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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (2): Coronary artery disease

Coronary artery calcification in chronic kidney disease: An update

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Abstract

Arterial calcification is a well-recognized complication of advanced atherosclerosis. Chronic kidney disease (CKD) is characterized by significantly more pronounced, disseminated and fast-progressing calcification of the vascular system, including the coronary arteries. New computed tomography-based imaging techniques allow for the noninvasive assessment and monitoring of calcification in different vascular sites. Coronary artery calcification (CAC) develops early in the course of CKD and is tightly associated with mineral and bone disorders, which include but are not limited to secondary hyperparathyroidism. In this review, recent data on the pathogenesis of CAC development and progression are discussed, with a special emphasis on fibroblast growth factor 23 and its co-receptor, klotho. The prevalence, progression and prognostic significance of CAC are reviewed separately for patients with end-stage renal disease treated with dialysis, kidney transplant recipients and patients with earlier stages of CKD. In the last section, therapeutic considerations are discussed, with special attention paid to the importance of treatment that addresses mineral and bone disorders of CKD.

Key words: Chronic kidney disease; Dialysis; Kidney transplantation; Vascular calcification; Coronary artery calcification; Coronary artery calcification score; Agatston units

Core tip: Vascular calcification, a common feature of advanced atherosclerosis in the general population, is extremely advanced in patients with chronic kidney disease (CKD). CKD is associated with very fast progression of vascular (and in particular coronary) calcification. Pathogenetic aspects, clinical consequences and prognostic significance of coronary artery calcification in different CKD populations are discussed in this review. Therapeutic strategies used to limit the extent of vascular calcification and to improve the prognosis of patients with CKD are also discussed.

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INTRODUCTION

The importance of pathological calcification of soft tissue in chronic uremia has been recognized for a long time. The new era of research is associated with the introduction of new tools, allowing for noninvasive, quantitative assessment of mineral depositions in soft tissues, and electron-beam computed tomography (CT) and multi-slice CT (MSCT). A milestone study in the field was published in 1996 by Braun *et al*[1] which documented an extremely high coronary artery calcium score (CACS) of 4290 ± 1509 Agatston units in patients on long-term hemodialysis (for comparison, a value of 400 Agatston units is associated with an extremely high risk of coronary artery disease in a general population). Many stud-

ies that followed this seminal paper reported advanced coronary and other cardiovascular calcification in patients with chronic kidney disease (CKD) in the pre-dialysis period, on hemodialysis, peritoneal dialysis and following kidney transplantation. Several studies also documented progression of arterial calcification in patients who remained on dialysis or progressed from earlier to more advanced stages of CKD. We were among the first who demonstrated such a progression in patients treated with peritoneal dialysis and attenuation of progression following kidney transplantation[2-4]. Several experimental and clinical studies attempted to highlight mechanisms of development and progression of vascular calcification under the setting of chronic uremia. In this review, the pathophysiological background of coronary artery calcification (CAC) is discussed and the recent literature in the field of CAC in CKD reviewed.

CURRENT UNDERSTANDING OF PATHOPHYSIOLOGY OF CAC IN CKD

Calcium and phosphate

Mineral and bone disorders of CKD (CKD-MBD) develop early in the course of CKD. The hallmark of these disorders is hyperphosphatemia; levels of calcium and parathyroid hormone (PTH) are variable, *i.e.,* decreased, normal or elevated. Phosphate plays two important roles in the development of artery mineralization. It certainly serves as a substrate that is deposited within the tunica media or intimal layer of the vessel. It also acts as a mediator activating transcription of certain genes in vascular smooth muscle cells (VSMC) and pericytes which results in their transformation into osteoblast-like cells. The term "ossification" used sometimes with regards to pathological calcification is fully justified since this is not just a passive deposition of minerals within the vessel wall, but a precisely regulated process that mirrors bone formation. Macrophages resembling osteoclasts can also be found in an area of vascular mineralization; they become silenced upon challenge with phosphates, so the process of "bone formation" within the blood vessel is not counterbalanced with "bone resorption"[5,6]. It should be emphasized that phosphate, considered a uremic toxin responsible for several adverse effects on cardiovascular system (CVS) in CKD, now has also been identified as such a toxin in the general population. Several population-based studies (such as the Framingham Offspring Study) showed that a high-normal serum phosphate level is also associated with a worse outcome and a higher risk of CV end-points[7-9]. Low normal serum phosphorus in patients with normal renal function is associated with less calcification within coronary arteries[10].

PTH

Changes in plasma PTH are linked to poor survival of patients with CKD, although the normal PTH level for a given level of glomerular filtration rate (GFR) is the matter of ongoing debate. Although recently published Kidney Disease: Improving Global Outcomes (KDIGO) guidelines on CKD-MBD expanded the upper acceptable value in CKD stage 5 to as high as nine times above the reference value for normal subjects, recent studies indicate that mortality increases markedly when plasma PTH decreases below 150 or exceeds 300 pg/mL (according to most laboratories, the upper normal level for a healthy population oscillates around 70 pg/mL)[11,12]. It seems that low plasma PTH is even more significantly associated with progression of vascular calcification than high PTH. Low bone turnover resulting from low PTH leads to decreased ability of bone to uptake calcium and phosphate delivered with diet since renal function is severely compromised and there is no "safety valve" by means of hypercalciuria and hyperphosphaturia; excess minerals activate pathological calcification and serve as substrates to this process[13].

As in the case of phosphates, PTH is also considered cardiotoxic in uremia[14,15]. High-normal plasma PTH is also considered a risk factor for increased CV morbidity in patients with normal renal function[16,17].

Calcium sensing receptor

The discovery of calcium sensing receptor (Ca-SR) allowed for a more precise understanding of regulation of PTH synthesis and release in the course of calcium-phosphate metabolism disorders. Although its expression was originally thought to be limited to parathyroid cells, now it has become apparent that Ca-SR is present in several cell types. These include endothelial cells, cardiomyocytes and VSMC. Stimulation of Ca-SR on parathyroid gland cells strongly suppresses PTH synthesis and release. Ca-SR located in cardiovascular (CVS) structures seems to protect against their pathological calcification, decreased expression of this receptor observed in chronic uremia promotes osteoblastic transformation of VSMC and accelerates vessel wall calcification. Drugs designed to sensitize Ca-SR (*i.e.,* to enhance the receptor response even in lower serum calcium level, calcimimetics) were demonstrated to limit development and progression of vascular calcification in several experiments[5,18-20]. This is in agreement with observations made in a general population suggesting that a high calcium diet is cardioprotective[20]. Two distinct protective mechanisms of these drugs can be considered: better control of hyperparathyroidism and direct interaction with the vessel wall. Data from clinical studies using calcimimetics to control secondary (renal) hyperparathyroidism are equivocal, although these drugs tend to slow down the progression of coronary artery and heart valve calcification[21].

Fibroblast growth factor 23 and klotho

The current era of investigation on vascular mineralization can be called the "era of Fibroblast growth factor (FGF)23 and klotho". FGF23 was recently described as the hormone that acts as a strong phosphaturic agent in line with PTH. This protein is synthesized and released by osteocytes and represents the family of proteins re-

ferred to as phosphatonins. Both PTH and FGF23 are released upon stimulation by a high serum phosphate level. Although PTH and FGF23 act synergistically on the proximal tubular epithelial cells where they limit phosphate reabsorption (and thus enhance phosphaturia), their effects in other pathways is rather opposite. PTH enhances renal activation of active vitamin D (calcitriol) and thus increases intestinal absorption of calcium and phosphate; FGF23 decreases calcitriol synthesis and stimulates its degradation, in turn resulting in decreased GI absorption of calcium and phosphate[22,23].

FGF23 starts to increase much earlier than PTH in the course of CKD. Its increase can already be noticed when the GFR decreases from 90 to 60 mL/min per 1.73 m^2; thereafter, this increase is even steeper. Changes in serum calcitriol level follow FGF23. It starts to decrease when GFR falls below 60-70 mL/min per 1.73 m^2. PTH elevation is a rather late event; it occurs in the GFR range between 45 and 50 mL/min per 1.73 m^2. Increased serum phosphate can be noticed usually when GFR drops below 40 mL/min per 1.73 m^2[24]. This sequence of events indicates the efficacy of phosphaturic agents in elimination of phosphate *via* the kidney (they significantly increase single nephron phosphaturia which is sufficient to keep a normal serum phosphate level despite progressive loss of the total nephron number).

FGF23 has been identified as a very powerful predictor of poor prognosis, both all-cause and cardiovascular mortality. This predictive value applies to the whole population with CKD, including end-stage renal disease (ESRD), CKD stages 2-4 and kidney transplant recipients[24-30]. FGF23 remains an independent predictive factor after correction for possible confounders, such as plasma phosphate, calcitriol or PTH. As in the case of high normal phosphate and PTH, borderline elevated or high normal FGF23 is also associated with a worse CV prognosis (this has been demonstrated, for example, in the Heart and Soul Study)[31]. An association between CV outcome and plasma FGF23 can at least in part be explained by stimulation of vascular calcification; some data may indicate that this phosphatonin stimulates more tunica media calcification (Monckeberg calcification or arteriosclerosis that translates into increased arterial stiffness, left ventricular hypertrophy and heart failure) rather than intimal calcification (localized mostly within atherosclerotic lesions, atherosclerosis)[32-35]. A predominance of Monckeberg-like lesions may in general explain why advanced CAC does not directly translate into coronary events (linked rather to calcification of lumen-narrowing atherosclerotic plaques). FGF23 was found to predict the severity of coronary artery disease in a large group of 1263 males and 813 females patients subjected to coronary angiography due to an acute coronary syndrome. FGF23 was an independent and strong predictor of stenosis score (that combined both severity of stenosis of an individual vessel and the number of vessels involved) and was also correlated with the extent of atherosclerosis and plaque calcification, as assessed with IVUS and virtual histology. There were 368 patients with eGFR < 60 mL/min per 1.73 m^2. FGF23 appeared to predict the extent of stenosis and number of stenotic vessels (integrated together into stenosis score) in the whole study group and separately in patients with normal (> 60 mL/min per 1.73 m^2) and reduced eGFR. FGF23 was inversely correlated with eGFR, but remained an independent predictor of coronary artery disease severity on angiography and the extent of atherosclerosis and plaque calcification on IVUS and virtual histology[36].

Klotho is one of the most fascinating proteins discovered in relation to vascular calcification and FGF23 function. This protein is considered to have an important anti-aging potential and to protect against CVS disease[37,38]. Since klotho is expressed mostly in renal tubular cells and parathyroid glands, this emphasizes the paramount importance of phosphate balance for cardiovascular health. Klotho facilitates normal phosphaturic function of FGF23 in the kidney and acts as its co-receptor. In experimental models of klotho, knockout FGF23 loses its phosphaturic potential even if renal function is preserved. Renal content of klotho possibly decreases early in the course of CKD and triggers upregulation of FGF23, even when other abnormalities of mineral balance (such as hyperphosphaturia) are not yet apparent[39]. It is important to mention that several tissue receptors for FGF23 can be localized without klotho co-expression, possibly elevated FGF23 overstimulates these receptors leading to adverse CVS effects. Indeed, receptors for FGF23 can be found in cardiomyocytes and experimental studies demonstrate that FGF23 leads to left ventricular hypertrophy. This may suggest a direct cardiotoxic effect of FGF23[32,33]. Klotho deficiency leads to increased expression of sodium-phosphate co-transporters Pit1 and Pit2 which facilitate phosphate transport into VSMC and stimulate their osteoblastic transformation. Runx2, a transcription factor that governs this transformation, is also upregulated in klotho deficiency[40,41].

Vitamin D and vitamin K; matrix Gla protein

In many experiments, very high doses of vitamin D were shown to induce disseminated vascular calcification; these doses are never used in humans[42]. Vitamin D receptor deficiency and a low vitamin D diet stimulate vascular calcification in mice[43]. Experiments also demonstrated that vitamin D analogues [vitamin D receptor agonists (VDRA) modified in order to decrease their hypercalcemic effect] may protect against pathological calcification. Patients with CKD (and especially those with end-stage renal disease) suffer from profound vitamin D deficiency. Dietary regimes, lack of skin exposure to sun, failure to hydroxylate vitamin D in 1α-position in failing kidneys, as well as the impact of high serum FGF23 contribute to such a deficiency[44]. Low plasma level of 25-hydroxy-vitamin D is associated with poor survival in patients with ESRD and CKD, as well as with the risk of progression to ESRD[45-47]. An association between low vitamin D status and adverse outcome in CKD may possibly be

explained in part by the risk of vascular calcification, inversely associated with plasma vitamin D (calcidiol)[48]. Multiple clinical observational or registry studies demonstrated that supplementing 1α-hydroxy-vitamin D is beneficial for the outcome of patients with end-stage renal disease; even better results can be achieved with novel analogues, such as paricalcitol. Unfortunately, these trials do not allow a conclusion of what the impact of vitamin D and other VDRA on vascular calcification in the clinical setting is.

Disseminated calcification of microcirculation that leads to necrotic lesions of skin and subcutaneous tissue, and ultimately to a fatal outcome has been well documented in ESRD (mostly on the level of case reports or case series) and is called calciphylaxis or calcifying uremic arteriolopathy (CUA). This phenomenon was demonstrated mostly in patients using warfarin and other drugs that antagonize vitamin K[49,50]. Vitamin K is responsible for γ-carboxylation of several proteins, not only those of the clotting cascade. It contributes to post-translational modification of matrix Gla protein (MGP), a protein synthesized by VSMC which acts as a potent inhibitor of vascular calcification. This biochemical pathway was supposed to link development of CUA and the use of warfarin[51,52]. Based on these observations, it has been hypothesized that vitamin K may have certain cardioprotective effects. The data from observational studies suggested a relationship between a higher intake of vitamin K (or biochemical measures suggesting high intake of this vitamin) and better CVS outcome, although a direct cardioprotective effect of vitamin K has not been proven to date[53]. A high percentage of ESRD patients suffer from vitamin K deficiency; supplementing them with menaquinone 7 (vitamin K2) decreases the level of circulating uncarboxylated MGP. This observation may provide a rationale for the therapeutic use of vitamin K in order to prevent cardiovascular disease (possibly by limiting advancement of vascular calcification)[54]. Low levels of carboxylated MGP were shown to predict a poor outcome in patients on maintenance dialysis[55].

Inflammation

Chronic inflammation is a well-recognized factor that accelerates atherosclerosis and vascular calcification. Chronic inflammation is one of the hallmarks of uremia. It is triggered by the uremic status itself but also results from multiple co-morbid conditions activating inflammation (such as periodontal disease, activity of autoimmune systemic diseases, infection of vascular access for hemodialysis, presence of other foci of infection, *etc.*)[56]. Several proinflammatory cytokines, such as interleukin 1, interleukin 6 or tumor necrosis factor alpha (TNFα), were shown to promote vascular calcification in experimental models of uremia and in uremic patients. C-reactive protein, the marker most commonly measured to assess inflammation, also correlated with the advancement of vascular and coronary calcification in patients with CKD[3,4,57-60].

The anti-inflammatory potential of human serum seems to be essential in protecting patients against vascular calcification. One of the best recognized protective mechanisms is serum fetuin A. This is a "negative" (anti-inflammatory) acute phase protein synthesized by hepatocytes. It was hypothesized some years ago that fetuin A prevents precipitation of calcium and phosphate in serum. Uremic serum is supersaturated with calcium and phosphate, which suggests their ability to precipitate spontaneously in the absence of inhibitors. Fetuin A forms colloidal complexes with calcium apatite and other crystals (called calciprotein particles), thus preventing from their precipitation within soft tissues[61]. Serum fetuin A was shown to predict prognosis in patients with advanced CKD; patient survival was inversely correlated with serum fetuin A[62]. Recent years have brought new insight into the role of fetuin A in vascular calcification. Data concerning the association between serum fetuin A and soft tissue calcification are equivocal: some studies reported such an association, whereas others failed to demonstrate it[63,64]. Hamano *et al*[65] found, in an animal model of uremia and in humans with CKD, that centrifugation of serum at 16000 *g* can separate fetuin A into two fractions: pellets in sediment, containing fetuin A, fibronectin-1, albumin, fibrinogen, Igκ light chains and Igμ heavy chains; and apolipoprotein A- I and "free" fetuin fraction in supernatant. The pellets are also enriched with calcium. The authors found that the serum level of fetuin A before centrifugation is higher compared to supernatant fetuin A after centrifugation in patients with different stages of CKD (including ESRD and dialysis); such a difference was not observed in healthy controls. CACS did not correlate with fetuin A; however, it was correlated with the reduction ratio of fetuin A (*i.e.,* reduction in fetuin A level in supernatant after sedimentation, reflecting the amount of fetuin complexed with calcium and other proteins in the calciprotein particle). These results were confirmed and extended by Smith *et al*[66], who also identified two fractions of fetuin in sera of patients with CKD, free and contributing to calciprotein particle formation. They found that high fetuin A in the calciprotein complex was positively associated with aortic pulse wave velocity, which reflects media calcification of arteries. In addition, they highlighted the importance of fetuin A molecule phosphorylation as a prerequisite to form calciprotein particles.

Epicardial fat as a new factor regulating CAC

Obesity and body mass index (BMI) were identified as important predictors of CAC both in the general population and in patients with CKD. Several cytokines such as TNFα that were implied in the development of CAC can be synthesized in adipose tissue; in addition, adipose tissue may be the source of more specific mediators (adipocytokines). The most important include leptin, adiponectin, visfatin and resistin. They were also shown to correlate with the degree and progression of CAC[3,58,67]. Recently, a fascinating observation has been made, name-

ly, that similar to fat present in other body regions, epicardial fat is also characterized with certain metabolic and proinflammatory functions and the hormonal cross-talk between epicardial adipose tissue (EAT), myocardium and coronary artery exists[68-73]. It is important to emphasize that adipose tissue in this location can be assessed quantitatively using similar techniques that are used to identify CAC (for example MSCT). Studies revealed an association between the amount of epicardial fat and the presence of CAC in post-menopausal women[74]. Recently, the series of studies on such a link was published in CKD patients. Kerr *et al*[75] searched for a correlation between CAC and epicardial fat volume in 94 stage 4-5 (pre-dialysis) CKD patients and found that CAC strongly and independently correlates with epicardial fat volume in this patient group. In addition, the amount of EAT was correlated with plasma interleukin 6, which confirms its inflammatory activity. A similar association was found in ESRD patients. Recent publications from the Turkish study group indicated that both CAC and EAT deposits were significantly more prevalent and more advanced in patients on renal replacement therapy compared to controls. These studies revealed an independent relationship between EAT and advancement of malnutrition, inflammation, atherosclerosis-calcification (MIAC) syndrome. MIAC integrates signs of malnutrition, enhanced "non-specific" inflammation of uremia, accelerated atherosclerosis and the presence of arterial calcification in one score. It cannot be concluded from the manuscript if there was a correlation between the amount of EAT and CACS[76].

PREVALENCE AND PROGRESSION OF CAC IN DIFFERENT GROUPS OF CKD PATIENTS AND ITS ASSOCIATION WITH OUTCOME

In this part of the review, the recent, most important publications dealing with CAC and its clinical and laboratory associations in different groups of renal patients are discussed.

Dialysis patients

As mentioned previously, the phenomenon of an extremely advanced CAC was first identified and explored in patients treated with hemodialysis; these publications were followed by investigation in the field of peritoneal dialysis. In recent years, a series of publications were issued by the Italian independent study group. These authors aimed to analyze if randomization to different types of phosphate binders (sevelamer HCl *vs* aluminum or calcium-containing salts) have any impact on the progression of CAC. The study was performed in patients new to hemodialysis (which is important, since previously many were performed in prevalent patients, *i.e.,* with different dialysis vintage before inclusion). The 24 mo observation period was completed by 132 patients (23% diabetics); 70.4% had evidence of CAC at the study entry (although the initial CAC score was relatively low and equaled 286 ± 744 Agatston units). About 61% of patients experienced progression in CACS; it was independently and positively associated with the presence of diabetes, increasing serum LDL-cholesterol and C-reactive protein; randomization to sevelamer decreased the risk of progression by 34% ($P < 0.001$). This study also demonstrated that an increment in CACS correlates with progression of pulse wave velocity and worsening in cardiac repolarization, as measured with QT dispersion. As in most of the previous studies, it was also shown that baseline CACS is an important predictor of CACS progression; in contrast to several other studies, age did not predict the progression[77,78].

High prevalence and fast progression of CAC were also identified in children and young adults with advanced CKD[79,80]. This issue was analyzed recently by Srivaths *et al*[81], who examined the relationship between CAC and FGF23, discussed above as one of the key predictors of cardiovascular outcome in renal patients. Sixteen patients aged 16 ± 3.3 years were involved in this study; they were on dialysis for quite a long period of time given their young age, *i.e.,* for 27.3 ± 19.3 mo. Compared to earlier reports on young patients, CACS was relatively low (median, 19; range 1-49 Agatston units) and present in only 5. FGF23 and serum phosphate were identified as being independently associated with CACS, although the statistical power in this small sized study must be considered very low. It should be emphasized that mean serum FGF23 level equaled 4024 pg/mL (in one of the recently published studies, the lowest quartile of FGF23 in patients with normal renal function was as low as < 40 pg/mL)[36,81]. Pencak *et al*[82], who recently analyzed correlations between CAC and a broad spectrum of calcification and bone turnover parameters (including FGF23, osteocalcin, osteoprotegerin, MGP, fetuin A, C-reactive protein, interleukin 6 and TNFα) in a large group of patients on hemodialysis, failed to reveal any association between CAC and any of the listed markers. Multiple logistic regression analysis allowed identification only of "classical" risk factors, namely age and time, on HD as independent predictors of CAC. FGF23 was not associated with the risk of CAC in the group of CKD patients (in stages 1-5) included in a recent Turkish study, although phosphatonin was related to valvular (aortic valve) calcification[83].

The impact of CAC on survival was analyzed in hemodialysis patients included into the prospective Nutritional and Inflammatory Evaluation of Dialysis Patients study that comprised of 166 subjects on hemodialysis (51% diabetics) who were followed prospectively and all-cause mortality was analyzed according to baseline CACS. More than 80% of patients were Hispanic or black and the majority was dialyzed for more than 2 years. Patients were divided according to baseline CACS into four groups (0, 1-100, 101-400, 400+ Agatston units). There was a statistically significant trend towards increasing

age, percentage of diabetics and value of the Charlson Comorbidity score with increasing CACS category; no differences in serum calcium, phosphate, cytokine profile or BMI were observed between the groups. Fifty deaths occurred during follow-up: 30 in 400+ CACS group and only 2 in patients with CACS 0 at baseline. This translated into 88.9% event-free survival rate in patients without CACS compared to 58.3% in those with CACS 400+. Cox proportional regression analysis with adjustment for case-mix variables has shown that the hazard ratio of death in three CASC groups (1-100, 101-400 and 400+ Agatston units) equaled 2.9, 8.5 and 13.3 compared to the reference group (CACS = 0). This analysis also revealed that CACS measured for each coronary artery (individual CACS) was also predictive for all-cause mortality (with significance decreasing from the left main through left anterior and left circumflex to right coronary artery)[84].

The predictive value of CAC for survival was also analyzed by the Italian group led by Prof. Gorgio Coen. 81 patients on maintenance hemodialysis for a very long time (82.5 ± 99.5 mo) at the time of baseline CAC assessment were included. In most of them (71 out of 81) CAC was found at baseline; the median value increased after one year from 481 to 528 Agatston units. Age and dialysis vintage were found to predict baseline CAC. A strong positive association was found between the baseline CAC and CAC increment over 12-18 mo observation period. In addition, calcium and PTH predicted the increment in CAC over this period of time, whereas fetuin A was shown to be protective. A total of 11 patients died during follow-up; mortality among those who progressed in terms of CACS increment equaled 72.7%. Agatston score was found to predict mortality during the follow-up[85].

In many previously published studies, a fascinating link between CAC and bone turnover was postulated: in clinical circumstances with excess bone resorption, a certain amount of mineral content from the skeletal system may deposit within soft tissues, including the vessel wall. The inverse relationship between vascular calcification, vascular stiffness and bone mineral density was described in the general population[86]. In CKD, characterized with bone and mineral disorders that are far more complicated that in osteoporosis, such a relationship was also documented[87]. So called "adynamic" bone disease (low bone turnover) was postulated to be a form of bone mineral disorders that is frequently associated with advanced and progressing vascular calcification in CKD patients[88]. Osteoprotegerin/receptor activator of NF-κB ligand (OPG/RANKL) axis, crucial in regulation of bone resorption, was also postulated to be involved in pathological soft tissue calcification in uremia. The possible link between this axis and CAC was recently addressed in a group of 78 HD patients, 44 CKD stage 4 subjects and 42 healthy volunteers in a prospective manner. Serum OPG was significantly higher in HD patients compared to stage 4 CKD or healthy controls; an opposite trend could be seen for RANKL and resulted in a significantly higher osteoprotegerin/RANKL ratio in HD patients compared to CKD stage 4 and healthy controls. Serum OPG and OPG/RANKL ratio were correlated with CAC at baseline and after one year; patients who progressed in CAC after one year (at least 10% and 50 Agatston units *vs* baseline) were characterized with a higher baseline and follow-up OPG and an increase in OPG during the one year observation period. Multivariate analysis confirmed an independent relationship between CAC progression and increase in serum OPG; high baseline CAC was also identified as another significant predictor of CAC progression. In the cited study, femoral bone mineral density was also measured but no correlation of BMD with baseline CAC or CAC progression was found[89].

Pre-dialysis patients

The burden of CAC in CKD subjects not yet on dialysis is also significant, although generally less advanced compared to dialysis patients. The prognostic significance of CAC in pre-dialysis, however, was not known until recently. Russo *et al*[90] analyzed the impact of baseline CAC and CAC progression on cardiac events in CKD patients not yet on dialysis (the study group comprised of the patients with CKD stages 2-5). They identified 181 patients with baseline CAC assessment who were followed prospectively and 54.7% of subjects were found to have CAC at baseline. The authors divided them into those with baseline CACS ≤ 100 and > 100 Agatston units and followed them until a cardiac event or end of the study, for a median period of 689 and 820 d, respectively (cardiac event was defined as cardiac death or myocardial infarction). Patients with higher baseline CACS were older, more frequently diabetic and had a longer duration of hypertension; interestingly, they did not differ in terms of GFR, mineral metabolism parameters, lipid profile or inflammatory markers. After adjustment for baseline differences, CACS > 100 Agatston units at the start of observation and accelerated progression of CAC (defined as annualized increment of CACS exceeding 75th percentile) were shown to predict cardiac events.

Another recent study addressed the issue of CAC progression in CKD patients not yet on dialysis. This study comprised of 103 CKD stage 3 and 4 patients with a baseline CAC assessment and who were then followed for 2 years. CAC was repeated after this period of time. Many other parameters, including a broad panel of biochemical markers and bone mineral density, were monitored. The study demonstrated that baseline CAC was higher in diabetic patients with CKD stage 3-4 compared to those without diabetes. Patients with diabetes were also more likely to progress in CAC compared to non-diabetics. The rate of progression was also faster among diabetics (although the increment in CAC was statistically significant within both groups). The prevalence of CAC greater than zero was also higher in diabetic CKD patients at baseline and follow-up (73% and 80%, respectively) compared to non-diabetics (46% and 60%). As in many previous reports, the most important predictors of

CAC progression were baseline CAC, BMI and serum phosphate level[91].

Proteinuric patients

Proteinuria is considered a powerful predictor of cardiovascular events (CVEs) and mortality due to CVS disease. To the best of my knowledge, no study has been performed to analyze the prevalence or extent of CAC among patients with proteinuria in the course of primary kidney disease (primary glomerulopathy). However, a study was performed in diabetic patients with CKD and overt proteinuria (mean eGFR 52 ± 26 mL/min per 1.73 m^2 and median urine protein loss 2.7 g/g of creatinine, *i.e.,* close to nephrotic). No correlation was found between CAC and proteinuria, or eGFR; there was also no association between CAC and parameters of mineral metabolism, including calcium, phosphate, PTH or 25-hydroxyvitamin D. Only age, male gender and ethnicity (being non-Latino white) were independently associated with advancement of CAC. In this study that involved 225 patients, 54 deaths occurred over the period of 39 ± 25 mo. CAC was an independent predictor of death in different statistical models and the hazard ratio of death equaled 1.49, 2.2 and 4.32 in patients with baseline CACS of 1-99, 100-399 and ⩾ 400 Agatston units, respectively, compared to patients with CACS = 0[92,93].

Renal transplant recipients

Several papers demonstrated that CAC is highly prevalent in transplant recipients and that successful kidney transplantation attenuates the rate of progression in CAC and mineralization within other vascular sites[2,4,94,95]. Papers that were published recently expand our knowledge of CAC after kidney transplantation.

Shu *et al*[96] analyzed the prevalence of CAC in a group of 99 renal transplant recipients from Taiwan. In 60% of patients CACS exceeded 10 Agatston units (mean and median values were not provided). CASC was independently associated with age and the presence of hypertension; female gender and high HDL-cholesterol were identified as protective factors in multivariate analysis.

Roe *et al*[97] were among the first who analyzed the impact of CAC on CVEs and mortality in renal transplant recipients. These authors selected a broad spectrum of inflammatory markers in addition to other "classical" clinical and biochemical risk factors of CVEs. The study group consisted of 112 renal transplant recipients (31.5% diabetics, 61% received kidney from a deceased donor) with age a mean 48.8 ± 12.5 years. Dialysis vintage before transplantation was relatively short (3 ± 2.7 years). Mean calcification score equaled 367.7 ± 682.3 Agatston units (median 70.5 units, no CAC found in 38 patients). These results correspond with values expected in wait-listed dialysis patients (usually healthier compared to non-selected dialysis population). The patients (n = 87) had CAC assessment repeated after the median period of 1.7 years; in 25.9% CAC progression was noted and 95.1% of patients with CAC < 100 units survived, whereas survival rate among those with CAC > 100 units was 82.3% (P = 0.03). The probability of remaining CVS event-free in respective CAC groups equaled 90.2% and 70.6%. Baseline CAC and CAC increments were shown to predict CVEs and mortality (depending on applied statistical approach, time spent on dialysis and if the presence of diabetes was predictive for CVS events or death).

Nguyen *et al*[98] recently published the observation of 281 renal transplant recipients in whom initial CAC and aortic calcification were measured and the predictive value of arterial calcification in these two localizations on development of CVE was analyzed. The patients had a very long history of ESRD since the main dialysis vintage before transplantation was 2.4 ± 2.4 years and the time between transplantation and baseline CAC analysis equaled 8.3 ± 6.9 years. They were much younger than an "average" dialysis cohort (53 ± 13 years). Higher CACS and previously experienced CVE were identified as independent predictors of future CVEs during the mean observation period of 2.3 ± 0.5 years. These two factors combined significantly decreased the chance of remaining CVE-free during the follow-up. Interestingly, in this study, "classical" factors such as age, male gender, obesity, lipid profile disorders and smoking, did not predict the onset of CVE.

Seyahi *et al*[99] analyzed the prevalence and progression of CAC in the group of renal transplant recipients a long time after transplantation (99.5 ± 54 mo) with well-preserved graft function (mean eGFR of 63.9 ± 18.1 mL/min per 1.73 m^2), who were earlier treated with dialysis for a mean period of two years. This Turkish population was much younger compared to an "average" Western dialysis or transplant cohort (38.7 ± 11.2 years) and, probably due to the young age, the prevalence and advancement of CAC was relatively low, despite a long history of renal replacement therapy (mean CACS 60 ± 174.8 Agatston units; median 0, range 0-1350; CAC present in 35.6% of patients). A very high percentage of patients (84%) received the kidney from a living donor. There were different methods of CAC progression defined in this study; depending on definition, progression in CAC was observed in 28%-38% of patients and prevalence of CAC-positive patients increased to 64.6% after 3 years. Baseline CAC and serum triglycerides were identified as independent predictors of CAC progression; in addition, bisphosphonate use was also independently associated with a 2.64-fold increased risk of CAC progression. The latter observation is very interesting and has been reported previously for other populations, for example, in a population-based Multi-Ethnic Study on Atherosclerosis. This study demonstrated that using bisphosphonates in post-menopausal osteoporotic women is associated with an increased risk of calcification in the aortic valve, aortic valve ring, mitral annulus, thoracic aorta and coronary arteries, especially in patients younger than 65 years[100].

One of the most interesting studies in the field is the paper reporting prevalence and progression of CAC in transplant recipients who were on dialysis due to lupus

nephritis. Systemic lupus erythematosus (SLE) is one of the most important causes of "secondary" glomerular diseases, especially among young females, and certain types of lupus nephritis are associated with poor renal outcome and a need for renal replacement therapy. SLE is a systemic inflammatory disease with a very high risk of atherosclerosis and CVS disease[101]. This includes a high prevalence of CAC in this patient group[102]. Patients with SLE on dialysis are excellent candidates for kidney transplantation (unless no disease activity is observed at the time of transplantation) and the outcome after transplantation is comparable with non-SLE subjects. Hence the importance of study performed by Norby *et al*[103] on CAC in renal transplant recipients should be acknowledged. These authors included 39 young renal transplant recipients with SLE (aged 34.1 ± 12.1 years, 74% female) in the study and identified a very high prevalence of CAC in MSCT (82%) and high mean and median CAC (894 ± 1679 and 135 Agatston units, respectively, with 36% of subjects with CAC exceeding 400 units). This important study identified the duration of SLE and BMI as independent predictors of CAC advancement; CAC was highly correlated with aortic pulse wave velocity (the measure of arterial stiffness and tunica media calcification). It should be emphasized that, in contrast to other papers in the field, the impact of dialysis on CAC in these patients was almost negligible: average time on dialysis was very short (13.2 ± 14.7 mo) and almost half of the recipients obtained a graft from a living donor[103]. Given the fact that CAC was shown to predict cardiovascular outcome in transplant patients, it is, however, sad to say that these young people (predominantly women) can be considered as high-risk patients.

THERAPEUTIC PERSPECTIVE

There are only a few prospective randomized trials available in the literature with therapeutic interventions aimed at controlling cardiovascular disease and improving survival in patients with advanced CKD. Their general message is rather pessimistic since most of the trials failed to prove that therapeutic interventions really change outcome (exceptions include one small study with carvedilol in patients with ESRD and heart failure, and another large trial demonstrating benefits of combined treatment with simvastatin and ezetimibe *vs* placebo in advanced CKD)[104,105]. Since there is an association between CKD-MBD, vascular calcification and mortality, mineral balance abnormalities became an obvious target for therapeutic interventions. Unfortunately, none of the interventions available in the field (including older and new phosphate binders, vitamin D and other VDRA, calcimimetics, low phosphate diet) was demonstrated to change patient prognosis and improve survival. This rather pessimistic notion was also upheld and emphasized by the most complex and comprehensive document in the field, namely, KDIGO clinical practice guidelines on CKD-MBD[106]. Unfortunately, since publication of the KDIGO guidelines, no additional data have been published to change this perspective. Probably the most disappointing news was the results of the EVOLVE trial; 3883 HD patients in this study were randomized to cinacalcet or placebo to test the hypothesis that treatment with cinacalcet would reduce the risks of death and nonfatal CVEs in this population. Unfortunately, no benefit was demonstrated from using the calcimimetic drug[107]. Several other studies were performed to demonstrate the usefulness of certain drugs to reduce the advancement of vascular (and coronary) calcification or at least to slow down the progression over time.

Phosphate binders

The most obvious therapeutic intervention in CKD-MBD is using phosphate-binding agents to reduce absorption of calcium and phosphate from GI (and thus limit the availability of substrates and stimulating agents for vascular calcification). Since the drugs traditionally used for this purpose, namely calcium containing phosphate binders (usually calcium carbonate, calcium acetate and citrate), may be the source of additional and unwanted calcium supply (which may promote vascular calcification, limit possibilities of using vitamin D and lead to parathyroid gland oversuppression)[108], most of the studies focused on the comparison between calcium-containing and calcium-free phosphate binders. The most important preparations in the field include lanthanum carbonate and synthetic compounds, sevelamer hydrochloride and sevelamer carbonate.

First, it is important to mention that in agreement with the KDIGO statement, other meta-analyses did not show survival benefit or attenuation in vascular calcification in patients using non-calcium containing phosphate binders *vs* those treated with calcium-based drugs[109]. Thus, early enthusiastic reports on the positive impact of sevelamer on CAC progression or even mortality could not be confirmed; they were also criticized as being underpowered to detect any outcome differences and influenced by the pharmaceutical industry[110-112]. In addition, other trials demonstrated similar efficacy of calcium acetate combined with a statin and sevelamer in control of CAC progression in patients on hemodialysis[113]. The newer studies in the field point on the higher efficacy of sevelamer in limiting the progression of CAC compared to calcium-containing phosphate binders, although these publications are also statistically underpowered due to small study samples and relatively short observation periods. Shantouf *et al*[114] found in a cross-sectional study that long-term sevelamer users on hemodialysis display lower values of CACS compared to those treated exclusively with calcium-containing phosphate binders. Barreto *et al*[115] assigned treatment with sevelamer or calcium acetate to 101 HD patients and followed them for one year, with baseline and follow-up bone biopsy and CAC assessment. They failed to demonstrate any difference both in terms of changes in bone turnover and CACS progression over 12 mo between the two treatment groups. A randomized study completed recently in Japan

included 183 HD patients with a relatively long (118 ± 89 mo) history of dialysis. They were randomly assigned in a 1:1 ratio to sevelamer or calcium carbonate. CACS increased significantly in both treatment arms after one year (in both groups with *P* value of < 0.001 *vs* baseline), although the increase of CACS was significantly lower in patients using sevelamer after adjustment for baseline differences between groups[116]. Similar results were also demonstrated for earlier stages of CKD. Russo *et al*[117] randomized 100 patients with CKD 3-5 (in stage 5 patients not yet on dialysis) to low-phosphate diet only, sevelamer or calcium carbonate. A significant increase of CACS was noted after an average observation period of two years in patients randomized to diet only and calcium carbonate (in both groups with $P < 0.001$ *vs* baseline), whereas it remained stable in those using sevelamer hydrochloride. An annualized progression in CACS equaled 205 ± 82 Agatston units in controls, 178 ± 40 units in the calcium carbonate group and 36 ± 32 units in the sevelamer group[117].

Sevelamer interacts with bile acid recirculation in the gut and may also influence lipid profile (with LDL-cholesterol lowering effect); some benefits of this polymer referred to this mode of action.

Lanthanum carbonate is a phosphate binder introduced to replace aluminum hydroxide in the treatment of hyperphosphatemia. In contrast to aluminum, GI absorption of lanthanum, a rare earth element, is considered negligible and thus it has been accepted as an effective phosphate binder without noticeable toxicity. In a recent study, it has been demonstrated that treatment with lanthanum carbonate is more effective compared to calcium carbonate in preventing the progression of CAC in patients on hemodialysis; in fact, regression by 6.4% was noticed in lanthanum-treated group *vs* 41.2% progression in those receiving calcium carbonate[118].

Statins

The above mentioned study of Qunibi[119] combined a statin with calcium acetate and demonstrated a similar efficacy in controlling CKD-MBD and CACS progression, as in the case of sevelamer. Lipid disorders are well-recognized triggers of atherosclerosis and they also contribute to arterial calcification[119,120]. There were attempts to control CACS progression with statins, although the results are equivocal and today there is no scientific background to conclude that these drugs really stop CAC progression[121-125]. Recently, Lemos *et al*[126] randomized 117 patients with CKD stage 3 and 4 (eGFR 36 ± 16.5 mL/min) to treatment with rosuvastatin, sevelamer or control group and found no difference between the three groups in terms of CACS progression *vs* baseline after two years. Statins are widely used in the general population in both primary and secondary prevention. Data on the beneficial influence of statins on cardiovascular health in non-renal patients are extrapolated to CKD patients and most of them are treated with these drugs; there are also some preliminary data on the usefulness of the benefits of statins in CKD[105,127]. Hence, preventing CAC would probably not be the primary indication to commence these drugs in CKD patients since they are already widely used.

VDRA, vitamin K, cinacalcet

Although there is some pathological background to believe that low vitamin D status is associated with CAC progression, there are no clinical trials on the therapeutic role of vitamin D (native, calcidiol, calcitriol) in the prevention of CAC progression. The same holds true for paricalcitol, the leading vitamin D analogue which controls hyperparathyroidism with a less pronounced action on calcium and phosphate absorption from the gastrointestinal tract. The results of the most important recent trial testing the impact of cinacalcet on CAC progression are somewhat inconclusive. A total of 360 patients in this study (known as ADVANCE) were randomized to cinacalcet with vitamin D or to vitamin D alone. After 5 wk, CACS increased by 24% as measured in Agatston units and by 22% as measured using the volume method in cinacalcet users, whereas in the vitamin D group the respective increases equaled 31% and 30%. The difference between treatment arms was non-significant when values in Agatston units were compared but became significant ($P = 0.009$) when the volume scoring was applied. Cinacalcet significantly attenuated the progression of aortic valve calcification but had no influence on mitral valve and thoracic aorta[21]. The results of this trial are difficult to interpret since VDRA were used in both treatment arms. ADVANCE was followed by publication of the EVOLVE trial, which demonstrated no impact of cinacalcet compared to placebo on mortality and major CVEs in the group of 3883 patients on maintenance dialysis[107]. As mentioned above, although there are multiple publications on the role of vitamin K-dependent proteins in the development of vascular calcification, to date no interventional study has been performed to show the benefit of vitamin K treatment in slowing down the progression of CAC.

Bisphosphonates

The role of bisphosphonates in the treatment of CKD-MBD is unknown since classical osteoporosis is not included in the classification of this disease[128]. In addition, a low value of GFR is a generally accepted contraindication for using these drugs. Small sample size trials performed in Japan some years ago suggested benefits associated with bisphosphonate use on CAC progression but it seems that this idea was abandoned since no further papers have emerged recently[129,130]. As mentioned in this review, there is a link between bone metabolism and soft-tissue calcification. Osteoporosis as a main indication for bisphosphonates may per se promote vascular calcification since calcium and phosphate mobilized from bone may serve as a source of substrates. Bisphosphonates interact with vitamin K metabolism and thus may decrease γ-carboxylation of MGP, a well-recognized inhibitor of

pathological calcification. Specifically in patients with CKD (including moderate CKD after kidney transplantation), low-turnover bone disease develops which may be additionally worsened with bisphosphonates. These mechanisms may explain why the increased risk of calcification in the aortic valve, aortic valve ring, mitral annulus, thoracic aorta and coronary arteries was found in a substantial percentage of post-menopausal women using bisphosphonates to treat or prevent osteoporosis[100]. On the other hand, bisphosphonates decrease expression of TNFα, down-regulate the inflammatory process and decrease the uptake of LDL-cholesterol by macrophages within atherosclerotic plaque; all these effects may potentially protect from calcification[131].

CONCLUSION

Soft tissue and especially arterial calcification is a dangerous process which may affect patients from the general population but poses a special threat to subjects with chronic (advanced) kidney disease. Although many risk factors of the development and progression of arterial calcification were identified, they are not universally confirmed across studies; only age seems to determine CAC in all studies and baseline CAC usually determines its progression over time. The extremely complex nature of uremic toxicity, additionally complicated by treatment (dialysis or transplantation), makes the identification of a single or main modifiable risk factor extremely difficult. In an attempt to prevent the development and progression of CAC, several pathological pathways (mostly related to mineral and bone disorders) are targeted but due to multi-factorial etiology many others remain unaddressed. This results in a very high prevalence and fast progression of CAC in patients with CKD, with potential consequences in terms of increased cardiovascular morbidity and mortality.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (2): Coronary artery disease

Myocardial ischemia is a key factor in the management of stable coronary artery disease

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Abstract

Previous studies demonstrated that coronary revascularization, especially percutaneous coronary intervention (PCI), does not significantly decrease the incidence of cardiac death or myocardial infarction in patients with stable coronary artery disease. Many studies using myocardial perfusion imaging (MPI) showed that, for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit, whereas for patients with no to mild ischemia, medical therapy is the main choice, and revascularization is associated with increased mortality. There is some evidence that revascularization in patients with no or mild ischemia is likely to result in worsened ischemia, which is associated with increased mortality. Studies using fractional flow reserve (FFR) demonstrate that ischemia-guided PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to decision-making for PCI. Complementary use of noninvasive MPI and invasive FFR would be important to compensate for each method's limitations. Recent studies of appropriateness criteria showed that, although PCI in the acute setting and coronary bypass surgery are properly performed in most patients, PCI in the non-acute setting is often inappropriate, and stress testing to identify myocardial ischemia is performed in less than half of patients. Also, some studies suggested that revascularization in an inappropriate setting is not associated with improved prognosis. Taken together, the presence and the extent of myocardial ischemia is a key factor in the management of patients with stable coronary artery disease, and coronary revascularization in the absence of myocardial ischemia is associated with worsened prognosis.

Key words: Coronary artery bypass surgery; Coronary revascularization; Fractional flow reserve; Myocardial ischemia; Myocardial perfusion imaging; Percutaneous coronary intervention

Core tip: Studies of myocardial perfusion imaging demonstrate that, for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit. For patients with no to mild ischemia, medical therapy is the main choice, and revascularization is associated with increased mortality probably because of worsened ischemia. Studies using fractional flow reserve demonstrate that ischemia-guided percutaneous coronary intervention (PCI) is superior to angiography-guided PCI, and the presence of ischemia is the key factor in decision-making for PCI. Thus, myocardial ischemia is a key factor in the management of patients with stable coronary artery disease.

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INTRODUCTION

Coronary artery disease is a leading cause of mortality and morbidity in developing and developed countries[1-5]. In approximately half of patients with newly diagnosed coronary artery disease, the first presentation is either acute myocardial infarction or sudden cardiac death[6,7].

The development of percutaneous coronary intervention (PCI) has enhanced the management of patients with acute coronary syndrome, and the prognosis of these patients has been considerably improved[8-15]. However, in patients with stable coronary artery disease, coronary revascularization decreases angina symptoms but does not significantly prevent cardiac death or myocardial infarction[16-21]. Recent studies suggest that the presence and extent of myocardial ischemia determine the prognosis of patients with stable coronary artery disease. Coronary revascularization is associated with improved prognosis in patients with moderate or severe ischemia, but is associated with worsened prognosis in patients with no or mild ischemia[22,23]. In this article, studies with myocardial perfusion imaging (MPI) and fractional flow reserve (FFR) on the effects of coronary revascularization on prognosis are reviewed.

CLINICAL OUTCOMES UTILIZING REVASCULARIZATION AND AGGRESSIVE DRUG EVALUATION TRIALS

Previous studies demonstrated that coronary revascularization does not significantly decrease the incidence of cardiac death and myocardial infarction in patients with stable coronary artery disease[16-21]. In particular, the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) study had a tremendous impact on our management of patients with stable coronary artery disease[24]. COURAGE trial is a randomized trial involving 2287 patients who had objective evidence of myocardial ischemia and significant coronary artery disease. The investigators assigned 1149 patients to undergo PCI with optimal medical therapy (PCI group) and 1138 to receive optimal medical therapy (OMT group) alone. The 4.6-year cumulative primary outcome (death from any cause and nonfatal myocardial infarction) rates were 19.0% in the PCI group and 18.5% in the OMT group (HR for the PCI group: 1.05; 95%CI: 0.87-1.27; P = 0.62). There were no significant differences between the PCI group and the OMT group in the composite of death, myocardial infarction, and stroke (20.0% *vs* 19.5%, HR = 1.05; 95%CI: 0.87-1.27; P = 0.62); hospitalization for acute coronary syndrome (12.4% *vs* 11.8%, HR = 1.07; 95%CI: 0.84-1.37; P = 0.56); or myocardial infarction (13.2% *vs* 12.3%, HR = 1.13; 95%CI: 0.89-1.43; P = 0.33). They concluded that as an initial management strategy in patients with stable coronary artery disease, PCI did not reduce the risk of death, myocardial infarction, or other major cardiovascular events when added to OMT.

However, the COURAGE Trial Nuclear Substudy tells another story[25]. This study enrolled 314 patients who underwent MPI performed before treatment and 6 to 18 mo after randomization. At follow-up, the reduction in ischemic myocardium was greater with PCI than with OMT (-2.7% *vs* -0.5%; $P < 0.0001$). More PCI patients exhibited significant ischemia reduction (33% *vs* 19%; $P = 0.0004$), especially patients with moderate to severe pretreatment ischemia (78% *vs* 52%; $P = 0.007$). Patients with ischemia reduction had lower ischemia-unadjusted risk of death or myocardial infarction ($P = 0.037$; risk-adjusted $P = 0.26$), particularly if baseline ischemia was moderate to severe ($P = 0.001$; risk-adjusted $P = 0.08$). Death or myocardial infarction rates ranged from 0% to 39% for patients with no residual ischemia to ⩾ 10% residual ischemia on follow-up MPI ($P = 0.002$; risk-adjusted $P = 0.09$). Thus this study showed that adding PCI to OMT resulted in a greater reduction in ischemia compared with OMT alone, although the effect of PCI on death or myocardial infarction was borderline significant probably because of the small number of patients.

MPI

MPI is the most commonly used test to assess the presence and the extent of myocardial ischemia. Many studies demonstrated that the presence and extent of myocardial ischemia was closely related to adverse cardiac events[26-36]. Hachamovitch *et al*[36] identified 5183 patients who underwent MPI and were followed up for the occurrence of cardiac death or myocardial infarction. Over a mean follow-up of 642 ± 226 d, 119 cardiac deaths and 158 myocardial infarctions occurred, giving an annual cardiac death rate of 3.0% and annual myocardial infarction rate of 2.3%. In patients with no [summed stress score (SSS) 0-3], mild (SSS 4-8), moderate (SSS 9-13), and severe (SSS > 13) ischemia, the annual cardiac death rate was 0.3%, 0.8%, 2.3%, and 2.9%, respectively. Similarly, in patients with no, mild, moderate, and severe ischemia, the annual myocardial infarction rate was 0.5%, 2.7%, 2.9%, and 4.2%, respectively. Thus increased myocardial ischemia is associated with more frequent cardiac events.

Many studies also showed that coronary revascularization has a beneficial effect in patients with moderate to severe ischemia[22,23,37]. Hachamovitch *et al*[22] studied 10627 patients without known coronary artery disease who underwent MPI and were followed up for 1.9 ± 0.6 years. Within 60 d after MPI, 671 patients underwent revascularization therapy and 9956 patients underwent medical therapy (MT). On the basis of the Cox proportional hazards model predicting cardiac death, patients undergoing MT demonstrated a survival advantage over patients undergoing revascularization in the setting of no or mild ischemia (% total myocardial ischemia less than 10%), whereas patients undergoing revascularization had an increasing survival benefit over patients undergoing MT when moderate ischemia (% total myocardial ischemia 11%-20%) to severe ischemia (% total myocardial ischemia more than 20%) was present. In 2011, the same

authors expanded their sample to 12329 patients and studied the interaction between the extent of ischemia and myocardial scar after revascularization on patient survival[23]. In the absence of prior coronary artery disease, increasing amounts of ischemia were associated with lower HRs with early revascularization. In the setting of little or no ischemia, early revascularization was associated with an approximately 50% greater risk than MT, whereas, with increasing ischemia, a progressive improvement in risk with early revascularization compared with MT was found. In the setting of extensive ischemia (> 20% myocardium), a 30% reduction in risk of all-cause death was present with the use of early revascularization compared with MT. Equipoise between the two strategies was present with approximately 10%-15% of the myocardium ischemic. As for patients with < 10% fixed defect, the risk reduction was 12.5% with MT and for patients with prior revascularization but no prior myocardial infarction it was 7.5%. Thus, these studies demonstrate that for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit, whereas for patients with no to mild ischemia MT is the main choice and revascularization is associated with increased mortality.

WHY IS CORONARY REVASCULARIZATION IN PATIENTS WITH NO OR MILD ISCHEMIA ASSOCIATED WITH INCREASED MORTALITY?

There is some evidence that revascularization in patients with no or mild ischemia is not associated with improved ischemia, but rather associated with worsened ischemia. Safley *et al*[38] identified 301 patients who underwent PCI for chronic total occlusion and in whom MPI was performed within 12 ± 3 mo before PCI and a follow-up study within 12 ± 3 mo after PCI. The change in % ischemia was +5.39% ($P = 0.006$), -1.70% ($P = 0.008$), -6.32% ($P < 0.001$), and -16.26% ($P < 0.001$) in patients with no/minimal (< 5% ischemic myocardium), mild (5%-9.9%), moderate (10%-16%), and severe (> 16%) ischemia, respectively. The percentage of patients with improved ischemic myocardium ≥ 5% was 0%, 34.7%, 68.5%, and 86.7% in patients with no/minimal, mild, moderate, and severe ischemia, respectively ($P < 0.001$). The percentage of patients with worsened ischemic myocardium ≥ 5% was 87.3%, 34.7%, 19.2%, and 9.2% in patients with no/minimal, mild, moderate, and severe ischemia, respectively ($P < 0.001$). Kaplan-Meier survival in patients with *vs* without improvement in ischemia showed a survival advantage in patients with improved ischemic myocardium ≥ 5% (87% *vs* 78%, $P = 0.018$). Receiver operating characteristics curve (ROC) analysis identified a 12.5% ischemic burden as the optimal cut-point to predict improvement in ischemia following PCI (sensitivity 80%, specificity 80%). This 12.5% ischemic burden is almost the same as that in the 2011 study by Hachamovitch *et al*[23]. Also ROC analysis identified a 6.25% ischemic burden as the optimal cut-point to predict worsening in ischemia following PCI (sensitivity 75%, specificity 80%). Thus, this study demonstrated that revascularization had no survival benefit and harms patients with no to mild ischemia, although the study was limited to patients who underwent PCI for chronic total occlusion.

Myocardial infarction associated with PCI (periprocedural myocardial infarction) is classified as type 4a by the third universal definition of myocardial infarction[39]. The prevalence of periprocedural myocardial infarction is 7.3% to 17.9% defined by CK-MB isoenzyme elevation > 3x upper limit of normal (ULN) and 15.0% to 44.2% defined by cardiac troponin > ULN[40-55]. The results of several studies suggested that any elevation in CK-MB was associated with reduced long-term survival and that there was a direct correlation between the magnitude of myonecrosis and mortality. Other studies have shown that only large myocardial infarctions were predictive of a poor long-term outcome[40-46]. Similarly, some studies showed that the serum concentration of cardiac troponin was an independent predictor of survival, others did not[47-55]. However two recent meta-analyses concluded that an elevated cardiac troponin levels after PCI does provide prognostic information[56,57]. Risk factors of periprocedural myocardial infarction are those which identify patients with increasing atherosclerotic disease burden, increased thrombotic risk, and with neurohormonal activation that predispose to either macrovascular complications (side branch occlusion or macroembolization) or microvascular obstruction (distal embolization of microparticles)[58].

In the era of coronary angioplasty, many studies reported that numerous "false positive" reversible perfusion defects occurred early after angioplasty, possibly as a result of inadequate early vessel remodeling or sustained abnormalities of coronary vasomotor tone. However, a significant percentage of patients showed persistent abnormalities in the later period[59-66]. In one study, 76% of patients without prior myocardial infarction showed improvement in perfusion abnormalities after angioplasty, but only 34% had completely reversible ischemia[60]. In the other study of 15 patients 1 to 2 wk after angioplasty, 7 had a reversible perfusion defect, of whom only 4 subsequently normalized by 4 to 6 wk[61]. These studies suggested that an improved or normalized perfusion abnormality does not necessarily occur after coronary angioplasty in every patient. Taken together, revascularization in patients with no or mild ischemia is likely to result in worsened ischemia, which is associated with increased mortality.

ISCHEMIA-GUIDED REVASCULARIZATION

There are some studies which showed that the ischemia-

guided (IG) strategy resulted in a better prognosis[67-70]. Farzaneh-Far *et al*[67] identified 1425 consecutive patients with coronary artery disease who underwent two serial MPI. They were followed for a median of 5.8 years after the second MPI. Patients were included in the PCI or coronary artery bypass graft (CABG) group on the basis of the first revascularization procedure occurring within 60 d of the first MPS scan. Thus patients were divided into a MT group, PCI group, and CABG group. The incidence of patients with worsening of the ischemic myocardium by ⩾ 5% was more frequent in the MT group (15.6%) compared with the PCI (6.2%) and CABG groups (6.7%) ($P < 0.001$). After adjustment for established predictors, ⩾ 5% ischemia worsening remained a significant independent predictor of death or myocardial infarction (HR = 1.634; $P = 0.0019$). Thus, this study showed that ischemia worsening was an independent predictor of death or myocardial infarction, and revascularization was associated with more frequent improvement in myocardial ischemia compared with MT.

Kim *et al*[68] studied the importance of IG revascularization. From a registry of 5340 patients with multivessel coronary artery disease, comprising 2587 PCI and 2753 CABG. MPI was performed in 42.3% of patients and IG revascularization was performed in 17.3%. The MPI was defined as abnormal if the SSS was 3 or greater. The incidence of major adverse cardiac and cerebrovascular events (MACCE) was significantly lower in the IG group than in the non-IG group [16.2% *vs* 20.7%, adjusted HR (aHR) = 0.73; 95%CI: 0.60-0.88; $P = 0.001$], primarily driven by the lower repeat revascularization rate (9.9% *vs* 22.8%, aHR = 0.66; 95%CI: 0.49-0.90; $P = 0.009$). Subgroup analysis showed that IG reduced the risk of MACCE in PCI patients (17.4% *vs* 22.8%, aHR = 0.59; 95%CI: 0.43-0.81; $P = 0.001$) but not in CABG patients (16.0% *vs* 18.5%, aHR = 0.87; 95%CI: 0.67-1.14; $P = 0.31$). Thus IG revascularization with MPI, particularly in PCI-treated patients, seems to decrease the risk of repeat revascularization and MACCE in patients with multivessel disease. Taken together, these studies suggest that the IG strategy is associated with improved prognosis.

FFR

FFR (the ratio of maximal blood flow in a stenotic artery to normal maximal flow), is now a gold standard for invasive assessment of coronary artery stenosis[71-80]. In Fractional Flow Reserve *vs* Angiography in Multivessel Evaluation (FAME) study, investigators randomly assigned 1005 patients with multivessel coronary artery disease to PCI with implantation of drug-eluting stents guided by angiography alone or guided by FFR measurements in addition to angiography[81]. Patients assigned to angiography-guided PCI underwent stenting of all indicated lesions, whereas those assigned to FFR-guided PCI underwent stenting of all indicated lesions only if the FFR was 0.80 or less. The primary endpoint was the rate of death, nonfatal myocardial infarction, and repeat revascularization at 1 year. The number of indicated lesions per patient was 2.7 ± 0.9 in the angiography group and 2.8 ± 1.0 in the FFR group ($P = 0.34$). The number of stents used per patient was 2.7 ± 1.2 and 1.9 ± 1.3, respectively ($P < 0.001$). The 1-year event rate was 18.3% in the angiography group and 13.2% in the FFR group ($P = 0.02$). The rate of death and myocardial infarction was 11.1% in the angiography group and 7.3% in the FFR group ($P = 0.04$). Pijls *et al*[82] reported the 2-year follow-up results of the FAME study. The 2-year rates of mortality or myocardial infarction were 12.9% in the angiography-guided group and 8.4% in the FFR-guided group ($P = 0.02$). Combined rates of death, nonfatal myocardial infarction, and revascularization were 22.4% and 17.9%, respectively ($P = 0.08$). For lesions deferred on the basis of FFR > 0.80, the rate of myocardial infarction was 0.2% and the rate of revascularization was 3.2% after 2 years, which is a very low rate. Thus, routine measurement of FFR in patients with multivessel coronary artery disease who undergo PCI with drug-eluting stents significantly reduced the rate of death, nonfatal myocardial infarction, and repeat revascularization for up to 2 years.

Tonino *et al*[83] studied the angiographic *vs* functional severity of coronary artery stenosis in the FAME study. Of the 1414 lesions (509 patients) in the FFR-guided arm of the FAME study, 1329 were successfully assessed by the FFR. Before FFR measurement, these lesions were categorized into 50%-70%, 71%-90%, and 91%-99% diameter stenosis by visual assessment. In the category 50%-70% stenosis, only 35% were functionally significant. In the category 71%-90% stenosis, 80% were functionally significant and in the category of subtotal stenoses, 96% were functionally significant. Of all 509 patients with angiographically defined multivessel disease, only 235 (46%) had functional multivessel disease.

In FAME 2 study, investigators enrolled patients with stable coronary artery disease for whom PCI was being considered, and assessed all stenoses by measuring FFR[84]. Patients in whom at least one stenosis was functionally significant (FFR ⩽ 0.80) were randomly assigned to FFR-guided PCI plus the best available MT (PCI group), or the best available MT alone (MT group). Patients in whom all stenoses had an FFR of more than 0.80 were entered into a registry and received the best available MT. The primary endpoint was a composite of death, myocardial infarction, or urgent revascularization. Recruitment was halted prematurely after enrollment of 1220 patients (888 who underwent randomization and 332 enrolled in the registry) because of a significant between-group difference in the percentage of patients who had a primary endpoint event: 4.3% in the PCI group and 12.7% in the MT group (HR with PCI: 0.32; 95%CI: 0.19-0.53; $P < 0.001$). The difference was driven by a lower rate of urgent revascularization in the PCI group than in the MT group (1.6% *vs* 11.1%; HR = 0.13; 95%CI: 0.06-0.30; $P < 0.001$). Among patients in the registry, 3.0% had a primary endpoint event, which was not significantly different from the PCI group. Thus, in

patients with stable coronary artery disease and functionally significant stenoses, FFR-guided PCI plus the best available MT, as compared with the best available MT alone, decreased the need for urgent revascularization. In patients without ischemia, the outcome appeared to be favorable with the best available MT alone. The main reason why there was no significant difference in death and myocardial infarction between the PCI group and MT group seems to be the relatively small number of patients and short-term follow-up period (mean duration of follow-up was 213 ± 128 d in the PCI group and 214 ± 127 d in the MT group).

Pijls *et al*[80] explain why FFR-guided PCI decreases the rate of death and myocardial infarction in the FAME study. From many studies it is known that the death and myocardial infarction rates are less than 1% per year for a functionally nonsignificant stenosis if treated appropriately by medication, between 5% and 10% per year for a functionally significant stenosis if only treated by medication, and approximately 3% per year for a stented lesion whether it was functionally significant or not. Thus, stenting a functionally significant stenosis improves outcome, but stenting a functionally nonsignificant stenosis worsens outcome. Taken together, these studies suggest that IG PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to the decision-making for PCI.

APPROPRIATENESS CRITERIA

For many years, the American College of Cardiology (ACC) and American Heart Association (AHA) have jointly published and updated guidelines for PCI and CABG[85,86]. Recently, the ACC Foundation/Society for Cardiovascular Angiography and Interventions/Society for Thoracic Surgeons/American Association for Thoracic Surgery/AHA/American Society of Nuclear Radiology released appropriateness criteria for coronary revascularization to serve as a supplement to the ACC/AHA guideline documents[87].

Hannan *et al*[88] studied the appropriateness of PCI and CABG performed in New York for patients without acute coronary syndrome or previous CABG. Of the 8168 patients undergoing CABG, 90.0% were appropriate for revascularization, 1.1% were inappropriate, and 8.6% were uncertain. Of the 33970 PCI patients, 28% lacked sufficient information to be rated. Of the patients who could be rated, 36.1% were appropriate, 14.3% were inappropriate, and 49.6% were uncertain. A total of 91% of the patients undergoing PCI who were classified as inappropriate had one- or two-vessel disease without proximal left anterior descending artery disease, and had no or minimal anti-ischemic MT. Chan *et al*[89] studied 500154 patients enrolled in the National Cardiovascular Data Registry. For 355417 patients with acute indications, 98.6% were classified as appropriate, 1.1% as inappropriate, and 0.3% as uncertain. For 144737 patients with nonacute indications, 50.4% were classified as appropriate, 11.6% as inappropriate, and 38.0% as uncertain. The majority of inappropriate PCIs for nonacute indications were performed in patients with no angina (53.8%), low-risk ischemia on noninvasive stress testing (71.6%), or suboptimal (≤ 1 medication) antianginal therapy (95.8%). Furthermore, although variation in the proportion of inappropriate PCI across hospitals was minimal for acute procedures, there was substantial hospital variation for nonacute procedures (mean hospital rate for inappropriate PCI, 10.8%; interquartile range, 6.0%-16.7%).

Lin *et al*[90] studied the frequency and predictors of stress testing prior to elective PCI in a Medicare population of 23887 patients. Only 44.5% of patients underwent stress testing within 90 d prior to elective PCI. There were wide regional variations among the hospital referral regions, with stress testing ranging from 22.1% to 70.6% (mean, 44.5%, interquartile range 39.0%-50.9%). Female sex [adjusted OR (aOR) = 0.91; 95%CI: 0.86-0.97], age 85 years or older (aOR = 0.83; 95%CI: 0.72-0.95), a history of congestive heart failure (aOR = 0.85; 95%CI: 0.79-0.92), and prior cardiac catheterization (aOR = 0.45; 95%CI: 0.38-0.54) were associated with a decreased likelihood of prior stress testing. Thus, these studies demonstrated that, although PCI in the acute setting and CABG are properly performed in most patients, PCI in the nonacute setting is often inappropriate, and stress testing to identify myocardial ischemia is performed in less than half of patients.

Some studies also showed that revascularization in an inappropriate setting is not associated with improved prognosis. Ko *et al*[91] assessed the appropriateness of coronary revascularization (PCI or CABG) and examined its association with longer-term outcomes. In 1625 patients with stable coronary artery disease, coronary revascularization was performed in only 69% in the appropriate category, 45% in the inappropriate category, and 54% in the uncertain category. In patients in the appropriate category, coronary revascularization was associated with a lower adjusted hazard of death or acute coronary syndrome (aHR = 0.61; 95%CI: 0.42-0.88; P = 0.0087) at 3 years compared with MT. No significant differences in death or acute coronary syndrome were observed between coronary revascularization and MT in the inappropriate category (aHR = 0.99; 95%CI: 0.48-2.02) and the uncertain category (aHR = 0.57; 95%CI: 0.28-1.16; P = 0.12).

FUTURE PERSPECTIVE

Both MPI and FFR clearly identify the presence or absence of myocardial ischemia, and IG revascularization is associated with improved prognosis. However, the FFR value which is concordant with a 10% ischemic myocardium by MPI remains to be determined. A cut-off value of 0.75 was determined by the positive or negative results of three noninvasive stress tests; bicycle exercise test, thallium scintigraphy, and stress echocardiography with dobutamine[92]. A FFR value between

0.75 and 0.80 is deemed to be in the gray zone. MPI has limitation in identification of the highest risk subsets, left main coronary artery disease and three-vessel coronary artery disease, because of "balanced ischemia"[93-98]. One study showed that in patients with left main coronary artery disease, MPI results were normal in 5% and low-risk in 10% of patients[93]. The other study showed that in patients with triple-vessel coronary artery disease, MPI results were normal in 12% and single-vessel in 28% of patients[94].

Some studies compared MPI and FFR in patients with multivessel coronary artery disease. Ragosta *et al*[99] performed angiography, FFR, and MPI in 36 patients (88 arteries), and determined the association between FFR and perfusion for each vascular zone. Concordance between angiography, FFR, and MPI was seen in 61 of 88 zones (69%). Discordance was seen in the remaining 27 zones (31%), and was predominantly related to the finding of a FFR < 0.75 or total occlusion despite no defect on MPI. Melikian *et al*[100] performed MPI and FFR in 67 patients (201 vessels) with angiographic two- or three-vessel coronary artery disease. In 42% of patients, MPI and FFR detected identical ischemic areas (mean number of areas 0.9 ± 0.8 for both, $P = 1.00$). In the remaining 36% MPI underestimated the number (MPI = 0.46 ± 0.6, FFR = 2.0 ± 0.6, $P < 0.001$) and in 22% overestimated the number (MPI = 1.9 ± 0.8, FFR = 0.5 ± 0.8, $P < 0.001$) in comparison with FFR. Thus, MPI has poor concordance with FFR and tends to underestimate or overestimate the functional importance of coronary stenosis in comparison with FFR in patients with multivessel disease. In patients with multivessel coronary artery disease, FFR is the preferred method to identify myocardial ischemia. Therefore, complementary use of noninvasive MPI and invasive FFR would be important to compensate for each method's limitations.

CONCLUSION

MPI studies demonstrate that for patients with moderate to severe ischemia, revascularization is the preferred therapy for survival benefit. For patients with no to mild ischemia, MT is the main choice and revascularization is associated with increased mortality probably because of worsened ischemia. FFR studies demonstrate that IG PCI is superior to angiography-guided PCI, and the presence of ischemia is the key to decision-making for PCI. Studies of appropriateness criteria demonstrate that, although CABG and emergency PCI are appropriately performed in most patients, use of elective PCI is often inappropriate. Some studies also suggest that revascularization in an inappropriate setting is not associated with improved prognosis. Taken together, myocardial ischemia is a key factor in the management of patients with stable coronary artery disease.

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TOPIC HIGHLIGHT

WJC 6th Anniversary Special Issues (5): Myocardial infarction

Clinical significance of glycated hemoglobin in the acute phase of ST elevation myocardial infarction

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Abstract

In population-based studies, including diabetic and nondiabetic cohorts, glycated hemoglobin A1c (HbA1c) has been reported as an independent predictor of all-cause and cardiovascular disease mortality. Data on the prognostic role of HbA1c in patients with acute myocardial infarction (MI) are not univocal since they stem from studies which mainly differ in patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency. The present review is focused on available evidence on the prognostic significance of HbA1c measured in the acute phase in patients with ST-elevation myocardial infarction (STEMI) submitted to primary percutaneous coronary intervention (PCI). We furthermore highlighted the role of HbA1c as a screening tool for glucose intolerance in patients with STEMI. According to available evidence, in contemporary cohorts of STEMI patients submitted to mechanical revascularization, HbA1c does not seem to be associated with short and long term mortality rates. However, HbA1c may represent a screening tool for glucose intolerance from the early phase on in STEMI patients. On a pragmatic ground, an HbA1c test has several advantages over fasting plasma glucose or an oral glucose tolerance test in an acute setting. The test can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. We therefore proposed an algorithm based on pragmatic grounds which could be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities from the early phase. The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping in identifying patients at risk for the development of glucose intolerance after MI. Further validation of this algorithm in prospective studies may be required in the contemporary STEMI population to resolve some of these uncertainties around HbA1c screening cutoff points.

Key words: Glycated hemoglobin; ST-elevation myocardial infarction; Prognosis; Hyperglycemia; Glucose intolerance

Core tip: Data on the prognostic role of glycated hemoglobin A1c (HbA1c) in patients with acute myocardial infarction (MI) are not univocal since they stem from studies which mainly differ in patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency. According to available evidence, in contemporary cohorts of ST-elevation myocardial infarction (STEMI) patients submitted to mechanical revascularization, HbA1c does not seem to be associated with short and long term mortality. However, in STEMI patients, HbA1c, even measured in the early phase, may represent a screening tool for glucose intolerance since its measurement can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. We therefore proposed an algorithm based on pragmatic grounds

which could be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities from the early phase. The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping in identifying patients at risk for the development of glucose intolerance after MI.

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INTRODUCTION

Discovered more than forty years ago by Rahbar *et al*[1], the breakthrough for glycated hemoglobin A1c (HbA1c) was achieved when it was discovered in the Diabetes Control and Complications Trial in 1993 that the concentration of HbA1c was an excellent predictor of diabetes-related long-term complications[2].

In population-based studies[3], including diabetic and nondiabetic cohorts, HbA1c has been reported as an independent predictor of all-cause and cardiovascular disease (CDV) mortality[4-6]. Among individuals with diabetes, every 1% rise in HbA1c is associated with a 30% increase in all-cause mortality and a 40% increase in CVD mortality[7]. In the Reykjavik Study and in a meta-analysis of other Western prospective studies, fasting and post-load glucose levels were modestly associated with coronary heart disease (CHD) risk in people without diabetes[8], while associations of HbA1c with CHD risk in such people appeared somewhat stronger (a RR for CHD of 1.20 per 1% higher HbA1c). In a community-based population study, elevated HbA1c has been recently reported to be predictive for CDV and mortality in patients without diabetes mellitus, regardless of fasting glucose levels[9].

Data on the prognostic role of HbA1c in patients with acute myocardial infarction (AMI) stem from studies which mainly differ for patients' selection criteria, therapy (thrombolysis *vs* mechanical revascularization) and number consistency.

The present review is focused on available evidence on the prognostic significance of HbA1c measured in the acute phase in patients with ST-elevation myocardial infarction (STEMI) submitted to primary percutaneous coronary intervention (PCI). We furthermore highlighted the role of HbA1c as a screening tool for glucose intolerance in these patients.

GLYCATED HEMOGLOBIN AS A PROGNOSTIC TOOL IN STEMI PATIENTS

Glycated hemoglobin and patients without known diabetes and with ST elevation myocardial infarction

Only small studies assessed the prognostic role of HbA1c in STEMI patients without a history of diabetes and results are not univocal due to differences in patients' selection criteria and methods[10-13]. In 150 non diabetic patients with myocardial infarction (MI), mortality rate and the risk of cardiogenic shock increased with HbA1c[10]. In a high-risk MI population[12]. HbA1c was a risk marker of death at follow-up in patients without a history of diabetes and not in diabetic patients, while, in a small group of MI patients (diabetic and not diabetic) treated with thrombolysis[11], there were significant relationships between admission glucose, HbA1c level and mortality at follow-up. Similarly, in 374 STEMI patients (diabetic and not diabetic), after adjusting for baseline characteristics, HbA1c remained a strong independent predictor of in-hospital mortality (OR = 1.412; 95%CI: 1.031-1.935, $P = 0.03$)[14].

On the other hand, in 504 unselected, consecutive non diabetic STEMI patients submitted to PCI, hyperglycemia (not glycated hemoglobin) was a predictor of 30-d outcome[13]. We recently[15] assessed the prognostic role of HbA1c for mortality at short and long terms in 518 consecutive STEMI patients without previously known diabetes, all submitted to mechanical revascularization. Patients with HbA1c $\geqslant$ 6.5% showed higher values of admission, peak and discharge glucose ($P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively) and a higher incidence of acute insulin resistance [as inferred by the Homeostatic Model Assessment index (HOMA)] ($P = 0.001$) as well as higher values of fibrinogen ($P < 0.001$) and triglycerides ($P = 0.001$) and lower values of HDL ($P = 0.018$). No differences in short and long-term mortality rates and in the use of devices were detectable between patients with HbA1c < 6.5% and those with HbA1c $\geqslant$ 6.5%. At multivariate backward logistic regression analysis HbA1c was not associated with in-hospital death (OR = 7.210, 95%CI: 0.75-69.69, $P = 0.088$). At follow-up [median 39.7 (22.2-57.1) mo], a Kaplan-Meier survival curve documented no significant differences between patients with HbA1c < 6.5% and those with HbA1c $\geqslant$ 6.5%. In our study population, patients with HbA1c levels higher than 6.5% did not show a higher infarct size (as indicated by TnI and left ventricular ejection fraction) or a more critical illness (as inferred by the use of devices). Discrepancies with previous papers are mainly related to number consistency[10], population selection criteria[11] and type of revascularization[13]. As a difference from previous studies[10,11,13], we observed that higher HbA1c values help in identifying a subset of patients who, in the early phase of STEMI, show an abnormal glucose response to stress as indicated by higher values of glucose, worse glycemic control during Intensive Cardiac Care Union (ICCU) stay (peak glycemia) and a higher incidence of acute insulin resistance (HOMA index). All these factors have been associated with increased risk of early death as reported by Deedwania *et al*[16] and by us in previous reports[17-21]. Patients with HbA1c > 6.5% also showed an increased inflammatory activation (increased values of fibrinogen), suggesting a link between acute glucose

dysmetabolism and inflammatory activation in the early phase of STEMI[16].

Similar results were recently reported by Tian *et al*[22] in an observational multicenter study performed in 608 STEMI patients submitted to primary PCI. The study population was stratified according to the new American Diabetes Association criteria, into three groups: Ⅰ, HbA1c 5.6% or less (n = 262); Ⅱ, HbA1c 5.7%-6.4% (n = 182); and Ⅲ, HbA1c at least 6.5% (n = 164). The 7-d mortality was similar (P = 0.179) between groups Ⅰ (1.9%), Ⅱ (2.2%), and Ⅲ (0.0%) as well as the 30-d mortality (P = 0.241) between groups Ⅰ (3.8%), Ⅱ (2.2%), and Ⅲ (1.2%). Major adverse cardiac events at the 7-d and 30-d follow-up were not significantly different between the three groups either (P > 0.05). After adjusting the baseline characteristics, HbA1c was not an independent predictor of short-term outcomes (HR = 0.431; 95%CI: 0.175-1.061, P = 0.067).

Glycated hemoglobin and patients with known diabetes and with STEMI

In patients with AMI and diabetes, the two Diabetes Insulin Glucose in AMI studies both showed that increasing HbA1c levels increased mortality in diabetic patients with MI[23,24]. Conversely[12], in Optimal Trial in Myocardial Infarction with the Angiotensin Ⅱ Antagonist Losartan trial (including patients with MI complicated by heart failure) the level of HbA1c had no impact on mortality among the patients with well-known diabetes. Similarly, in consecutive diabetic patients undergoing PCI[25], HbA1c was not a predictor of cardiac events at one-year follow-up.

In a recent investigation[26], which includes the largest series of consecutive STEMI patients with known diabetes submitted to mechanical revascularization, we observed that HbA1c was not associated with mortality in either the short or the long term. Nevertheless, higher HbA1c values (which were detectable in about half of the entire population) helped to identify a subset of patients who, in the early phase of STEMI, showed an abnormal glucose response to stress as indicated by higher values of glucose, a worse glycemic control during ICCU stay (as inferred by peak glycemia) and a higher incidence of acute insulin resistance (as indicated by HOMA index). This subset of patients may deserve a more aggressive treatment for glucose management, since previous studies performed by other intestigators[16] and by us[17-21,26,27] showed that admission glycemia and peak glycemia are independent predictors for in-hospital mortality in STEMI patients.

Glycated hemoglobin and long term mortality in STEMI patients

In the thrombolytic era, in two small studies both excluding patients with newly diagnosed diabetes[28,29], an independent effect on mortality of HbA1c was reported in nondiabetic patients with MI. HbA1c levels higher than 6.5% were associated with higher ischemic score in patients with MI (diabetic and non diabetic) submitted to thrombolysis[13], and significant relationships were observed between admission glucose, HbA1c level and mortality at follow-up. Glycated hemoglobin was a potent risk marker of death at follow-up only in MI patients without a history of diabetes but not in diabetic patients[12]. Conversely, elevated admission glucose (and not glycated hemoglobin) was an important predictor of 30-d outcome after STEMI in 504 unselected, consecutive non diabetic patients with STEMI submitted to PCI[11]. Chan *et al*[30] reported, in a small cohort of 317 diabetic patients with acute coronary syndrome, that HbA1c levels before admission were not associated with short-term cardiovascular outcome (all-cause mortality, cardiovascular mortality, symptom driven revascularization, rehospitalization for angina, and hospitalization for heart failure).

On the other hand, Timmer *et al*[31] observed that increasing quartiles of HbA1c (even below the diagnostic threshold for diabetes mellitus) were associated with increased mortality rates over an average 3.3 years of follow-up in 4176 consecutive STEMI patients without known diabetes submitted to PCI. This finding was partially related to the fact that increasing HbA1c levels were associated with adverse baseline characteristics such as a higher cardiovascular risk profile.

In a large contemporary cohort of 1205 consecutive patients with STEMI submitted to PCI, we recently[32] assessed the impact of increased HBA1c (≥ 6.5%) on long term mortality. In our series 276 patients with previously diagnosed diabetes (276/1205, 22.9%, Group A), 78 patients without previously known diabetes and HbA1c ≥ 6.5% (78/1205, 6.5%, Group B) and 851 patients without previously known diabetes and HbA1c < 6.5% (851/1205, 70.1%, Group C). At Cox regression analysis, HbA1c ≥ 6.5% was not related to 1-year post discharge mortality in patients with previously diagnosed diabetes (Group A) nor in those without previously known diabetes (Group B and C). Kaplan-Meier survival curve analysis showed that patients in Group A exhibited the lowest survival rate, while patients in Group B (that is patients without previously known diabetes and with HbA1c ≥ 6.5%) showed a significant reduction in their survival rate since 6-mo after discharge. In conclusion, in our investigation HBA1c levels were not related with outcomes at multivariable analysis in a large cohort of unselected STEMI patients submitted to PCI.

GLYCATED HEMOGLOBIN AS A SCREENING TOOL FOR GLUCOSE INTOLERANCE IN STEMI PATIENTS IN THE ACUTE PHASE

More than 18 million people in the United States have diabetes mellitus, and approximately 35% of the population is prediabetic[33]. Another 7 million Americans have undiagnosed diabetes and are at high risk of developing

Table 1 Prevalence of glucose intolerance in patients with acute myocardial infarction

Ref.	Patients	Methods	Prevalence	Results
Norhammar *et al*[40], 2002	81 non diabetic AMI patients	OGTT	Diabetes: 31% IGT: 35%	HbA1c on admission was independent predictor of glucose intolerance at 3 mo (P = 0.024)
Ishihara *et al*[53], 2006	200 non diabetic patients with AMI	OGTT	Diabetes: 27%	Fasting glucose and HbA1c were independent predictors of abnormal glucose tolerance, but admission glucose was not.
Gustafsson *et al*[12], 2007	2841 patients with heart failure complicating AMI	HbA1c	History of diabetes: 17% HbA1c < 4.9%: 58% HbA1c 4.9%-5.1%: 15% HbA1c > 5.1%: 10%	In non diabetic patients, a 1% absolute increase in HbA1c level at baseline resulted in a 24% increase in mortality In diabetic patients, the level of HbA1c had no impact on mortality
Rasoul *et al*[11], 2007	504 non diabetic STEMI	HbA1c	HbA1c < 6.0%: 82.5% HbA1c > 6.0%: 17.5%	HbA1c was not associated with 30-d mortality
Cakmak *et al*[13], 2008	100 non diabetic patients with AMI treated with thrombolysis; patients on antidiabetic therapy excluded	HbA1c	HbA1c 4.5-6.4%: 25% HbA1c 6.5-8.5%: 28% HbA1c > 8.5%: 47%	Admission HbA1c was significantly correlated with mortality (P = 0.009)
Knudsen *et al*[47], 2009	224 non diabetic STEMI	OGTT	Abnormal glucose regulation: 46.9% in the early phase 24.9% at 3 mo	High levels of HbA1c and admission plasma glucose in-hospital significantly predicted abnormal glucose regulation at 3 mo (P < 0.001)
Timmer *et al*[31], 2011	4176 non diabetic STEMI patients	HbA1c quartiles	IQR1 ⩽ 5.35%: 27% IQR2 5.6%-5.54%: 24% IQR3 5.55%-5.80%: 25% IQR4 ⩾ 5.81%: 24%	HbA1c (hazard ratio, 1.2 per interquartile range; P < 0.01), but not glucose, was independently associated with long-term mortality
Lazzeri *et al*[15], 2012	518 non diabetic STEMI patients	HbA1c	HbA1c < 6.5%: 90.4% HbA1 c ⩾ 6.5%: 9.6%	HbA1c was not associated with short and long term mortality
Tian *et al*[22], 2013	608 STEMI	Hb1c groups	I: HbA1c ⩽ 5.6%: 43% II: HbA1c 5.7%-6.4%: 30% III: HbA1c ⩾ 6.5%: 27%	After adjusting the baseline characteristics, HbA1c was not an independent predictor of short-term outcomes (HR = 0.431; 95%CI: 0.175-1.061, P = 0.067)
Lazzeri *et al*[32], 2013	1204 STEMI patients	HbA1c	Diabetic patients: 22.9% patients without known diabetes: HbA1c < 6.5%: 70.1% HbA1c ⩾ 6.5%: 6.5%	At Cox regression analysis, HbA1c ⩾ 6.5% was not related to 1-yr post discharge mortality in diabetic and in non diabetic patients

HbA1c: Glycated hemoglobin A1c; OGTT: Oral glucose tolerance test; AMI: Acute myocardial infarction; IGT: Impaired glucose tolerance; STEMI: ST-elevation myocardial infarction; IQR: Interquartile range.

diabetic complications, including CDV[34,35]. These numbers are expected to continue to rise in the United States and worldwide in large part due to the growing obesity epidemic[36-38]. In 2010, an estimated 6.4% of the world's adult population (approximately 285 million individuals) had diabetes, and the prevalence is projected to increase to 7.7% (approximately 439 million individuals) by 2030[39].

Prevalence of glucose intolerance in STEMI patients

In the glucose tolerance in AMI study[40], HbA1c independently predicted glucose intolerance (OR = 2.58 95%CI: 1.17-6.09, P = 0.024) in people with acute coronary syndrome without known diabetes, correlating closely with the 2-h plasma glucose in an oral glucose tolerance test (r = 0.39, P < 0.0001). Furthermore, an HbA1c ⩾ 30 mmol/mol (4.9%) had sensitivity and specificity of 79% and 49% for detecting undiagnosed diabetes, respectively, with the area under curve of 0.685 (P = 0.001). In the Euro Heart Survey on diabetes, 22% of people admitted to hospital as emergency cases because of coronary artery disease were found to have undiagnosed diabetes after a glucose tolerance test, with a further 36% found to have impaired glucose tolerance[41].

It has been recently observed among patients with high-risk non-ST-segment elevation acute coronary syndrome (NSTE ACS)[42] that a substantial proportion of patients admitted with high-risk NSTE ACS had previously undiagnosed diabetes mellitus (12.2%) or prediabetes (10.8%) as defined by fasting glucose or HbA1c after hospital admission.

Table 1 shows the prevalence of glucose intolerance according to existing investigations on this topic in patients with AMI. These studies were selected by a PubMed search matching "acute myocardial infarction/STEMI/acute coronary syndrome" and "glucose intolerance/hyperglycemia/glycated hemoglobin".

The prevalence of STEMI patients with glucose intolerance, as detected mainly by HbA1c measured in the early phase, varies, ranging from 10% to more than 40%. Differences can be mainly related to the chosen value of HbA1c. More recently, in an observational multicenter study, Tian *et al*[22] stratified the study population according to HbA1c values and observed that the percentage of patients with HbA1c > 5.7% accounted for more than 50%.

On a clinical ground, in STEMI patients, early diag-

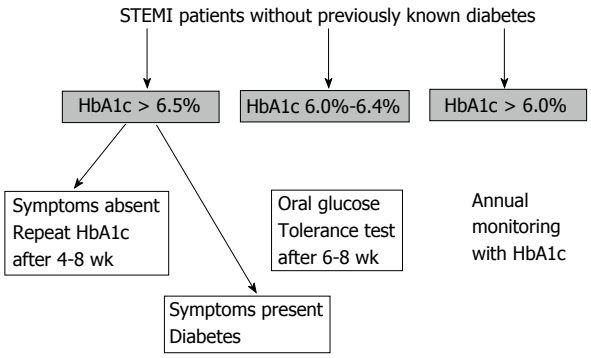

Figure 1 A screening algorithm for glucose intolerance based on glycated hemoglobin. STEMI: ST-elevation myocardial infarction; HbA1c: Glycated hemoglobin A1c.

nosis of unknown type 2 diabetes or impaired glucose regulation allows initiation of treatment or lifestyle interventions, including diet and exercise to prevent type 2 diabetes and associated complications. Gaining information on family history for diabetes could help in identifying subjects with undiagnosed diabetes or at risk[43,44].

However, in the acute phase of STEMI, the identification of glucose intolerance is quite difficult since the common finding of hyperglycemia, irrespective of underlying diabetic status, is to be related mainly to the acute stress response[16-21,26] to myocardial ischemia[45].

Strategy for screening for glucose intolerance in STEMI patients according to glycated hemoglobin

Recently, National Institute for Health and Clinical Excellence (NICE) guidelines on the management of hyperglycaemia in acute coronary syndrome have advocated any hyperglycaemia (blood glucose > 11.0 mmol/L) without known diabetes be followed up with an HbA1c measurement before discharge and fasting plasma glucose test 4 d after the onset of acute coronary syndrome[46]. NICE recommend against routine use of the oral glucose tolerance test in patients with acute coronary syndrome and with fasting plasma glucose and HbA1c in the normal range. However, guidance on categorization of glycaemic status of those with elevated HbA1c and fasting plasma glucose, as well as screening for diabetes in those without hyperglycaemia, is less clear. As a consequence, the lack of simple strategy for early identification of glucose intolerance in acute coronary syndrome is potentially leaving many people undiagnosed and under-treated, especially after the cardiac event.

The oral glucose tolerance test is performed infrequently in the acute setting[41], since it is time consuming, not always well tolerated and it does not seem to provide reliable information on long-term glucometabolic state[47].

In the early phase of STEMI, fasting plasma glucose can be acutely elevated and therefore unreliable in the first 2 d of an acute event and in a large MI[48]. NICE has suggested fasting plasma glucose testing should not be conducted within the first 4 d of the acute event. However, in the current era of early reperfusion therapies, many patients with acute coronary syndrome are discharged earlier.

On a pragmatic ground, an HbA1c test has several advantages over fasting plasma glucose or an oral glucose tolerance test in an acute setting. The test can be performed in the non-fasting state and reflects average glucose concentration over the preceding 2-3 mo. Therefore, in our opinion, glycated hemoglobin should be measured in all patients with STEMI.

Measuring HbA1c assumes International Federation of Clinical Chemistry standardized laboratory assays are used. Furthermore, conditions precluding accurate measurement of HbA1c concentration for diagnosis should be excluded, including abnormalities of red cell turnover, chronic renal or liver failure and chronic use of certain medications.

We therefore proposed an algorithm (Figure 1) based on pragmatic grounds (and our experience) which should be applied in STEMI patients without known diabetes in order to detect glucose intolerance abnormalities since the early phase.

Above HbA1c > 6.5%, individuals should be assessed for symptoms of diabetes (*i.e.*, increased thirst, polyuria, unexplained weight loss, blurred vision, extreme fatigue), ruling out other causes, for example polyuria attributable to diuretic therapy. In those with unequivocal symptoms the diagnosis is confirmed[49]. Conversely, those with ambiguous or absent symptoms should undergo a confirmatory HbA1c measurement 4-8 wk post-discharge for consistency and to counteract any potential laboratory errors on the first occasion.

Patients with HbA1c between 6.0% and < 6.4% should undergo an oral glucose tolerance test after 6-8 wk.

STEMI patients without known diabetes and HbA1c < 6.0% should undergo annual surveillance with HbA1c as incident impaired glucose regulation and diabetes is higher compared with the general population[50].

The main advantage of this algorithm is that it may help in tailoring the follow-up program, by helping to identify patients at risk for the development of glucose intolerance after MI.

Further validation of this algorithm in prospective studies may be required in the contemporary STEMI population to resolve some of these uncertainties around HbA1c screening cut points.

Given the increasing focus on managing multiple coexisting illnesses affecting cardiovascular patients[51], the assessment of glycosylated hemoglobin (HbA1c) in patients with STEMI could be an important opportunity to improve care for these patients[52].

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TOPIC HIGHLIGHT

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Duration of dual antiplatelet treatment in the era of next generation drug-eluting stents

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Abstract

Current percutaneous coronary intervention guidelines recommend dual antiplatelets (aspirin 100 mg + clopidogrel 75 mg daily) for at least 12 mo following drug-eluting stent (DES) implantation if patients are not at high risk of bleeding. Several reports have tried to shorten the dual antiplatelet therapy to 3-6 mo, especially following next-generation DES implantation, for cost-effectiveness. However, the clinical results are inconsistent and the data regarding next-generation DESs limited. In this report, recently published important pivotal reports regarding the optimal duration of dual antiplatelets following DES implantation are summarized.

Key words: Drug-eluting stent; Dual antiplatelet treatment; Percutaneous coronary intervention

Core tip: Recently published important pivotal reports regarding the optimal duration of dual antiplatelets following drug-eluting stent implantation are summarized.

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INTRODUCTION

Multiple randomized clinical trials have shown the efficacy of drug-eluting stents (DES) in reducing restenosis and the need for target lesion revascularization (TLR) compared with bare-metal stents (BMS)[1,2]. Despite the reduced incidence of recurrence, safety issues related to DESs, such as stent thrombosis, late stent malapposition, aneurysm, stent fracture, endothelial dysfunction and restenosis, have been reported elsewhere, particularly with first-generation DESs. Furthermore, some observational studies have shown that the risk of death or myocardial infarction was even higher with DESs than BMSs, possibly due to a higher incidence of late or very late stent thrombosis[3].

Early or premature discontinuation of dual antiplatelet therapy has been reported as an important risk factor for late stent thrombosis following DES implantation[4,5]. Thus, current percutaneous coronary intervention (PCI) guidelines recommend dual antiplatelets (aspirin + clopidogrel 75 mg daily) for at least 12 mo following DES implantation if patients are not at high risk of bleeding[6]. Several reports have tried to address this issue but the results are inconsistent and the data regarding second-generation DESs limited. In this report, the important pivotal reports regarding the optimal duration of dual antiplatelets following DES implantation, particularly in patients who underwent PCI with next generation DESs, are summarized.

OPTIMAL DURATION OF DUAL ANTIPLATELET THERAPY WITH DESs

Major clinical trials for duration of dual antiplatelets after DES implantation

REAL-LATE and ZEST-LATE trial: (Aspirin +

Table 1 Clinical outcomes at 12 mo and 24 mo[1]

Clinical outcomes	At 12 mo		At 24 mo		HR (95%CI)[2]	*P*
	Clopidogrel + aspirin	Aspirin alone	Clopidogrel + aspirin	Aspirin alone	Clopidogrel + aspirin	Aspirin alone
Primary end point: MI or death from cardiac causes	0.7	0.5	1.8	1.2	1.65 (0.80-3.36)	0.17
Secondary end points						
Death from any cause	0.5	0.5	1.6	1.4	1.52 (0.75-3.50)	0.24
MI	0.4	0.3	0.8	0.7	1.41 (0.54-3.71)	0.49
Stroke	0.3	0.3	1.0	0.3	2.22 (0.68-7.20)	0.19
Stent thrombosis, definite	0.2	0.1	0.4	0.4	1.23 (0.33-4.58)	0.76
Repeat revascularization	1.7	1.1	3.1	2.4	1.37 (0.83-2.27)	0.22
MI or death from any cause	0.8	0.8	2.3	1.7	1.57 (0.85-2.88)	0.15
MI, stroke, or death from any cause	1.1	1.1	3.2	1.8	1.73 (0.99-3.00)	0.05
MI, stroke, or death from cardiac causes	1.0	0.8	2.7	1.3	1.84 (0.99-3.45)	0.06
Major bleeding, according to TIMI criteria	0.2	0.1	0.2	0.1	2.96 (0.31-28.46)	0.35

[1]For the total number of events for each type of end point, only the first event is counted. Cumulative rates of events are based on Kaplan-Meier estimates. All deaths were considered to be from cardiac causes unless an unequivocal noncardiac cause could be established; [2]Hazard ratios are for the dual-therapy group as compared with the aspirin-alone group. MI: Myocardial infarction; TIMI: Thrombolysis in myocardial infarction. (Modified from Ref. [7]).

clopidogrel *vs* aspirin alone after 1 year). A randomized trial from South Korea showed that dual antiplatelets for longer than 12 mo following DES implantation was not significantly more effective than aspirin monotherapy[7]. In two trials (REAL-LATE and ZEST-LATE trials were merged), a total of 2701 patients who had received DESs and had been free of major adverse cardiac or cerebrovascular events and major bleeding for a period of at least 12 mo were randomly assigned to receive clopidogrel plus aspirin or aspirin alone.

In this trial, more than half of the patients received a sirolimus-eluting stent (SES, Cypher, Cordis) and the other half received a paclitaxel-eluting stent (PES, Taxus, Boston Scientific) or a zotarolimus-eluting stent (ZES, Endeavor, Medtronic). Thus, the study population underwent PCI with predominantly first-generation DESs.

The median duration of follow-up was 19.2 mo. The cumulative incidence of primary outcomes (composite of myocardial infarction or death from cardiac causes) at 2 years was 1.8% with dual antiplatelet therapy compared with 1.2% with aspirin monotherapy (HR = 1.65; 95%CI: 0.80-3.36; $P = 0.17$). The individual risks of myocardial infarction, stroke, stent thrombosis, need for repeat revascularization, major bleeding and death from any cause did not differ between the two groups. However, in the dual therapy group, there was a non-significant increase in the composite risk of myocardial infarction, stroke or death from any cause (HR = 1.73, $P = 0.051$) and in the composite risk of myocardial infarction, stroke or death from cardiac causes (HR = 1.84, $P = 0.06$, Table 1). This trial concluded that the use of dual antiplatelets for longer than 12 mo following DES implantation was not more effective than aspirin monotherapy in reducing the rate of myocardial infarction or death from cardiac causes.

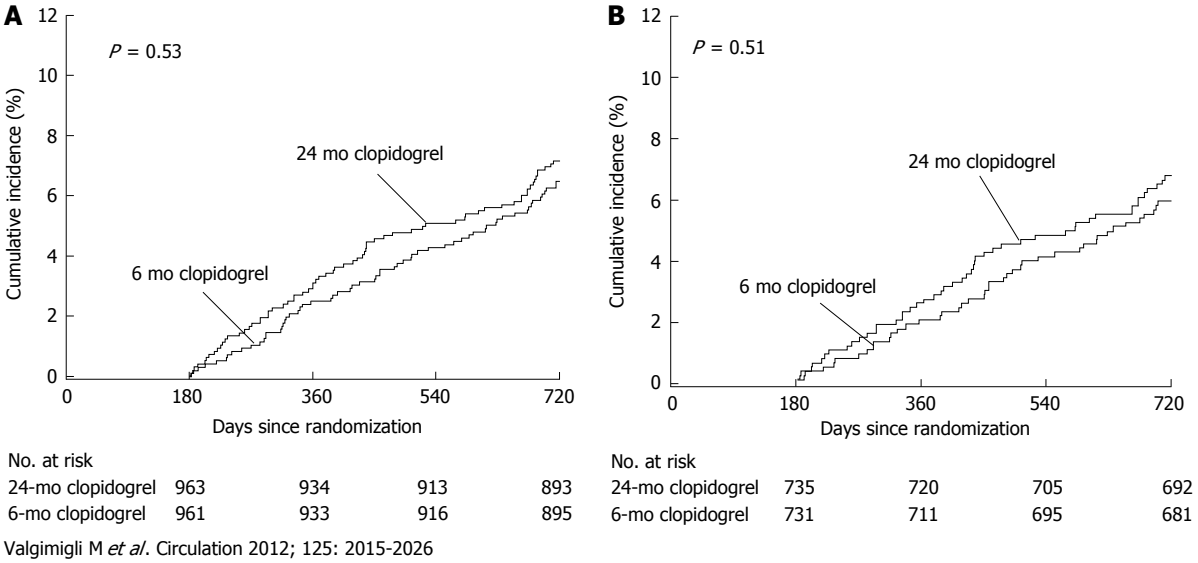
Recently, the DES-LATE trial reported that in the patients who were on 12 mo dual antiplatelet therapy without complications, an additional 24 mo of dual antiplatelet therapy *vs* aspirin alone did not reduce the risk of major composite hard endpoints (cardiac deaths, myocardial infarction or stroke)[8].

The EXCELLENT trial: (Dual antiplatelet 6 mo *vs* 12 mo). Some previous registry data suggested that dual antiplatelets for less than 12 mo after DES implantation does not increase major adverse cardiac events (MACE) and that there was no apparent clinical benefit from dual antiplatelets for longer than 6 mo[9-11]. Data comparing a shorter duration of dual antiplatelets compared with 12 mo of dual antiplatelets are very limited. The EXCELLENT (Efficacy of Xience/Promus *vs* Cypher to Reduce Late Loss After Stenting) trial from South Korea compared 6 mo *vs* 12 mo dual antiplatelet therapy following DES implantation[12].

Following DES implantation, 1443 patients were randomly assigned to receive 6 mo or 12 mo dual antiplatelets. The primary endpoint was a target vessel failure (composite of cardiac death, myocardial infarction or ischemia-driven target vessel revascularization) at 12 mo.

The rate of target vessel failure at 12 mo was 4.8% in the 6 mo dual antiplatelet group and 4.3% in the 12 mo group (the upper limit of 1-sided 95%CI: 2.4%; $P = 0.001$ for non-inferiority with a predefined non-inferiority margin of 4.0%). Although stent thrombosis tended to occur more frequently in the 6 mo dual antiplatelets group than 12 mo group (0.9% *vs* 0.1%, HR = 6.02; 95%CI: 0.72-49.96; $P = 0.10$), the risk of death or myocardial infarction did not differ in the two groups. In the prespecified subgroup analysis, target vessel failure occurred more frequently in the 6 mo dual antiplatelet group (HR = 3.16; 95%CI: 1.42-7.03; $P = 0.005$) in diabetic patients (Table 2).

This study population predominantly received an everolimus-eluting stent (EES, Xience or Promus, 74.8%)



Valgimigli M *et al*. Circulation 2012; 125: 2015-2026

Figure 1 Landmark analyses of PRODIGY Trial[13]. Cumulative rates of composite of death, myocardial infarction or cerebrovascular accident in all recruited patients (A) or in patients randomly allocated to the drug-eluting stent groups (B) using the 6 mo landmark analysis.

Table 2 Clinical outcomes of EXCELLENT trial *n* (%)

Clinical outcomes	6-mo DAPT (*n* = 722)	12-mo DAPT (*n* = 721)	HR[1] (95%CI)	*P*
Target vessel failure[2]	34 (4.8)	30 (4.3)	1.14 (0.70-1.86)	0.60
Total death	4 (0.6)	7 (1.0)	0.57 (0.17-1.95)	0.37
Cardiac death	2 (0.3)	3 (0.4)	0.67 (0.11-3.99)	0.66
Myocardial infarction	13 (1.8)	7 (1.0)	1.86 (0.74-4.67)	0.19
Death/myocardial infarction	17 (2.4)	14 (1.9)	1.21 (0.60-2.47)	0.58
Target vessel myocardial infarction	12 (1.7)	6 (0.8)	2.00 (0.75-5.34)	0.16
Cerebrovascular accident	3 (0.4)	5 (0.7)	0.60 (0.14-2.51)	0.48
Target lesion revascularization	17 (2.4)	18 (2.6)	0.94 (0.49-1.83)	0.86
Target vessel revascularization	22 (3.1)	22 (3.2)	1.00 (0.56-1.81)	0.99
Any revascularization	43 (6.2)	43 (6.2)	1.00 (0.66-1.53)	0.99
Stent thrombosis	6 (0.9)	1 (0.1)	6.02 (0.72-49.96)	0.10
Any bleeding	4 (0.6)	10 (1.4)	0.40 (0.13-1.27)	0.12
TIMI major bleeding	2 (0.3)	4 (0.6)	0.50 (0.09-2.73)	0.42
MACCE[3]	56 (8.0)	60 (8.5)	0.94 (0.65-1.35)	0.72
Safety end point[4]	24 (3.3)	21 (3.0)	1.15 (0.64-2.06)	0.64

The percentages shown are Kaplan-Meier estimates from the intention-to-treat analysis. [1]HRs are for the 6 mo *vs* 12 mo DAPT group; [2]Target vessel failure was a composite of cardiac death, myocardial infarction or target vessel revascularization; [3]MACCE was a composite of death, myocardial infarction, stroke or any revascularization; [4]Safety end point was a composite of death, myocardial infarction, stroke, stent thrombosis or TIMI major bleeding. (Modified from Ref. [12]). DAPT: Dual antiplatelet therapy; TIMI: Thrombolysis in myocardial infarction; MACCE: Major cardiocerebral event.

and rest of the patients received SES (25.2%). The study population was heterogeneous in terms of different DESs, particularly first *vs* second generation DESs.

They concluded that 6 mo of dual antiplatelets did not increase the risk of target vessel failure at 12 mo after DES implantation compared with 12 mo of dual antiplatelets.

Although 6 mo of dual antiplatelets cannot be recommended in the general population on the basis of this trial, this may be helpful for physicians to decide the duration of dual antiplatelets case by case in clinical practice.

PRODIGY trial: (Dual antiplatelets 6 mo *vs* 24 mo). The purpose of the PRODIGY trial (Prolonging Dual Antiplatelets Treatment After Grading Stent-Induced Intimal Hyperplasia) was to assess the effect of dual antiplatelets for 6 mo *vs* 24 mo on long-term clinical outcomes after PCI in a broad all-comers patient population receiving a balanced DES or base-metal stent (BMS)[13].

They randomly assigned 2013 patients to receive BMS, ZES, PES or EES. At 30 d, each stent group was randomly allocated to receive up to 6 mo or 24 mo of clopidogrel therapy in addition to aspirin.

The cumulative risk of the primary outcome (composite of death of any cause, myocardial infarction or cerebrovascular accident) at 2 years was 10.1% in the 24 mo dual antiplatelet group compared with 10.0% in the 6 mo group (HR = 0.98; 95%CI: 0.74-1.29; *P* = 0.91, Figure 1). The individual risks of death, myocardial infarction, cerebrovascular accident or stent thrombosis did not differ between the two groups; however, there was a consistently greater risk of hemorrhage in the 24 mo group. They concluded that a regimen of 24 mo of clopidogrel therapy in patients who had received a balanced mixture of DES or BMS was not significantly more effective than a 6 mo regimen in reducing the composite of death from any cause, myocardial infarction or cerebrovascular accident.

Table 3 Two year clinical outcomes of TWENTE trial *n* (%)

	Resolute ZES (*n* = 695)	Xience V EES (*n* = 692)	Difference (95%CI)	*P*
Target vessel failure	75 (10.8)	80 (11.6)	-0.8 (-4.1 to 2.6)	0.65
Death				
Any cause	29 (4.2)	33 (4.8)	-0.6 (-2.8 to 1.6)	0.59
Cardiac cause	11 (1.6)	19 (2.7)	-1.2 (-2.7 to 0.4)	0.14
Target vessel–related myocardial infarction				
Any	37 (5.3)	39 (5.6)	-0.3 (-2.7 to 2.1)	0.80
Q-wave	8 (1.2)	9 (1.3)	-0.2 (-1.3 to 1.0)	0.80
Non–Q-wave	29 (4.2)	30 (4.3)	-0.2 (-2.3 to 2.0)	0.88
Clinically indicated target vessel revascularization				
Any	39 (5.6)	35 (5.1)	0.6 (−1.8 to 2.9)	0.65
Target lesion failure	73 (10.5)	68 (9.8)	0.7 (−2.5 to 3.9)	0.68
Clinically indicated target lesion revascularization				
Any	34 (4.9)	18 (2.6)	2.3 (0.3 to 4.3)	0.03
Death from cardiac causes or target vessel myocardial infarction	46 (6.6)	53 (7.7)	-1.0 (-3.8 to 1.7)	0.45
Major adverse cardiac events[1]	90 (12.9)	82 (11.8)	1.1 (-2.4 to 4.6)	0.53
Patient-oriented composite endpoint[2]	114 (16.4)	118 (17.1)	-0.7 (-4.6 to 3.3)	0.75
Stent thrombosis				
Definite (0-720 d)	6 (0.9)	1 (0.1)	0.7 (-0.0 to 1.5)	0.12
Definite or probable (0-720 d)	8 (1.2)	10 (1.4)	-0.3 (-1.5 to 0.9)	0.63
Definite, probable, or possible (0-720 d)	14 (2.0)	20 (2.9)	-0.9 (-2.5 to 0.8)	0.29
Very late definite or probable (361-720 d)	2 (0.3)	2 (0.3)	0 (-0.6 to 0.6)	1.00

Values are *n* (%). [1]Major adverse cardiac events is a composite of all-cause death, any myocardial infarction, emergent coronary artery bypass surgery and clinically indicated target lesion revascularization; [2]Patient-oriented composite endpoint is a composite endpoint of all-cause death, any myocardial infarction and any revascularization. (Modified from Ref. [17]). ZES: Zotarolimus-eluting stent; EES: Everolimus-eluting stent.

TWENTE Trial: (Discontinuation of dual antiplatelets after 12 mo in ZES and EES). Second-generation DESs, such as EES (Xience V, Abbott Vascular, Santa Clara, California) and ZES (Resolute ZES, Medtronic Inc, Santa Rosa, California), were developed to improve clinical outcomes by overcoming the limitations of first generation DESs[14,15]. The randomized TWENTE (The Real-World Endeavor Resolute *vs* Xience V DES Study in Twente) trial is an investigator-initiated study performed in a population with many complex patients and lesions and only limited exclusion criteria[16]. Patients were randomly assigned 1:1 to ZES (*n* = 697) or EES (*n* = 694).

Two year follow up information was available on all patients. A strict policy of discontinuation of dual antiplatelets after 12 mo was followed, which is of interest for the present pre-specified 2 year analysis of clinical outcomes[17]. The rate of continuation of dual antiplatelets beyond 12 mo was very low (5.4%). The primary endpoint of target vessel failure, a composite of cardiac death, target vessel-related myocardial infarction and target vessel revascularization, did not differ between ZES and EES (10.8% *vs* 11.6%, *P* = 0.65), despite fewer TLRs in patients with EES (2.6% *vs* 4.9%, *P* = 0.03). The patient-oriented composite endpoint was similar (16.4% *vs* 17.1%, *P* = 0.75). Two year rates of definite or probable stent thrombosis were 1.2% and 1.4%, respectively (*P* = 0.63). Very late definite or probable stent thrombosis only occurred in 2 patients in each study arm (0.3% *vs* 0.3%, *P* = 1.00, Table 3).

They concluded that after 2 years of follow-up and stringent discontinuation of dual antiplatelets beyond 12 mo, Resolute ZES and Xience V EES showed similar results in terms of safety and efficacy for treating patients with a majority of complex lesions and off-label indications for DESs.

Other recent clinical reports

Kotani *et al*[18] recently reported 5 year follow up results after SES implantation. They analyzed a prospective registry of 2050 patients with SES during a 5 year follow-up. A total of 1691 patients were divided into two groups: dual antiplatelets ≤ 12 mo, *n* = 749 and dual antiplatelets > 12 mo, *n* = 942 and compared the clinical outcomes using a landmark analysis. The frequencies of MACE (15.6% *vs* 18.2%), death (10.0% *vs* 11.5%), myocardial infarction (2.3% *vs* 2.1%), TLR (4.5% *vs* 11.5%) and stent thrombosis (0.8% *vs* 0.8%) were similar between the two groups. However, with regards to bleeding, an increase in the frequency of hemorrhage events was observed after 4 years from the index procedure in the dual antiplatelets > 12 mo group. They concluded that dual antiplatelets beyond 12 mo was associated with an increased frequency of bleeding complications and does not prevent the incidence of MACE, including stent thrombosis, during 5 years follow-up after SES implantation.

A recently published meta-analysis also supports a shorter duration of dual antiplatelets for both safety and efficacy following DES implantation[19]. They searched for randomized controlled trials that compared longer *vs* shorter dual antiplatelet duration after DES implantation from the database inception to December 2011. Three randomized controlled trials comparing 5622 patients were included. Compared with short-term therapy, longer dual antiplatelet duration had a pooled OR of 1.26 (95%CI: 0.88-1.80; *P* = 0.21, random-effects) for the primary outcomes of cardiac death, myocardial infarction or stroke; OR = 1.29 (95%CI: 0.85-1.93; fixed-effects) for all-cause death; 1.23 (95%CI: 0.78-1.93; fixed-effects) for cardiac death; 0.91 (95%CI: 0.58-1.42; random-effects) for myocardial infarction; and 1.93 (95%CI: 1.01-3.69; fixed-effects) for stroked and 2.51 (95%CI: 1.10-5.71, fixed-effects) for thrombolysis in myocardial infarction major bleeding. The number needed to treat for an additional harmful outcome was 217.6 for stroke and 243 for thrombolysis in myocardial infarction major bleeding. This meta-analysis provides no evidence of benefits with longer dual antiplatelet duration compared with a shorter

course of therapy. It also reports significant harm with respect to major bleeding and stroke associated with prolonged dual antiplatelet use.

Another new clinical trial (OPTIDUAL; OPTImal DUAL antiplatelet therapy trial) is ongoing to assess the efficacy and safety of 12 *vs* 48 mo of dual antiplatelet therapy after DES implantation[20].

Lastly, regarding clinical events associated with stent thrombosis, P2Y12 and thromboxane receptor are not the sole therapeutic measure to prevent the thrombotic risk. There must be different pathways leading to thrombotic events, including hypersensitivity reactions[21,22].

CONCLUSION

Despite the latest PCI guidelines recommending at least 1 year of dual antiplatelet therapy, recent randomized clinical trials, registries and meta-analysis data have shown that a shorter duration of dual antiplatelet therapy is as effective as a longer duration of dual antiplatelets, regardless of DES type (whether first-generation or next generation). Furthermore, a shorter duration of dual antiplatelets was associated with less bleeding complications without increasing the incidence of stent thrombosis. Currently, at least 6 mo of dual antiplatelets following next-generation DES implantation appears to be safe and effective, even with the expanded indication in the contemporary PCI setting. However, caution should be exercised until enough clinical data is obtained, in particular in the subset of higher risk patients, including diabetes, aspirin and clopidogrel resistance or the very complex lesion subset expecting a vulnerability to stent thrombosis. In this review, we focused only on classical dual antiplatelets, aspirin and clopidogrel. However, more data is needed to define the role of newer generation P2Y12 inhibitors, including ticagrelor and prasugrel, especially in the acute coronary syndrome setting in the future.

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REVIEW

Arrhythmogenic ventricular cardiomyopathy: A paradigm shift from right to biventricular disease

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Abstract

Arrhythmogenic ventricular cardiomyopathy (AVC) is generally referred to as arrhythmogenic right ventricular (RV) cardiomyopathy/dysplasia and constitutes an inherited cardiomyopathy. Affected patients may succumb to sudden cardiac death (SCD), ventricular tachyarrhythmias (VTA) and heart failure. Genetic studies have identified causative mutations in genes encoding proteins of the intercalated disk that lead to reduced myocardial electro-mechanical stability. The term arrhythmogenic RV cardiomyopathy is somewhat misleading as biventricular involvement or isolated left ventricular (LV) involvement may be present and thus a broader term such as AVC should be preferred. The diagnosis is established on a point score basis according to the revised 2010 task force criteria utilizing imaging modalities, demonstrating fibrous replacement through biopsy, electrocardiographic abnormalities, ventricular arrhythmias and a positive family history including identification of genetic mutations. Although several risk factors for SCD such as previous cardiac arrest, syncope, documented VTA, severe RV/LV dysfunction and young age at manifestation have been identified, risk stratification still needs improvement, especially in asymptomatic family members. Particularly, the role of genetic testing and environmental factors has to be further elucidated. Therapeutic interventions include restriction from physical exercise, beta-blockers, sotalol, amiodarone, implantable cardioverter-defibrillators and catheter ablation. Life-long follow-up is warranted in symptomatic patients, but also asymptomatic carriers of pathogenic mutations.

Key words: Arrhythmogenic right ventricular dysplasia/cardiomyopathy; Arrhythmias; Ventricular tachycardia; Sudden cardiac death; Implantable cardioverter defibrillator

Core tip: This manuscript constitutes an updated overview about arrhythmogenic ventricular cardiomyopathy (AVC) and describes well the paradigm shift in the understanding of AVC from an isolated right-sided entity to biventricular disease that can present with multiple facets. The most recent advances in molecular and clinical research are discussed, with particular focus on genetic novelties and risk stratification. We believe that this review will help clinicians to better understand the pathomechanisms that lead to AVC, its diagnosis and state-of-the-art therapeutic decision making.

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INTRODUCTION

Arrhythmogenic ventricular cardiomyopathy (AVC), as

recently re-named by the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) consensus statement paper[1], is generally referred to as arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), constituting a hereditary cardiomyopathy usually with an autosomal-dominant inheritance pattern. Its first description by Giovanni Maria Lancisi, the Pope's physician, dates back to 1736 in his book "De Motu Cordis et Aneurysmatibus"[2]. The first comprehensive description of ARVC/D by Guy Fontaine in 1978 marks a milestone for our current understanding of this heterogeneous disease[3]. Initially, ARVC/D was thought to be an embryological aberration, such as Uhl's anomaly leading to the original designation of dysplasia[4]. However, further research shed light on the pathophysiology of ongoing genetically determined myocardial atrophy that did not support the theory of a congenital myocardial absence. Thus, in 1995, ARVC/D was assigned to the World Health Organization's definition and classification of primary cardiomyopathies[5]. Autopsy studies have been crucial in understanding AVC. Progressive atrophy of the ventricular musculature due to cumulative myocyte loss and infiltration by fibrous and adipose tissue can be observed.

The right ventricle (RV) is primarily affected in AVC, representing the most common form known as ARVC/D, and thus can be referred to as classic AVC[6]. At a later stage, the left ventricle (LV) can also be involved and is often associated with severe disease and a worse prognosis[7]. Advanced molecular genetic studies have identified causative mutations in genes encoding proteins of the intercalated disk, mainly desmosomal proteins[8] that lead to reduced electrical and mechanical stability of the myocardium[9,10]. Subsequent myocardial inflammation, apoptosis and necrosis may occur. Some of these histological changes are currently discussed as potential cases of myocarditis mimicking AVC[11-14]. Because of the genetic basis and the many facets of the disease, the term "ARVC" is somewhat misleading. Particularly as biventricular involvement and less often isolated LV involvement may be present in a substantial proportion of patients[15], a broader term such as "arrhythmogenic cardiomyopathy" should be preferred, as already suggested by Gallo *et al*[16] almost 20 years ago, and as recently proposed by the HRS and the EHRA[1]. However, the cardiology community is still reluctant to accept the proposed new nomenclature, probably because RV involvement constitutes a hallmark of the disease and non-classic forms are difficult to distinguish from non-ischemic dilated cardiomyopathies.

EPIDEMIOLOGY

In most parts of the world, phenotypic expression is more common in men than in women (2-3:1)[17,18]. AVC commonly manifests during late childhood or adolescence but can also emerge in the elderly[19,20]. With a general prevalence of 1:2000, which can be higher in certain geographical regions with enhanced genetic prevalence such as the Veneto region or the Greek island Naxos, it is not so rare[21,22]. Recent data indicates that the prevalence is even higher than initially estimated[23]. AVC is recognized as a leading cause of sudden cardiac death (SCD) in young adults ≤ 35 years of age and may account for up to 10% of cardiovascular deaths in the < 65 age group[24,25]. Of note, in one series from northern Italy, AVC accounted for up to 22% of SCD in all young adults ≤ 35 years of age[26-29]. AVC usually first manifests with ventricular tachyarrhythmias (VTA) or SCD. In its most common form ARVC/D, ventricular arrhythmias originate in the RV and thus have left bundle branch block (LBBB) morphology[28,30]. Less often, the primary manifestation can be heart failure without symptomatic arrhythmias. As LV function is often preserved at early stages, ventricular tachycardia (VT) may be asymptomatic as far as it does not degenerate into ventricular fibrillation (VF)[29]. An early concealed phase without gross structural abnormalities is unique among the primary cardiomyopathies. On the contrary, in hypertrophic cardiomyopathy, arrhythmic risk can be ascribed to the underlying myocardial disarray. In dilated cardiomyopathy (DCM), arrhythmias generally concur with significant LV systolic dysfunction[31]. Of note, early AVC may resemble myocardial channelopathies, such as Brugada syndrome (Bs)[32], thus making correct diagnosis and risk stratification difficult.

DISEASE SUBTYPES

Classification of AVC into three different subtypes is evolving. AVC in its classic right-dominant form is the most common and best known and referred to as ARVC/D. The non-classic forms were first described by pathologists on autopsy studies and in isolated clinical case reports[33,34]. Through intensive *in vivo* characterization of affected families, a link to hereditary mutations of the intercalated disk was established[35-37]. LV involvement is increasingly described with a prevalence of up to 76% of cases, which may be attributed to improved diagnostic methods such as genetic testing, high-resolution contrast-enhanced cardiac magnetic resonance tomography (CMR), and recently the new technology of echocardiographic strain imaging[38]. The proposed classification below is simplistic since due to genetic heterogeneity and epigenetic factors, a phenotypic continuum with right- and left-dominant subtypes at opposite ends has to be assumed.

In classic right-dominant ARVC, a dilated RV with fibro-fatty infiltration with no or only minimal LV involvement can be found at autopsy (Figure 1). This fibro-fatty infiltration typically begins subepicardially and may expand transmurally over time[39]. Papillary muscles and trabeculae are generally not involved in this process[25]. Yet, fatty infiltration alone does not constitute a pathognomonic sign of AVC, as a certain amount of epicardial and intramyocardial fat without an increase in fibrous tissue is present in both ventricles, more commonly in the RV, of persons without cardiovascular disease, particu-

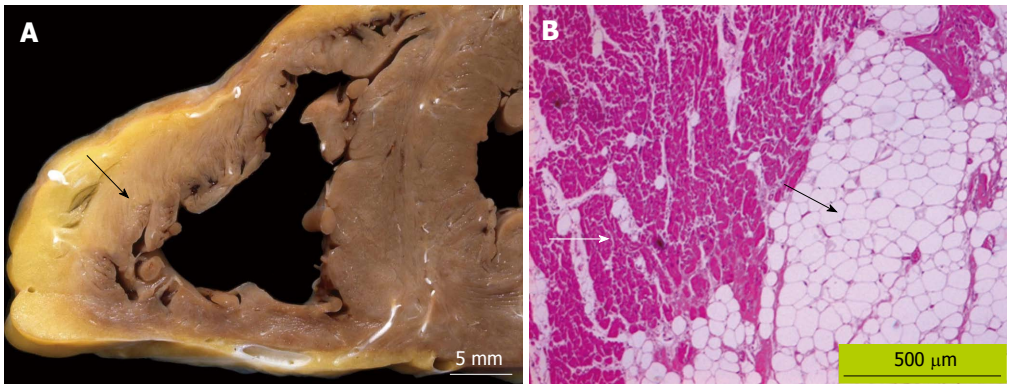

Figure 1 Typical pathology findings in arrhythmogenic ventricular cardiomyopathy/dysplasia. A: Macroscopic finding in a patient with arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). The myocardium of the right ventricular free wall is partially replaced by fibro-fatty tissue (black arrow) that typically begins in the epicardial region and at later stages expands transmurally; B: Endomyocardial biopsy from a patient with ARVC/D demonstrating fatty (black arrow) replacement of the right ventricular myocardium. Strands of myocardium are still visible (white arrow, heidenhain trichrome, magnification × 60).

larly in the obese and elderly[17,40,41]. Another consistent finding in AVC is myocardial atrophy. Myocardial wall thinning, but also thickening, can both be seen on macroscopic examination[22,40]. The subtricuspid region and the thin RV outflow tract (RVOT) are particularly prone to ventricular bulging and aneurysm formation that is present in 20%-50% of autopsy cases of ARVC/D[39]. The former concept of early RV apical involvement and the term "triangle of dysplasia" have recently been questioned[42]. Even although not very specific, ventricular aneurysms are strongly associated with the disease. The fact that the interventricular septum is rarely affected by fibro-fatty infiltration is an important disadvantage of endomyocardial biopsies, usually obtained from the septum, which may frequently yield false-negative results[43]. If an affected region can be obtained for histological evaluation, it may reveal both replacement fibrosis, a repair mechanism after myocyte loss, and interstitial fibrosis, a reactive process, *e.g.,* to inflammation[36,39].

Biventricular AVC is characterized by early and parallel involvement of both ventricles that can only be visualized by advanced imaging techniques such as contrast CMR or strain echocardiography[36,44]. Progressive disease is characterized by systolic impairment and biventricular dilation with clinical features of global congestive heart failure. In contrast to other cardiomyopathies with biventricular involvement, ventricular arrhythmias of both right bundle branch block (RBBB) and LBBB configuration are present at an early stage, with around 10% of patients presenting with both[31].

Left-dominant AVC (ALVC) has recently been suggested as a distinct form of AVC and is characterized by the early occurrence of LV involvement, while global RV function is preserved[36]. An overlap with idiopathic myocardial fibrosis (IMF) accounting for certain SCD cases in a post mortem series has been reported[45]. Typically, IMF features diffuse interstitial and replacement fibrosis with a predilection for the inferior LV wall in the absence of coronary artery disease and other structural abnormalities. Of note, myocardial infiltration by adipocytes is lacking in IMF. In biventricular disease or ALVC, ventricular arrhythmias may also originate from the LV and thus show a RBBB configuration. Structural and electrocardiographic (ECG) findings are the left-sided analogues to those observed in ARVC/D (Table 1). The RV to LV ratio typically remains < 1.0. To better understand ALVC and its clinical course, future investigations will be required.

PATHOGENESIS

Genetically-determined disruption of intercalated-disk integrity is a key factor promoting the development of AVC and SCD. This is widely named the "defective desmosome" hypothesis[46,47]. Recent data indicates that loss of desmosomal integrity can substantially affect gap junctions, sodium channel function and electrical propagation at the micro- and nano-scale, thereby promoting ventricular arrhythmias in the absence of overt structural damage[48]. Accordingly, lethal arrhythmias such as VF and polymorphic VT often occur during these concealed early stages, while sustained monomorphic VT occur at later stages, where there is enough substrate for macro-re-entry. Delmar *et al*[9] thus have postulated that mutations in desmosomal genes may affect the integrity of other molecular complexes that reside in proximity to desmosomes, such as connexins and voltage gated sodium channels, and are crucial for electrical synchrony. This molecular complex and its interactions have been named the cardiac connexome[49,50]. Yet, genetic mutations in gap junctions such as connexin-43 have not been associated with AVC so far[10,51].

Currently, two theories for the understanding of progressive fibro-fatty replacement of the myocardium exist: (1) inflammation as a response to myocardial injury[4,25,39]. Lymphocytic interstitial infiltrates surrounding foci of necrotic or degenerative myocytes are observed on histopathology. Myocyte cell death may occur *via* apoptosis or necrosis underlying chronic inflammation. Acute myocyte cell death has also been reported, suggesting acute myocarditis during the disease course[52]. Periodic exacerbations of a previously quiescent disease may be

Table 1 Characteristics of arrhythmogenic ventricular cardiomyopathy

	Classic right dominant form (ARVC/D)	Left dominant form
12-lead surface ECG	Intraventricular conduction delay in V1-V3	Leftward QRS axis (< 0°)
	QRS complex prolongation V1-V3	ε like waves in inferior or lateral leads
	ε wave in V1-V3	LBBB
	(Incomplete) RBBB	Inverted T-waves in infero-lateral leads
	Inverted T-waves in V1-V3	Inverted T-waves V1-6 with biventricular involvement
	Inverted T-waves in V1-V6 with biventricular involvement	-
	ST elevation in V1-V3	-
	Poor R wave progression	
Signal-averaged ECG	Late potentials	-
Arrhythmia	PVC/VT of LBBB configuration	PVC/VT of RBBB configuration
Ventricular volumes	Mild to severe RV-dilation ± dysfunction	Mild to severe LV-dilation ± dysfunction
RV/LV volume ratio	⩾ 1.2, increases with disease expression	< 1.0
Other imaging abnormalities	Regional wall motion abnormalities in RV	Regional wall motion abnormalities in LV
	RV aneurysms	Non-compacted appearance
	Fat/LGE in RV myocardium	LGE in the subepicardial and midwall LV myocardium
Genetics	Affected genes currently known to be associated with AVC	Association with TMEM43 and phospholamban mutations[1]

Adapted from Jacoby *et al*[84]. ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; ECG: Electrocardiogram; ε: Epsilon; LBBB: Left bundle branch block; LGE: Late gadolinium enhancement; LV: Left ventricle; PVC: Premature ventricular contraction; RBBB: Right bundle branch block; RV: Right ventricle; VT: Ventricular tachycardia; AVC: Arrhythmogenic ventricular cardiomyopathy; TMEM43: Transmembrane protein 43.

triggered by such inflammatory episodes and are called "hot phases" of AVC. Occasionally, these phases may clinically present with chest pain, dynamic ECG changes and increased arrhythmic activity[31]. Strenuous physical activity can trigger inflammation as mechanical stress to the impaired intercalated disk leads to myocyte detachment and myocyte cell death[53]. It is important to keep in mind that isolated myocarditis, sarcoidosis, Bs and other diseases can mimic AVC[14], which may prompt further histological and molecular investigations. If molecular genetic analyses or pedigree analyses of affected family members are not performed, a biopsy specimen may be classified as focal myocarditis[36]. Yet, previous studies have indicated a link between AVC and a susceptibility to viral and bacterial myocarditis, particularly in non-hereditary forms[54,55]. The prevalence of viral genome in myocardial biopsies from AVC patients is reported with a broad range from 0% to 75%, but a causal association is difficult to prove. Presence of enteroviral RNA has been reported in tissue from patients with DCM, suggesting an innocent bystander role. Nevertheless, viral presence may play a secondary yet important role in disease progression[47]; and (2) apoptosis following disruption of the intercalated disc[56] with electromechanical instability, as indicated by detection of fragmented DNA, expression of protease CPP-32 by immunohistochemistry and positive Tc-annexin V scintigraphy *in vivo*[11-14,57,58]. These histological disarrangements create a substrate for electrical re-entrant phenomena and delayed ventricular activation triggering ventricular arrhythmias. Of note, as AVC can cause ventricular arrhythmias and SCD in the absence of gross macroscopic abnormalities, histological and molecular examinations are important to establish a post-mortem diagnosis[59]. Other investigators observed that epicardium-derived cell cultures obtained from neonatal hearts lacking plakophilin-2 (PKP2), an important desmosomal gene, revealed enhanced cell migration velocity and proliferation, leading to the hypothesis that desmosomal mutations may cause infiltration of fibroblasts and adipocytes from the epicardial cell layer into the myocardium[60]. This hypothesis is consistent with the frequent clinical observation that fibro-fatty infiltration progresses from the epicardium towards the endocardium.

GENETICS

Analyses of the first- and second-degree relatives of patients suggest that up to 50% of AVC cases are familial[61,62]. AVC is most commonly inherited as a Mendelian autosomal dominant trait with incomplete penetrance[46,47], although two autosomal recessive forms have been described[63-65]. To date, 12 different AVC loci are reported in the Online Mendelian Inheritance in Man (Table 2)[66]. Compound and digenic heterozygosity has been recently suggested, indicating that in some cases more than one pathogenic allele may be involved in the disease process[65,67,68]. As penetrance is incomplete, genetically affected relatives often demonstrate variable and mild phenotype and the prevalence of familial disease is often underestimated in clinical practice[31,62]. The fact that AVC can be inherited has been known since 1982 after the description of 24 adult cases, two in the same family, by Marcus *et al*[69]. Six years later, the autosomal dominant pattern of inheritance with incomplete penetrance and variable expression was demonstrated in a study of nine Italian families[26]. As patients with fully penetrant cardiomyopathy and readily discernible features of the palms, plantar fascia and hair were clustered in families on the Greek island Naxos, an autosomal recessive mutation in the desmosomal protein junction plakoglobin (JUP) was finally discovered, which became known as Naxos disease. Myocytes and epidermal cells share similar intercalated disks (desmosomes and fascia adherens) and are both exposed to high shear stress, the

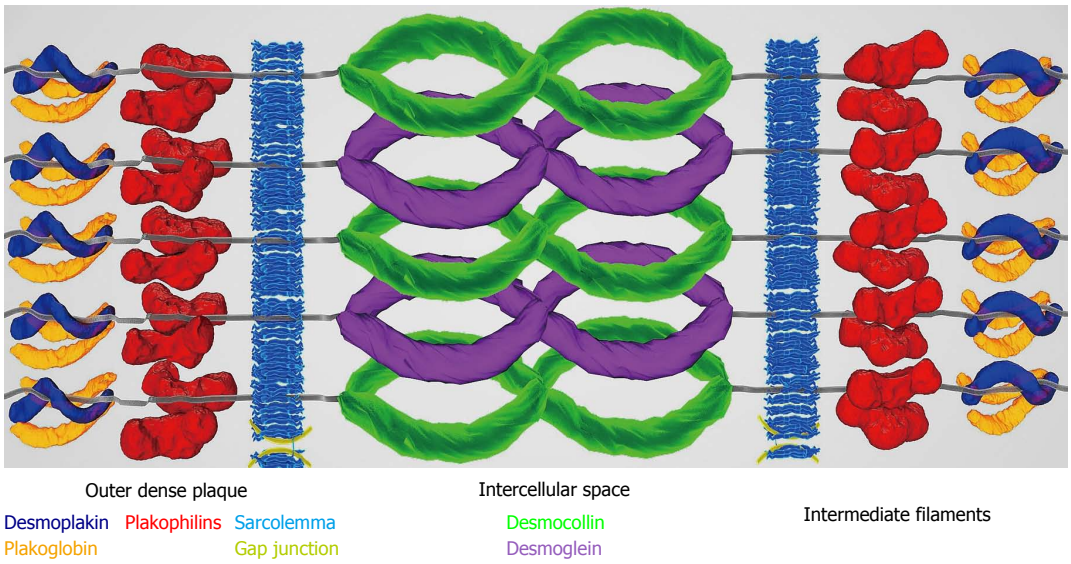

Figure 2 Molecular model of the desmosome: in the desmosomal complex the intermediate filaments of the cytoskeleton (desmin in the heart) are linked to the transmembranous cadherins (desmocollin and desmoglein) *via* armadillo proteins (plakoglobin and plakophilin) and desmoplakin. This interaction is crucial for myocardial mechanical and electrical stability. Mutations in arrhythmogenic right ventricular cardiomyopathy mostly affect desmosomal proteins.

Table 2 Arrhythmogenic ventricular cardiomyopathy classification, from OMIMTM Online Mendelian inheritance in Man

AVC subtype	Chromosome/locus	Mode of transmission	Encoded protein
ARVC/D 1	14q23-q24	Autosomal-dominant	TGFβ3
ARVC/D 2	1q42-q43	Autosomal-dominant	RyR2
ARVC/D 3	14q12-q22	Autosomal-dominant	-
ARVC/D 4	2q32	Autosomal-dominant	TTN
ARVC/D 5	3p23	Autosomal-dominant	TMEM43
ARVC/D 6	10p12-p14	Autosomal-dominant	-
ARVC/D 7	10q22	Autosomal-dominant	-
ARVC/D 8	6p24	Autosomal-dominant	DSP
ARVC/D 9	12p11	Autosomal-dominant	PKP2
ARVC/D 10	18q12	Autosomal-dominant	DSG2
ARVC/D 11	18q12.1	Autosomal-dominant	DSC2
ARVC/D 12	17q21	Autosomal-dominant	JUP
Naxos disease	17q21	Autosomal-recessive	JUP

AVC: Arrhythmogenic ventricular cardiomyopathy; ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; TGF: Transforming growth factor; RyR2: Ryanodine receptor 2; TTN: Titin; TMEM43: Transmembrane protein 43; DSP: Desmoplakin; PKP2: Plakophilin-2; DSG2: Desmoglein-2; DSC2: Desmocollin-2; JUP: Junction plakoglobin.

heart particularly during strenuous physical activity and increased cardiac workload. Thus, it has been assumed that common genes encoding proteins of the intercalated disk might be responsible for AVC. In 1994, the first chromosomal locus (14q23-q24) for autosomal dominant AVC was reported in Italy[47]. Linkage analyses shed light on its genetic heterogeneity with sequential discovery of several loci on chromosomes 1, 2, 3, 6, 10, 12, 14, 17 and 18 (Table 2). Most frequently, mutations in genes encoding components of the cardiac desmosome, an important protein complex of the intercalated disk (Figure 2), are associated with AVC, resulting in impaired intercalated-disk integrity[62,67,68]. The pathogenic importance of desmosomal mutations was confirmed by electron microscopy and immunohistochemistry[2,56]. Intercellular junctions consist of a core region that mediates cell-cell adhesion and a plaque region that provides attachment to the intermediate filaments within the myocyte. Three groups of desmosomal proteins are known: (1) transmembrane desmosomal cadherins including desmocollins 2 and desmogleins 2 (DSG2); (2) desmoplakin (DSP), a plakin family protein that attaches directly to intermediate filaments (desmin in the myocardium); and (3) linker proteins such as armadillo family proteins including JUP (catenin-γ) and PKP2 that mediate interactions between the desmosomal cadherin tails and DSP[70]. In about 80% of cases with confirmed pathogenic mutations, PKP2, DSP and DSG2 are altered[22]. Besides desmosomal gene mutations, mutations in genes encoding proteins that interact with desmosomal proteins were found as well. These include: (1) the transforming growth factor β3 that conveys cytokine-stimulating fibrosis and modulates cell adhesion and growth[52]; (2) the human ryanodine receptor 2 (RyR2) that induces the release of calcium from the myocardial sarcoplasmic reticulum and that is also associated with catecholaminergic polymorphic VT (CPVT)[71]; (3) the transmembrane protein 43 (TMEM43) discovered in the Canadian Newfoundland founder population and Europe[72] that functions as a PPAR-γ response element, an adipogenic transcription factor; (4) the intermediate filament desmin; (5) the tumor protein 63; and (6) recently, titin (TTN) that bridges the sarcomere along its longitudinal axis and forms a continuous filament along the myofibril[66]. As TTN binds to the transitional junction of the intercalated disk, this may explain a functional link to the desmosome[66,73,74]. Current molecular studies are screening other components of the desmosome and related proteins, such as plectin and pinin (Table 3)[75]. NFκB interacting protein-1 is another extra-desmosomal gene of interest, which has been isolated in Poll-Here-

Table 3 Future candidate proteins for arrhythmogenic ventricular cardiomyopathy

Encoded protein
Components of the desmosome
Plectin
Emerin
Components of the adherens junction
β-catenin
α-catenin
N-cadherin
Components of the gap junction
Connexin 43
Myotonic dystrophy protein kinase-1
Laminin receptor-1
Components of dystrophin-glycoprotein complex

ford cattle with recessive AVC and woolly hair coat syndrome[76]. Yet, the pathogenic role of NFκB interacting protein-1 mutations in humans has to be demonstrated in future studies.

MODIFIER GENES AND ENVIRONMENTAL FACTORS

Although a plethora of pathogenic mutations exists, these mutations cannot account for the entire broad spectrum of disease expression. Data from the Newfoundland founder population and populations from the Dutch and Swiss ARVC/D registries show a strong male predominance of disease expression[77]. A modifier effect of testosterone has been discussed. Yet, this male predominance has not been confirmed in the Johns Hopkins ARVC/D cohort, which may be associated with similar exercise levels among males and females in the United States. Nevertheless, outcomes were strongly gender dependent in all of those cohorts, with male gender constituting an independent risk factor for adverse outcomes[37,62,72,78,79]. In one study, 67% of family members showed discordant disease patterns between RV and LV involvement[31]. Recent data pointing at the importance of compound and digenic heterozygosity indicates that modifier genes may account for residual variation and disease severity[68,80]. The first evidence for environmental influences in AVC arose from monozygotic twin studies, where differences were reported in symptom onset, structural severity and arrhythmic risk. Strenuous physical activity seemed to play an important role in these four cases[81]. These preliminary observations were confirmed in two recent studies, in which endurance training and frequent exercise were associated with earlier disease manifestation and disease severity[31,82]. Future studies will be crucial to distinguish between pathogenic mutations and innocent bystander mutations and to define the role of epigenetic factors in disease manifestation and progression. As recently proposed by the HRS/EHRA consensus statement, genetic testing should only be performed if the signal-to-noise ratio is expected to be > 10[1].

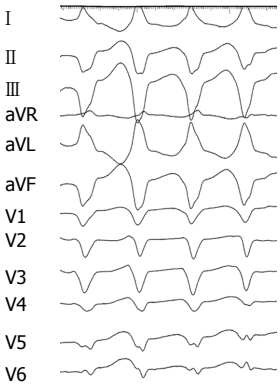

Figure 3 Monomorphic sustained ventricular tachycardia with left bundle branch block morphology and superior axis (Ⅱ, Ⅲ, aVF negative), a major criterion for arrhythmogenic right ventricular cardiomyopathy/dysplasia according to the revised 2010 task force criteria.

CLINICAL PRESENTATION

AVC has a reported community-based prevalence of 1 in 2000 and thus cannot be classified as a "rare" disease according to the 2007 European definition. These numbers reflect the importance of appropriate diagnostic tools as it is often underdiagnosed, particularly in early and mild cases. The above mentioned non-classic subtypes are usually not considered or misattributed as DCM. Some forms mimic myocarditis. Early disease with arrhythmias but without overt structural changes may be misjudged as idiopathic VT or ventricular ectopy[36,46]. In the elderly, AVC is rarely considered as a differential diagnosis, which is certainly a false assumption. All these aspects infer that real-world prevalence is higher. In the following section, we provide an overview of clinical symptoms and signs that shall increase awareness of the disease, particularly in non-classic forms, for timely diagnosis and prevention of SCD. AVC should be suspected if the following symptoms or signs occur: (1) palpitations; (2) presumably arrhythmic presyncope or syncope; (3) VT with LBBB morphology; (4) aborted SCD. Palpitations and (pre)syncope are the most frequent symptoms[17]. A high clinical suspicion should be raised if these symptoms correlate with premature ventricular contractions (PVC) or VT with LBBB morphology, particularly with a superior axis (Figure 3). However, ALVC or biventricular disease can present with VT with RBBB morphology or both (Table 1, Figure 3). The presence of monomorphic VT is associated with late disease stages, although gross structural changes are not mandatory[28,83]. Recently, disease severity, VT frequency and early onset of VT have been associated with the presence of common desmosomal mutations, particularly if more than one pathogenic variant was present[62,67,84]. Up to 25% of patients present with supraventricular tachycardia (SVT), most frequently atrial fibrillation, which is associated with male gender, increasing age and left atrial enlargement in AVC[85]. SVT are very important as they are associated with inappropriate implantable cardioverter defibrillator (ICD) shocks

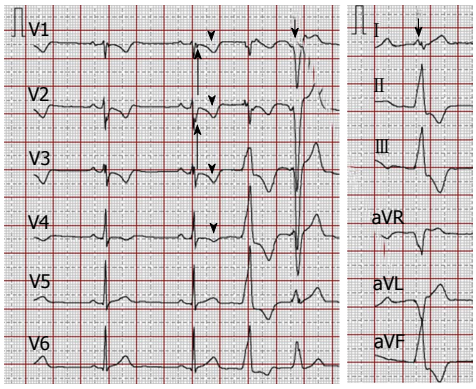

Figure 4 Electrocardiographic findings. A 12-lead surface electrocardiogram (25 mm/s, 10 mm/mV) showing typical depolarization abnormalities (prolonged terminal activation duration in V1-V2, a minor criterion according to 2010 task force criteria, long arrows) and repolarization abnormalities (T-wave inversions V1-V4 in the absence of complete right bundle branch block, a major criterion according to 2010 task force criteria, arrowheads), and premature ventricular contractions with two different morphologies (short arrows).

and an increased risk of both heart failure and death. Furthermore, atrial arrhythmias present at a younger age than in the general population[86]. It is not rare that AVC first manifests as SCD, with some authors reporting an annual incidence of 9%[87]. Whereas some authors report that SCD occurs preferentially during strenuous physical activity[25,87,88], according to others it may often occur in the sedentary state[13,25]. In ARVC/D caused by TMEM43 mutations, enhanced sympathetic activity as a trigger for lethal arrhythmias is established[71]; (5) chest pain with or without dynamic ST elevation/T-wave changes on 12-lead surface ECG ± rise in cardiac biomarkers; and (6) presumed DCM with early onset and frequent ventricular arrhythmias. Precordial T-wave inversions beyond V1 after puberty (Table 1, Figure 4) and T-wave inversions in the right precordial leads V1-V3 may potentially be benign, particularly before puberty. Their prevalence among athletes and sedentary controls is similar[89], suggesting that this is not a training-related phenomenon. According to recent recommendations, a further evaluation with transthoracic echocardiography (TTE) may be performed after puberty. If imaging is inconclusive, regular follow-up by serial clinical examinations, ECG and TTE can be performed as structural alteration may become apparent after several years[90,91]. RV failure with dyspnea and signs of right sided heart failure are rather rare and reported in up to 6% of patients at initial presentation. If the LV is involved, congestive heart failure may occur. Importantly, the clinician should be aware that AVC cannot be excluded by the absence of structural abnormalities as arrhythmias often occur in the "concealed phase" and structural abnormalities may follow after years. In a review reporting 37 families with AVC index patients, only 151 of 365 family members had clinically manifested disease and 17 family members were healthy despite a pathogenic mutation[28]. Thus, genetic screening of family members may help to identify AVC, although a negative test does not exclude it.

DIAGNOSIS

Revised 2010 task force criteria

Currently, no gold standard to establish or exclude the diagnosis of AVC exists. In 2010, the original 1994 task force criteria (TFC) for diagnosis of ARVC/D by Marcus *et al*[92] were revised in order to enhance diagnostic sensitivity and particularly to improve identification of affected asymptomatic family members[93]. The importance of pathogenic mutations was acknowledged and precise cut-off values for imaging and histological evaluation were provided. The impact of these changes is currently being evaluated. Some investigators report an increased diagnostic yield with the revised TFC[94,95], while others could not demonstrate a benefit[96,97]. It is important to keep in mind that these TFC only apply to ARVC/D with or without LV involvement. The revised TFC assign the findings into six categories (Table 4): (1) global and/or regional myocardial dysfunction and structural abnormalities; (2) histological characterization; (3) repolarization abnormalities on 12-lead surface ECG; (4) depolarization abnormalities on 12-lead surface ECG; (5) arrhythmias; and (6) family history and genetics.

Definite diagnosis requires 2 major criteria, 1 major and 2 minor criteria, or 4 minor criteria from different categories. ARVC/D is considered "borderline" if 1 major and 1 minor criterion, or 3 minor criteria are present. ARVC/D is still "possible" if 1 major criterion or 2 minor criteria are present. For each individual, comprehensive non-invasive evaluation is necessary. This includes a thorough clinical history and examination, pedigree analysis, 12-lead surface ECG, TTE with detailed assessment of the RV, CMR, stress testing in order to induce arrhythmias, and Holter ECG monitoring. If suspicion remains high and symptoms are rare, event recorders and invasive procedures may be needed.

Physical examination

Fifty percent of patients will have a normal physical exam. The other 50% will show abnormalities such as giant a-waves on the jugular veins, tricuspid regurgitation murmur, a fixed splitting of S2, and right-sided S3-S4 at the left sternal border with augmentation during inspiration in case of RV dilation[88,98].

12-lead surface ECG and signal-averaged ECG

An abnormal 12-lead surface ECG will be present in about 50% of patients with ARVC/D. In one study, ECG was abnormal in 90% of patients after a follow-up period of 6 years[99]. Abnormalities include epsilon waves, a QRS duration ⩾ 110 ms in V1-V3, and T-wave inversions in the right precordial leads (Figure 4). A prolonged terminal activation duration (measured from the nadir of the S wave until the end of the QRS complex) in V1-V3 ⩾ 55 ms is considered as a minor criterion for ARVC/D and has been reported as the first sign in young asymptomatic family members[45,62,100]. However, interpretation of ECG findings, apart from T-wave inversions, significantly var-

Table 4 Revised (2010) task force criteria for diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia, adapted from Marcus *et al*[92]

	Structural alterations
Major	TTE regional RV akinesia, dyskinesia, or aneurysm and 1 of the following criteria (end diastole)
	PLAX RVOT ≥ 32 mm [(PLAX/BSA) ≥ 19 mm/m^2]
	PSAX RVOT ≥ 36 mm [(PSAX/BSA) ≥ 21 mm/m^2]
	Or RV fractional area change ≤ 33%
	CMR regional RV akinesia, dyskinesia, or dyssynchronous RV contraction and 1 of the following criteria (end diastole)
	RV end-diastolic volume/BSA ≥ 110 mL/m^2 (♂) or ≥ 100 mL/m^2 (♀)
	Or RV ejection fraction ≤ 40%
	RV angiography regional RV akinesia, dyskinesia, or aneurysm
Minor	TTE regional RV akinesia, or dyskinesia and 1 of the following criteria (end diastole)
	PLAX RVOT ≥ 29-31mm [(PLAX/BSA) ≥ 16-18 mm/m^2]
	PSAX RVOT ≥ 32-35 mm [(PSAX/BSA) ≥ 18-20 mm/m^2]
	RV fractional area change > 33%-39%
	CMR regional RV akinesia, dyskinesia, or dyssynchronous RV contraction and 1 of the following criteria (end diastolic)
	RV end-diastolic volume/BSA ≥ 100-109 mL/m^2 (♂) or ≥ 90-99 mL/m^2 (♀)
	Or RV ejection fraction > 40%-44%
	Histopathology (endomyocardial biopsy)
Major	Residual myocytes < 60% by morphometric analysis with fibrous replacement of the RV free wall myocardium ≥ 1 sample, with or without fatty replacement
Minor	Residual myocytes 60%-75% by morphometric analysis with fibrous
	Replacement of the RV free wall ≥ 1 sample
	Repolarization abnormalities (> 14 years of age)
Major	T-wave inversions V1-V3 or beyond (in absence of complete RBBB)
Minor	T-wave inversions V1-V2 or V4-V6 (in absence of complete RBBB)
	T-wave inversions V1-V4, if complete RBBB present
	Depolarization abnormalities
Major	Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T-wave) in V1 to V3
Minor	SAECG with late potentials (if QRS complex on standard surface ECG < 110 ms) or terminal activation duration of QRS ≥ 55 ms in V1, V2 or V3
	Arrhythmias
Major	VT of LBBB morphology with superior axis
Minor	VT of RVOT configuration, LBBB morphology with inferior axis or of unknown axis
	> 500 PVC per 24 h (holter)
	Family history
Major	ARVC/D in a first-degree relative who meets current TFC
	ARVC/D confirmed pathologically at autopsy or surgery in a first-degree relative
	Identification of a pathogenic mutation categorized associated with ARVC/D in an index patient
Minor	Suspected ARVC/D in a first-degree relative-premature SCD (< 35 years of age) due to suspected ARVC/D in a first-degree relative
	ARVC/D confirmed pathologically or by current TFC in second-degree relatives

Definite diagnosis: two major or one major and two minor criteria or four minor from different categories; Borderline diagnosis: one major and one minor or three minor criteria from different categories; Possible diagnosis: one major or two minor criteria from different categories. BSA: Body surface area; CMR: Cardiac magnetic resonance tomography; LV: Left ventricle; PLAX: Parasternal long-axis view; PSAX: Parasternal short-axis view; RBBB: Right bundle branch block; RVOT: Right ventricular outflow tract; RV: Right ventricle, TTE: Transthoracic echocardiogram, PVC: Premature ventricular contraction VT: Ventricular tachycardia; SAECG: Signal-averaged electrocardiographic; LBBB: Left bundle branch block; ARVC/D: Arrhythmogenic right ventricular cardiomyopathy/dysplasia; TFC: Task force criteria; SCD: Sudden cardiac death.

ies among observers (unpublished data as yet from our group). This is particularly true for what is considered an epsilon wave. A limitation of T-wave inversions is the fact that they can also be found in healthy individuals, patients with anterior ischemia or RV hypertrophy[90,101]. A recent study highlighted the importance of serial ECG evaluations as dynamic ECG changes occurred in 23% of patients over a median follow-up period of 34 mo, but these were not paralleled by structural abnormalities[102]. Fibro-fatty infiltrations disrupt the electrical continuity of myocardial fibers. This leads to fragmentation and delay of ventricular depolarization (zig-zag pathways). On the surface, this may be visible as QRS fragmentation[103], late ventricular potentials of small amplitude such as epsilon waves[104], or late potentials recorded by signal-averaged ECG (SAECG)[87,105]. An abnormal SAECG (a minor criterion) indicates progressive disease and may predict VT, although a recent study has questioned the latter[28,106]. SAECG may not be sensitive enough to detect early forms of AVC[28].

Stress testing

Exercise can induce ventricular arrhythmias and is important in patients with suspected AVC. However, VT with LBBB morphology and inferior axis can occur in both ARVC/D and idiopathic RVOT-VT without underlying structural abnormalities[107]. A recent study has proposed ECG criteria and a scoring system to distinguish between the two entities[108].

Transthoracic echocardiography

In many centers, TTE constitutes the initial imaging tool for evaluation of patients with suspected AVC and for screening family members as it is readily available and

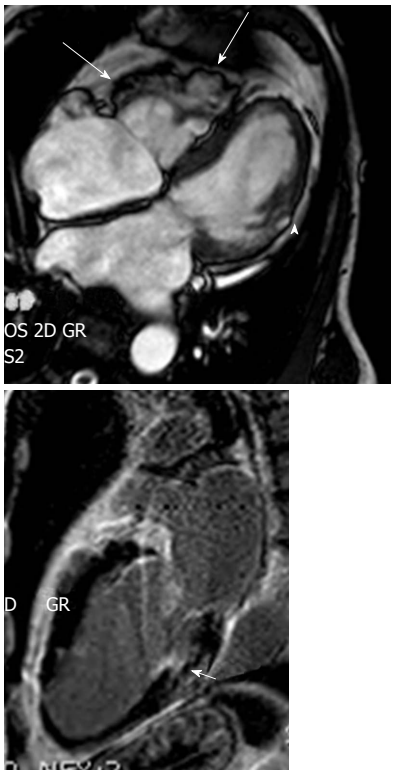

Figure 5 Regional right ventricular dyskinesia of the right free wall detected by cardiac imaging are considered as a major criterion for right ventricular cardiomyopathy/dysplasia according to the revised task force criteria if additionally right ventricle dilation or impaired right ventricle ejection fraction are present. These cardiac magnetic resonance images (upper panel 4-chamber view, lower panel 2-chamber view late sequences) show aneurysms of the RV free wall (long arrows), and LV involvement detected by a small akinetic region (arrowhead) and late gadolinium enhancement of the posterior LV wall (short arrow), confirming biventricular involvement. ARVC/D: arrhythmogenic right ventricular cardiomyopathy/dysplasia; RV: Right ventricle; LV: Left ventricle.

rapidly informative. It may demonstrate RV enlargement or multiple areas of dilation and regional contraction abnormalities, mainly in the subtricuspid region, RVOT and RV apex[109]. According to the revised TFC, evaluation and measurements of the RVOT are crucial for diagnosis[110]. The LV can also be affected, particularly in non-classic forms displaying hypokinesia and a reduced ejection fraction, although in most cases LV structural abnormalities are localized in the posterolateral region[111,112].

CMR

CMR has emerged as the non-invasive diagnostic tool of choice for assessing the RV over the past 15 years[45,113]. Besides highly accurate assessment of right sided volumes, myocardial mass and systolic and diastolic function, the contrast-enhanced CMR can reveal intramyocardial fibrosis by late gadolinium enhancement (LGE)[114]. Yet, intramyocardial fat and fibrosis as diagnostic targets in AVC were not integrated in the revised TFC because of the limited specificity of these findings, particularly in the absence of regional wall motion abnormalities, significant intra- and inter-observer variability, and the need for highly specialized interpreters in visualizing the RV myocardium[45,115,116]. In fact, it can be challenging to be certain of LGE within the RV myocardium because of the thin RV and possible confusion with fat. The main difference in CMR criteria compared to the 1994 criteria constitutes the quantification of RV dilation and RV function. CMR plays an important role in diagnosing AVC (Figure 5) but consensus guidelines for non-classic forms are eagerly awaited. Some authors emphasize the importance of combining TTE with CMR to increase diagnostic yield. New diagnostic tools for detection of early diastolic and systolic abnormalities such as three-dimensional echocardiography, strain echocardiography and CMR tagging could facilitate early diagnosis of ACV[117-120]. The promising results of these preliminary studies[121,122] will have to be validated in large prospective studies.

RV angiography

RV angiography is considered a very useful test to diagnose classic forms of AVC and to evaluate RV function[123,124]. Its positive predictive value is above 85%, with a negative predictive value of 95%[88]. Technical aspects of the procedure can be found at *arvd.org*. Good quality images allow global and regional analyses of morphology and wall motion. RV angiography also has certain limitations that explain why it is not widely used in clinical practice. Clinicians want to offer non-invasive strategies without ionising radiation, particularly if patients are young. Additionally, serial follow-up RV angiographies for monitoring disease progression are difficult to perform. It is important to remember that according to the revised 2010 TFC, with all three imaging techniques, hypokinesia is no longer considered diagnostic.

Electrophysiological study and electroanatomical voltage mapping

Arrhythmias can be induced during an electrophysiological study (EPS) with programmed ventricular stimulation. Induction of clinical VT can guide ablation. The susceptibility for arrhythmias, arrhythmia detection, ICD treatment algorithms and efficacy of antiarrhythmic drugs can be assessed. Electroanatomical voltage mapping (EAM) is a technique using electrophysiological catheters to measure local myocardial voltages. After obtaining several hundred points, a voltage map can be reconstructed. According to several studies, healthy RV myocardium displays bipolar voltages > 1.5 mV[125-127]. In myocardium infiltrated by fibro-fatty tissue, abnormally low voltages with a longer duration, splitting and fractionation of signals can be found. Myocardial voltage maps are usually obtained from the endocardium but epicardial measurements after puncturing the pericardial sac are also feasible. EAM has been shown to be safe and to improve outcomes of VT ablation in ARVC/D[128-131]. The diagnostic and prognostic utility of EAM has not yet been implemented in the current TFC. Larger prospective studies may consolidate the role of EAM in the diagnostic armamentarium[125,132,133].

Endomyocardial biopsy

Endomyocardial biopsy (EMB) was considered the di-

agnostic gold standard for AVC for a long time. It may allow confirmation of AVC in an index patient and exclude potential differential diagnoses such as sarcoidosis or Chagas disease. However, EMB are commonly taken from the thicker RV septum to assure a safe procedure. It was recognized that the septum is often spared by fibro-fatty infiltration and thus often yields false-negative results[132,134]. Nevertheless, septal EMB can identify other conditions such as sarcoidosis, myocarditis and IMF. EMB from diseased regions is problematic as these regions are often difficult to reach, very thin and sample acquisition carries an increased risk of perforation and tamponade[4]. Histological analysis should best be performed by an expert cardiac pathologist who judges the amount of surviving myocytes and fibro-fatty replacement. The results can be allocated as one major or one minor criterion according to the revised TFC. As AVC is patchy, several biopsies should be obtained. EAM-guided biopsies taken from low-voltage areas may improve diagnostic yield and better distinguish between myocarditis or sarcoidosis[14,125,135]. Serious concerns remain about the hazards of sampling thin areas, although complication rates in preliminary studies were low[14]. Moreover, EAM-guided EMB may be of limited value in early stages of AVC when serious arrhythmias occur in the absence of gross structural abnormalities. Additional immunohistochemical staining of the intercalated disk, *e.g.*, with plakoglobin, may turn into a valuable tool for pathologists in the future but results very much depend on the protocols used[2]. Confirmation of typical histological changes by cardiac surgery or necropsy can help to confirm the diagnosis and exclude differential diagnoses.

Genetic testing

A consensus statement from the HRS and the EHRA regarding genetic testing in AVC was published recently[1]. The major purposes of genetic testing are to confirm AVC in probands with a high (Class Ⅱa recommendation, level of evidence C) or intermediate (at least 1 major or 2 minor criteria; Class Ⅱb recommendation, level of evidence C) clinical suspicion and to identify genetically-affected relatives harboring the pathogenic mutation (Class I recommendation, level of evidence C), particularly those without overt disease. Genetic testing in probands fulfilling only one minor criterion is not recommended. A family background and identification of a pathogenic mutation has been demonstrated in up to 50%, while in the remaining probands, an underlying familial disease with incomplete penetrance cannot be excluded. The most common mutations are found in PKP2 (80% of mutations in the Dutch and Northern American cohorts) and DSP (39% in the Italian cohort)[80], followed by DSG2[8,47,62,136,137]. It should be kept in mind that molecular genetic testing may only support a clinical diagnosis or suspicion. A negative test does not rule out AVC, because other causal genetic mutations and unknown environmental factors may also cause the disease[47,138]. Pathogenic mutations do not make a diagnosis of AVC itself, as multiple sources of diagnostic information such as ECG changes, ventricular arrhythmias and ventricular abnormalities have to be considered[56]. Yet, the identification of pathogenic mutations may be useful in the differential diagnosis of AVC and phenocopies, such as myocarditis, idiopathic RVOT tachycardia, DCM, muscular dystrophies, IMF or sarcoidosis[35]. Cascade genetic screening of relatives may offer another strategy to serial non-invasive cardiovascular evaluation of family members. Current guidelines[1,87] do not recommend genetic testing for risk stratification and therapeutic decision making in AVC because study results regarding the ability of genotyping to detect malignant mutations associated with an increased susceptibility to potentially lethal arrhythmias have been conflicting[8,62,64,90,139]. Recent large scale studies[8,62] indicate an association between positive mutation carrier status and early disease onset. Thus, genotyping of younger family members should strongly be encouraged. This might be particularly important for patients carrying digenic or compound heterozygote mutations that are reported in up to 18% of the AVC population studied and have been associated with a stronger phenotype[1]. Issues such as the availability of genetic counselling in a multidisciplinary setting[140], low-probability mutations[136], genetic testing for "low-probability" AVC, psychological repercussions of young patients, and costs need to be considered before performing genetic screening[75].

DIFFERENTIAL DIAGNOSIS

Idiopathic RVOT-VT is a major non-hereditary differential diagnosis that has to be distinguished from ARVC/D. This is often demanding, particularly in the early stages of AVC[141]. RVOT-VT is not associated with structural heart disease and thus has a more benign course. Its etiology is unclear, although in one study a somatic point mutation in the inhibitory G protein Gai2 was identified by EMB from the arrhythmic focus[142]. In RVOT-VT, 12-lead surface ECG and SAECG are normal during sinus rhythm. It is characterized by repetitive monomorphic VT of a single morphology with LBBB morphology and an inferior axis. Similar VT morphologies can be found in patients with ARVC/D. 12-lead ECG scoring systems to differentiate both types of VT have recently been proposed[108]. In ARVC/D the duration of the QRS complex during VT is usually longer (≥ 120 ms in lead I)[143]. Notching of the QRS and precordial transition in lead V6 may exclusively be seen in ARVC/D[144]. RVOT-VT is difficult to induce by programmed ventricular stimulation during EPS, particularly in the absence of isoproterenol[87]. It responds well to beta-blockers or verapamil and ablation after successful mapping is usually curative. EAM demonstrates normal voltages. CPVT is caused by mutations in the *RyR2* gene, which has also been described in ARVC/D subtype 2. CPVT is characterized by effort-induced polymorphic VT in patients with structurally normal hearts. Genetic analysis, a positive family history, EAM and EMB can help to differentiate AVC and regional myocarditis[14]. Myocardial involvement in sarcoidosis can mimic ARVC/D and the

current TFC do not reliably distinguish between them. In a prospective study of patients with suspected ARVC/D, evaluated by a protocol including EMB, a surprisingly high incidence (15%) of cardiac sarcoidosis was verified[145]. Sarcoidosis with cardiac involvement thus always needs to be considered, particularly if respiratory and systemic symptoms, high-grade atrioventricular conduction block, and no family disease are present. Similar clinical presentations and imaging findings can pose a challenge in the absence of histological diagnosis. Features favoring cardiac sarcoidosis include early septal involvement, reduced LV function, a wide QRS during VT, right-sided apical VT and more inducible forms of monomorphic VT[146]. Diagnosis is usually confirmed by EMB[147]. In patients who survive SCD, ischemic heart disease and an anomalous origin of the coronary arteries have to be excluded. DCM is particularly difficult to distinguish from non-classic forms of AVC. Palpitations, (pre)syncope and ventricular arrhythmias are present at an early stage in AVC, often in the absence of gross structural abnormalities, which is usually not the case in DCM. Subepicardial LGE on CMR, particularly in the posterobasal LV wall, also favors AVC[36]. Atrioventricular conduction block is more common in DCM, but mutations in lamin A/C can cause AVC with conduction defects[148]. Bs may mimic ARVC as RV conduction delay has been demonstrated in both and recently a genetic overlap between these two entities has been proposed[94,149]. The presence of gross structural abnormalities favors AVC and mutations in SCN5A are very rare in AVC. Further differential diagnoses include RV infarction, pulmonary hypertension, congenital left-to-right shunts, Chagas disease and Uhl's disease (congenital hypoplastic RV).

DISEASE COURSE AND PROGNOSIS

Although AVC is a progressive disease, the individual disease course can vary considerably. The mortality rate is currently estimated to be around 1%-3% per year. In one study, after 8 years of mean follow-up, total mortality was approximately 20% and the mean age at death 54 ± 19 years. Most patients died of progressive heart failure (59%) and VTA (29%)[150]. Embolic stroke may lead to death in a smaller proportion of patients.

AVC occurs in four phases[2]: (1) concealed phase, during which patients are asymptomatic and structural abnormalities are absent or subtle. Nevertheless, AVC can present with SCD as the primary manifestation; (2) occurrence of symptomatic arrhythmias; (3) early heart failure symptoms; and (4) end-stage heart failure necessitating a ventricular assist device or cardiac transplantation. One study has shown that 7% of AVC patients received cardiac transplantation after a mean follow-up period of 10 years and severe LV involvement is often present in this population[7]. Strenuous physical activity often leads to early disease manifestation and rapid disease progression. Young competitive athletes with AVC have a 5-fold increased risk of SCD compared to non-athletes and identification of affected athletes by pre-participation screening has substantially reduced mortality in this cohort[64,151]. Interestingly, in one study, mutation-carrying female relatives were less frequently affected than male relatives. This has been interpreted as prevention of apoptosis in cardiac myocytes by estradiol but could also be related to more life-long physical activity in men[152].

RISK STRATIFICATION

SCD in patients with AVC is difficult to predict and often occurs without alarming symptoms. The only reliable strategy for SCD prevention is the implantation of an ICD, with an annual incidence of appropriate ICD interventions among AVC patients of 5%-22%, demonstrating its importance for these patients. Thus, in secondary prevention after aborted SCD, VF or sustained VT, ICD implantation is recommended[87,147]. Besides aborted SCD, VF and sustained VT, other potential risk factors for SCD or appropriate ICD therapy (a surrogate marker for SCD) have been suggested: (1) syncope (DARVIN 2 study)[93]; (2) left ventricular dysfunction[7,56,153]; (3) young age at presentation[62,63,67] and young age per se[47,64]; (4) RV structural abnormalities fulfilling 2010 TFC[47,154]; (5) severe tricuspid regurgitation[7]; (6) particular genetic variants[8,72]; (7) presence of non-sustained VT[155]; (8) male gender[79]; (9) proband status[79]; (10) frequent PVC[79]; and (11) presence of precordial T-wave inversions[79].

It is important to recognize that the use of appropriate ICD therapy due to sustained VT or VF as a surrogate for SCD can result in an overestimation of this endpoint. Whether in the absence of arrhythmic syncope or significant ventricular arrhythmias the other potential risk factors are consistently related to an adverse arrhythmic outcome and require prophylactic ICD therapy remains to be determined by future studies. Of note, young patients may suffer from neurocardiogenic syncope, making differential diagnosis difficult and its prognostic value elusive. T-waves in the precordial and inferior leads often become negative with progression of AVC and a greater extent of precordial negative T-waves are associated with more severe RV dilation and dysfunction[100]. Recently, the Johns Hopkins group found that 88% of patients with documented sustained VTA exhibited an abnormal ECG. A total of 122 (84%) subjects demonstrated T-wave inversions in the precordial leads with 97 of them extending to lead V3 and beyond, while depolarization abnormalities such as epsilon waves were present only in a minority of patients[156]. The same group found that the presence of T-wave inversions in ≥ 3 precordial ECG leads was an independent predictor of adverse events during follow-up[79]. An Italian group has also demonstrated a link between the extent of negative T-waves and ventricular arrhythmic events during follow-up[157]. Although a class Ⅱb recommendation, the role of EPS with programmed ventricular stimulation for risk stratification in AVC is less well established and conflicting data about its prognostic significance exist[45,64,90,158]. Differ-

ences in the studied patient population may be influenced by disease severity[159] and differences in study design may have led to discrepant results. A positive family history of SCD in asymptomatic patients does not seem to increase their individual risk for lethal arrhythmias. Guidelines do not support genetic testing for risk stratification in AVC[1] and genotype-phenotype correlation studies so far have not consistently been able to show that genotyping is able to detect mutations specifically associated with an increased susceptibility to life-threatening arrhythmic events. However, recent data indicates that certain pathogenic mutations (*e.g.*, plakoglobin in Naxos disease, RyR2 and TME-43) may increase the risk for SCD[8,62,67]. These preliminary results have to be confirmed in larger studies and more precise risk stratification tools for asymptomatic patients are needed. Novel imaging modalities such as strain and three-dimensional echocardiography could help to further improve risk stratification[160].

Based on the available data from observational studies, we suggest classifying patients into three risk categories[79,161]: (1) high risk: aborted SCD, sustained VT and VF, arrhythmic syncope; (2) moderate risk: non-sustained VT, severe structural abnormalities of RV and/or LV, presence of cardiac symptoms, $\geqslant$ 3 leads with T-wave inversions, frequent PVCs (*i.e.*, > 760 PVC/24 h Holter) and severe disease onset age < 35 years; and (3) low risk: asymptomatic family members (also despite a positive family history of SCD), < 10 PVC/24 h Holter.

The risk factors listed here have focused largely on patients with right-dominant disease. Prognostic factors in non-classic disease still remain elusive. Patients should be astute for symptoms. Dynamic T-wave inversions, ST segment elevation and myocardial biomarker release mimicking myocardial infarction should alert the treating physicians to think of a "hot phase" of AVC. Clinical evaluation starting at age 10-12 is suggested for all first- and second-degree relatives of AVC index patients until age 60[140]. If SCD occurs at age < 35, a full postmortem autopsy by an expert cardiac pathologist including molecular autopsy screening for genetic variants should be performed.

THERAPY

Physical activity restriction

It is a general consensus that strenuous physical activity should be avoided in symptomatic patients with AVC. There is no consensus that physical activity should be avoided in asymptomatic healthy gene carriers. A recent study has shown that endurance exercise and frequent exercise increase the risk of VT/VF and heart failure in patients, but also in healthy family members carrying a pathogenic desmosomal mutation, supporting exercise restriction for these patients[82]. We prudently advise all symptomatic patients and healthy gene carriers to refrain from practicing competitive sports and strenuous physical exercise, not only for reducing the risk of ventricular arrhythmias, but also to prevent disease onset and progression.

Pharmacological therapy

Beta-blockers, amiodarone and sotalol can be effective for treatment of sustained VT or VF in patients with AVC. However, they have no proven prognostic benefit such as ICD therapy. Wichter *et al*[107] proved that sotalol is highly effective to suppress VT by programmed ventricular stimulation with an efficacy of 68% and 83%, respectively, but had no effects on prognosis and SCD. Amiodarone was not superior to sotalol in this study and is not considered first-line therapy by many clinicians because of frequent side effects during long term therapy, particularly in young patients. However, recent data from the Northern American ARVC registry demonstrated amiodarone to confer the greatest efficacy in preventing ventricular arrhythmias when compared to sotalol or beta-blockers. However, mean sotalol doses were lower than in the study from Wichter *et al*[107] and only ten patients were treated with amiodarone in the American study. In clinical practice, beta-blockers, sotalol or amiodarone are often used as an adjunctive therapy to reduce arrhythmia burden in patient with an ICD and amiodarone is sometimes combined with beta-blockers in order to reduce sympathetic tone and mechanical wall stress[162]. Co-administration of sotalol and amiodarone is not recommended due to QT interval prolongation. Hiroi *et al*[163] suggest that carvedilol may control arrhythmias and improve LV function in some patients with biventricular AVC. Calcium antagonists such as verapamil and mexiletin may be effective in some patients to suppress VT but data is anecdotal. If heart failure occurs, standard therapy with beta-blockers, angiotensin converting enzyme-inhibitors and a diuretic should be established, although there are no specific studies in patients with AVC[46]. Brain natriuretic peptide, C-reactive protein, IL-1β and TNF-α as surrogate biomarkers for disease activity, inflammation and prognosis have been advocated in AVC but await further validation[3,58,164]. AVC patients at later stages have an increased risk for thromboembolism[43]. The annual incidence of thromboembolic complications, including pulmonary embolism, RVOT thrombosis and cerebrovascular events, was 0.5% in a retrospective study of 126 patients followed up for a mean period of 99 ± 64 mo[56]. Anticoagulation is often started by clinicians in the presence of severe ventricular dilation, dysfunction and aneurysm, although existing studies do not support prophylactic use in those with RV aneurysms. Data for the non-classic subtypes are lacking.

Implantable cardioverter-defibrillator

According to the ACC/AHA/HRS 2008 Guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities and its recent update[165,166], ICD implantation is indicated in patients with structural heart disease who have experienced a sustained VTA (secondary prevention, Class I indication). It is also stated that ICD implantation is reasonable in AVC patients who have at least one

risk factor for SCD (Ⅱ A indication, level of evidence C). Thus, ICDs constitute a cornerstone for those patients and can prolong survival in this population. In fact, a large number of studies has demonstrated that patients with AVC who undergo ICD implantation have a high likelihood for appropriate ICD therapies[167]. However, many questions remain regarding AVC patients and their relatives who are at low to moderate risk for SCD. In these patients, a lifelong risk for lethal arrhythmias has to be weighed against the complication rates of ICDs, inadequate interventions (up to 24% within 5 years), psychological burden and economic costs of this therapy. However, complication rates seem to have declined since the use of third- or fourth-generation defibrillators. Active and young patients are at particular risk of lead displacement and inappropriate discharges for sinus tachycardia, including painful shocks and multiple invasive procedures. Thus, indiscriminate device implantation cannot be endorsed. Instead, reliable risk stratification is of paramount importance. An ICD with dual chamber detection algorithms may be wise in young patients to discriminate VT or SVT from sinus tachycardia. The use of antiarrhythmic agents can also reduce the number of inadequate interventions due to supraventricular tachyarrhythmias. Furthermore, programming of higher VT/VF cut-offs and longer detection intervals can avoid inappropriate ICD shocks[168]. Complications of ICD therapy include a risk for perforation caused by thinning of the RV wall, lead dislodgement, R wave under-sensing and high pacing thresholds. As patients are young and mobile, these risks need particular consideration, although in one study, short and long-term risks of ICD therapy were similar to patients without AVC[45].

In our clinical routine, we recommend ICD implantation for all AVC patients who have experienced a sustained VTA but we also carefully evaluate ICD implantation for primary prevention in probands and family members without documented sustained VTA. Therefore, we evaluate whether a particular patient (1) has high-risk features for SCD during follow-up (see list above), (2) whether the patient is willing to take his medication regularly and to stop competitive sports (*i.e.,* competitive individual events like triathlon or participation in a competitive sports team), and (3) the patient's preferences. Our threshold for ICD implantation is higher in family members and asymptomatic patients owing to the fact that previous studies have consistently shown that family members are at lower risk of experiencing sustained VTA. A possible explanation for this finding is that diagnosis occurs earlier in the disease course and once diagnosed, family members are encouraged to give up competitive sports. However, more data obtained from different well characterized AVC cohorts are necessary to assist clinicians in guiding ICD therapy.

Catheter ablation

Catheter ablation was first applied to treat drug-resistant VT. The application of direct current (DC) termed fulguration, used DC from a defibrillator to burn myocardial sites responsible for abnormal ventricular activation. The electric voltage was directly delivered through a catheter to the origins of VT. However, this procedure was associated with a significant risk of complications and thus rapidly abandoned. Currently accepted indications for radiofrequency catheter ablation in patients with AVC include drug-refractory VT or incessant VT with frequent ICD shocks. It should be kept in mind that, unlike in patients with idiopathic VT where catheter ablation is curative, catheter ablation in patients with AVC can only improve quality of life by decreasing the number of VT episodes and PVCs[169]. Catheter ablation can follow a trial of beta-blocker therapy and antiarrhythmic therapy. In some patients who do not wish long-term therapy with beta-blockers, sotalol and particularly amiodarone, catheter ablation can be performed as first line therapy. Elimination of clinical tachycardia can relieve symptoms but may not prevent SCD.

Over the last years, mapping and ablation techniques have made outstanding progress and nowadays include activation, pace and entrainment mapping during VT and substrate-based ablation using EAM that can be performed *via* an endocardial and epicardial approach[170]. Substrate-based ablation of PVCs and VT is particularly important when conventional mapping during tachycardia is not possible due to hemodynamic instability or multiple VT morphologies[171]. Although the initial approach involved extensive mapping to identify critical zones of slow conduction during VT, this approach has recently been replaced by a substrate-based approach. Preliminary studies have shown promising results regarding safety, arrhythmia-free survival and reduction of ICD discharges, particularly if an endocardial and epicardial approach are combined[128-131]. In one recent study from the Johns Hopkins cohort, the overall freedom from VT was 47%, 21% and 15% at 1, 5 and 10 years, respectively. Following epicardial VT ablation, the cumulative freedom from VT was 64% and 45% at 1 and 5 years. Of note, the VT burden decreased from a median of 0.16 VT episodes per month pre ablation to 0.08 episodes per month post ablation[172]. Mid-term and long-term success and safety of these methods have to be demonstrated in future studies with larger cohorts.

Surgical methods

Total surgical electrical RV disconnection carries an important risk of postoperative RV failure and has been practically abandoned[173]. If severe therapy refractory heart failure occurs, ventricular assist devices or heart transplantation have to be considered for isolated LV or biventricular failure and less frequently isolated RV failure. Some authors suggest that right heart catheterization should be performed in all cases with suspected severe RV dysfunction. If increased filling pressures suggest a Fontan-type physiology, the patient may be considered for heart transplantation[174].

CONCLUSION

During the last three decades, our understanding of AVC from a developmental RV dysplasia with substitution by adipose tissue has remarkably changed to a mostly inherited polygenic disease of the intercalated disk with a broad phenotypic spectrum. Although AVC predominantly affects the RV, non-classic forms affecting the LV or both ventricles are increasingly recognized. A hallmark is the early propensity to ventricular arrhythmias associated with SCD at a young age. Enormous progress in unravelling the genetic and molecular basis of this complex disease, in which environmental factors seem to play a pivotal role, has been made in the last years. While progress in imaging and device therapy has facilitated clinical diagnosis and prevention of SCD, today's challenges include discovery of novel genetic and environmental factors, early detection of asymptomatic patients, improved risk stratification, catheter ablation strategies and causal therapies to cure the disease[175]. Multicenter, large, prospective follow-up studies are planned to improve our understanding of the complex underlying molecular mechanisms of AVC, which may facilitate diagnosis, risk stratification and causal therapy.

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MINIREVIEWS

High-sensitivity cardiac troponins in everyday clinical practice

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Abstract

High-sensitivity cardiac troponin (hs-cTn) assays are increasingly being used in many countries worldwide, however, a generally accepted definition of high-sensitivity is still pending. These assays enable cTn measurement with a high degree of analytical sensitivity with a low analytical imprecision at the low measuring range of cTn assays (coefficient of variation of < 10% at the 99th percentile upper reference limit). One of the most important advantages of these new assays is that they allow novel, more rapid approaches to rule in or rule out acute coronary syndromes (ACSs) than with previous cTn assay generations which are still more commonly used in practice worldwide. hs-cTn is also more sensitive for the detection of myocardial damage unrelated to acute myocardial ischemia. Therefore, the increase in early diagnostic sensitivity of hs-cTn assays for ACS comes at the cost of a reduced ACS specificity, because more patients with other causes of acute or chronic myocardial injury without overt myocardial ischemia are detected than with previous cTn assays. As hs-cTn assays are increasingly being adopted in clinical practice and more hs-cTn assays are being developed, this review attempts to synthesize the available clinical data to make recommendations for their everyday clinical routine use.

Key words: Cardiac troponin; High-sensitivity; Diagnosis; Acute myocardial infarction; Acute coronary syndrome; Review

Core tip: High-sensitivity cardiac troponin (hs-cTn) assays enable cardiac troponin measurement with a high degree of analytical sensitivity with a low analytical imprecision at the low measuring range. One of the most important advantages of these new assays is that they allow novel, more rapid approaches to rule in or rule out acute coronary syndromes (ACSs). The increase in early diagnostic sensitivity of hs-cTn assays for ACS comes at the cost of a reduced ACS specificity, because more patients with other causes of acute or chronic myocardial injury without overt myocardial ischemia are detected than with previous cTn assays.

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INTRODUCTION

Cardiac troponin I (cTnI) and cTnT are the biomarkers of choice for the diagnosis of myocardial damage, because they are the most sensitive and cardiac-specific biomarkers currently available[1,2]. Recommendations for the use of cTn measurement in acute cardiac care[1] and practical clinical considerations in the interpretation of cTn elevations[2] have been published recently. Over the years the analytical sensitivity of cTn assays has been continuously improved, and more recently a new generation of cTn assays, *i.e.*, the high-sensitivity (hs)-cTn assays, have been introduced into routine clinical practice[3]. It is important to note, that these assays measure the same analyte as previous assay generations but with

substantially improved analytical sensitivity and assay precision at the low measuring range[3-6]. It is also important to note because of discrepancies in routine use[7,8], that, regardless of how assays are named by manufacturers, hs-cTn assays should be only designated as hs-cTn assays, if the below listed analytical characteristics are met by an assay also in routine use together with publication of its hs analytical characteristics in peer-reviewed literature[7,8].

From a clinical perspective it has been noted that the improved analytical performance of hs-cTn assays also increased their clinical ability to detect small amounts of myocardial damage and to precisely identify small differences in cTn concentrations in serial testing compared with previous cTn assay generations[8]. It is expected that hs-cTn assays, if used appropriately, will improve both early diagnosis and short and long-term risk stratification. In this review recommendations for the clinical interpretation of hs-cTn test results are proposed based on the currently available clinical evidence, and it is also indicated where sufficient clinical data are still lacking.

ANALYTICAL CHARACTERISTICS OF HS-CTN ASSAYS

The analytical characteristics of hs-cTn assays are summarized in Table 1. The analytical lower limit of detection (LoD) is in the range of single digits of ng/L or even below[7-11]. Therefore, it is recommended that hs-cTn assay results are reported as ng/L (= pg/mL), and cTn values below the LoD should not be reported as numbers[8]. hs-cTn assays must have high precision in routine use at lower concentration ranges with total analytical coefficient of variation (CV) < 10% at the 99th percentile concentration of the reference population, which is the recommended upper reference limit (URL). Despite increased analytical sensitivity hs-cTn assay must maintain analytical specificity for the detection of cardiac troponin isoforms. There have not been reports of major analytical interferences with hs-cTn assays, but they are possible and thorough evaluations of possible analytical interferences is needed before approval for routine use[7,8]. In contrast to conventional cTn assays, hs-cTn assays permit measurement of cTn concentrations in a significant proportion of apparently pathology-free individuals, which favours a precise calculation of the URL[1,7]. There is still no consensus on a specific percentage of detectable cTn concentrations in the reference population which is required for the label hs as long as all the other criteria are fulfilled, but usually > 50% are recommended[7]. There are reports on sex-specific URLs which are higher for men than women for hs-cTn assays including the already commercially available hs-cTnT and hs-cTnI assays from Roche and Abbott Diagnostics[3,5,7-11], and it may turn out that sex-specific URLs should be used in routine as well. The underlying mechanisms for cTn release from normal hearts are still uncertain and remain to be established. Since analytical interferences can be ruled out[3,5,10], a constant limited turnover of cardiomyocytes appears to be present in normal hearts as well.

Table 1 Analytical characteristics of high-sensitivity cardiac troponin assays

The analytical lower limit of detection is in the range of single digits of ng/L and is markedly lower than the upper reference limit
Hs-cTn assays have high precision in routine use at lower concentration ranges with analytical CV < 10% at the 99th percentile concentration of the reference population
Hs-cTn assays enable detection of cTn in a significant proportion of the reference population, thereby allowing for a more accurate calculation of the 99th percentile URL with its 95% confidence interval
Hs-cTn assays must be highly specific for the detection of cardiac cTn isoforms

Hs-cTn: High-sensitivity cardiac troponin; CV: Coefficient of variation; URL: Upper reference limit.

EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

Hs-cTn assays detect cTn release at an earlier time point than the previous generations of cTn assays leading to an improved early sensitivity for acute myocardial infarction (AMI) diagnosis within 3 h of presentation[12-15]. Most but not all studies demonstrated a higher diagnostic accuracy of hs-cTn assays for early AMI diagnosis when compared to previous cTn assay generations on admission to the emergency department[16]. However, scrutiny is needed when evaluating studies on this topic as differences between assays often have been overstated by use of different medical decision limits for the older and newer cTn assays, *e.g.,* 10% CV concentration limit *vs* 99th percentile URL. This leads to apparent higher specificity and lesser sensitivity with non hs-cTn assays and magnifies the differences in early sensitivities at patient presentation observed with the hs-cTn assays[16]. However, guidelines recommend the use of the URL as a medical decision limit even when it cannot be measured with a CV of < 10%[17]. Thus early sensitivities must be compared by using the 99th percentile URL as a medical decision limit for standard and hs-cTn assays. In addition, some patients may not have AMI diagnosed because their standard cTn values do not increase above the cut-off value but do so with the hs-cTn assay. Thus, a significant number of patients with unstable angina may migrate from that designation to the AMI category if reclassified using the hs-cTn test results. Studies of the diagnostic performance of hs-cTn assays in more heterogeneous populations are also still needed because most present studies have been done in pre-selected emergency department populations presenting with cardiac symptoms or chest pain unit populations. Study design influences the sensitivity and the specificity of cTn, the optimal blood sampling regimens, and optimal decision limits for absolute or relative changes in serial testing. Statistical analyses are also heterogeneous. Most studies determine optimal decision limits according to receiver operating characteristic curve analysis which weighs sensitivity and specificity equally, while others have optimized cut-off values for specificity. The selection of criteria for change limits for AMI diagnosis will also differ depending on whether there is

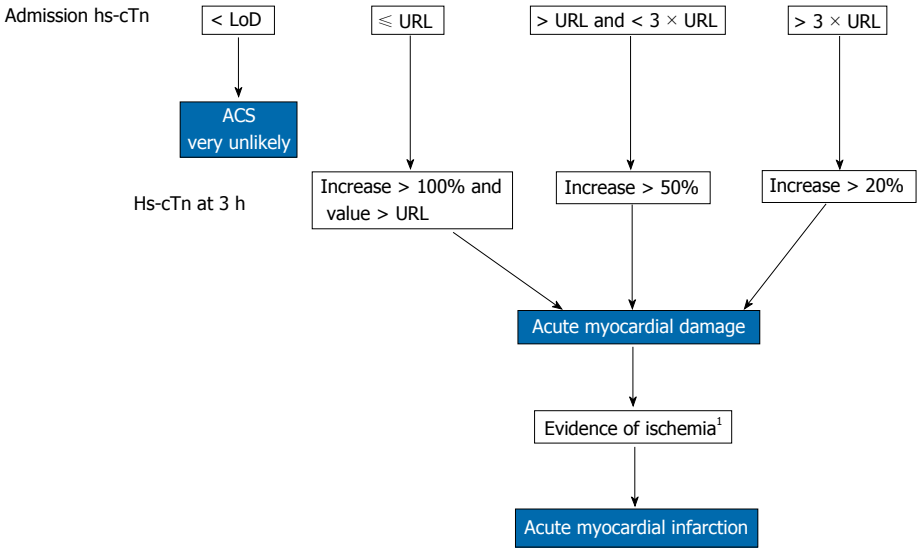

Figure 1 Algorithm for the rapid evaluation of clinically suspected acute myocardial infarction with high-sensitivity cardiac troponin testing. This algorithm is based on best current knowledge and may have to be modified with upcoming new data. This approach at least guarantees that the changes will be above the analytical and biological variation. It is important to note that hs-cTn changes over a 3 h period in patients presenting late after AMI onset may be less than 20%. For hs-cTnT some studies favour absolute changes over relative concentration changes. [1]Evidence of acute myocardial ischemia by new ECG changes and/or new imaging corroborations. Hs-cTn: High-sensitivity cardiac troponin; URL: 99th percentile upper reference limit of healthy controls; ACS: Acute coronary syndrome; LoD: Lower limit of detection; AMI: Acute myocardial infarction; ECG: Electrocardiogram.

a need for high specificity at the cost of lower sensitivity or increased sensitivity at the cost of lower specificity. Clinicians must be aware of this trade off in evaluating individual patients. For all these reasons, the pooling of study data from the literature is currently problematic.

Clinically relevant hs-cTn assay concentration changes in serial testing

Key to the use of hs-cTn assays is the need to evaluate cTn kinetics with serial testing in the clinical evaluation of chest pain patients[18,19]. At least two measurements of hs-cTn test results to verify a kinetic pattern are required to comply with the Universal Definition of Myocardial Infarction[20]. Even in patients with increased hs-cTn values a significant change must be documented by serial measurements.

In general, most AMI patients have substantial and obvious changes in hs-cTn values. It must be emphasized that dynamic changes are not specific for AMI but are rather indicative of acute myocardial damage. An algorithm for the use of hs-cTn serial measurements for the evaluation of AMI in patients presenting with symptoms suggestive for an acute coronary syndrome (ACS) based on the currently available clinical data is shown in Figure 1. Previous recommendations on change criteria just considered analytical variation and advocated based on a total CV < 10% any change in serial testing of > 20% to be significant[21]. The precision necessary to implement this approach is not present within the reference range for hs-cTn assays either[11]. In addition, biological variation needs to be considered. Changes of hs-cTn measurements near the 99th percentile URL must exceed conjoint analytical and biological variation to be of clinical significance. This is done by calculation of the so-called reference change values (RCV). Such values can be calculated only for reference individuals, but the theory of biological variation postulates the same process in patients with disease. These calculated RCV values are assay and analyte specific and must be obtained separately for each commercially available hs-cTn assay. For many assays, short-term RCVs are in the 40%-60% range[22-24], although one report has values as high as 86%[25]. Data on short- and long-term variation of hs-cTn concentrations in clinically stable patients with chronic cardiac diseases are very limited[26], but the reported variation is in the range of healthy individuals. A recently published study evaluating serial changes using a pre-marketing version of the Abbott® hs-cTnI assay in pre-selected chest pain unit patients, suggested that increases above the 99th percentile URL with relative increases of > 250% over a 3 h period in patients with baseline values < URL and increases > 50% with modestly increased baseline values optimize specificity for the diagnosis of AMI[15]. However, AMI diagnosis in this study was based on clinical criteria and an increase in a conventional local cTnI assay > 99th percentile URL with a > 20% change over a 6 h period. As expected, higher cTnI sensitivities were found at lower percentage changes.

Whether the diagnostic performances of percentage change differ from an absolute change of cTn concentrations, has been tested with the hs-cTnT assay in recent clinical studies[27,28]. It has been described at hs-cTnT values below or close to the 99th percentile URL that an absolute increase of hs-cTnT values (*e.g.*, > 7 ng/L over 2 h) is superior to a relative percentage changes from baseline. Other hs-cTn assays may require different metrics, because data on absolute changes in serial testing are assay specific. Undetectable hs-cTn ruled out ACS with a

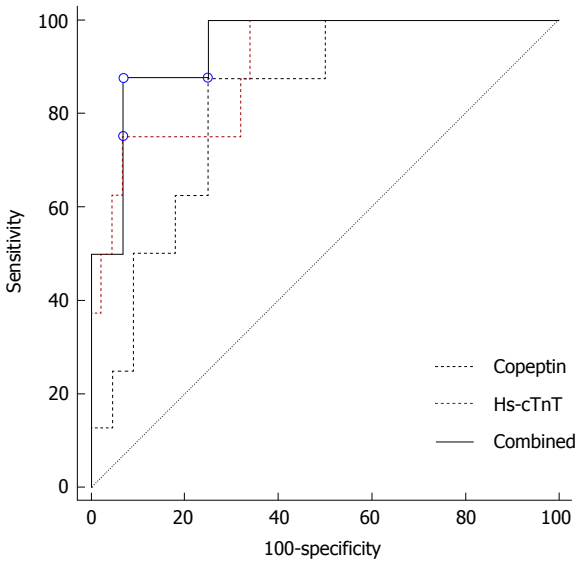

Figure 2 Diagnostic performances of high-sensitivity troponin T and copeptin for the diagnosis of acute myocardial infarction in chest pain patients. Own unpublished results, the area under receiver operator characteristic curves of the combination of copeptin with hs-cTnT (0.94) was not significantly different from the area under hs-cTnT curve (0.90). The worthless test is indicated as reference line. Hs-cTnT: High-sensitivity cardiac troponin T.

negative predictive value > 99% on ED admission[15,16].

Timing of hs-cTn measurements in serial testing

According to the recent European guideline for the management of ACS, blood samples should be obtained at the time of presentation and 3 h after admission when using hs-cTn assays[19]. There is recent evidence suggesting that many patients with an AMI can be reliably identified within 3 h after admission with close to 100% sensitivity and negative predictive value using a hs-cTn assay, which indicates that observation time in the emergency department may be reduced for the rule out of AMI[12-15]. However, most of these studies based the diagnosis of AMI on the prior less sensitive cTn assays and ignored AMI only detected with hs-cTn assays. Thus, if the clinical situation is ambiguous and the pre-test likelihood of disease is high, additional subsequent sampling (*e.g.,* at 6 h and even beyond) is still necessary in individual patients.

Myocardial infarction after percutaneous coronary interventions or aortocoronary bypass grafting

There are still no data on hs-cTn decision limits in these clinical settings. In acute percutaneous coronary interventions (PCI) or nowadays rarely performed acute coronary artery bypass grafting (CABG) for evolving AMI acute myocardial damage is caused by AMI itself and the potential additional myocardial damage caused by PCI or CABG cannot be differentiated from cTn release caused by ongoing AMI. In elective PCI or CABG, by contrast, baseline cTn values are usually within the normal range and potential myocardial damage caused by these interventions can be reliably detected by hs-cTn measurements. However, in these elective patients hs-cTn decision limits for periprocedural AMI are also still not available. Thus, only the limits recommended by the universal definition of AMI can be currently used[20], *i.e.,* increase > 5-times URL after PCI and > 10-times URL after CABG. However, these limits are still very controversially discussed in the communities of interventionists and cardiac surgeons, because it appears from the available data that periprocedural cTn increases in clinically uncomplicated patients must be substantially higher to be of prognostic significance[29].

DO WE NEED ADDITIONAL BIOMARKERS FOR AMI DIAGNOSIS WHEN HS-CTN ASSAYS ARE USED?

The most recently advertised markers for the early diagnosis of AMI are heart-type fatty acid binding protein (H-FABP) and copeptin. However, in the vast majority of studies these markers were compared only with previous, less sensitive cTn assays and comparative data with hs-cTn assays are still limited.

H-FABP

Despite its name this protein is not a cardiac-specific marker as it is also expressed, although in much lower amounts, in several other tissues. It is cleared by the kidneys and thereby increased in case of renal failure[30]. H-FABP increases rapidly in ACS[31], but more recent data do not support a benefit when combined with hs-cTn[15,32].

Copeptin

Copeptin is the 39 amino acids long c-terminal part of pro-arginine-vasopressin and a stable surrogate marker of vasopressin secretion[33]. It is a marker of stress[33] and has been proposed for early AMI diagnosis on emergency department admission[34]. More recent data do not support a benefit when combined with hs-cTn (Figure 2)[15,32].

In summary, when hs-cTn assays are used instead of standard cTn assays both H-FABP and copeptin do not add to the early diagnosis of AMI, particularly, if the LoD is used as an AMI rule-out limit for hs-cTn in chest pain patients. However, in case of point-of-care testing where the criteria for hs are very difficult to be fulfilled for cTn assays a combination with these markers may be useful.

DISEASES WITH POTENTIAL HS-CTN ELEVATIONS OTHER THAN AMI

Given the high frequency of detectable and slightly elevated hs-cTn values in the community[35-37], especially in patients with cardiovascular comorbidities, it is important to note that an increased hs-cTn concentration alone is not sufficient to make the diagnosis of AMI[20]. hs-cTn increases must, therefore, be interpreted in relation to the clinical presentation (Table 2). Thus, a recent publication suggested that it may be advisable to use a higher cut-point (about 3-fold the 99th percentile URL) as a decision

Table 2 Elevations of high-sensitivity cardiac troponin in the absence of significant coronary artery disease

Acute myocardial damage related to secondary myocardial ischemia (AMI type 2)	Tachycardia or bradycardia (*e.g.*, rapid pacing during transcutaneous aortic valve replacement)
	Aortic dissection with involvement of coronary ostia
	Severe aortic valve stenosis
	Hypertrophic cardiomyopathy
	Hypo- or hyper-tension (*e.g.*, hemorrhagic shock, hypertensive emergency)
	Acute heart failure without significant concomitant CAD
	Severe pulmonary embolism or pulmonary hypertension
	Coronary vasculitis, *e.g.*, systemic lupus erythematosus
	Coronary endothelial dysfunction (spasm) without significant CAD, *e.g.*, cocaine abuse
	Coronary embolism
Acute myocardial damage not related to myocardial ischemia	Cardiac contusion
	Cardiac incisions with surgery
	Radiofrequency or cryoablation therapy for arrhythmias
	Rhabdomyolysis with cardiac involvement
	Myocarditis
	Cardiotoxic agents, *e.g.*, anthracyclines, CO poisoning, severe burns affecting > 30% of body surface
Indeterminate or multiform group	Apical ballooning syndrome
	Renal failure
	Severe acute neurological diseases, *e.g.*, stroke, trauma
	Infiltrative diseases, *e.g.*, amyloidosis, sarcoidosis
	Extreme exertion
	Sepsis
	Acute respiratory failure
	Frequent defibrillator shocks
Analytical interferences	Rare, *e.g.*, by high titres of auto- or hetero-philic antibodies

AMI: Acute myocardial infarction; CO: Carbone monoxide; CAD: Coronary artery disease.

limit for AMI in > 70 year-old patients[38]. However, it is likely that most of elevations in the elderly are caused by comorbidities. Thus, the use of higher cut-off values decreases early sensitivity for AMI in older patients without comorbidities. Regardless of the cut-off value used, the critical distinction that remains to be made is to determine whether there is a significant rising pattern of hs-cTn values in serial testing as an indicator of acute myocardial damage. Thus, clinical judgement still remains essential.

With hs-cTn assays, elevations above the 99th percentile URL are common in patients with structural heart disease (Table 2), including patients with stable coronary artery disease[39-43]. In patients with putative stable angina, a hs-cTnT value > 99th percentile URL is found in 37% of those with coronary plaques that are thought to be more labile or vulnerable[39,40]. In stable heart failure patients, the median concentration for hs-cTnT is 12 ng/L, which is very close to the 99th percentile URL of 14 ng/L for this assay[42,43]. However, regardless of the cause, elevations of hs-cTn values are associated with an adverse clinical outcome in most clinical conditions, as in patients with AMI, stable CAD, heart failure, pulmonary embolism or chronic pulmonary arterial hypertension[35,37,39,41-45].

Cardiac specificity of cTnT vs cTnI

A recent report again raised concerns regarding the cardiac-specificity of the current generation cTnT assay in patients with chronic skeletal muscle disorders due to potential reexpression of cTnT isoforms or expression of an immunoreactive protein in skeletal muscle myopathies. In patients without evidence of myocardial injury increases of creatine kinase MB (CKMB) isoenzyme and cTnT without concomitant increases in cTnI were found[46]. A potential release of cTnT from skeletal muscle with normal cTnI in patients with chronic skeletal muscle damage is also highlighted by an own case in whom we measured cTnT and cTnI with hs assays (Figure 3). The most cardiac-specific marker in this rare patient population with chronic skeletal muscle damage (*e.g.*, muscular dystrophies) is cTn. Based on our experience patients with unexplained increased cTnT with normal cTnI should be also evaluated for possible, clinically still asymptomatic chronic skeletal muscular diseases.

RISK STRATIFICATION BY HS-CTN TESTING-IS THERE ADDITIONAL VALUE COMPARED WITH HIGH-SENSITIVITY C-REACTIVE PROTEIN OR NATRIURETIC PEPTIDE TESTING?

There are no studies to date evaluating hs-CRP or natriuretic peptides together with hs-cTn assays for risk stratification in non-ST-segment elevation myocardial infarctions. Patients in the community who have elevated values of hs-cTn have underlying cardiovascular disease and thus are in the long run at increased risk for ischemic events and heart failure, and hs-cTn was also described as an independent risk marker in the general population[35-37,39,43,46,47]. However, despite robust statistical predictive value, hs-cTn is similar to hs-CRP and natriuretic peptide testing in the sense that when added to traditional risk factors, it only modestly improves risk stratification and reclassification. There are still insufficient data to as-

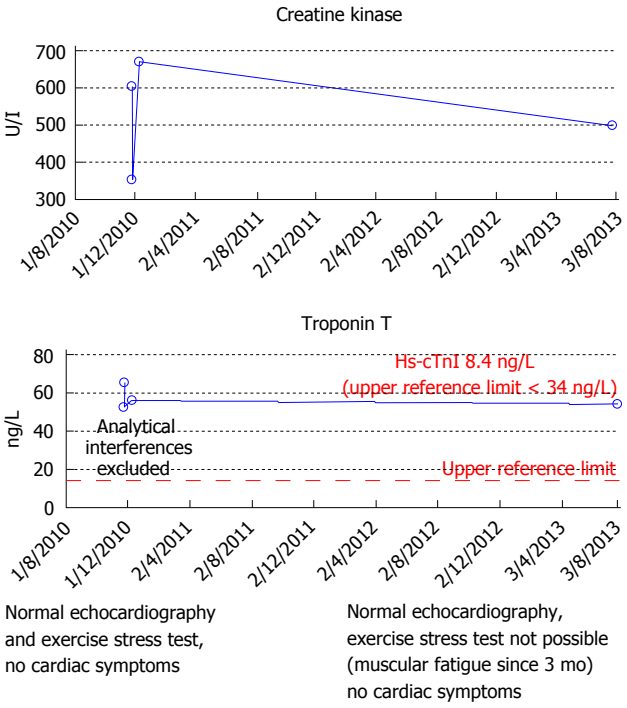

Figure 3 Creatine kinase and high-sensitivity cardiac troponin T and I in a 72-year-old male with late onset limb-girdle muscular dystrophy. This patient presented first to our outpatient clinic in 2010 because of clinically unexplained increased high-sensitivity cardiac troponin T (hs-cTnT) concentrations. The echocardiography, exercise stress test and renal function were completely normal, the patient was free of any cardiac symptoms. In 2010 analytical interferences with the hs-cTnT assay were excluded by serial dilution experiments and an interference by heterophilic antibodies could be ruled out by addition of antibody blocking agents to the sample. There was no evidence for macro creatine kinase as well. As the patient had no cardiac symptoms or symptoms suggesting skeletal muscle disease no further work-up was done. In 2013 the patient developed typically symptoms of muscular dystrophy and was again seen in our outpatient clinic. The electrocardiogram and echocardiogram remained normal, he still had no cardiac symptoms but an exercise stress test was no longer possible because of skeletal muscle fatigue. At this visit we found a marked discrepancy between hs-cTnT (moderately increased) and hs-cTnI (normal) suggesting a release of hs-cTnT from chronically injured skeletal muscle by previously already described reexpression of cardiac cTnT isoforms. In retrospect, unexplained hs-cTnT increase in this patient was an early sign of late onset muscular dystrophy.

sess which of these biomarkers is best for risk stratification or whether a multimarker panel including hs-cTn is significantly superior to single marker testing.

CONCLUSION

The 99th percentile concentration of the reference population should be used as the cTn URL and as the medical decision limit. In patients with clinically suspected AMI, the LoD of hs-cTn assays is a useful rule out decision limit with a negative predictive value > 99% even on emergency department admission. The diagnosis of acute myocardial damage requires a significant change with serial hs-cTn testing. At low cTn baseline concentrations (≤ 99th percentile URL) the change in serial testing in order to be clinically significant requires to be a marked (> 100%) increase together with an increase above the URL. In case of borderline increased baseline values (> URL and ≤ 3 times URL) only relative changes > 50% should be considered as clinical significant. In the case of markedly elevated baseline values (> 3 times URL), a minimum change > 20% in follow-up testing is required. It may turn out that for some hs-cTn assays absolute hs-cTn concentration changes perform better than relative changes. Additional testing of other early markers of acute myocardial necrosis, such as myoglobin, CKMB isoforms, or H-FABP is no longer needed. Copeptin testing adds very little as well, particularly, if the LoD is used as a ACS rule out limit on emergency department admission for the hs-cTn assays. Blood sampling in patients with suspicion of AMI should be performed on admission and 3 h later at a minimum. Measurements of hs-cTn should be repeated at 6 h after admission in patients of whom the 3 h values are unchanged but in whom the clinical suspicion of AMI is still high. According to the Universal Definition of Myocardial Infarction[20] in chest pain patients presenting after 6 h subsequent blood sampling (*e.g.*, after 12 h) is also needed to document a troponin rise or fall as a sign for acute myocardial damage. Blood sampling only at a single time point for troponin measurement is not recommended. cTn is a marker of myocardial necrosis but not a specific marker of AMI. AMI should only be diagnosed when there is a rise and/or fall of cTn together with characteristic symptoms, and/or electrocardiogram or imaging evidence of acute myocardial ischemia. Besides myocardial ischemia one should consider also other alternative causes of acute myocardial damage (*e.g.*, acute heart failure, myocarditis, pulmonary embolism) whenever an elevated hs-cTn test result is obtained. Direct myocardial trauma (*e.g.*, ablation therapy for arrhythmias, surgical incisions of the myocardium, myocardial contusion) also lead to troponin leakage from the myocardium. Stable or inconsistently variable troponin elevations without significant dynamic changes are likely markers of chronic structural heart disease, if analytical interferences (which are rare) have been ruled out.

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ORIGINAL ARTICLE

Molecular phenotypes of human parvovirus B19 in patients with myocarditis

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Abstract

AIM: To investigate molecular phenotypes of myocardial B19V-infection to determine the role of B19V in myocarditis and dilated cardiomyopathy (DCM).

METHODS: Endomyocardial biopsies (EMBs) from 498 B19V-positive patients with myocarditis and DCM were analyzed using molecular methods and functional experiments. EMBs were obtained from the University Hospitals of Greifswald and Tuebingen and additionally from 36 German cardiology centers. Control tissues were obtained at autopsy from 34 victims of accidents, crime or suicide. Identification of mononuclear cell infiltrates in EMBs was performed using immunohistological staining. Anti-B19V-IgM and anti-B19V-IgG were analyzed by enzyme-linked immunosorbent assay (ELISA). B19V viral loads were determined using in-house quantitative real-time polymerase chain reaction (PCR). For B19V-genotyping a new B19V-genotype-specific restriction fragment length polymorphism (RFLP)-PCR was established. B19V-genotyping was verified by direct DNA-sequencing and sequences were aligned using BLAST and BioEdit software. B19V P6-promoter and HHV6-U94-transactivator constructs were generated for cell culture experiments. Transfection experiments were conducted using human endothelial cells 1. Luciferase reporter assays were performed to determine B19V-replication activity. Statistical analysis and graphical representation were calculated using SPSS and Prism5 software.

RESULTS: The prevalence of B19V was significantly more likely to be associated with inflammatory cardiomyopathy (iCMP) compared to uninflamed DCM (59.6% *vs* 35.3%) ($P < 0.0001$). The detection of B19V-mRNA replication intermediates proved that replication of B19V was present. RFLP-PCR assays showed that B19V-genotype 1 (57.4%) and B19V-genotype 2 (36.7%) were the most prevalent viral genotypes. B19V-genotype 2 was observed more frequently in EMBs with iCMP (65.0%) compared to DCM (35%) ($P = 0.049$). Although there was no significant difference in gender-specific B19V-loads, women were more frequently infected with B19V-genotype 2 (44.6%) than men (36.0%) ($P = 0.0448$). Coinfection with B19V and other cardiotropic viruses was found in 19.2% of tissue

samples and was associated with higher B19V viral load compared to B19V-monoinfected tissue (P = 0.0012). The most frequent coinfecting virus was human herpes virus 6 (HHV6, 16.5%). B19V-coinfection with HHV6 showed higher B19V-loads compared to B19V-monoinfected EMBs (P = 0.0033), suggesting that HHV6 had transactivated B19V. In vitro experiments confirmed a 2.4-fold increased B19V P6-promoter activity by the HHV6 U94-transactivator.

CONCLUSION: The finding of significantly increased B19V loads in patients with histologically proven cardiac inflammation suggests a crucial role of B19V-genotypes and reactivation of B19V-infection by HHV6-coinfection in B19V-associated iCMP. Our findings suggest that B19V-infection of the human heart can be a causative event for the development of an endothelial cell-mediated inflammatory disease and that this is related to both viral load and genotype.

Key words: Myocarditis; Dilated cardiomyopathy; Parvovirus B19; B19V-genotypes; B19V co-infection

Core tip: Human parvovirus B19 (B19V) has recently been shown to be an emerging pathogen for inflammatory cardiomyopathy (iCMP). We showed that B19V replication intermediates could be detected in acute and ongoing myocarditis. B19V-genotypes 1 and 2 were predominant although B19V-genotype 2 was more prevalent in iCMP. Further analyses revealed that B19V-coinfection with other cardiotropic viruses does occur, most frequently with human herpes virus 6 (HHV6). In vitro experiments showed that the HHV6 U94-transactivator element could transactivate the B19V-P6-promoter. We suggest that long-term persistence of B19V DNA in the human heart occurs and that active/reactivated B19V-replication can be associated with iCMP in a viral load and genotype-dependent manner.

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INTRODUCTION

It has been shown that parvovirus B19 (B19V) infection of the myocardium can cause potentially lethal acute myocarditis in infants and adults[1-3]. Acute B19V-infection of endothelial cells is accompanied by the intravascular accumulation, adhesion and penetration of inflammatory cells in to vessel walls, leading to an impairment of the myocardial micro-circulation with secondary myocyte necrosis that can mimic myocardial infarction[1,2]. B19V has been found in high copy numbers in myocardial endothelial cells of small vessels but not in myocytes[1]. We recently reported that B19V-loads greater than 500 GE/μg of isolated nucleic acid identified in endomyocardial biopsies (EMBs) argue for virus-induced myocarditis. In contrast, a low viral load detected in uninflamed hearts has been associated with a latent-type of B19V-infection[4]. As expected, high viral loads of approximately 3 × 10^5 GE/μg were detected in acute myocardial B19V-infection, while approximately 700 GE/μg were found to be characteristically associated with chronic myocarditis[4]. Notably, a growing number of reports suggests an association between B19V-infection and the development of chronic myocarditis, as well as isolated endothelial and/or diastolic dysfunction[5-8]. However, the frequent detection of B19V genomes in EMBs of patients clinically suspected of having myocarditis and dilated cardiomyopathy (DCM) and the potential pathogenic role of B19V remains controversial and warrants studies to differentiate viral pathogenic effects from harmless latent B19V-infection[9,10].

Infection with human parvovirus B19 (B19V) is common, with approximately 70% to 90% of adolescents having anti-B19V IgG detectable in serum[11]. B19V-infection is usually benign and in children it most commonly manifests with erythema infectiosum (fifth disease)[12]. The genome of B19V consists of a single stranded DNA molecule of approximately 5.5 kb that contains three major open reading frames coding for the two capsid proteins VP1 and VP2 and the nonstructural protein NS1. Genome diversity divides the genus erythrovirus of the parvoviridae family into three pathogenic human genotypes: PVBAu (genotype 1), Lali-like (genotype 2) and V9-like (genotype 3) viruses[13]. The three erythrovirus genotypes show different geographical and temporal distributions. Whereas B19V-genotype 1 and 2 can be detected in most populations, genotype 3 seems to be prevalent only in Ghana, France and Brazil[13-15]. Interestingly, the age distribution of B19V-genotype 1 and 2 infections is different, with B19V-genotype 1 occurring most frequently in individuals born after 1955 while B19V-genotype 2 is predominantly found in individuals older than 50 years[16,17].

Recent reports have indicated that coinfection with different cardiotropic viruses of the human heart is common[7,8,18,19]. Human herpes virus 6 (HHV6) has been identified as an important coinfecting pathogen with B19V of the myocardium and resulting in fatal myocarditis in infants[1,20]. It has been reported that HHV6 is able to transactivate human immunodeficiency virus (HIV) and human cytomegalovirus (HCMV)[19,21].

In the present study, we explored molecular phenotypes of myocardial B19V-infection in association with patient age, gender, B19V replicative mRNA intermediates, B19V genotype and B19V-coinfection to gain fur-

ther insight into the pathogenesis of B19V-myocarditis as an endothelial-cell mediated inflammatory disease.

MATERIALS AND METHODS

Ethical approval

The study was approved by the ethics committee of the University Hospital of Tuebingen (297/2005). All patients gave written informed consent for EMB analysis to investigate a possible etiology for their disease.

Study population

This cardiopathological clinical and experimental study was designed as a retrospective evaluation of B19V-positive EMBs of 498 consecutive patients (341 male, 157 female, mean age 46.9 ± 15.85 years, ejection fraction < 45%, 2-3 biopsies/patient) with histologically proven myocarditis and DCM who were diagnosed at our institution between 2003 and 2010 (Table 1). In addition to the University Hospitals of Greifswald and Tuebingen, EMBs were obtained from 36 German cardiology centers. Control tissues were obtained at autopsy from 34 victims of accidents, crime or suicide (median age 29 years, kindly provided by Professor Dr. Wehner, Institute of Forensic Medicine, University of Tuebingen). In addition, myocardial tissue obtained from 57 unselected consecutive autopsies at our institute from patients (median age 67.2 years) dying of cardiovascular, cardiopulmonary or tumor-related diseases served as control tissue samples after exclusion of myocarditis and DCM.

Immunohistochemistry and serological testing

Immunohistological staining of paraffin-embedded tissue sections was performed using an avidin-biotin-immunoperoxidase method according to the manufacturer's protocol (Vectastain Elite ABC Kit, Vector, Burlingame, California). The following monoclonal antibodies were used for identification of mononuclear cell infiltrates: CD3 for T cells (Novocastra Laboratories, Newcastle on Tyne, United Kingdom), PGM1 (CD68) for macrophages and natural killer cells, and HLA-DR-Antigen, alpha chain (DAKO, Hamburg, Germany) to assess HLA class Ⅱ expression on professional antigen-presenting immune cells. According to the World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies, EMBs were considered to have significant inflammation if immunohistochemical staining revealed the presence of focal or diffuse mononuclear infiltrates with > 14 leukocytes per mm^2 ($CD3^+$ T lymphocytes and/or $CD68^+$ macrophages) in the myocardium, in addition to enhanced expression of HLA class Ⅱ molecules[1].

Anti-B19V-IgM and anti-B19V-IgG (VP1/VP2) were analyzed by enzyme-linked immunosorbent assay (ELISA) (Parvovirus B19-IgM; B19-IgG, DxSelect™ FocusDiagnostics, Germany) according to the manufacturer's instructions.

Table 1 Baseline characteristics of the study population *n* (%)

Characteristic	Value[1]	*n*[3]
Age, yr	46.9 ± 15.8[2]	498
Male	341 (68.5)	498
Female	157 (31.5)	498
Molecular findings		
B19V-genotype 1	286 (57.4)	498
B19V-genotype 2	183 (36.7)	498
Endomyocardial biopsy results[4]		
Acute myocarditis	25 (5.0) (3.2 × 105 GE/μg)[5]	498
Inflammatory cardiomyopathy	297 (59.6) (709 GE/μg)[5]	498
Dilated cardiomyopathy	176 (35.3) (392 GE/μg)[5]	498
Uninflamed control hearts		
B19V-detection	7 (7.7) (84 GE/μg)[5]	91
Age (at death), yr	48.1 ± 20.82	91
Male	49 (53.9)	91
Female	42 (46.1)	91

[1]Values are number, absolute and relative frequency of patients; [2]Values are expressed as mean and ± SD; [3]Total number of patients; [4]Histopathology according to the Dallas criteria[41] supplemented by immunohistochemistry for detection of CD3-positive T-lymphocytes, CD68-positive macrophages and natural killer cells, and HLA class Ⅱ expression in professional antigen-presenting immune cells as described[18]; [5]Values are expressed as mean of B19V-genome equivalents per microgram isolated nucleic acids[4].

Nucleic acids extraction from EMBs and polymerase chain reaction amplification of viral genomes

Nucleic acids from RNAlater (Qiagen, Hilden, Germany) fixed EMBs and of controls from formalin fixed tissue were extracted as described previously[1,6]. Polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR was performed to detect parvovirus B19 (B19V), enteroviruses (EV) (including coxsackieviruses and echoviruses), adenoviruses (ADV), HCMV, Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV6) as previously described[1]. B19V mRNA was detected using nucleic acids isolated from EMBs. After extensive RNase-free-DNase digestion (20 U; Qiagen, Hilden, Germany) of 30 μL nucleic acid solution for 2 h at 37 ℃, the DNAse was inactivated for 15 min at 75 ℃. 5 μL of the DNAse-digested samples were analyzed for removal of B19V DNA by B19V-specific PCR using primer pairs PVB3 and PVB4 and nested PCR primer pairs PVB1 and PVB2 as previously described (Table 2)[1]. RT-PCR for the detection of B19V-RNA was performed using a one-step RT-PCR reaction kit (Qiagen, Hilden, Germany) and the following primer pairs: first/RT-PCR NS-25 and NS-30 and nested PCR NS-27 and NS-32 (Table 2). RT-PCR reaction was done at 50 ℃ for 30 min followed by 95 ℃ for 15 min. PCR was for 35 cycles at 94 ℃ for 30 s, 53 ℃ for 30 s, and 72 ℃ for 45 s, followed by a final extension for 5 min at 72 ℃. Nested PCR was performed with an initial denaturation step at 95 ℃ for 2 min followed by 29 cycles at 95 ℃ for 30 s, 53 ℃ for 30 s, and 72 ℃ for 45 s, followed by a final extension for 5 min at 72 ℃. Five μL of each reaction was analyzed using RNase-free agarose gel electrophoresis. Sample processing (DNA/RNA-extraction, template preparation, master-mix preparation)

Table 2 Primer sequences

No	Primer name	Sequences (5′ to 3′)	Position (numbering according M13178)	1st, 2nd (RT/RFLP)-PCR
1	PVB1	GCTAACTCTGTAACTTGTAC	3221-3240	Sense B19V-VP2-PCR (2nd PCR)
2	PVB2	AAATATCTCCATGGGGTTGAG	3373-3393	As B19V-VP2-PCR (2nd PCR)
3	PVB3	AGCATGTGGAGTGAGGGGGC	3191-3210	Sense B19V-VP2-PCR (1st PCR)
4	PVB4	AAAGCATCAGGAGCTATACTTCC	3458-3480	As B19V-VP2-PCR (1st PCR)
5	NS-25	AAATGCGTGGAAGTGTAGCT	1628-1647	Sense B19V-NS1-PCR (RT/1st PCR)
6	NS-27	ATGCGTGGAAGTGTAGCTGT	1630-1649	As B19V-NS1-PCR (2nd PCR)
7	NS-30	CCAACTAACAGTTCACGAAAC	2172-2192	Sense B19V-NS1-PCR (RT/1st PCR)
8	NS-32	TAACAGTTCACGAAACTGGTC	2168-2187	As B19V-NS1-PCR (2nd PCR)
9	NS-38	ATTCCACAAATTGCTGATACAC	2498-2519	As RFLP-PCR (1st PCR)
10	NS-40	AATTGCTGATACACAGCTTTAG	2490-2511	As RFLP-PCR (2nd PCR)
11	G2170	CAGTTTCGTGAACTGTTAGT	2170-2189	Sense RFLP-PCR (1st PCR)
12	G2176	CGTGAACTGTTAGTTGGGGTTGA	2176-2198	Sense RFLP-PCR (2nd PCR)

As: Antisense; RFLP: Restriction fragment length polymorphism; RT-PCR: Reverse transcriptase-polymerase chain reaction.

and PCR were done in separate laboratory rooms, which are all certified for molecular diagnostics using standard precautions to prevent assay contamination.

Quantitative real-time PCR

B19V viral load was determined using quantitative real-time PCR (qPCR) and calculated according to genome equivalents per microgram isolated myocardial nucleic acid (GE/μg) as described previously[1,4,6]. Dilutions of B19V plasmid DNA and the World Health Organization international B19V DNA standard (code 99/800) were included to standardize the assay. A qPCR of the adenosine triphosphate synthase-6 gene was performed as a control for the addition of equivalent amounts of human DNA as described previously[6]. All samples were analyzed in duplicate.

DNA sequence analysis and B19V genotype analysis

DNA fragments spanning the B19V NS1/VP1/VP2 coding region (nt 602 to 5014; 4413 nt; numbering according to GenBank accession no. AF162273) were amplified by PCR using primer pairs as described previously[22]. DNA sequencing was performed in duplicate with purified PCR products in 2 mL BigDye Terminator cycle sequencing mix (Perkin Elmer) and 15 pmol of forward and reverse primers as described previously[22]. B19V sequences were aligned using BLAST (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/ blast/blast.cgi). The reliability of alignment was checked using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Prototype B19V sequences from GenBank were used as reference sequences (GenBank accession numbers: genotype 1: AB030694, AF113323, AF162273, M13178, DQ225148, DQ225149, DQ225150 and DQ225151; genotype 2: AY064476, AY044266, AY661663 and AY661664; genotype 3: AX003421 and AY083234).

Restriction fragment length polymorphism-PCR for B19V genotyping

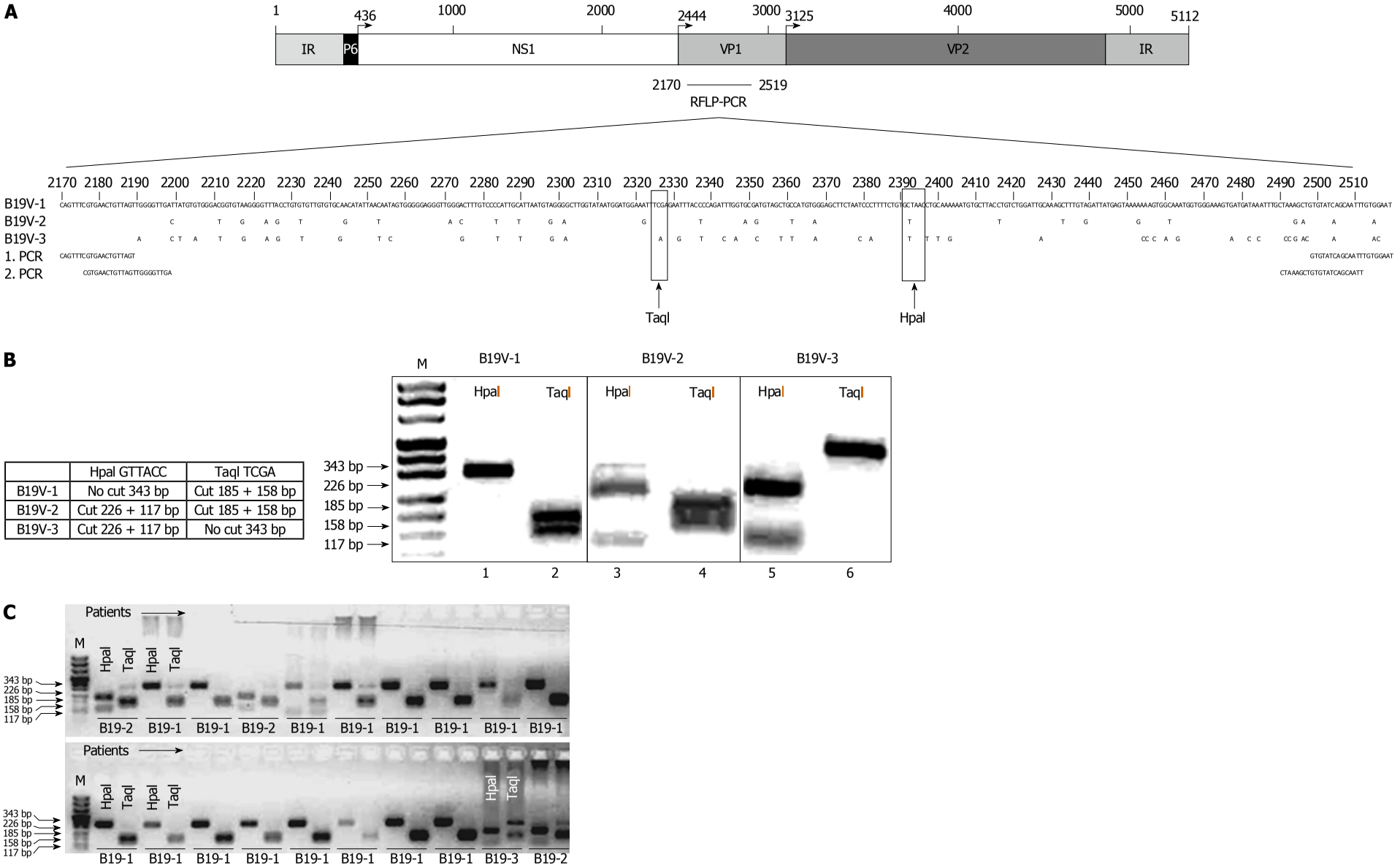
In order to determine B19V genotypes, a restriction fragment length polymorphism PCR (RFLP-PCR) was developed using nested PCR. The following primer pairs were used for the first PCR, G2170F and NS-38, and for the second PCR, G2176F and NS-40 (Table 2, Figure 1). Reactions were initially denatured at 94 ℃ for 4 min followed by 35 cycles of 94 ℃ for 30 s, 52 ℃ for 30 s, and 72 ℃ for 30 s, followed by a final extension for 10 min at 72 ℃. Nested PCR was performed using the primer pair G2176F and NS-40 (Table 2). DNA amplicons (343 bp) were digested with the restriction enzymes HpaI and TaqI in separate reactions (New England Biolabs, Frankfurt, Germany) for 30 min at 37 ℃ and analyzed by agarose gel electrophoresis. The restriction recognition site for HpaI is only present in B19V-genotype 2 and B19V-genotype 3, resulting in fragment sizes of 117 and 226 bp, respectively, while the TaqI restriction site is only present in B19V-genotype 1 and B19V-genotype 2 (resulting in fragment sizes of 158 and 185 bp) (Figure 1).

Luciferase reporter gene assay

After transfection of human endothelial cells 1 cells with luciferase plasmid constructs containing the B19V P6-promoter region to a final mass of 5 μg per plasmid containing 0.2 μg of the β-galactosidase reporter pCMVb-Gal as an internal standard, cells were cultured for 48 h. Cells were co-transfected with an HHV6 U94 expressing vector construct (kindly provided by Dr. S. Aberle, University of Tuebingen, Germany). To measure luciferase, activity cells were harvested, lysed and measured as described previously[23].

Statistical analysis

Statistical analysis and graphical representation were performed by two-tailed *T*-test and non-parametric Mann-Whitney *U*-test using SPSS statistical software version 16.0 and GraphPad Prism, version 5 (GraphPad Software Inc, San Diego, United States). One-way ANOVA followed by post hoc testing and Tukey's multiple comparison test were also performed. The results were expressed as mean ± SD. Values below significance level of 0.05 were considered statistically significant.



	HpaI GTTACC	TaqI TCGA
B19V-1	No cut 343 bp	Cut 185 + 158 bp
B19V-2	Cut 226 + 117 bp	Cut 185 + 158 bp
B19V-3	Cut 226 + 117 bp	No cut 343 bp

Figure 1 Generation of B19V-genotype 1 to 3 specific restriction fragment length polymorphism-polymerase chain reaction. A: Schematic representation of the B19V genome showing localization of the B19V-genotype-specific restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) in the B19V NS1-VP1u region. Sequences of B19V-1, B19V-2 and B19V-3 showing the RFLP-PCR fragment and HpaI and TaqI restriction enzyme sites (lower panel). Primer positions for 1st and 2nd RFLP-PCR are indicated (1. PCR and 2. PCR, see also Table 2); B: Expected fragment size and digestion pattern after HpaI and TaqI digestions (right panel). Agarose gel electrophoresis showing respective PCR-fragments after HpaI and TaqI digestion for each B19V-genotype (left panel); C: Representative agarose gel electrophoresis of patient-specific B19V RFLP-PCRs. B19V-1: B19V-genotype 1.

RESULTS

Study population

EMBs of 498 B19V-positive patients described in this retrospective study were obtained from 38 clinical centers in Germany between 2003 and 2010 for cardiopathological diagnosis of myocarditis and DCM. In addition, 91 uninflamed hearts without cardiac failure served as a control group. The baseline data of the patients, molecular and cardiopathological findings of the EMBs are provided in Table 1. Quantitative assessment of B19V loads has been described previously, with viral loads of more than 500 GE/μg in EMBs as a clinically relevant threshold for the maintenance of myocardial inflammation[4]. Patients were relatively young (mean age 46.9 ± 15.9 years) and approximately two thirds were men (68.5%) (Table 1). With regards to clinical history, the majority of patients had cardiac symptoms for longer than six months, consistent with chronic heart disease.

Figure 2 illustrates typical histological and immunohistological findings in acute and chronic myocarditis, as well as in uninflamed DCM in B19V-positive human hearts. As expected, acute B19V-myocarditis had a low prevalence of 5% in our study (Figure 2A and 2B, Table 1), while the majority of patients (59.6%) had chronic B19V-myocarditis (Figure 2C and 2D, Table 1). By contrast, chronic DCM without inflammation (Figure 2E and 2F) exhibiting a latent type of B19V-infection was found in 35.3% of our study population (Table 1). Only 7% of 91 uninflamed control hearts were found to harbor latent B19V genomes (Figure 2G and 2H, Table 1).

Replication activity of B19V in myocarditis

In order to determine replication activity of B19V in infected endothelial cells of the myocardium, we determined B19V RNA replication intermediates of 114 randomly selected patients of our cohorts using RT-PCR to amplify the NS1 and VP1 regions of the B19V genome (2 acute, 54 chronic myocarditis, 51 DCM and 7 control hearts). As expected, B19V replicative RNA intermediates could be confirmed in acute B19V-myocarditis as described[4] (2/2), but also in EMBs of patients with chronic myocarditis harboring viral loads greater than 500 GE/μg (16/51) (Figure 3). In contrast, B19V-mRNA intermediates were not observed in EMBs of DCM-patients with uninflamed hearts and viral loads < 100 GE/μg, indicating a latent-type of B19V-infection. In addition, B19V-mRNA intermediates were found to be absent in latent B19V-infected normal hearts without inflammation.

B19V-specific IgG and IgM antibodies were detected in the serum by ELISA almost exclusively in B19V-DNA positive EMBs of patients with acute myocarditis (11.4%) but not with DCM or in controls. Overall, B19V-IgG antibodies, but not IgM, were detected in the serum of 91% of patients with chronic myocarditis and in 60.9% with DCM.

Detection of different B19V genotypes in EMBs

Recent reports have shown an association between B19V-infection of myocardial endothelial cells and the development of viral myocarditis[1,4,24-26]. However, it is not clear whether B19V-genotypes modulate the outcome of the disease.

In order to determine the B19V genotype confirmed in B19V-positive EMBs, we developed a new RFLP-PCR method spanning the NS1/VP1u region (nt 2170 to 2519; Figure 1A). As shown in Figure 1B, the RFLP-PCR method is capable of discriminating between the known B19V genotypes 1 to 3 by using Hpa1 and Taq1 restriction enzyme digestions. RFLP-PCR patterns of representative patient-specific samples are shown in Figure 1C. In addition, direct sequencing and phylogenetic analysis was performed to verify the specificity of the results by RFLP-PCR. B19V-genotype 1 (286/498; 57.4%) and B19V-genotype 2 (183/498; 36.7%) were the most frequently detected genotypes in persistently infected patients, whereas twenty-five patients with acute B19V-infection showed B19V-genotype 1 (5.0%) (Table 1). In contrast, B19V-genotype 3 infection of the human heart was found to be rare in Germany and only detectable in 0.8% (4/498) of our patients.

B19V genotype-dependent correlation with inflammatory cardiomyopathy

The prevalence of B19V-genotype 1 and B19V-genotype 2 in EMBs was investigated in order to establish if a correlation exists between B19V-genotypes and the occurrence of myocarditis and DCM. B19V-genotype 1 was detected in 176/286 of EMBs (61.5%) with iCMP and in 110/286 samples (38.5%) of DCM-patients without myocardial inflammation, respectively (Figure 4A). A strong trend was observed in the more frequent prevalence of B19V-genotype 2 genomes (total 183/498) in EMBs with iCMP (119/183, 65.0%) compared to DCM (64/183, 35%; $P = 0.048$) (Figure 4A). We found comparable viral loads in B19V-positive EMBs for both B19V-genotype 1 (584 ± 695 GE/μg) and B19V-genotype 2 (613 ± 715 GE/μg) (P = ns; Figure 4B).

Determination of B19V-genotype specific viral loads in EMBs of patients with iCMP and DCM showed that B19V-genotype 1 load was significantly higher in iCMP samples (706 ± 821 GE/μg) compared to DCM (389 ± 343 GE/μg; $P < 0.0002$). In keeping with this finding, significantly higher viral loads were observed in B19V-genotype 2 positive EMBs with iCMP (723 ± 846 GE/μg) compared to DCM samples (408 ± 270 GE/μg; $P = 0.0039$) (Figure 4C).

Age-dependent distribution of B19V-genotype 1 and B19V-genotype 2

Recent publications have shown an association between age and B19V genotype prevalence[16]. B19V-genotype 2 is considered to be an "older" B19V-variant which is more often found in tissues of patients over the age of 60 years, whereas B19V-genotype 1 is more frequently observed in younger people. In accordance with recent reports, we determined a comparable age-dependent distribution of B19V-genotypes in human hearts[16,17]. B19V-

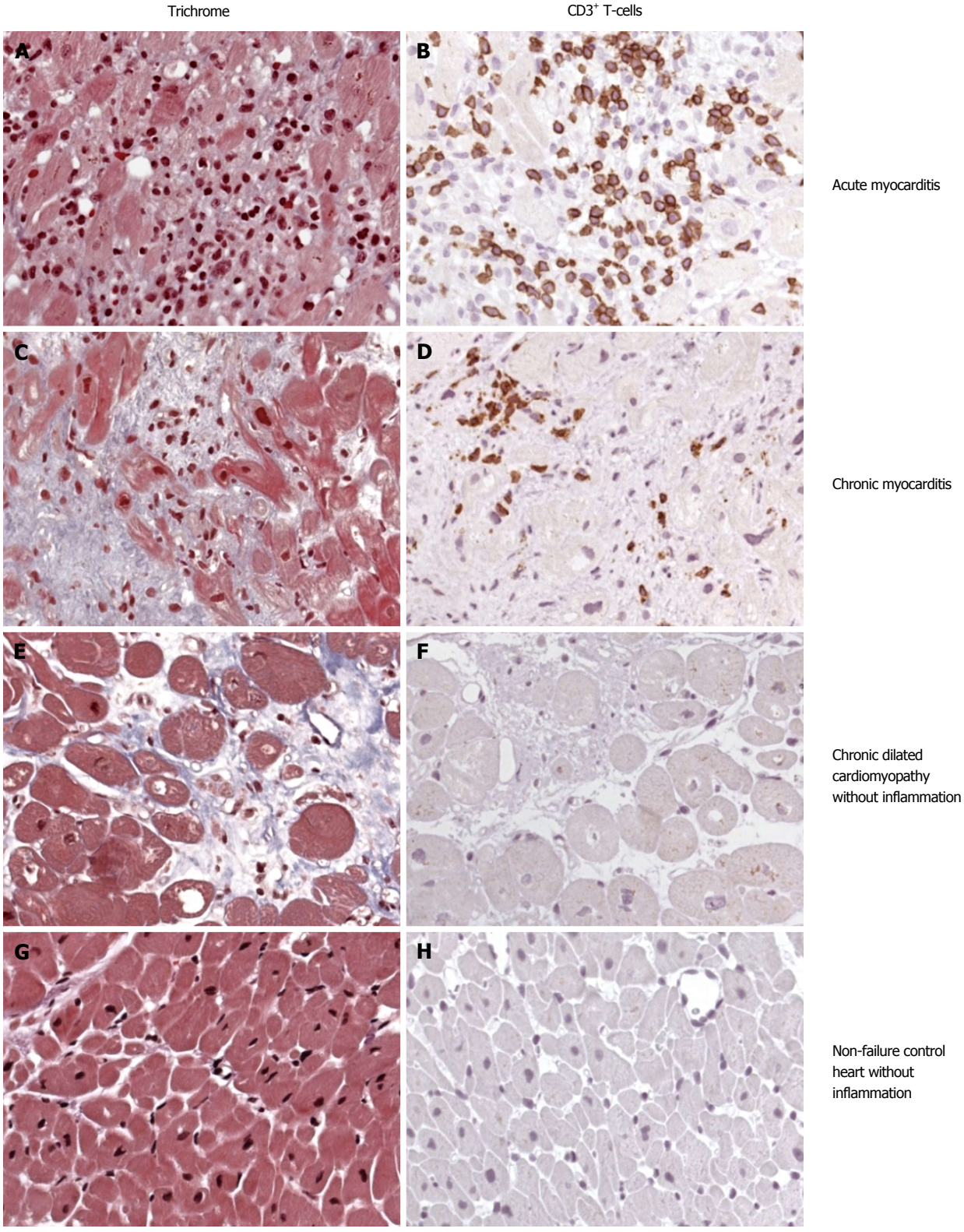


Figure 2 Typical histopathological and immunohistological findings in acute myocarditis (A and B), chronic myocarditis/inflammatory myocarditis (C and D), chronic dilated cardiomyopathy without inflammation (E and F), and non-failure control hearts (G and H). Masson trichrome staining (A, C, E and G) and immunohistological detection of $CD3^+$ T-lymphocytes (B, D, F and H).

genotype 1 was predominantly detected in individuals born after 1955, with an average of 43 ± 14 years ($P < 0.0001$), while B19V-genotype 2 was predominantly observed in individuals born before 1955, with an average age of 61 ± 12 years (Figure 5A).

Gender-dependent distribution of B19V-genotype 1 and B19V-genotype 2

We next explored the role of gender in B19V-associated myocarditis. 341/498 (68.5%) EMBs were obtained from male and 157/498 (31.5%) from female patients. There

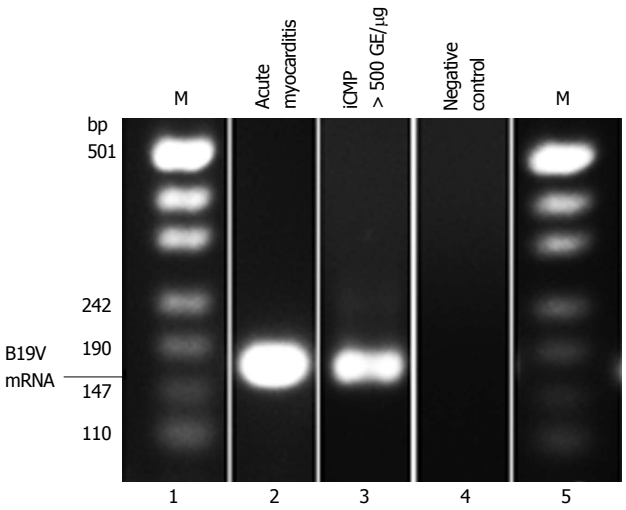


Figure 3 Representative B19V-specific reverse transcription-polymerase chain reaction showing B19V mRNA replication intermediates isolated from endomyocardial biopsies of patients with acute myocarditis (lane 2) and chronic myocarditis/iCMP (lane 3). iCMP: Inflammatory cardiomyopathy.

was no significant difference in age between women (49 ± 16 years) and men (46 ± 16 years; P = ns) (Figure 5B). Furthermore, there was no significant difference in the occurrence of iCMP or DCM in B19V-positive male and female patients (P = ns). A comparison of the B19V-loads in EMBs from men and women showed no significant differences (men 586 ± 679 GE/μg *vs* women 602 ± 711 GE/μg) (P = ns). However, there was a trend towards women being more frequently infected with B19V-genotype 2 (66/148, 44.6%) compared to men (117/325; 36.0%; P = 0.0448), whereas this was not the case for B19V-genotype 1 infection of men (198/325; 60.9%) and women (81/148; 54.7%; P = ns) (Figure 5C).

Coinfection with cardiotropic viruses detected in B19V-positive EMBs

Recent reports have shown that coinfection of B19V with other cardiotropic viruses, such as HHV6, HCMV, EBV and EV, is not infrequent in viral myocarditis[18,27]. However, the impact of coinfection on B19V replication has not been determined. Hence, the frequency of B19V-coinfections in EMBs of 473/498 B19V-positive patients with iCMP and DCM was analyzed excluding the twenty-five acute cases of B19V-myocarditis (Table 1). 382/473 (80.8%) EMB samples were infected with B19V alone, while 91/473 (19.2%) were coinfected with other cardiotropic viruses (Figure 6A). The most prevalent coinfecting virus was HHV6 (78/473, 16.5%). EBV (5/473, 1.1%), EV (4/473, 0.8%) and HCMV (1/473, 0.2%) were detected less frequently (Figure 6B). 3/473 (0.6%) showed evidence of triple-infection with HHV6, EBV and/or HCMV. Only HHV6 subtype B was detected in B19V-positive EMBs. There was no statistically significant difference in the frequency of coinfection with either B19V-genotype 1 or 2 (46.2% *vs* 53.8%; P = ns). Determination of B19V loads in EMBs revealed a statistically significant higher viral load in B19V-coinfection in comparison to

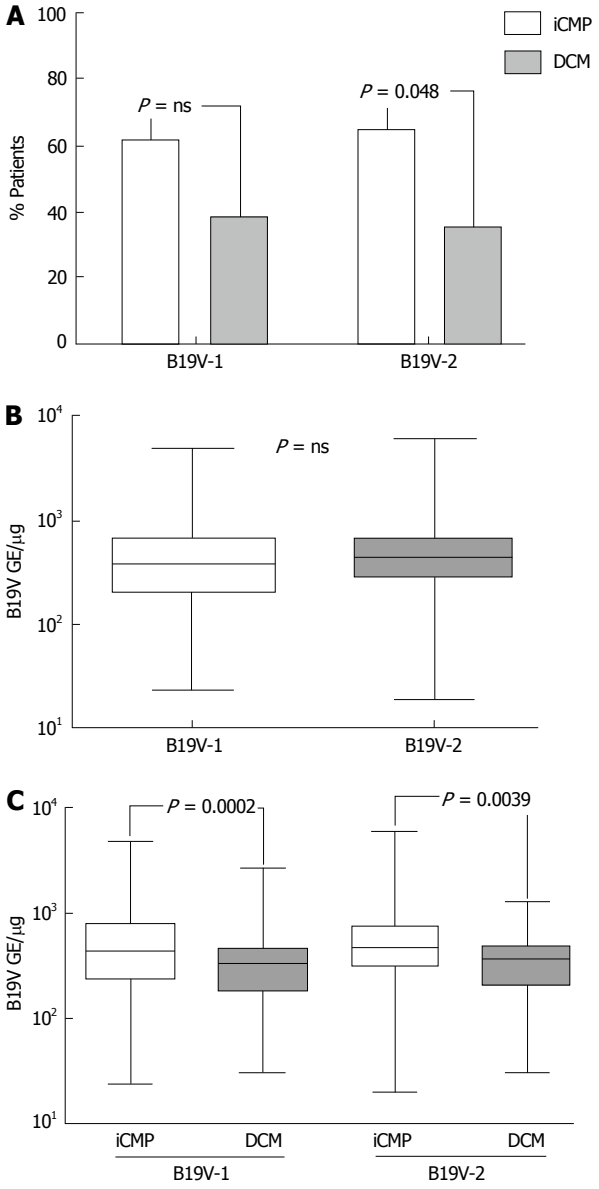


Figure 4 Genotype specific myocardial B19V loads of patients with chronic myocarditis. A: Prevalence of B19V-genotype 1 (B19V-1) and B19V-2 in endomyocardial biopsies of patients with myocarditis [inflammatory cardiomyopathy (iCMP), grey columns] and dilated cardiomyopathy (DCM, white columns). Patient number is given in %; B: qPCR of myocardial of B19V-1 and B19V-2 loads in endomyocardial biopsies (EMBs); C: B19V genotype-specific myocardial viral loads in EMBs of patients with chronic myocarditis (iCMP, white columns) and DCM (grey columns) determined by qPCR. One-way Anova was highly significant (P < 0.0001). P < 0.05 is statistically significant (two-tailed T-test). qPCR: Quantitative real-time polymerase chain reaction.

B19V-monoinfection (754 ± 967 *vs* 552 ± 615 GE/μg; P = 0.0012) (Figure 6C). Analysis of the prevalence of B19V genotypes showed that B19V-genotype 2 was more frequently associated with B19V coinfection in comparison to B19V-monoinfection (36.7% *vs* 48.3%; P = 0.023), whereas this was not the case for B19V-genotype 1 (63.3% *vs* 51.7%; P = ns) (Figure 6D). Interestingly, B19V loads were significantly increased in iCMP samples of B19V-coinfected in comparison to B19V-monoinfected patients with iCMP (934 ± 1122 *vs* 650 ± 723 GE/μg; P = 0.0157) (Figure 6E). No difference in B19V loads was found in

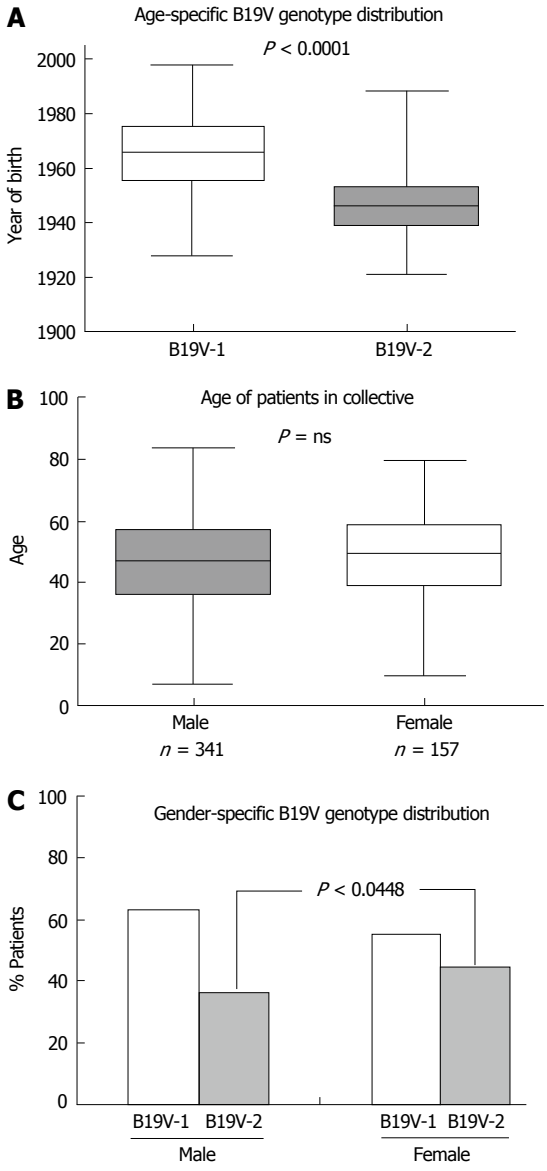


Figure 5 Age and gender dependent distribution of B19V-genotypes in endomyocardial biopsies of patients with myocarditis. A: Distribution of B19V-genotype 1 (B19V-1) and B19V-2 according to year of birth; B: Gender-specific mean age of our patient cohort; C: Gender-specific distribution of B19V-1 (white columns) and B19V-2 (grey columns). $P < 0.05$ is statistically significant (two-tailed T-test).

DCM samples of B19V mono- and coinfected patients (396 ± 336 *vs* 371 ± 205 GE/μg; P = ns) (Figure 6E). Further analysis showed that the increased B19V load was mainly due to coinfection of B19V with HHV6 (869 ± 992 *vs* 552 ± 615 GE/μg; $P = 0.0006$) in comparison to B19V coinfections with EV or EBV (466 ± 254 and 350 ± 218 GE/μg, respectively; P = ns) (Figure 6F). Functional analysis demonstrated that the HHV6 U94 transactivator, which shows similarities to parvovirus NS1/Rep transactivator[28], is able to transactivate the P6-promoter of B19V by 2.4 fold using luciferase promoter activity assays (Figure 6G).

DISCUSSION

Parvovirus B19 has been reported to be the causative agent for a wide variety of clinical conditions, ranging from asymptomatic infection to fifth disease in childhood (erythema infectiosum), hydrops fetalis and transient aplastic anemia[12,29,30]. In addition to EV, the classical causative pathogens of myocarditis[8,27], B19V has also been described to account for inflammatory cardiomyopathy (iCMP)[1,24,25,27]. However, the relatively high prevalence of B19V in EMBs has led to controversy around the role and mechanism of B19V in the pathogenesis of myocarditis[9,31].

It has been demonstrated that B19V infects endothelial cells of small myocardial blood vessels, which can result in the impairment of myocardial microcirculation[1,2,24,25], endothelial dysfunction[6] and secondary necrosis of myocardial cells[2]. A recent report by Streitz *et al*[32] demonstrated the presence of anti-B19V NS1-specific T-cell response in B19V-associated myocarditis, supporting the notion that B19V-infection has a pathogenic role in the development of iCMP. It has been shown that active persistent B19V-infection is responsible for triggering inflammatory response in the myocardium[18]. Recently, we demonstrated that B19V and the viral proteins of B19V play an important pathophysiological role in modulating inflammatory signaling, regulation of pro-apoptotic processes and modulation of the intracellular Ca^{2+}-activity leading to endothelial dysfunction[26,33,34].

It is important to note that B19V DNA is more frequently found in EMBs of patients with chronic myocarditis (59.6%) compared to control cardiac tissue samples from individuals without heart failure (7.7%), a finding consistent with other reports[2,35]. Based on the assessment of B19V-myocarditis and DCM, a viral load threshold of greater than 500 B19V-GE/μg has been suggested to be of clinical significance for the maintenance of myocardial inflammation[4]. This correlates with the finding of B19V RNA replication intermediates predominantly in myocardial tissue of patients with acute and chronic myocarditis, but not in uninflamed hearts (Figure 2). However, the detection of B19V-RNA replication intermediates in EMBs is challenging because of sampling errors and RNA copy numbers that may be below limit of detection.

In order to determine the B19V-genotype distribution in EMBs of patients with iCMP/DCM, we developed a RFLP-PCR technique to discriminate between the three B19V-genotypes (Figure 3). In line with recent publications, we found that B19V-genotype 1 was the most common genotype, followed by B19V-genotype 2, while B19V-3 was rarely found[17]. This is not surprising as B19V-genotype 3 is most commonly found in Ghana, Brazil and France[13,14,36].

While B19V was detected more frequently in EMBs of patients with iCMP compared to DCM that lacked inflammatory cell infiltrates, B19V viral loads of both B19V-genotypes 1 and 2 were significantly higher in iCMP samples (approximately 700 GE/μg) compared to DCM (approximately 400 GE/μg) (Figure 4). These findings indicate that regardless of the B19V-genotype, B19V-iCMP is associated with significantly higher viral

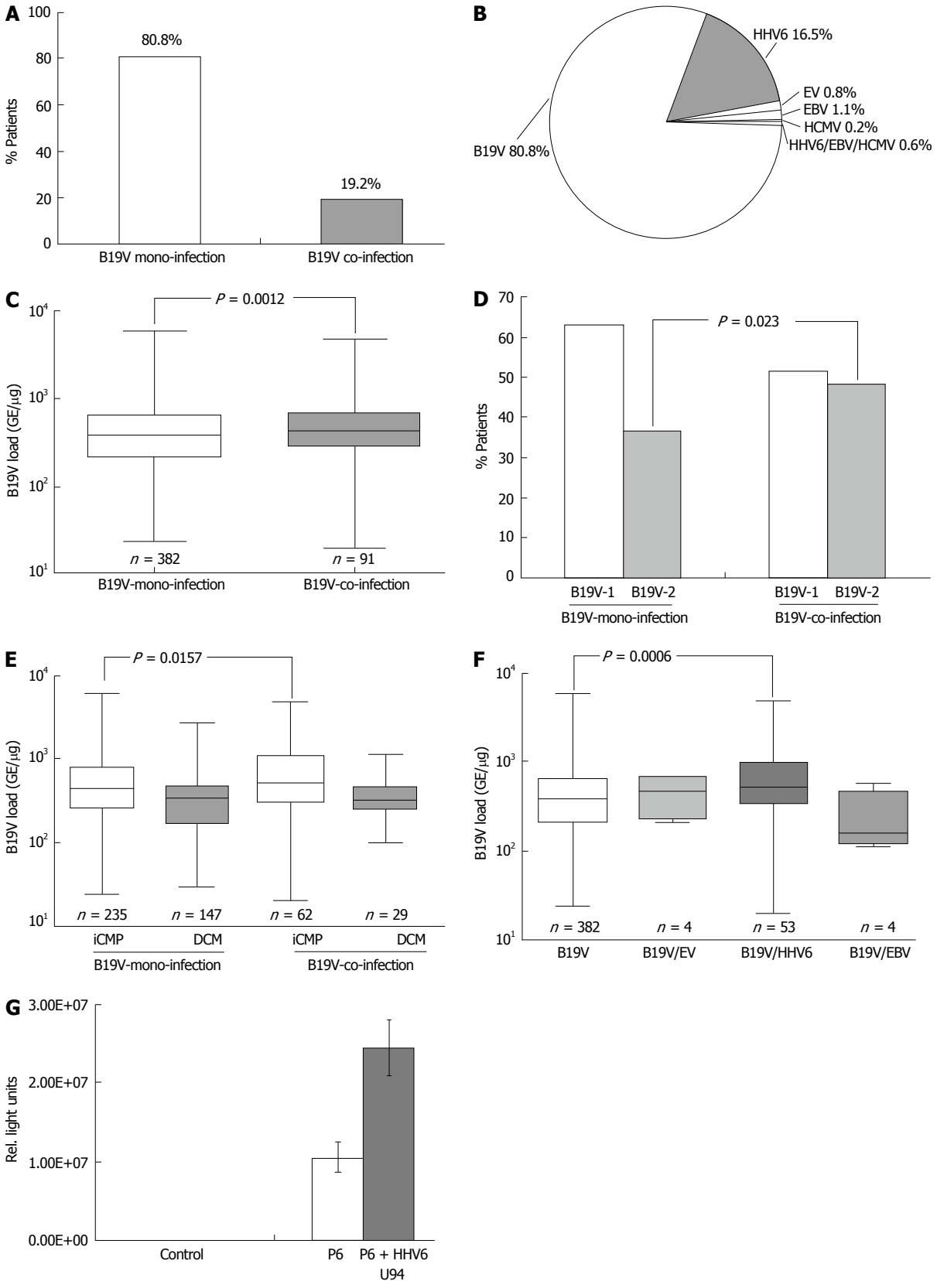


Figure 6 Distribution of B19V-coinfection with cardiotropic viruses. A: In endomyocardial biopsies determined by virus-specific nPCR; B: Frequency of B19V-coinfection with cardiotropic viruses; C: qPCR of B19V loads in B19V mono- and co-infection; D: Distribution of B19V-genotype 1 and 2 in B19V mono- and co-infection in endomyocardial biopsies (EMBs); E: B19V loads of B19V mono- and co-infection in EMBs of patients with iCMP and DCM. One-way Anova was highly significant ($P < 0.0001$); F: B19V loads of B19V mono- and co-infection with cardiotropic viruses. One-way Anova was highly significant ($P = 0.0091$); G: Luciferase reporter assay to determine transactivation capacity of the HHV6-U94 transactivator on the B19V P6-promoter activity. $P < 0.05$ is statistically significant (two-tailed T-test). HHV6: Human herpesvirus 6; EV: Enterovirus; HCMV: Human cytomegalovirus; EBV: Epstein-Barr virus; DCM: Dilated cardiomyopathy; iCMP: Inflammatory cardiomyopathy; qPCR: Quantitative real-time polymerase chain reaction.

loads than B19V-DCM.

As with the age-dependent distribution of B19V-genotype 1 and 2 in various tissue samples (*e.g.*, skin, synovium, liver, and heart)[15-17], we found that B19V-genotype 1 was predominantly detected in younger people (mean age 43 years), while B19V-genotype 2 was mostly observed in patients older than 60 years (Figure 5). This age-associated difference in frequency of genotypes is thought to be due to the reported lifelong persistence of viral genomes in humans[15,16,29]. Analysis of gender dependency of B19V genotype distribution and viral loads did not show any significant differences. However, a significantly higher proportion of women were found to be persistently infected with B19V-genotype 2 in contrast to men ($P < 0.05$) (Figure 5).

It can be hypothesized that B19V can be reactivated from long-term persistent or latent infection by viral and/or host-specific determinants. B19V-coinfection with other cardiotropic viruses like EV, ADV and HHV6 may contribute to the severity of B19V-myocarditis[4,7,8,20], possibly by reactivating B19V replication and thereby enhancing virus specific host immune responses, tissue inflammation and the progression to chronic heart failure[37]. This is reminiscent of the increased replication of HIV caused by coinfection with HHV6 and HHV8[21,38]. Our finding show that coinfection with B19V and other cardiotropic viruses, particularly HHV6, is not infrequent and is associated with higher B19V loads in EMBs. HHV6 cannot only infect and replicate in endothelial cells[39], but also encodes a viral transactivator[37] called U94 which is homologous to the B19V-NS1 transactivator[28]. By using promoter activity assays, we have shown that the HHV6 U94 transactivator also transactivates the B19V-P6 promoter, resulting in enhanced B19V loads in B19V/HHV6 coinfection (Figure 6).

Our data shows that B19V is capable of long-term persistence in the human heart, even lasting for decades, and that active B19V replication is associated with the development of iCMP. To establish a clinically relevant link between B19V-infection and the development of iCMP, it is important to develop molecular diagnostic techniques to determine myocardial viral loads and sequence analysis to verify the association between myocardial disease and genotype-specific B19V isolates. Notably, persistently low replicating and latent B19V-infection may be reactivated by coinfecting cardiotropic viruses, especially HHV6. Our findings together with other recently published data[1,6,18,25,26,40] argue an important role for B19V in the development of iCMP.

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COMMENTS

Background

Viral and post viral myocarditis is the major cause of acute and chronic dilated cardiomyopathy (DCM). Human parvovirus B19 has been identified as a new emerging pathogenic agent in the etiology of inflammatory cardiomyopathy (iCMP). However, the role of B19V-infection in the development of chronic myocarditis is still unclear. The authors have recently demonstrated that endothelial cells but not cardiac myocytes are B19V-specific target cells in patients with B19V-associated myocarditis. Furthermore, B19V was not detected frequently in patients with unexplained isolated diastolic dysfunction. Molecular phenotypes such as patient age, gender, B19V replicative mRNA intermediates, B19V genotype and B19V-coinfection of myocardial B19V-infection contributing to the etiopathogenesis of B19V-myocarditis as an endothelial-cell mediated inflammatory disease have not been described so far.

Research frontiers

The identification of various viral nucleic acids by polymerase chain reaction in the myocardium of patients with suspected myocarditis led to the hypothesis that acute and chronic myocarditis may develop as a result of infection, not only with enteroviruses (Coxsackievirus B3), but also with other cardiotropic viruses, such as parvovirus B19. In this regard, the research hotspot is how molecular phenotypes can contribute to the pathogenic role of myocardial B19V-infection in myocarditis and DCM.

Innovations and breakthroughs

The study results showed that B19V RNA replication intermediates could be determined mainly in acute and ongoing myocarditis, indicating active replication of B19V. B19V-genotypes 1 and 2 were predominant with a more frequent prevalence of B19V-genotype 2 in iCMP. Furthermore, B19V-coinfections with other cardiotropic viruses can occur, most frequently with human herpes virus 6 (HHV6). Functional experiments showed that the HHV6 U94-transactivator could transactivate the B19V-P6-promoter. From these findings it is suggested that long-term persistence of B19V DNA in the human heart occurs and that active/reactivated B19V-replication can be associated with iCMP in a viral load and genotype-dependent manner.

Applications

To establish a clinically relevant link between B19V-infection and the development of iCMP, it is important to pursue molecular diagnostic techniques to determine myocardial B19V loads and to verify genotype-specific B19V isolates. Notably, persistent low replicating and even latent B19V-infection may be reactivated by coinfecting cardiotropic viruses, especially HHV6.

Terminology

Parvovirus B19 was assumed to be an agent of human disease when its association with erythema infectiosum (fifth disease), hydrops fetalis and transient aplastic anemia was demonstrated in the 1980s. During the last few years, a growing number of reports have been published demonstrating an association between B19V and many other clinical diseases, like arthritis, myocarditis, various vasculitis syndromes, hepatitis and neurological disorders. A growing number of reports have suggested an association between B19V infection with acute and chronic cardiac diseases. Myocarditis is the term used to indicate infectious, toxic or autoimmune processes causing inflammation of the heart and represents a non-ischemic inflammatory heart disease with a highly variable clinical outcome. In most cases this disease is self-limiting; however, it may also lead to acute heart failure, resulting in early death or heart transplantation. Myocarditis can also mimic acute myocardial infarction. ICMP was included as a subtype of the specific cardiomyopathies and defined "as myocarditis in association with cardiac dysfunction" by the World Health Organization.

Peer review

The paper deals with the interesting subject of molecular phenotypes of human parvovirus B19 in patients with myocarditis. Both the specific virus, which is a cause of important pathologies, as well as myocarditis, an entity that can affect great portions of a population, among them young, otherwise healthy individuals, are very interesting subjects with an impact on the general practice of internists, cardiologists, general physicians, pathologists, biologists and genetic scientists. The paper deals with the aforementioned interesting subject in a thorough way.

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ORIGINAL ARTICLE

Coronary artery disease in congenital single coronary artery in adults: A Dutch case series

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Abstract

AIM: To assess the current diagnostic and therapeutic management and the clinical implications of congenital single coronary artery (SCA) in adults.

METHODS: We identified 15 patients with a SCA detected from four Dutch angiography centers in the period between 2010 and 2013. Symptomatic patients who underwent routine diagnostic coronary angiography (CAG) for suspected coronary artery disease and who incidentally were found to have isolated SCA were analyzed.

RESULTS: Fifteen (7 females) with a mean age of 58.5 ± 13.78 years (range 43-86) had a SCA. Conventional CAG demonstrated congenital isolated SCA originating as a single ostium from the right sinus of Valsalva in 6 patients and originating from the left in 9 patients. Minimal to moderate coronary atherosclerotic changes were found in 4, and severe stenotic lesions in another 4 patients. Seven patients were free of coronary atherosclerosis. Runs of non-sustained ventricular tachycardia were documented in 2 patients, one of whom demonstrated transmural ischemic changes on presentation. Myocardial perfusion scintigraphic evidence of transmural myocardial ischemia was found in 1 patient due to kinking and squeezing of the SCA with an interarterial course between the aorta and pulmonary artery. Multi-slice computed tomography (MSCT) was helpful to delineate the course of the anomalous artery relative to the aorta and pulmonary artery. Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA.

CONCLUSION: SCA may be associated with transient transmural myocardial ischemia and aborted sudden death in the absence of coronary atherosclerosis. The availability and sophistication of MSCT facilitates the delineation of the course of a SCA. We present a Dutch case series and review of the literature.

Key words: Congenital heart disease; Coronary artery anomaly; Coronary angiography; Single coronary artery; Coronary artery disease; Multi-slice computed tomography

Core tip: A Dutch case series of 15 adult patients with congenital isolated single coronary artery (SCA) are presented. Conventional coronary angiography demon-

strated congenital isolated SCA originating as a single ostium from the right sinus of Valsalva in 6 patients and originating from the left in 9 patients. SCA may be associated with symptomatic transient transmural myocardial ischemia, non-sustained ventricular tachycardia, and aborted sudden death in the absence or presence of coronary atherosclerosis. The availability of multi-slice computed tomography (MSCT) and cardiovascular magnetic resonance imaging facilitates the delineation of the course of the anomalous vessel. MSCT was helpful to delineate the course of the anomalous artery relative to the aorta and pulmonary artery. Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA. The literature addressing SCA is reviewed.

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INTRODUCTION

A single coronary artery (SCA) is defined as a single aortic orifice or origin providing for all of the coronary blood perfusion of the entire myocardium[1-3]. In 1967, Halperin *et al*[4] reported the first ante mortem angiographic diagnosis of SCA arising from the left sinus of Valsalva (LSV). SCA is a rare congenital anomaly and occurs as an incidental finding in approximately 0.066% of the coronary angiography (CAG) population[5]. SCA has been reported in association with and without atherosclerotic changes[6,7] or in association with coronary artery fistulas[8,9], bicuspid aortic valves, and with hypertrophic cardiomyopathy[1,7,10,11].

An equal distribution is found between SCA originating from the right sinus of Valsalva (RSV) and the LSV[2,12]. Exact delineation of the course of the abnormal coronaries relative to the aorta and pulmonary artery is of major importance as myocardial ischemia during exertion can be caused by kinking or squeezing of the branches of the anomalous SCA between the aorta and pulmonary artery. CAG is the first diagnostic tool in the detection of a SCA. Once abnormal coronary arteries are suspected, multi-slice computed tomography (MSCT) and cardiac magnetic resonance (CMR) imaging[13] scans are excellent tools for non-invasive determination of the course of the abnormal coronaries relative to the aorta and pulmonary artery[14]. Determination of the course of incidentally found congenital coronary anomalies during routine CAG without the direct availability of CMR or MSCT scanning is challenging.

We discuss the clinical presentation and angiographic findings of 15 adult symptomatic patients with congenital isolated SCA incidentally found during routine CAG.

MATERIALS AND METHODS

Between 2010 and 2013, 15 adult patients with a mean age of 58.5 ± 13.78 years (range 43-86) were diagnosed with a SCA during CAG in 4 Dutch angiography centers (Hospital Group Twente, Almelo; St. Lucas Andreas Hospital, Amsterdam; St. Anna Hospital, Geldrop; Hospital Group Twente, Hengelo; and Gerle Hospital, Zutphen). Indications for CAG were angina pectoris, dyspnea, and syncope.

The angiograms were reviewed by at least two experienced cardiologists who reached a consensus on the origin and course of the SCA. The angiographic variations and the course of the anomalous artery were defined according to the classification of Lipton *et al*[1]. The definition of SCA was adopted from Angelini *et al*[15] and defined as an isolated coronary artery arising from the sinus of Valsalva through a single ostium and with no evidence of a second ostium, thus being responsible for supplying blood to the entire myocardial tissue, regardless of its distribution.

Significant atherosclerosis was defined as luminal narrowing of ⩾ 75% detected in a main branch of the epicardial coronary arteries. Patients were categorized as having significant single, double, or triple vessel disease when a significant lesion was found in one or more coronary artery branches arising from the SCA and supplying the right coronary artery (RCA), circumflex (Cx), or left anterior descending coronary artery regions. A 12-lead ECG was performed in all patients.

An exercise tolerance test (ETT) was performed in 10 patients, myocardial perfusion test [methoxy-isobutyl-isonitrile (MIBI) scan] in five patients, and ^{13}ammonia-adenosine positron emission tomography (positron emission tomography-computed tomography) scan in one patient. MSCT was performed in 6 patients using a retrospective ECG-gated procedure (128-slice, Philips Medical Systems, Best, The Netherlands).

RESULTS

Patients comprised 8 males and 7 females, aged between 43 and 86 years (mean 58.5 ± 13.78). Effort angina pectoris was found in 6 patients, 4 had dyspnea on exertion, 4 complained of atypical chest pain, fainting and pre-syncope, 1 had recurrent syncopal attacks, and 2 presented with acute coronary syndrome. Patients' characteristics are presented in Table 1. Between 2010 and 2013, 8917 coronary angiograms were performed in the 4 Dutch angiography centers all together, with an incidence of 15/8917 (0.017%).

On the resting ECG, all patients were in sinus rhythm and had normal PR intervals. Two patients (patients 3 and 15) had a complete left bundle branch block. One patient (patient 5) had inverted T-waves in the inferior leads. In another patient (patient 8), ECG evidence of an old infero postero lateral infarction was shown. Short

Table 1 Patients' characteristics

Case /gender/age	Clinical presentation	Rest ECG	Risk factors	ETT	MIBI scan	CAD	Management	CAG [1]classification	MSCT
1/F/45	AP, DOE	SR	-	Inconclusive	NA	None	CMM	R-ⅡP	NA
2/M/56	DOE	SR RD	+	Positive Ischemia IL	NA	Intermediate lesion FFR 0.93	CMM	R- I	Overestimation of the Cx-lesion
3/F/60	AP	SR LBBB	-	NA	NA	Mild	CMM	R-Ⅲ	NA
4/M/86	ACS	SR	+	Negative	NA	Significant	PCI	L- I	NA
5/M/63	Effort AP	SR Negative T Inferior leads	+	Positive	NA	Significant	PCI	L-ⅡA	NA
6/F/43	ACP, fainting and pre-syncope	SR	+	Inconclusive	Positive ^{13}N-adenosine PET-CT: normal	None	CABG	L-ⅡB	Course: between aorta and pulmonary artery
7/M/48	AP, syncope	SR NSVT (5 beats)	+	Negative	Negative	None Ergonovine test: No spasm	CMM	L- I	NA
8/F/53	DOE, palpitation	SR RD NSVT (20 beats)	+	NA	Positive	Intermediate lesion	CMM	R-ⅡA	NA
9/M/46	AP, palpitation	SR	-	NA	NA	None	CMM	R-ⅡA	NA
10/M/63	AP	SR	+	Positive	NA	Significant	CABG	L-ⅡB	Course: between aorta and pulmonary artery
11/F/83	NSTEMI	SR	+	NA	NA	Significant	PCI	R-Ⅲ	NA
12/F/47	ACP	SR	+	Negative	NA	None	CMM	L-ⅡA	NA
13/F/53	CP syncope	SR	-	Negative	Negative	None	CABG	L-ⅡB	Course: between aorta and pulmonary artery
14/M/72	DOE	SR	+	NA	NA	Intermediate lesion	CABG	L-ⅡB	Course: between aorta and pulmonary artery
15/M/41	ACP	SR LBBB	+	Negative	Negative	None	CMM	L-ⅡA	Benign course

[1]Classification according to Lipton *et al*[1]. A: Anterior; AP: Angina pectoris; ACP: Atypical chest pain; ACS: Acute coronary syndrome; B: Between aorta and pulmonary artery; CAD: Coronary artery disease; CMM: Conservative medical management; ETT: Exercise tolerance test; F: Female; FFR: Fractional flow reserve; M: Male; CABG: Coronary artery bypass grafting; DOE: Dyspnoea on exertion; N: Normal; NA: Not available; P: Posterior to the aorta; PCI: Percutaneous coronary intervention; R: Right; L: Left; LBBB: Left bundle branch block; MSCT: Multi-slice computed tomography; SR: Sinus rhythm; -: Absent; +: Present (hypertension, smoking, obesity, hypercholesterolemia); PET-CT: Positron emission tomography-computed tomography; NSVT: Non-sustained ventricular tachycardia; RD: Repolarization disturbances; Cx: Circumflex; CAG: Coronary angiography; NSTEMI: Non-ST elevation myocardial infarction; MIBI: Methoxy-isobutyl-isonitrile; CP: Chest pain.

runs of non-sustained ventricular tachycardia (NSVT), varying from 5 to 20 beats/min with a frequency of 176/min and duration of 1800 ms, were documented in 2 patients (patients 7 and 8) (Figure 1).

ETT was inconclusive in 2 patients (1 and 6). Diagnostic CAG showed no significant coronary artery lesions in either patient. In patient number 6, the PET scan was positive due to kinking and squeezing of the SCA with a course between the aorta and pulmonary artery. This patient underwent coronary artery bypass grafting (CABG) whereby a mammary arterial graft was anastomosed to the RCA. In 3 patients (2, 5 and 10), the ETT was positive for myocardial ischemia. Of the 3 patients with positive ETT, 1 had significant CAD and underwent percutaneous coronary intervention (PCI). The other 2 patients demonstrated an intermediate lesion distally located in the coronary arterial tree and were managed medically. The ETT was negative in 5 patients (4, 7, 12, 13 and 15). Despite a negative ETT, patient number 4 showed a significant coronary lesion on CAG and underwent PCI. Patient number 7 had no significant coronary artery lesions and the ergonovine test was negative. Patient number 8 had a positive MIBI scan and CAG showed an intermediate lesion, which was managed medically. MSCT scan of 5 patients (6, 10, 13, 14 and 15) demonstrated an interarterial course and they underwent CABG, whereby a mammary arterial graft was anastomosed to the RCA in 4 and the fifth showed a benign course.

Conventional CAG demonstrated a SCA originating as a single ostium from the RSV in 6 patients and SCA originating as a single ostium from the LSV in 9 patients (Figures 2 and 3).

MSCT was performed in 6 patients (2, 6, 10, 13, 14 and 15). In patient 2, MSCT confirmed the diagnosis of SCA but gave an overestimation of the severity of the coronary lesion in the Cx trajectory, which was not significant (0.93) by fractional flow reserve measurement (Figure 4). In 4 patients (6, 10, 13 and 14), SCA was also proven by MSCT depicting clearly the course of the SCA running between the aorta and the pulmonary artery

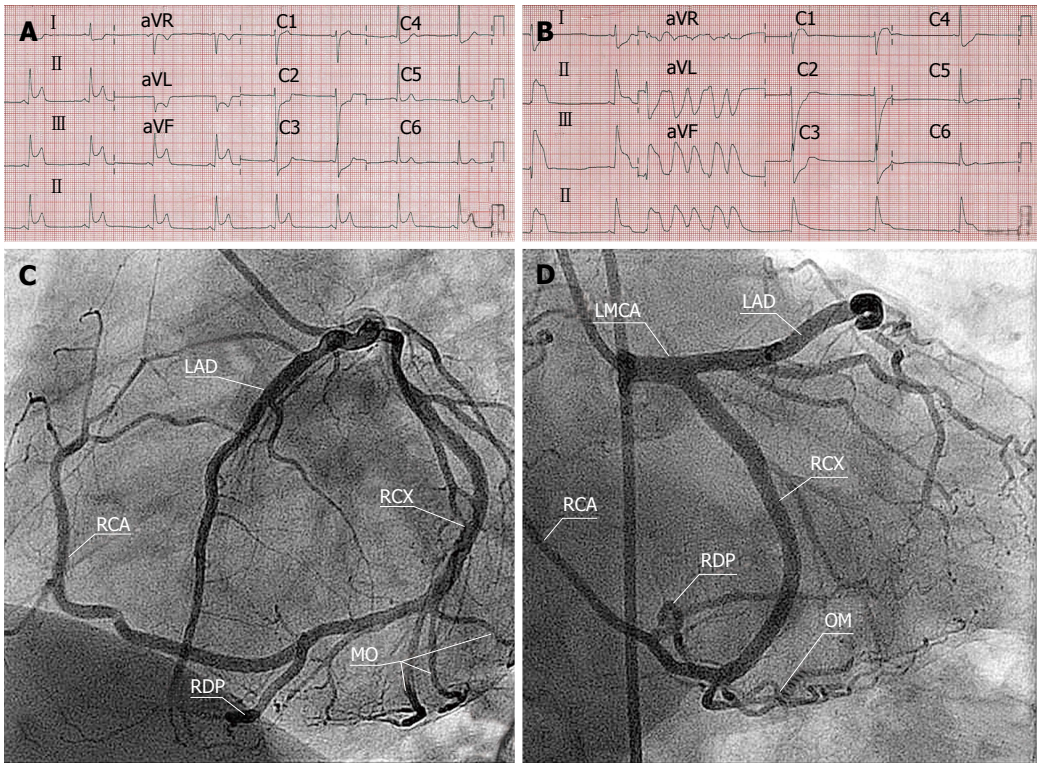

Figure 1 Resting electrocardiograph. A: Electrocardiograph during chest pain depicting transmural ischemia in the infero-posterior leads; B: Followed by a non-sustained monomorphic ventricular tachycardia; C: Coronary angiography showed absence of the right coronary ostium and a single coronary artery arising from the left sinus of Valsalva with normal origin of the left coronary artery (LCA) having normal anatomical course of the left main stem, the left anterior descending, and the circumflex artery (Lipton L-Ⅰ); D: The LCA supplies the entire myocardial tissue. No significant stenoses were found. The right coronary artery (RCA) appeared as a continuation of the distal left circumflex artery to the right atrioventricular groove and terminated near the RSV (Lipton L-Ⅰ). LAD: Left anterior descending; LMCA: Left main coronary artery; RCX: Ramus circumflexus; RDP: Ramus descending posterior; OM: Obtuse marginal.

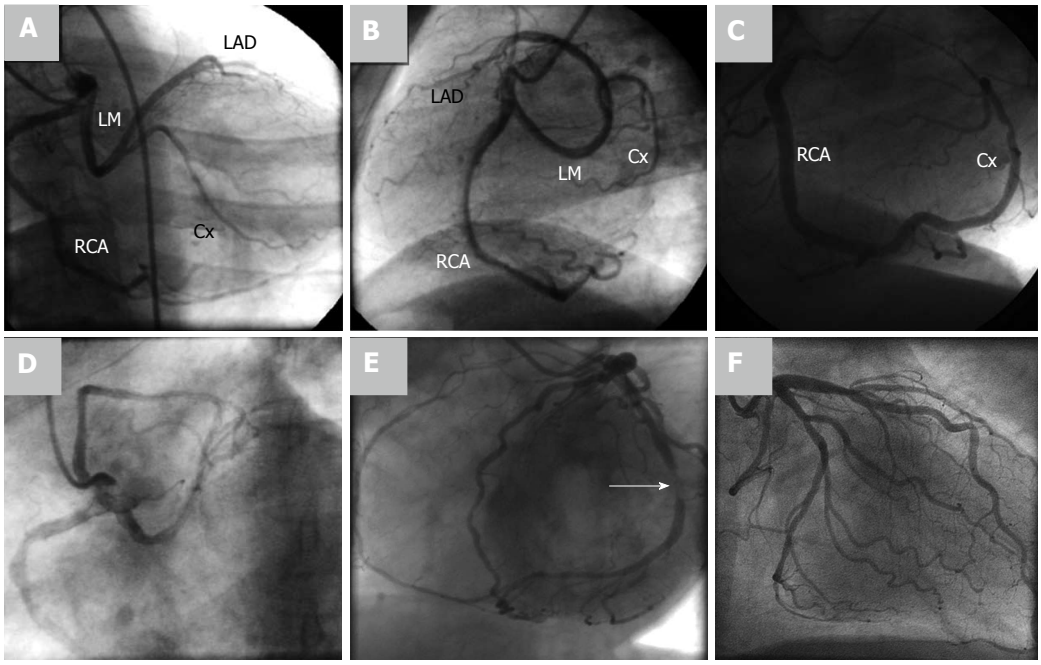

Figure 2 Coronary angiography frame. A: Coronary angiography frame of right anterior oblique projection with cranial angulation; B: Left lateral (LL) projection showing a single origin of the right and left coronary arteries from a common right coronary ostium (Lipton R-ⅡP), the long curved left main stem and right dominancy are delineated; C: Coronary angiography frame in LL projection demonstrating a single coronary artery originating from the right sinus of Valsalva (RSV) giving the left anterior descending (LAD) and continued as the circumflex artery (Lipton R-Ⅰ); D: Coronary angiography frame in left anterior oblique view demonstrating a single coronary artery arising from RSV as a single unique ostium (Lipton R-Ⅲ); E: Coronary angiography frame in left anterior oblique view showing a single coronary artery originating from the left sinus of Valsalva. The terminal branch of circumflex artery represented the right coronary artery (Lipton L-Ⅰ). Significant stenosis of the mid circumflex artery is demonstrated (white arrow); F: Coronary angiography frame demonstrates appearance of both right and left coronary arteries on injection of left sinus of Valsalva, as a single common ostium (Lipton L-ⅡA). Cx: Circumflex artery; RCA: Right coronary artery.

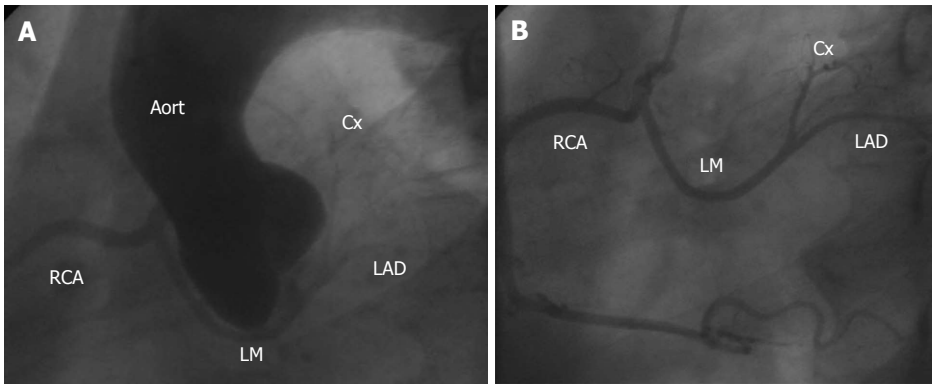

Figure 3 Angiography. A: Supravalvular aortogram in left anterior oblique projection illustrating a single origin of the coronary arteries originating from the right sinus of Valsalva (Lipton R-ⅡA); B: Selective coronary angiography frame in left anterior oblique view showing a single coronary artery from the right sinus of Valsalva. Cx: Circumflex artery; LAD: Left anterior descending; RCA: Right coronary artery.

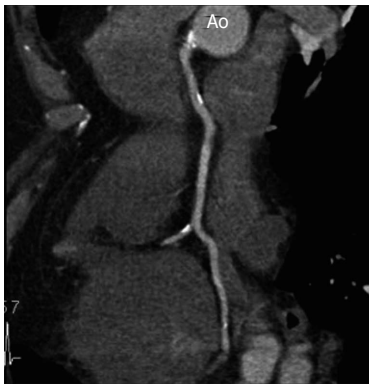

Figure 4 Transverse Multi-slice computed tomography scan in subtype (Lipton R-Ⅰ) demonstrating the origin of the single coronary artery arising from the right sinus of Valsalva supplying the whole heart.

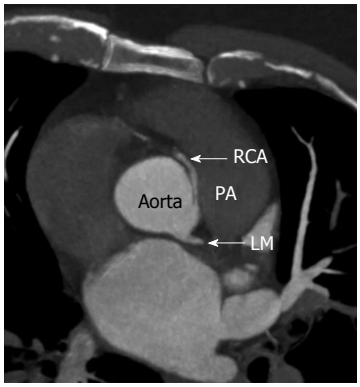

Figure 5 Volume-rendered image in subtype (Lipton L-ⅡB) demonstrating the inter-arterial course of the right coronary artery between the aorta and pulmonary artery.

(Figures 5, 6). MSCT in patient 15 demonstrated a benign course of the SCA (Figure 7).

DISCUSSION

Historical background of classifications

The coronary arterial circulation may rarely be supplied by a SCA arising from either the right, left or posterior

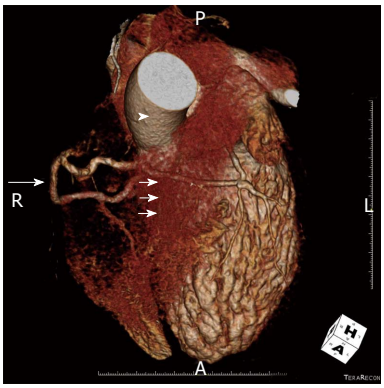

Figure 6 Three-dimensional volume-rendered image in subtype (Lipton L-ⅡB) demonstrating the inter-arterial course of the right coronary artery (long arrow) between the aorta (arrowhead) and semitransparent pulmonary artery (short arrows).

sinus of Valsalva[16]. The course of the SCA can be highly variable. In the last century, different classification systems for SCA based on necropsy findings and angiographic variants were suggested in the fifties by Smith[3] (3 types), in the seventies by Lipton *et al*[1], in the eighties by Roberts[17], and finally through the nineties by Shirani *et al*[2] and Roberts *et al*[18].

Recently, a clinically useful classification scheme has been published, using either subgroups based on the site of origin and course of the anomalous coronary artery or descriptive anatomic terminology. In 2005, Rigatelli *et al*[19,20] based his classification on clinical significance of the anomaly and launched a global practical classification of four categories (class A: benign; class B: relevant due to fixed myocardial ischemia; class C: severe, involved in sudden cardiac death (SCD); and class D: critical due to worsened clinical picture). The clinical significance and management of the various types of SCA are different as shown in Table 2.

Cheitlin *et al*[21] expressed the pathological significance of a SCA or of both coronary arteries originating from the RSV when the anomalous artery that supplies the left coronary distribution passes leftward with an inter-arterial course between the aorta and pulmonary trunk, rendering

Table 2 Clinical-significance-based classification of coronary artery anomalies by Rigatelli *et al*[19,20]

Class	Subtypes	Clinical significance	Current series
A	*E.g.*, ectopic origin of Cx from RSV[1]	Benign natural history, asymptomatic careful follow-up with conservative medical management or percutaneous intervention	Patients: none[1]
B	Ectopic origin of Cx from the RCA	Relevant, related to myocardial ischemia	Patients: 1, 2, 3, 7, 8, 9, 12, 15
	R-Ⅰ, R-Ⅱ, R-Ⅲ anterior/posterior course[2]	Careful follow-up with conservative medical management or percutaneous intervention	
C	L-Ⅰ, L-Ⅱ, L-Ⅲ anterior/posterior course[2]	Severe, potentially related to sudden cardiac death	
	R-Ⅰ, R-Ⅱ, R-Ⅲ between/interseptal course[2]	Requires surgical treatment	Patients: 6, 10, 13, 14
D	L-Ⅰ, L-Ⅱ, L-Ⅲ between/interseptal course[2]	Critical, class B or C with superimposed coronary artery atherosclerotic disease	Patients: 4, 5, 11
	B or C subgroups with concomitant coronary atherosclerosis	Requires urgent percutaneous management or surgical treatment	

[1]Not included in the current paper; [2]Classification according to Lipton *et al*[1]. Cx: Circumflex coronary artery; L: Left; R: Right; RCA: Right coronary artery; RSV: Right sinus of Valsalva.

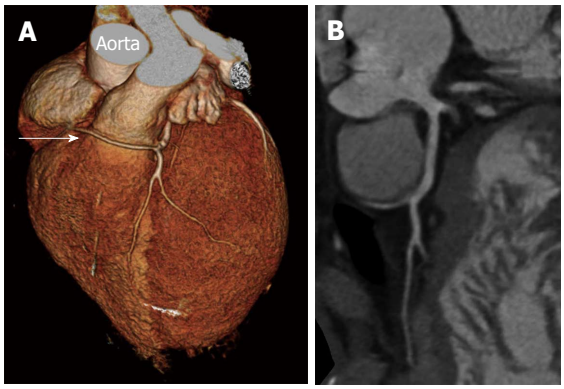

Figure 7 Single coronary artery. A: Three-dimensional volume-rendered image of benign course of right coronary artery (arrow) from left sinus of Valsalva (Lipton L-IIA); B: Transverse multi-slice computed tomography scan in subtype (Lipton L-IIA) demonstrating the origin of the single coronary artery arising from the left sinus of Valsalva supplying the whole heart.

it prone to compression and kinking on physical exercise. This variant is considered malignant since it is associated with SCD in adolescents and young adults, especially on the athletic arena. It has been found that anomalous origin of the left coronary artery from the right aortic sinus is consistently related to sudden death in more than half of the cases (53%)[18].

On the other hand, when a SCA originating from the LSV or both coronary arteries arising from separate ostia located in the LSV with the RCA passing inter-arterially (between the aorta and pulmonary trunk, *e.g.*, subtype Lipton L-ⅡB) is less deleterious even though compression can occur but SCD is a rare event. Four of our 15 patients (patients 6, 10, 13 and 14), having the above-mentioned subtype, underwent successful arterial bypass grafting to the RCA.

In a necropsy series, SCA was found in 18% of subjects. Fifty percent arose from the RSV and 50% originated from the LSV. Sudden death was twofold more frequently associated with the SCA arising from the RSV (18%) compared with those from the LSV (9%)[12].

Coronary artery anomalies are associated with life threatening symptoms and may cause SCD during or after strenuous exercise. The most common congenital coronary artery anomalies causing SCD involve an anomalous origin of either the right or left coronary artery arising from the left or the RSV, respectively[22]. SCD is common (82%) when the anomalous LCA has an inter-arterial course passing between the aorta and main pulmonary artery[12]. Moreover, SCD may rarely occur after surgical repair[23]. The incidence is very low and estimated at 0.024% to 0.098% in the general population[1,5,9,19,24]. The incidence of all coronary artery anomalies in the necropsy series is approximately 0.23% and varying from 0.3% to 13% in the angiographic series[1,9,25,26]. Recently, the incidence of SCA, using dual-source computer tomography angiography, was estimated at 0.05% in the Chinese adult population[27]. SCA may be associated with longevity and has been reported in an octogenarian[28].

Diagnostic modalities: The correct diagnosis of a SCA and its course is not always easily made based on conventional CAG only. Precise delineation of anatomical and functional characteristics requires further complementary diagnostic modalities such as MSCT or CMR[29-31].

Conventional CAG: Isolated SCA may be incidentally detected on routine CAG[32], as was the case in our current series. Even with multiple projections and different angiographic views and the use of a pulmonary artery catheter, the identification of the origin and proximal course of the vessel can be difficult[13]. Serota *et al*[33] proposed an angiographic technique (the dot-and-eye method) for rapid identification of the course of SCA but even with this method, identification remains difficult.

MSCT CAG: MSCT has been very useful in the diagnosis and identification of the origin and course of SCA[28,32]. Although the radiation dose using new algorithms is decreasing, this rapidly developing non-invasive technique still has the disadvantage of radiation exposure. However, the spatial resolution (0.4-0.6 mm^3) is higher than CMR and the temporal resolution of 64-slice double source MSCT is around 83 ms[30,31,34-37]. In 5 of our patients (patients 6, 10, 13, 14 and 15) of the current series, 128-slice

MSCT confirmed the diagnosis of a SCA with clear demonstration of the inter-arterial course of the RCA originating from the LSV in four (Figure 6) and a benign course of the RCA from LSV in one (Figure 7).

Cardiovascular MR imaging: This technique has the advantage of not using ionizing radiation and has no need for the use of iodinated nephrotoxic ionic or non-ionic contrast agents. Image acquisition occurs with fairly good spatial and temporal resolution, but acquisition and imaging time is long, which makes routine use difficult and time consuming. Cardiovascular magnetic resonance proved to be useful in determining the anatomy and functional significance of SCA[38]. Both the MSCT and the CMR imaging techniques have the additional advantage of 3-D reconstruction of the areas of the coronaries relative to the aorta and pulmonary artery. This makes a definitive diagnosis of squeezed aberrant coronary arteries between the great vessels feasible[13].

Treatment

The detection of atherosclerotic coronary artery disease (CAD) in the presence of coronary anomalies is of practical importance, especially when a decision between PCI and CABG has to be made. For diagnostic and therapeutic reasons, the knowledge of possible variations of the coronary anatomy, their different origin, and their course is of pivotal importance. Symptomatic patients with associated significant CAD may be treated with routine interventions such as PCI or CABG[6,39]. Angiographic recognition of coronary artery anomalies prior to surgery is of great importance. During operation, surgical complications may occur if an unrecognized anomalous vessel is excluded from perfusion during cardiopulmonary bypass or if the surgeon inadvertently damages an artery with an anomalous pathway.

Because of the reported high mortality, the occurrence of "symptomatic or asymptomatic" squeezing of SCA, regardless of the degree of atherosclerosis or site of origin, justifies arterial grafting, as was shown in 4 of our series (patients 6, 10, 13 and 14).

Significant atherosclerotic CAD[37] in association with coronary artery anomalies has been reported in 26%-60% of cases[1,2,40-42]. Rigatelli *et al*[43] suggested that benign coronary artery anomalies are not associated with or involved in the development of premature atherosclerotic CAD. Indeed the high percentage of coronary artery stenosis could be biased by the indication to perform CAG as SCA is mainly found during this diagnostic procedure. Only 4 of our 15 (27%) patients (patients 4, 5, 10 and 11) had significant CAD and 3 of them required percutaneous intervention. When the SCA does not course between the aorta and pulmonary artery, it is not vulnerable to acute angulations or kinking of the coronaries. SCA may be associated with longevity and patients in the 7th and 8th decade of life have been reported[3,12,13,44-47], as was the case in 2 octogenarians from our current series (patients 4 and 11).

Although a SCA is often a benign congenital anomaly, in which sudden death is a rare complication, different diagnostic modalities should be used to exclude an inter-arterial course between the aorta and pulmonary artery to detect patients at risk for serious complications.

Congenital coronary artery anomalies, detected at necropsy, associated with sudden death and without antecedent signs have been recognized in calves[48]. SCA is not limited to the human race, it has also been reported in other mammals such as horses[49], syrian hamsters[50] and minipigs[51].

As was shown in our patient's population, SCA can be associated with longevity. It has been documented up till the 8th decade of life. In the adult population, SCA-isolated or in association with acquired atherosclerotic changes-may cause severe sequelae. In some cases without CAD, the course of the SCA may be malignant.

SCA may be associated with symptomatic transient transmural myocardial ischemia, NSVT, and aborted sudden death in the absence or presence of coronary atherosclerosis. The availability of MSCT and CMR facilitates the delineation of the course of the anomalous vessel. The accurate delineation of the course of the anomalous vessel is of great importance even in patients without CAD and in cases of surgical intervention where anatomic details of the course of the vessel are of importance.

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COMMENTS

Background

Single coronary artery (SCA) is a rare congenital anomaly and occurs as an incidental finding in approximately 0.066% of the coronary angiography (CAG) population. SCA has been reported in association with and without atherosclerotic changes or in association with coronary artery fistulas, bicuspid aortic valves, and with hypertrophic cardiomyopathy.

Research frontiers

CAG is the first diagnostic tool in the detection of a SCA. Once abnormal coronary arteries are suspected, multi-slice computed tomography (MSCT) and cardiac magnetic resonance (CMR) imaging scans are excellent tools for non-invasive determination of the course of the abnormal coronaries relative to the aorta and pulmonary artery. Determination of the course of incidentally found congenital coronary anomalies during routine CAG without the direct availability of CMR or MSCT scanning is challenging.

Innovations and breakthroughs

Percutaneous coronary intervention was successfully performed in 3 patients. Eight patients were managed medically. Arterial bypass graft was performed in 4 patients with the squeezed SCA. The literature addressing SCA is reviewed.

Applications

Congenital coronary artery anomalies, detected at necropsy, associated with sudden death and without antecedent signs have been recognized in calves. SCA is not limited to the human race, it has also been reported in other mammals such as horses, syrian hamsters and minipigs.

Peer review

This paper showed that the availability and sophistications of MSCT facilitated the delineation of the course of a SCA. The authors presented a Dutch case series and review of the literature. This is an interesting report for clinical practice. Overall the report appears to be carefully examined and data adequately discussed.

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RETROSPECTIVE STUDY

Prognostic value of increased carbohydrate antigen in patients with heart failure

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Abstract

AIM: To study the prognostic value of carbohydrate antigen 125 (CA125) and whether it adds prognostic information to N-terminal pro-brain natriuretic peptide (NT-proBNP) in stable heart failure (HF) patients.

METHODS: The predictive value of CA125 was retrospectively assessed in 156 patients with stable HF remitted to the outpatient HF unit for monitoring from 2009 to 2011. Patients were included in the study if they had a previous documented episode of HF and received HF treatment. CA125 and NT-proBNP concentrations were measured. The independent association between NT-proBNP or CA125 and mortality was assessed with Cox regression analysis, and their combined predictive ability was tested by the integrated discrimination improvement (IDI) index.

RESULTS: The mean age of the 156 patients was 72 ± 12 years. During follow-up (17 ± 8 mo), 27 patients died, 1 received an urgent heart transplantation and 106 required hospitalization for HF. Higher CA125 values were correlated with outcomes: 58 ± 85 KU/L if hospitalized *vs* 34 ± 61 KU/L if not ($P < 0.05$), and 94 ± 121 KU/L in those who died or needed urgent heart transplantation *vs* 45 ± 78 KU/L in survivors ($P < 0.01$). After adjusting for propensity scores, the highest risk was observed when both biomarkers were elevated *vs* not elevated (HR = 8.95, 95%CI: 3.11-25.73; $P < 0.001$) and intermediate when only NT-proBNP was elevated *vs* not elevated (HR = 4.15, 95%CI: 1.41-12.24; $P < 0.01$). Moreover, when CA125 was added to the clinical model with NT-proBNP, a 4% ($P < 0.05$) improvement in the IDI was found.

CONCLUSION: CA125 > 60 KU/L identified patients in stable HF with poor survival. Circulating CA125 level adds prognostic value to NT-proBNP level in predicting HF outcomes.

Key words: Heart failure; Prognosis; Carbohydrate antigen 125; Brain natriuretic peptides; Survival

Core tip: Increased carbohydrate antigen 125 (CA125) has prognostic implications in acute heart failure (HF). The aim of this study was to assess the prognostic value of increased CA125 and whether it adds prognostic information to N-terminal pro-brain natriuretic peptide (NT-proBNP) in stable HF patients. Higher CA125 values correlated with outcomes. The highest risk was observed when both biomarkers CA125 and NT-proBNP were elevated *vs* not elevated (HR = 8.95, 95%CI: 3.11-25.73; $P < 0.001$). CA125 > 60 KU/L identified patients in stable HF with very poor survival. Circulating CA125 level adds prognostic value to NT-proBNP level in predicting HF outcomes.

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INTRODUCTION

Heart failure (HF) is a disease with high mortality[1] and an estimated prevalence up to 3% in the European population[2]. This prevalence increases exponentially with age, especially in patients older than 75 years. As the main cause of hospitalization, in patients over 65, HF is also associated with high costs. Despite recent advances in treatment, mortality is still high (12% per year) in stable patients, and there is a high rate of hospital readmissions due to worsening HF[3,4]. Even though a high number of clinical parameters have been associated with poor outcome in patients with stable HF, the assessment of prognosis is still a challenge[5,6]. Multiple biomarkers have been suggested to identify patients with worse prognosis, such as some interleukins, natriuretic peptides, endothelin, ST2, and several fibrosis markers[7-11]. Although most of these effectively select patients with high risk of death, only circulating levels of natriuretic peptides have proven useful in clinical practice. Natriuretic peptides are released when ventricular wall stress and ventricular end-diastolic pressure increase. Thus, an elevated level of either brain natriuretic peptide (BNP) or the amino terminal portion of N-terminal pro-BNP (NT-proBNP) in peripheral blood is associated with decompensated HF. The negative and positive predictive values of these peptides to diagnose HF have been widely studied[12-14]. Moreover, they are useful in the assessment of prognosis after a HF admission[8]. However, certain limitations affect the more extensive use of natriuretic peptides[15].

Carbohydrate antigen 125 (CA125) is a biomarker previously used in the detection and monitoring of some cancers, especially ovarian cancer[16]. It is a high-molecular-weight glycoprotein synthesized by epithelial cells of the serosa when there is inflammation and increased interstitial fluid; therefore, its level is elevated in the presence of pleural or pericardial effusion and ascites. Elevated CA125 has also been found in patients with HF, with or without fluid retention[16-19]. Although its release mechanisms are not yet well understood, they correlate with increased left ventricular end-diastolic pressure, higher BNP level, and worse New York Heart Association (NYHA) functional class[20,21]. Moreover, previous studies of BNP and CA125 in acute HF have demonstrated an additive prognostic value of CA125; therefore, the determination of both biomarkers would improve risk stratification[22]. The advantages of CA125 determination with respect to natriuretic peptides are its higher stability in the circulation and lower cost[23]. The usefulness of CA125 measurement to assess prognosis in patients admitted with acute decompensated HF has been previously demonstrated[19-22]. However, information is lacking on its value in assessing the prognosis of patients with stable chronic HF. The aim of this study was to analyze the prognostic implications of increased CA125 concentration in peripheral blood and whether it adds prognostic information to NT-proBNP in stable HF patients.

MATERIALS AND METHODS

Study population

The population was a prospective cohort of 156 patients diagnosed with HF and remitted to the outpatient HF unit for monitoring from 2009 to 2011. Patients were included in the study if they had a previous documented episode of HF and received HF treatment. The diagnosis of HF was made following the Clinical Practice Guidelines of the European Society of Cardiology[24]. HF treatment included angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin Ⅱ receptor blockers (ARBs) if ACEIs were contraindicated, beta blockers, diuretics, and aldosterone antagonists, with individualized assessment of treatment indication and optimized doses per recommendations of the Clinical Practice Guidelines of the European Society of Cardiology[24].

We excluded patients in unstable HF (NYHA functional class Ⅳ), patients with hemodynamic instability, or those diagnosed with cancer or systemic diseases that could shorten life expectancy. Patients with valve heart disease waiting for surgery repair were also excluded from the study. A 12-lead electrocardiogram (ECG) chest radiography and echocardiogram were performed in all patients to establish the HF etiology. Left ventricular systolic dysfunction was considered when ejection fraction was < 50%[25] and ventricular hypertrophy when ventricular septum or posterior left ventricular wall thickness was > 11 mm. Ischemic heart disease was diagnosed if pathologic Q waves were observed on the ECG or significant coronary lesions on the coronary angiography. Valve heart disease was diagnosed when a biological or mechanic valve prosthesis had been implanted or if the valve dysfunction

was considered significant by echocardiography.

The hospital ethics committee approved the study, patients gave informed consent to participate, and the protocol conformed to the principles outlined in the Declaration of Helsinki.

Laboratory measurements

To obtain routine laboratory determinations and NT-proBNP concentrations, fasting serum blood samples were obtained during the first visit to the HF unit. CA125 and NT-proBNP concentrations were measured with commercial electrochemiluminescence assays in a Modular E170 analyzer (both from Roche Diagnostics, Basel, Switzerland). The CA125 assay used a pair of monoclonal anti-CA125 antibodies, one labeled with biotin, the other linked to ruthenium chelates. The analyzer had a sensitivity of 0.60 KU/L, and the total imprecision at different concentrations was $\leqslant$ 2.5%, expressed as a coefficient of variation.

Statistical analysis

During follow-up, mortality or need for urgent heart transplantation and new hospital admissions due to worsening HF were assessed. The primary end-point was all-cause mortality, which included need for urgent heart transplantation. The secondary end-point was worsening HF requiring hospital admission.

Continuous variables are expressed as mean ± SD and categorical variables as percentages. Continuous variables were compared using Student's *t* test. The χ^2 or Fisher exact test was used for categorical variables. The Kruskal-Wallis test was used when necessary. Variables without homogeneous distribution were analyzed with the non-parametric Mann-Whitney test. To normalize CA125 and NT-proBNP measurements, the neperian logarithm of CA125 and NT-proBNP values was used, creating two new variables: ln-CA125 and ln-NT-proBNP. Without a clear cut-off for NT-proBNP in the literature, 3100 ng/L was selected because it was close to the mean NT-proBNP value of our population and it had the best cut-off according to area under the curve (AUC) calculation, with a sensitivity of 62% and a specificity of 82% to predict mortality. A CA125 cut-off of 60 KU/L was used, in keeping with the best predictor of mortality identified by a previous study in patients with acute HF[22]. Based on these data, a new dichotomous variable was created for a CA125 value of 60 KU/L. Also, both variables were categorized for clinical practice and better interpretation.

To avoid overfitting multivariate models, two propensity score models[26,27] were used to adjust for confounding factors. Propensity score analysis was performed using logistic regression for computing probability to fall in NT-proBNP 3100 ng/L or CA125 60 KU/L categories. This analysis was based on seven variables associated to end-points with the objective of eliminating differences in baseline patient characteristics that might affect event comparison. The AUC was 0.75 (0.66-0.84); $P < 0.001$ for NT-proBNP categories and 0.70 (0.61-0.80); $P < 0.001$ for CA125. Variables included in the propensity score were left atrial diameter, age, atrial fibrillation, left ventricular ejection fraction, estimated glomerular filtrate rate (eGFR), hemoglobin and interventricular septum thickness.

The independent associations between CA125 and NT-proBNP and survival were assessed with the Cox regression analysis. The models were adjusted for the two propensity scores performed previously.

Two Cox models were compared to test improvement in risk stratification: Cox-model 1 (adjusted model with NT-proBNP 3100 ng/L) *vs* Cox-model 3 (adjusted model with 4 groups according to the cut-off concentrations of CA125 and NT-proBNP) (Table 1): The four groups in Cox model 3 were patients with both markers below the cut-off values, with only one elevated level (assessed for each biomarker), and with both markers above the cut-offs. The increase in the prognostic utility of NT-proBNP and NT-proBNP combined with CA125 when adding sequentially to the model was evaluated by the integrated discrimination improvement (IDI) index. When two nested models are compared, IDI quantifies the increment in the predicted probabilities for the subset of patients experiencing the event and the decrease for those not experiencing the event. In simpler terms, it reflects an improvement in the average of the true positive rate without sacrificing its average true negative rate[28]. The proportionality assumption for the hazard function over time was tested by means of the Schoenfeld residuals. The discriminative ability of the final model was assessed by Harrell's *C*-statistic, and the calibration ability was assessed by the Gronnesby and Borgan test[29]. A two-sided $P < 0.05$ was considered statistically significant for all analyses. All analyses were performed using SPSS version 19.0 (Statistical Package for the Social Sciences, Chicago, Illinois) and R (R: A Language and Environment for Statistical Computing at http://www.R-project.org).

RESULTS

Baseline clinical characteristics

The mean age of the studied population was 72 ± 12 years; 63% were male. Clinical characteristics of the studied population and HF treatments according to the specified 4 groups are shown in Table 1. Mean ejection fraction was 48% ± 17%, and half (77) of the patients had preserved ejection fraction. Ischemic heart disease was the most frequent etiology of HF, followed by hypertension and valve disease. The percentage of patients with HFrEF treated with ACEI/ARB was 85%, with beta-blockers 76% and with mineral corticoid receptor antagonists 56%, while in the HFpEF group the percentage of patients treated with ACEI/ARB was 69%, with beta-blockers 42% and with mineral corticoid receptor antagonists 39%. The percentage of patients treated with furosemide was similar in both groups, 84%.

Outcomes

No patient was lost during the mean follow-up of 17 ± 8 mo (range 2 to 32 mo). Monitoring of patients was blind-

Table 1 Baseline characteristics by carbohydrate antigen 125 and N-terminal pro-brain natriuretic peptide class

Variable	CA125 < 60 NT-proBNP < 3100 (*n* = 95, 61%)	CA125 > 60 NT-proBNP < 3100 (*n* = 17, 11%)	CA125 < 60 NT-proBNP > 3100 (*n* = 24, 15%)	CA125 > 60 NT-proBNP > 3100 (*n* = 20, 13%)	*P*
Male	55 (58%)	11 (65%)	19 (79%)	14 (70%)	0.240
Age	71 ± 12	69 ± 10	76 ± 12	73 ± 15	0.309
Hypertension	73 (77%)	10 (59%)	18 (75%)	14 (70%)	0.471
Diabetes	38 (40%)	7 (41%)	11 (46%)	8 (40%)	0.964
Dyslipidemia	46 (48%)	5 (29%)	11 (46%)	7 (35%)	0.406
Atrial fibrillation	47 (49%)	9 (53%)	17 (71%)	11 (55%)	0.316
NYHA class Ⅱ	85 (89%)	7 (41%)	13 (54%)	11 (55%)	< 0.001
NYHA class Ⅲ	10 (11%)	10 (59%)	11 (46%)	9 (45%)	< 0.001
HF Etiology					
Hypertension	26 (27%)	1 (6%)	5 (21%)	3 (15%)	0.212
Ischemic heart disease	30 (32%)	7 (41%)	8 (33%)	9 (45%)	0.641
Dilated cardiomyopathy	8 (8%)	2 (12%)	1 (4%)	2 (10%)	0.768
Valve heart disease	15 (16%)	5 (29%)	4 (17%)	5 (25%)	0.439
Congenital	0 (0%)	1 (6%)	1 (4%)	1 (5%)	0.058
Others	16 (17%)	1 (6%)	5 (21%)	0 (0%)	0.110
Systolic BP (mmHg)	129 ± 18	118 ± 20	119 ± 21	114 ± 12	0.001
Diastolic BP (mmHg)	74 ± 12	67 ± 8	73 ± 14	71 ± 9	0.156
LVEF (%)	51 ± 16	46 ± 16	46 ± 22	38 ± 18	0.015
LVDD (mm)	54 ± 8	52 ± 9	56 ± 13	57 ± 11	0.290
LAD (mm)	49 ± 9	49 ± 9	51 ± 9	54 ± 12	0.184
IVS (mm)	12 ± 3	12 ± 2	13 ± 3	11 ± 3	0.158
LVPW (mm)	11 ± 2	10 ± 2	11 ± 2	10 ± 2	0.461
Na^+ (mEq/dL)	140 ± 3	138 ± 4	140 ± 4	139 ± 5	0.227
K^+ (mEq/dL)	4.2 ± 0.5	4.3 ± 0.6	4.4 ± 0.7	4.2 ± 0.5	0.453
GF (mL/min per 1.73 m^2)	61 ± 20	62 ± 16	49 ± 22	53 ± 20	0.029
Hemoglobin (g/dL)	131 ± 20	118 ± 25	126 ± 21	129 ± 24	0.111

LVEF: Left ventricular ejection fraction; NYHA: New York Heart Association; HF: Heart failure; LVDD: Left ventricular diastolic diameter; LAD: Left auricular diameter; IVS: Interventricular septum; LVPW: Left ventricular posterior wall; BP: Blood pressure; Na: Sodium; K: Potassium; GF: Glomerular filtration; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

Table 2 Univariate analysis, variables significantly associated with mortality or need for urgent cardiac transplantation

Variable	Deaths	Alive	*P*
Age	78 ± 12	70 ± 12	0.004
LVEF (%)	53 ± 16	47 ± 17	0.08
IVS (mm)	13.7 ± 3.4	11.9 ± 2.5	0.005
LA (mm)	54 ± 12	49 ± 9	0.01
GF (MDRD)	46 ± 18	61 ± 19	0.0001
Hemoglobin (g/L)	121 ± 21	130 ± 21	0.02
Na^+ (mEq/mL)	140 ± 4	139 ± 3	0.54
K^+ (mEq/mL)	4.3 ± 0.6	4.2 ± 0.5	0.54
Systolic BP (mmHg)	120 ± 19	125 ± 19	0.24
Diastolic BP (mmHg)	70 ± 12	73 ± 12	0.25
CA125 (KU/L)	94 ± 121	45 ± 78	0.01
NT-proBNP (pg/dL)	6613 ± 8437	2326 ± 2823	0.02
AF (%)	71	50	0.05
NYHA class Ⅲ (%)	70	30	0.03

LVEF: Left ventricular ejection fraction; IVS: Interventricular septum; LA: Left atrial; GF: Glomerular filtration; Na: Sodium; K: Potassium; BP: Blood pressure; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide; AF: Atrial fibrillation; NYHA: New York Heart Association; MDRD: Modification of diet in renal disease.

ed to CA125 values. During follow-up, 27 patients died (17%), and 1 had progressive HF deterioration requiring urgent heart transplantation at one year. All 27 deaths were due to cardiovascular causes, 23 (85%) of them to progressive HF (3 of which required hospital admission for decompensated HF) and 3 to cerebrovascular accident. The remaining patient had a diagnosis of ischemic heart disease and was lost to sudden death while being treated with chemotherapy for rectal cancer that appeared during follow-up. Mortality was similar for women (19%) and men (18%). There was a high incidence of worsening, new HF episodes: 106 patients required an admission for decompensated HF (68%), and nearly half (50 patients) were admitted more than once for HF during follow-up.

Prognostic implications of CA125

Older age, increased ventricular septum thickness and left atrial diameter, low eGFR and hemoglobin concentration, higher CA125 and NT-proBNP levels, the presence of atrial fibrillation, and worse NYHA functional class were all associated with mortality or need for urgent heart transplantation by univariate analyses (Table 2). Hospitalization for worsening HF was associated with low eGFR, higher CA125 and NT-proBNP values, presence of atrial fibrillation, and worse NYHA functional class by univariate analyses (Table 3). Patients who died and those who required admission for decompensated HF had significantly lower eGFR, had higher CA125 and NT-proBNP, and more frequently had atrial fibrillation and NYHA functional class Ⅲ. In univariate analyses, ln-CA125 was positively associated with ln-NT-proBNP ($\beta = 0.39$, $P =$

Table 3 Univariate analysis, variables significantly associated with need for hospitalization for decompensated heart failure

	Hospitalization	No hospitalization	*P*
LVEF (%)	48 ± 18	49 ± 17	0.7
IVS (mm)	12 ± 3	11 ± 3	0.14
LA (mm)	50 ± 9	48 ± 10	0.15
GF (MDRD)	56 ± 19	64 ± 21	0.01
Hemoglobin (g/L)	127 ± 22	132 ± 20	0.16
Na^+ (mEq/mL)	139 ± 3.7	140 ± 3.6	0.4
K^+ (mEq/mL)	4.2 ± 0.5	4.3 ± 0.6	0.3
Systolic BP (mmHg)	124 ± 18	125 ± 20	0.9
Diastolic BP (mmHg)	73 ± 11	72 ± 13	0.7
CA125 (KU/L)	58 ± 85	34 ± 61	0.01
NT-proBNP (pg/dL)	3431 ± 4792	2031 ± 3234	0.03
AF (%)	61	39	0.01
NYHA class Ⅲ (%)	97	3	0.0001

LVEF: Left ventricular ejection fraction; IVS: Interventricular septum; LA: Left atrial; GF: Glomerular filtration; Na: Sodium; K: Potassium; BP: Blood pressure; CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide; AF: Atrial fibrillation; NYHA: New York Heart Association.

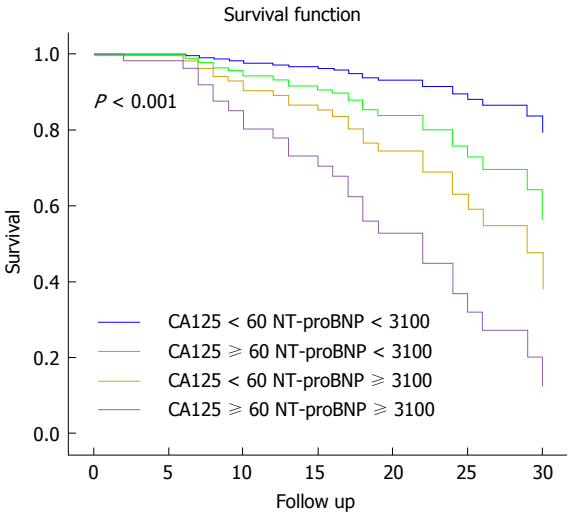

Figure 1 Kaplan-Meier survival curves in the 4 groups defined by N-terminal pro-brain natriuretic peptide < or ⩾ 3100 ng/L and carbohydrate antigen 125 < or ⩾ 60 KU/L. Patients with both biomarkers elevated presented the worst survival. CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

0.0001) and negatively with left ventricular ejection fraction (β = -0.23, *P* = 0.003) and sodium concentration (β = -0.24, *P* = 0.003). There was no relationship between ln-CA125 and eGFR or hemoglobin. The receiver operating characteristic curve analysis showed similar values for CA125 and NT-proBNP; the AUC for mortality prediction was 0.699 (95%CI: 0.59-0.80) for CA125 and 0.710 (95%CI: 0.59-0.82) for NT-proBNP.

The relationship between CA125 and outcomes was evaluated in multivariate Cox proportional analyses. Cox-model 1 identified NT-proBNP ⩾ 3100 ng/L and Cox-model 2 identified CA125 ⩾ 60 KU/L as independent predictors of mortality (Table 4). Two Cox models were compared to test improvement in risk stratification: Cox-model 1 (adjusted model with NT-proBNP ⩾ 3100 ng/L) *vs* Cox-model 3 (adjusted model with 4 groups according to the cut-off concentrations of CA125 and NT-proBNP). Cox-model 1 had good discriminative ability (Harrell's *C*-statistic = 0.774), and Cox-model 3 had slightly better discriminative ability (Harrell's *C*-statistic = 0.777) and good calibration ability (*P* = 0.39, Gronnesby and Borgan test). However, the *C*-statistic is not sensitive to changes when a new factor is included in a model. Therefore, when we compared Cox-model 1 *vs* Cox-model 3 by the IDI index, the discrimination slope of the Cox-model 3 was 4 percentage points higher than model 1 (*P* < 0.05). Survival curves adjusted by propensity scores from Cox-model 3 (Figure 1) showed risk stratification when HF patients were classified into the 4 groups defined by NT-ProBNP and CA125 cut-offs. Patients with both biomarkers elevated presented the worst survival.

Table 4 Multivariate analysis for global mortality

	HR	95%CI	Sig
Cox-model 1 variable			
NT-proBNP (3100 ng/L)	4.95	2.11-11.62	< 0.001
Cox-model 2 variable			
CA125 (60 KU/L)	3.32	1.50-7.37	0.003
Cox-model 2 variable			
CA125 < 60 NT-proBNP < 3100	1		
CA125 > 60 NT-proBNP < 3100	2.49	0.65-9.53	0.18
CA125 < 60 NT-proBNP > 3100	4.15	1.41-12.24	< 0.01
CA125 > 60 NT-proBNP > 3100	8.95	3.11-25.73	< 0.001

Adjusted by propensity scores of NT-proBNP > 3100 ng/L and CA125 > 60 KU/L categories. CA125: Carbohydrate antigen 125; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

DISCUSSION

In our study, a concentration of CA125 > 60 KU/L was identified as an independent predictor of mortality or of the need for urgent heart transplantation at mid-term follow-up. This biomarker also added prognostic information beyond that provided by NT-proBNP concentration. Patients with NT-proBNP ⩾ 3100 ng/L and CA125 ⩾ 60 KU/L had extremely low survival during follow-up.

CA125 and HF

CA125 is a cancer marker that can be synthesized in mesothelial cells from the peritoneum and pleura[30]. CA125 concentration is increased in patients with pericardial, pleural, and peritoneal effusions[30]. Increased CA125 concentration in patients with HF is believed to be multifactorial and secondary to the increased end-diastolic and pulmonary venocapillary pressure that causes interstitial pulmonary edema[31], an inflammatory substrate present in HF. In addition, the appearance of pleural effusion and signs of congestion have been associated with increased CA125[32].

The good correlation of CA125 with natriuretic peptides suggests a similar release mechanism related to an increase in intracavitary pressures and stress of both atrial and ventricular walls. It has also been suggested that

mesothelial cells can secrete CA125 in response to activation of cytokines. Thus, the activation of either TNF-alpha or interleukin-6 has been associated with elevated CA125, and the increase of these cytokines correlates with the severity of HF[7,18]. It has been suggested that increased CA125 can be a sign of both congestion and inflammation; both parameters have been associated with poor prognosis in HF patients. Furthermore, due to the long half-life of CA125 (about 12 d) it can be used as an indirect marker of fluid retention in the weeks preceding blood extraction, in both acute and chronic HF. Especially in patients with stable HF, CA125 ⩾ 60 KU/L can be an early sign of congestion that would identify patients with worse prognosis.

Previous studies

Numerous studies have examined the value of natriuretic peptides to assess the prognosis of HF[33-36], most of them performed in the emergency room or in patients with acute decompensated HF requiring hospitalization. Few of the studies focus on stable chronic HF[37,38]. Earlier studies in acute HF patients associated increased CA125 with worse NYHA functional class, greater left atrial size, higher BNP, and poor prognosis at 6 mo follow-up[19,20,22]. A recent study by Ordu *et al*[37] reports that increased CA125 and NT-proBNP were similar in their capacity to predict prognosis in patients with stable HF[38]. Although our population differed from theirs, which included only patients with systolic dysfunction and elevated NT-proBNP, the CA125 concentrations were similar in both studies. Our patients were better controlled, with higher proportions receiving ACEI/ARB, beta-blockers and spironolactone therapy. Both studies report a significant correlation between CA125 and NT-proBNP. However, Ordu *et al*[37] used a higher NT-proBNP cut-off value for patient stratification than previous studies[11,15]. It is possible that with this high cut-off value the addition of CA125 did not improve upon the predictive value obtained with only NT-proBNP. However, lower NT-proBNP is frequently found in stable HF patients. In our study, CA125 ⩾ 60 KU/L added prognostic information to that obtained from the ⩾ 3100 ng/L NT-proBNP cut-off. In fact, when both biomarkers were elevated, the prognosis was very poor. In another recent study performed in stable patients with left ventricular dysfunction, a value of CA125 ⩾ 60 KU/L was associated with an increased risk of cardiovascular death and hospitalization for HF[22]. Our study did not select patients according to the degree of left ventricular dysfunction; all patients diagnosed with HF were included. A high level of CA125 allowed the identification of patients with worse prognosis independently of their ejection fraction. This is important because almost half of all patients diagnosed with HF in the majority of epidemiological studies have preserved ejection fraction[39]. Furthermore, new biomarkers are under development to improve diagnosis and prognosis assessment in HF. Recently, experimental studies have suggested that changes in circulating microRNAs can be used as a biomarker of disease[40,41]. However, these new molecular markers are still under investigation, On the contrary, more than 10 years of experience with natriuretic peptides and CA125 have already been reported[12,20].

Although the value of natriuretic peptides for the diagnosis of HF has been widely demonstrated, its usefulness to assess prognosis in some cases remains controversial. Thus, in advanced HF, there is controversy whether BNP concentration helps identify patients requiring heart transplantation[42,43]. Similarly, in asymptomatic individuals with HF and those in pre-HF stages, NT-proBNP failed to predict the patients who presented with clinical HF during follow-up[44]. Other limitations to the use of natriuretic peptides exist, particularly with NT-proBNP since cut-off points for assessing prognosis have not been clearly established. Therefore, the addition of CA125 may help to improve prognosis assessment in patients with stable HF.

Limitations

In this study the inclusion criterion was the diagnosis of HF. The predictive value of CA125 possibly could have been even greater if left ventricular dysfunction had been a selection criterion. Although the cohort was relatively small, mortality and hospitalization rates were similar to previous studies analyzing stable HF patients. When the study population was divided according to CA125 and NT-proBNP values, some subgroups were quite small. Nonetheless, increased CA125 concentration was effective in identifying stable HF patients at high risk of death and new admissions for worsening HF, and despite having a relatively small sample size the results are consistent with those obtained in previous studies in acute HF.

In conclusion, CA125 is an excellent marker of prognosis in patients with stable HF. CA125 ⩾ 60 KU/L identified patients at high risk of death or need for urgent heart transplantation. CA125 added prognostic information to the predictive value of NT-proBNP. Its easy determination and low cost may encourage its expanded use.

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COMMENTS

Background

Heart failure (HF) is the final phase of many heart diseases. The estimated prevalence is up to 3% in the European population. This prevalence increases exponentially with age. As the main cause of hospitalization, in patients over 65, HF is also associated with high costs. Despite recent advances in treatment, mortality is still high (12% per year) in stable patients, and there is a high rate of hospital readmissions due to worsening HF. Even though a high number of clinical parameters have been associated with poor outcome in patients with stable HF, the assessment of prognosis is still a challenge.

Research frontiers

Several biomarkers have been used to assess the prognosis of patients with HF. Although the natriuretic peptides are considered the most widely used bio-

markers, they still have some limitations. Increased concentrations of natriuretic peptides have been associated with worse prognosis in patients hospitalized for worsening HF. However, its utility to identify patients at high risk of death in stable HF has been less studied. Furthermore, although new molecular biomarkers are under development to improve prognosis assessment in HF, they are not ready for clinical use. On the other hand, more than 10 years of experience with natriuretic peptides and carbohydrate antigen 125 (CA125) have already been reported.

Innovations and breakthroughs

This study provides the results of a clinical investigation demonstrating that the combination of two well-known biomarkers, CA125 and N-terminal pro-brain natriuretic peptide (NT-proBNP), is useful to select patients with stable HF and poor outcome. CA125 concentration, when added to NT-proBNP, provides relevant prognostic information.

Applications

CA125 concentration can be routinely added to NT-proBNP assessment in stable HF patients, at a low cost, to identify patients at high risk of death or a worsening HF episode.

Terminology

CA125 is a biomarker previously used in the detection and monitoring of some cancers. It is a high-molecular-weight glycoprotein synthesized by epithelial cells of the serosa when there is inflammation and increased interstitial fluid. Elevated CA125 has also been found in patients with HF, with or without fluid retention.

Peer review

The authors report on the prognostic valve of CA125 in patients with stable chronic HF. This biomarker has proven its validity in previous studies, in this manuscript has additive value, due to the combination of the new biomarker with the established brain natriuretic peptide-value.

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CASE REPORT

Cardiac embolism after implantable cardiac defibrillator shock in non-anticoagulated atrial fibrillation: The role of left atrial appendage occlusion

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Abstract

Cardioembolic events are one of the most feared complications in patients with non-valvular atrial fibrillation (NVAF) and a formal contraindication to oral anticoagulation (OAC). The present case report describes a case of massive peripheral embolism after an implantable cardiac defibrillator (ICD) shock in a patient with NVAF and a formal contraindication to OAC due to previous intracranial hemorrhage. In order to reduce the risk of future cardioembolic events, the patient underwent percutaneous left atrial appendage (LAA) occlusion. A 25 mm Amplatzer™ Amulet was implanted and the patient was discharged the following day without complications. The potential risk of thrombus dislodgement after an electrical shock in patients with NVAF and no anticoagulation constitutes a particular scenario that might be associated with an additional cardioembolic risk. Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a very valid alternative for patients with NVAF and a formal contraindication to OAC.

Key words: Left atrial appendage; Implantable cardiac defibrillator; Defibrillator; Atrial fibrillation

Core tip: The present case report discusses the treatment of a patient with atrial fibrillation and contraindication to anticoagulation who presented with a massive peripheral embolism after an implantable cardiac defibrillator shock. The manuscript describes the successful management of the patient and discusses a clinical setting that might be associated with an increased cardioembolic risk.

Freixa X, Andrea R, Martín-Yuste V, Fernández-Rodríguez D, Brugaletta S, Masotti M, Sabaté M. Cardiac embolism after implantable cardiac defibrillator shock in non-anticoagulated atrial fibrillation: The role of left atrial appendage occlusion. *World J Cardiol* 2014; 6(4): 213-215 Available from: URL: http://www.wjgnet.com/1949-8462/full/v6/i4/213.htm DOI: http://dx.doi.org/10.4330/wjc.v6.i4.213

INTRODUCTION

Cardioembolic events are one of the most feared complications in patients with non-valvular atrial fibrillation (NVAF) and a formal contraindication to oral anticoagulation (OAC). In these patients, the risk of stroke can generally be predicted using the CHA_2DS_2VASc score[1]. However, factors not contemplated in the CHA_2DS_2-

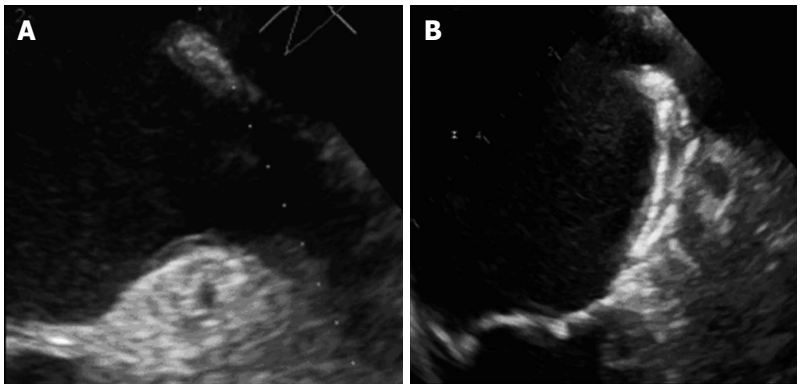

Figure 1 Left atrial appendage before and after occlusion with an Amplatzer Amulet. Left atrial appendage before (A) and after (B) percutaneous occlusion.

VASc score may also play a relevant role. One of these factors might be the presence of implantable cardiac defibrillators (ICD) and the potential risk of thrombus dislodgement after electrical shocks. In the following report, we describe a case of massive peripheral embolism after an ICD shock in a patient with NVAF and a formal contraindication to OAC. In order to reduce the risk of new cardioembolic events, the patient underwent percutaneous left atrial appendage (LAA) occlusion. Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a valid alternative for patients with NVAF and a formal contraindication to OAC.

CASE REPORT

This was a 61-year-old male with a previous history of hypertension, diabetes, stroke, dilated cardiomyopathy and ICD for secondary prevention. The patient also presented chronic NVAF with a CHA_2DS_2VASc of 5 treated initially with OAC. Anticoagulation was, however, discontinued after an episode of intracranial hemorrhage and single aspirin treatment was started. Six months after OAC discontinuation, the patient was admitted with a massive abdominal embolism after an appropriate ICD shock requiring mechanical aspiration of emboli in the right hepatic and superior mesenteric arteries. After consultation with the neurology department, reintroduction of OAC was not recommended as a result of the risk of recurrent intracranial bleeding. Transesophageal echocardiography (TEE) showed no thrombus in the LAA and a mean diameter of 22 mm at the landing area. Considering the high risk of thrombus formation as a result of the slow LAA blood flow velocity (0.3 m/s) and the risk of cardioembolic recurrence after another potential ICD shock, percutaneous LAA occlusion with a 25 mm Amplatzer™ Amulet™ was conducted without complications (Figure 1). The patient was discharged the following day under dual antiplatelet therapy. At 3 mo, TEE showed complete LAA sealing and the patient was left on single antiplatelet therapy again.

DISCUSSION

In patients with NVAF, cardioembolic strokes are generally more disabling and more lethal than strokes from other sources[2]. Although OAC has been shown to be highly effective in reducing the rate of cardioembolic events and deaths[3], between 30% and 50%[4] of patients present a formal contraindication for OAC, have unstable international normalized ratios or are not fully compliant. Currently, percutaneous LAA occlusion represents a valid alternative in patients with NVAF and a formal contraindication for OAC, but it might also be considered for those at high risk of bleeding or drug cessation (Ⅱ-b indication)[5]. Although the presence of an ICD is not contemplated in the CHA_2DS_2VASc score, the authors believe that it should be taken into consideration when assessing the cardioembolic risk in patients with NVAF and no anticoagulation. In fact, the incidence of cardioembolic events after electrical shocks remains high in these patients, ranging between 5% and 7% with every shock[6]. In addition, the CHA_2DS_2VASc score in patients with ICDs is generally high as a result of the increased cardiovascular comorbidity. The usual high CHA_2DS_2VASc score of this population, the unpredictable formation of thrombus in the LAA without anticoagulation, and the increased risk of thrombus dislodgement after ICD shocks constitute a particular scenario that might be associated with a high risk of cardioembolic events. In this sense, the occlusion of the LAA, an anatomical structure related with most cardioembolic events, might be a valid alternative. Although further evidence will be necessary to determine if the presence of ICD constitutes an independent predictor of cardioembolic events in patients with NVAF and the absence of OAC, the present case report is hypothesis generating as it highlights the specific risk of these patients and describes a potential alternative for their management.

COMMENTS

Case characteristics

Secondary prevention for cardiac embolism in a patient with previous peripheral embolism and non-anticoagulated atrial fibrillation.

Clinical diagnosis

Non-anticoagulated atrial fibrillation in a patient with previous implantable cardiac defibrillator and cardiac embolism after electrical shock.

Differential diagnosis

The potential risk of thrombus dislodgement after an electrical shock in patients with atrial fibrillation and no anticoagulation constitutes a particular scenario

that might be associated with an additional cardioembolic risk.

Imaging diagnosis

Previous intracranial hemorrhage.

Pathological diagnosis

Non-valvular atrial fibrillation (NVAF) with formal contraindication to anticoagulation due to previous intracranial bleeding.

Treatment

Percutaneous left atrial appendage (LAA) occlusion.

Experiences and lessons

Although LAA occlusion is a relatively new technique, its usage is rapidly expanding worldwide and constitutes a valid alternative for patients with atrial fibrillation and a formal contraindication to oral anticoagulation (OAC).

Peer review

The authors reported a case with non-valvular atrial fibrillation and a formal contraindication to OAC, who had undergone percutaneous LAA occlusion after the occurrence of massive abdominal embolism. This case report is interesting and suggestive but there are several questions to be solved.

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GENERAL INFORMATION

World Journal of Cardiology (*World J Cardiol*, *WJC*, online ISSN 1949-8462, DOI: 10.4330) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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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 PMCID: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]

Issue with no volume

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. *HRSA 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

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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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 Euro-

pean 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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Write as mean ± SD or mean ± SE.

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