorly (Figure 1).

As a result, pathology of the AV cusp not included in the view may go undetected and this may eventually result in misunderstanding of the underlying pathophysiological mechanism for aortic regurgitation and hence in unsuccessful repair.

Recent development of real-time 3D transoesophageal echocardiography (3D-TEE) allows multiple plane reconstruction (MPR) which lets visualize all the three coaptation planes between the AV cusps. Using MPR is possible to adjust the orthogonal imaging planes for op-





**Figure 3** Patient male, 53-year-old. A: On 2 D there is no clear demonstration of prolapse; B: Multiple plane reconstruction of the aortic valve with 3D transesophageal echocardiography: on reformatted image a prolapse of left coronary cusps is shown (right).

timal visualization of all three aortic coaptation lines. By moving the planes is possible to identify the points where the cusps come together and to obtain, for each one, an accurate determination of the eH (Figure 2). 3D-echo multi-plane reconstruction is a valuable tool in the assessment of valve geometry and it is highly recommendable before AV repair to accurately study the complex three dimensional cusps anatomy and their geometric interrelation with aortic root as a functional unit (Figure 3). This

is important since effective cusps height is a significant predictor of aortic valve repair failure. Indeed, an effective height > 8 mm is associated with 99.6% probability of clinically insignificant aortic regurgitation. Other significant predictors are residual regurgitation, a coaptation length < 4 mm and a level of cusp coaptation which is below the aortic annulus (type C)<sup>[5]</sup>.

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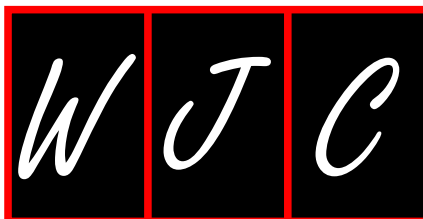
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- 2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

In press

- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

### Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

- 12 **Breedlove GK**, Schorheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee.

Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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