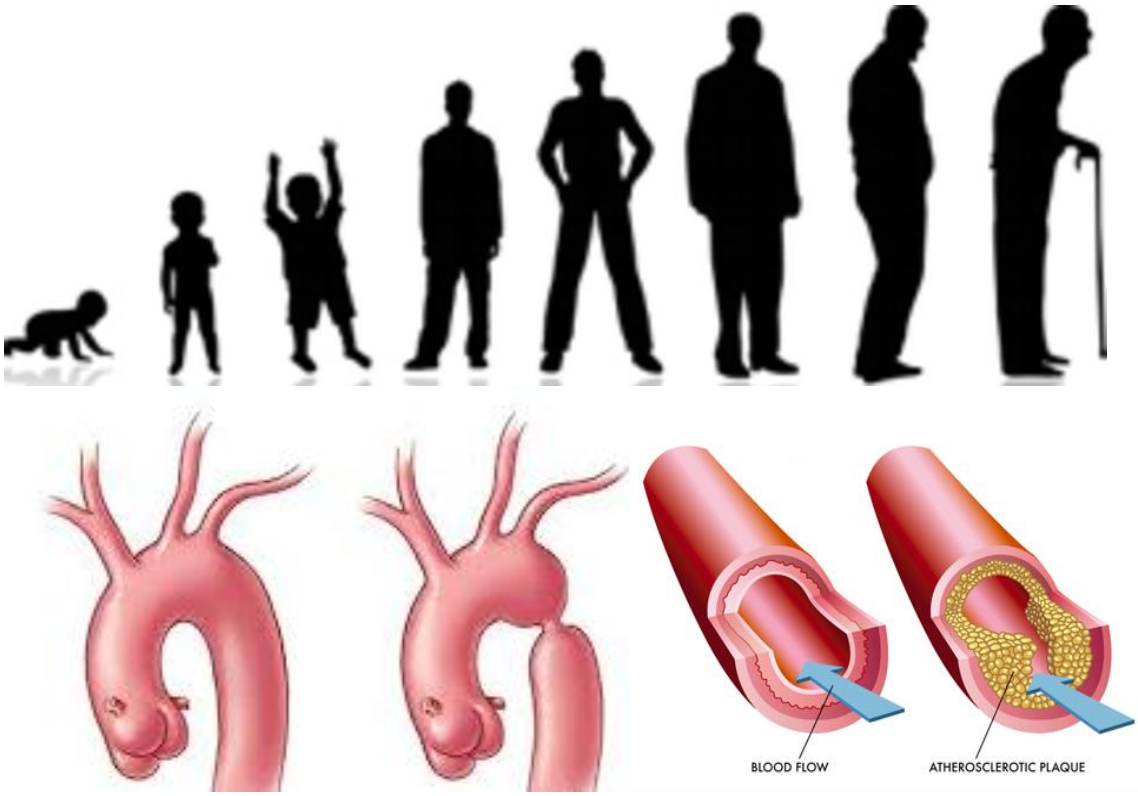
**“International PhD program in Cardiovascular Pathophysiology and Therapeutics – CardioPaTh”**




# CARDIOVASCULAR RISK ASSESSMENT AND OUTCOMES: FROM CHILDHOOD TO ADULTHOOD

**“Senectus ipsa est morbus?”**

**FABIO MARSICO**

# CARDIOVASCULAR RISK ASSESSMENT AND OUTCOMES: FROM CHILDHOOD TO ADULTHOOD

"Senectus ipsa est morbus?"

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# CHAPTER 1.

## 1. Introduction

Although the major part of cardiovascular (CV) events in childhood are characterized by congenital heart disease (CHD), there is a wide spectrum of CV events that start from conventional CV risk factors that are present also in children, arriving to atherosclerotic events, most of all in some particular populations (i.e. patients with rheumatic diseases, obese patients, patients with HIV, patients with kidney disease, etc.). On the other hand, the large use of some pharmacological agents can play a role in the development of CV events, generally atherosclerotic. This aspect, that normally involve adult patients, could affect also children. There is also a spectrum of causes related to heart failure, that generally involve adults, but that can start from childhood. However, CHD remain the most common CV presentation in childhood. Thanks to progress in surgery, a great part of these patients arrives to adulthood, where they are included in another classification called grown-up congenital heart disease (GUCH).

### *1.1. Cardiovascular diseases could start from childhood*

Atherosclerotic CV disease remains the leading cause of morbidity and mortality worldwide, but manifest disease in childhood and adolescence is rare (1). By contrast, risk factors responsible of atherosclerosis are present since childhood. The autopsy findings in The Bogalusa Heart Study and Pathobiological Determinants of Atherosclerosis in Youth showed a strong relationship of vascular disease to traditional CV risk factors (2,3). This

observation provided irrefutable evidence of the importance of act on modifiable CV risk factors, such as cigarette smoking, hypercholesterolemia, hypertension, obesity and diabetes (4–6). On the other hand, there are several emerging CV risk factors, that have not been widely studied (i.e. C reactive protein, homocysteine, small dense low density lipoprotein, oxidized low density lipoprotein, apoliproteins, lipoprotein a, fibrinogen) (7), and also some non-modifiable atherosclerosic risk factors (i.e. age, gender, CV family history), that don't permit to reduce completely the CV burden in childhood, resulting in a great CV risk in adulthood. Considered a relatively recently discovered CV risk factor, common carotid artery intima-media thickness (IMT) measured by ultrasound imaging, represents a marker of preclinical atherosclerosis, correlating with vascular risk factors (8–10), relating to the severity and extent of coronary artery disease (8). Several studies, in particular in some specific conditions (i.e. obesity, HIV), have shown an increase of IMT also in childhood (11,12). Finally, a more recent parameter of CV risk has been studied, epicardial adipose tissue (EAT), that is part of the visceral fat deposited within the pericardic sac around the heart. EAT shares a common embryological origin with the intra-abdominal visceral adipose tissue and, as such, it is a metabolically active adipose tissue (13,14). Hence, although EAT represents only 1% of the total body fat mass, it may be important in the pathophysiology of CV disease, in particular in obese patients and in HIV patients (13,14). Well known is the role of EAT in adult patients, instead weak is known about its possible role in CV risk in childhood, in particular in HIV young patients. These CV markers play an important role in a subclinical atherosclerosis, which can lead to

coronary artery disease. For this reason, very important is also the role of imaging techniques to detect very early subclinical coronary artery disease, to treat as soon as possible CV disease, for a better prognosis. There is also a large part of iatrogenic CV events, that could start from childhood, but that generally are more frequent in adult patients, due to the large use of some medications in adulthood. In particular, in patients with rheumatic diseases, that could be present also in childhood, there is a wide use of non-steroid anti-inflammatory drugs (NSAIDs), that have many side effects, not only on gastrointestinal system, but also at cardiac level (15), increasing the risk of ischemic disease and heart failure. Although heart failure is typically an "older disease", there are many situations related to its development that can start since childhood. First of all, many CHD could lead to heart failure, also HIV infection could be related to left ventricular dysfunction, but there are also many other causes of heart failure development, that go from some CV risk factors implicated not only in atherosclerotic events (i.e. diabetes) (16–18), to a deficiency of some factors (one of the most important is vitamin D) (19). There are two points to define in these cases: first of all, these two causes can start since childhood, leading to CV events very early, and secondary, there are new treatment approach for diabetes (i.e. inhibitors of sodium glucose co-transporter-2, and glucagon-like peptide-1 agonists) (16–18) and also vitamin D supplementation (20,21), that could be responsible of a regression of CV events in these patients.

### *1.2. Congenital heart disease: from childhood to GUCH*

CHD is the most common cause of major congenital anomalies, and 28% of all major

congenital anomalies consist of heart defects (22,23). Reported birth prevalence of CHD varies widely among studies worldwide. The estimate of 8 per 1,000 live births is generally accepted as the best approximation (22,24). A reliable classification of CHD should be the one that define a cyanotic pattern, or ductal-dependent classification, considering an important role of ductus arteriosus in the survival of these patients. In the group of congenital heart disease, there are also some known mutations, that involve the heart, leading to some pattern of cardiomyopathy that sometimes are incompatible with survival. One of these are the mitochondrial disorders, which are generally associated with congenital hypertrophic cardiomyopathy, with a fatal prognosis. Without a treatment, most defects of moderate or great complexity have a bleak prognosis (25). Thanks to the progress in cardiac surgery, since many years, a great part of patients born with CHD, reach adulthood, creating a completely new and steadily growing patient population: patients with grown-up congenital heart disease (GUCH) (22). It is estimated that about 90% of affected children are expected to survive to adulthood (26), with an estimated prevalence of CHD of 4 per 1,000 adults (27). Patients with GUCH often need long-term expert medical care and healthcare-related costs are high (28). In fact, it is recognized that CHD is associated with lifelong comorbidity that affect healthcare costs. The impact of ongoing disease burden includes atrial arrhythmias, pulmonary hypertension, arterial hypertension, heart failure, cerebrovascular events, coronary events, and a repeated need for surgery, which results in significant increase in health services utilization during childhood, transition years, adulthood, and geriatric age group (29). A particular mention

is due to pulmonary hypertension, that is present in most part of shunt-defects, and that is responsible of a bad prognosis in these patients and arterial hypertension, that is a common complication in patients with coarctation of the aorta (CoA), and that worsen the prognosis in these patients.

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# PART 1

**Cardiovascular risk in adults could start from childhood: how to detect early cardiovascular risk starting from conventional and non-conventional risk factors.**

**CHAPTER 2.**

**NSAIDs and cardiovascular risk**

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# NSAIDs and cardiovascular risk

Fabio Marsico, Stefania Paolillo and Pasquale P. Filardi

**NSAIDs are the most largely used class of drugs in the world, due to their large use in many diseases, in particular for the systemic inflammatory diseases. Nevertheless, today NSAIDs are less used for some of these diseases, due to several side-effects correlated to these drugs. The antiinflammatory mechanism of NSAIDs consist in the inibhition of two forms of cyclooxygenase, namely COX-1 (its block contributes to an antiplatelet effect) and COX-2 (its block has a greater antiinflammatory, antipyretic and analgesic effect). The COX-2 inhibition might reduce the risk of gastrointestinal toxicity, but several studies have shown the cardiovascular side effects of this inhibition. Mechanisms of the cardiovascular side effects are controversial yet, so the aim of this document is to review side-effects profile of NSAIDs and, specifically, to investigate cardiovascular consequences of NSAIDs use in clinical practice.**

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Keywords: cardiovascular events, cardiovascular risk, NSAIDs

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## Introduction

NSAIDs are a largely used class of drugs, maybe the most widely used in the world, because of implicated to treat pain in many different diseases. In many countries (i.e., the United States, Germany, Sweden, Spain, and others), some of them (most often ibuprofen, naproxen, and diclofenac) can also be purchased as over-the-counter medicines in supermarkets, at gas stations, and convenience stores without any expert advice on their use or potential side-effects.[1] In patients with systemic inflammatory diseases, NSAIDs play an important role as analgesics and anti-inflammatory effects.[2,3] Nevertheless, today NSAIDs are less used in systemic inflammatory diseases patients as first-line treatment because of their inability to control disease progression and several side-effects, particular at gastrointestinal[4] and cardiac[5] level. Aim of this document is to review side-effects profile of NSAIDs and, specifically, to investigate cardiovascular consequences of NSAIDs use in clinical practice.

## Mechanisms of action and side-effects profile

NSAIDs inhibit the two recognized forms of cyclooxygenase, namely COX-1 and COX-2, blocking prostaglandin and prostacyclin biosynthesis.[6] By inhibiting COX-1, NSAIDs as aspirin, reduce thromboxane production, and this contributes to their antiplatelet effect. By more effectively inhibiting COX-2, other NSAIDs, as ibuprofen, naproxen, diclofenac, and COX-2 selective inhibitors, known as coxibs, have relatively greater anti-inflammatory, antipyretic, and analgesic effects.[7] The adverse effect profile of nonselective NSAIDs primarily includes bleeding, particularly gastrointestinal bleeding, which is thought to result from gastric irritation, antiplatelet effects, and the loss of prostaglandin-mediated mucosal repair.[8]

COX-1 is expressed constitutively in most tissues, whereas COX-2 is induced primarily by inflammation and in response to shear stress on endothelial tissues.[9] Thus, the largest part of gastrointestinal side-effects of NSAIDs is because of COX-1 inhibition, whereas NSAIDs, which selectively inhibit COX-2, might reduce the risk of gastrointestinal toxicity. Coxibs were developed in the 1990s, and early trials comparing coxibs vs. traditional NSAIDs seemed to confirm similar analgesic efficacy and less gastrointestinal toxicity.[10,11] Unfortunately, subsequent placebo-controlled trials showed unequivocally that coxibs are associated with an increased risk of atherothrombotic vascular events.

Cardiovascular safety on NSAIDs is highly controversial. For several coxibs, randomized placebo-controlled clinical trials have demonstrated an increased risk of serious cardiovascular disease.[12–15] However, there is considerable uncertainty with regard to cardiovascular safety of the older traditional NSAIDs.[16] Meta-analyses of observational studies[17,18] suggested that cardiovascular risk varies for individual drugs in this class, with diclofenac associated with greater risk than naproxen. Otherwise, meta-analysis of clinical trials[16] reported a greater risk of both diclofenac and ibuprofen over naproxen (Table 1). Subsequent observational studies demonstrated an association between ischemic cardiovascular events and the use of nonselective and more commonly available NSAIDs, such as ibuprofen and diclofenac, especially when taken at higher doses and by patients with known cardiovascular disease.[16,19]



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**Table 1 Risk of cardiovascular events related to NSAIDs use**

| NSAID | Cardiovascular side-effect |
|---|---|
| Diclofenac | High risk |
| Ibuprofen | High risk |
| Rofecoxib | High risk |
| Celecoxib | Unclear/high risk |
| Naproxen | Low risk |

Many proposed pathophysiological mechanisms have been raised to explain the cardiovascular side-effects of NSAIDs. One possible explanation that has been recently validated in animal models is that the imbalance of vasodilatory prostacyclin and prostaglandin E2 vs. vasoconstrictive thromboxane A2, created in the endothelium with NSAIDs use, leads to thrombosis.[20,21] It has also been well established that COX-2 inhibition promotes sodium and water retention, exacerbates heart failure and hypertension, and increases adverse ventricular remodeling.[22–24] Indeed, NSAIDs use in patients with chronic heart failure has been associated with a significant increase in cardiovascular morbidity and mortality.[25] Furthermore, the beneficial effect of aspirin may be attenuated by concomitant administration of NSAIDs.[26,27]

An increase in thrombotic events, ischemic hospitalizations, and heart failure is expected consistent with the clinical and physiologic effects of NSAIDs. First, there is evidence that the homeostatic mechanisms that exist between prostacyclin-mediated vasodilation (blocked by COX-2 inhibitors) and tromboxane A2-mediated vasoconstriction is perturbed when (selective or nonselective) COX-2 inhibitors are used.[20] Second, NSAIDs may increase salt and water retention, blood pressure,[20,24,28] and afterload, which also can result in increased myocardial infarction (MI), stroke, and heart failure risk. Third, as already mentioned, these drugs have been shown to possibly attenuate the action of aspirin.[26,27]

## NSAIDs and cardiovascular side-effects

The rate of NSAIDs cardiovascular side-effects has been widely investigated in several studies.

Olsen *et al.*[29] in a cohort of 61 971 patients admitted for first-time MI, showed an increased rate of combined cardiovascular events (cardiovascular death, nonfatal recurrent MI, ischemic stroke, transient ischemic attack, and systemic arterial emboli) in patients treated with NSAIDs, than in patients without NSAIDs treatment [hazard ratio, 1.40 (95% CI, 1.30–1.49)] during a median follow-up of 3.5 years. Lamberts *et al.*[30] studied 150 900 patients with atrial fibrillation, of which 69.8% were treated with an antiplatelet or an oral anticoagulant, whereas only 5% of the whole population were treated with a concomitant NSAID. This study showed an increased absolute risk of serious bleeding (described as gastrointestinal and intracranial) and thromboembolism with NSAIDs treatment compared with no NSAIDs treatment [hazard ratio, 2.27 (95% CI, 2.15–2.40), hazard ratio, 1.36 (95% CI, 1.27–1.45), respectively]. In an analysis from the REduction of Atherothrombosis for Continued Health registry, a multinational registry of outpatients with stable atherothrombotic disease, Kohli *et al.*[9] examined 44 095 patients and reported the relationship between NSAIDs use and several cardiovascular endpoints at 4 years. These included the occurrence of the composite of cardiovascular death, nonfatal MI, nonfatal stroke and ischemic hospitalizations, the composite of cardiovascular death, MI and stroke, heart failure, and each individual component of composite endpoints. When analyzed in a univariate fashion, there were significantly higher rates of the composite of cardiovascular death/MI/stroke/hospitalization (32.6 vs. 30.3%), hospitalization for heart failure (11.4 vs. 8.2%), and hospitalization for ischemic events (25.3 vs. 22.3%) in individuals who used NSAIDs ($P \leq 0.001$ for all). Olsen *et al.*[31] in another study, considered a cohort of 99 187 patients with first time MI. They showed a consistently increased risk of composite outcome (all-cause death, coronary death, readmission for nonfatal MI) among patients receiving any NSAIDs during the 5-year follow-up. The risk remained virtually unchanged throughout all 5 years. Use of diclofenac was associated with the highest risk compared with ibuprofen, rofecoxib, celecoxib, and particularly naproxen that was the drug with the lowest relative risk of cardiovascular events.

According to these data, much attention has been given to the cardiovascular safety of NSAIDs. It is widely accepted that the use of common agents, including diclofenac, high doses of celecoxib, and ibuprofen, increase the risk of thrombotic events, whereas naproxen has not been associated with this reported increased risk (Table 1).[18,19,32,33]

Ray *et al.*[34] in a cohort of 48 566 patient, admitted for acute MI (40%), coronary revascularization procedures (40%), or unstable angina (20%), found the lowest adjusted rates of both serious coronary heart disease and serious cardiovascular disease/death from any cause in current users of naproxen compared with other NSAIDs, and no differences with nonusers. In particular, when compared with current users of naproxen, users of diclofenac showed an increased risk of serious coronary heart disease [1.44 (0.96 to 2.15), $P = 0.076$] and serious cardiovascular disease/death [1.52 (1.22 to 1.89), $P = 0.0002$], whereas ibuprofen users exhibited increased risk only of the latter endpoint [1.25 (1.02 to 1.53), $P = 0.032$]. The most commonly prescribed dose of naproxen was 1000 mg or greater, accounting for 77% of current use. Compared with nonusers of any NSAID, current users of naproxen showed no increased risk of either serious coronary heart disease [incidence rate ratio (IRR)_0.78 (0.55 to 1.10)] or serious cardiovascular disease/death [IRR_0.85 (0.71 to 1.03)]. Relative to high-dose naproxen, current users of high-dose

celecoxib (>200 mg) and rofecoxib (>25 mg) showed increased risk of serious coronary heart disease [IRRs of 1.61 (1.01 to 2.57) and 2.29 (1.24 to 4.22), respectively]. Similarly, Fosbøl *et al.*[1] studied 1 028 427 healthy persons, showing that the use of ibuprofen was associated with a significant increase in risk of coronary death or nonfatal MI and fatal or nonfatal stroke (only in high doses). Use of diclofenac was associated with a significant increase in risk of cardiovascular death, coronary death, or nonfatal MI, as well as fatal or nonfatal stroke (high doses). The results showed a clear dose-dependent relationship. The selective COX-2 inhibitor rofecoxib was significantly related to an increased risk of cardiovascular death and the composite of coronary death or nonfatal MI. Celecoxib was not related to excess cardiovascular death or fatal/nonfatal stroke, and the results showed no trend for a dose-dependent relationship. Finally, use of naproxen was neutral in terms of outcome except for fatal or nonfatal stroke, which showed a trend for increased risk.

Finally, a meta-analysis of clinical trials[33] showed that, compared with placebo, the risk of major vascular events was increased by about a third in patients allocated to a coxib compared with placebo (307 [1.15% per annum] vs 175 [0.82% per annum], respectively; rate ratio [RR] 1.37, 95% CI 1.14–1.66, $P=0.0009$) or diclofenac (1.41, 1.12–1.78, $P=0.0036$), chiefly because of an increase of about three-quarters in the risk of major coronary events (coxibs 1.76, 1.31–2.37, $P=0.0001$; diclofenac 1.70, 1.19–2.41, $P=0.0032$). Ibuprofen also significantly increased major coronary events (2.22, 1.10–4.48, $P=0.0253$), but not major vascular events (1.44, 0.89–2.33, $P=0.14$). By contrast with other traditional NSAIDs (heterogeneity $P=0.04$), high-dose naproxen was not associated with any significant excess risk of major vascular events (0.93, 0.69–1.27), and nor was there an increase in major coronary events (0.84, 0.52–1.35). There was no evidence that any NSAID significantly increased the risk of stroke. The risk of hospitalization because of heart failure was roughly doubled by all NSAIDs regimens studied (coxib 2.28, 95% CI 1.62–3.20, $P<0.0001$; diclofenac 1.85, 1.17–2.94, $P=0.0088$; ibuprofen 2.49, 1.19–5.20, $P=0.0155$; naproxen 1.87, 1.10–3.16, $P=0.0197$). The risk of vascular death was significantly increased by coxibs (1.58, 99% CI 1.00–2.49, $P=0.0103$) and diclofenac (1.65, 0.95–2.85, $P=0.0187$), nonsignificantly increased by ibuprofen (1.90, 0.56–6.41, $P=0.17$) and by naproxen (1.08, 0.48–2.47, $P=0.80$). The risk of death from any cause was significantly increased by around a quarter by allocation to a coxib (1.22, 1.04–1.44, $P=0.0139$), but despite a clear excess of vascular deaths, the corresponding excess was not significant for diclofenac (1.20, 0.94–1.54, $P=0.15$), and nor were there significant excesses of death from any cause for ibuprofen (1.61, 0.90–2.88, $P=0.11$) or naproxen (1.03, 0.71–1.49, $P=0.88$).

## Conclusion

In conclusion, NSAIDs use exhibits a great risk of major cardiovascular events occurrence and the safest drug in terms of cardiovascular side-effects is naproxen. Nevertheless, the adverse effects profile of NSAIDs and concern about the relationship between NSAIDs and higher rates of ischemic cardiovascular events, prompted clinical guidelines to recommend caution on the use of these agents in patients with a previous history of cardiovascular disease.[35,36]

However, because these medications are readily available, generally well tolerated, and often an alternative treatment in patients with severe or functionally impairing arthritis, physicians are often pushed to use them, despite their cardiovascular risk profile.[37] Thus, an accurate selection of patients and an adequate drugs' choice should be always advised and performed to minimize the risk of serious adverse events.

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**CHAPTER 3.**

## Multicentre multi-device hybrid imaging study of coronary artery disease: results from the EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischaemic Heart Disease (EVINCI) hybrid imaging population

Liga R, Vontebol J, Rovai D, Marinelli M, Caselli C, Pietila M, Teresinska A, Aguadé-Bruix S, Pizzi MN, Todiere G, Gimelli A, Chiappino D, Marraccini P, Schroeder S, Drosch T, Poddighe R, Casolo G, Anagnostopoulos C, Pugliese F, Rouzet F, Le Guludec D, Cappelli F, Valente S, Gensini GF, Zawaideh C, Capitanio S, Sambuceti G, **Marsico F**, Perrone Filardi P, Fernàndez-Golfin C, Rincon LM, Graner FP, de Graaf MA, Stehli J, Reyes E, Nkomo S, Mäki M, Lorenzoni V, Turchetti G, Carpeggiani C, Puzzuoli S, Mangione M, Marcheschi P, Giannessi D, Nekolla S, Lombardi M, Sicari R, Scholte AJ, Zamorano JL, Underwood SR, Knuuti J, Kaufmann PA, Neglia D, Gaemperli O; EVINCI study Investigators

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# Multicentre multi-device hybrid imaging study of coronary artery disease: results from the EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischaemic Heart Disease (EVINCI) hybrid imaging population

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**Aims** Hybrid imaging provides a non-invasive assessment of coronary anatomy and myocardial perfusion. We sought to evaluate the added clinical value of hybrid imaging in a multi-centre multi-vendor setting.

**Methods and results** Fourteen centres enrolled 252 patients with stable angina and intermediate (20-90%) pre-test likelihood of coronary artery disease (CAD) who underwent myocardial perfusion scintigraphy (MPS), CT coronary angiography (CTCA), and quantitative coronary angiography (QCA) with fractional flow reserve (FFR). Hybrid MPS/CTCA images were obtained by 3D image fusion. Blinded core-lab analyses were performed for CTCA, MPS, QCA and hybrid datasets. Hemodynamically significant CAD was ruled-in non-invasively in the presence of a matched finding (myocardial perfusion defect co-localized with stenosed coronary artery) and ruled-out with normal findings (both CTCA and MPS normal). Overall prevalence of significant CAD on QCA (>70% stenosis or 30-70% with FFR≤0.80) was 37%. Of 1004 pathological myocardial segments on MPS, 246 (25%) were reclassified from their standard coronary distribution to another territory by hybrid imaging. In this respect, in 45/252 (18%) patients, hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 42% of the cases. Hybrid imaging allowed non-invasive CAD rule-out in 41%, and rule-in in 24% of patients, with a negative and positive predictive value of 88% and 87%, respectively.

**Conclusion** In patients at intermediate risk of CAD, hybrid imaging allows non-invasive co-localization of myocardial perfusion defects and subtending coronary arteries, impacting clinical decision-making in almost one every five subjects.

**Keywords** Hybrid imaging • Myocardial perfusion scintigraphy • CT coronary angiography • Coronary artery disease

# Introduction

The risk of patients with stable coronary artery disease (CAD) varies considerably based on the extent of anatomical involvement and of myocardial ischaemia.[1] Unfortunately, there is disagreement between the angiographic severity of CAD and myocardial perfusion abnormalities.[2,3] Thus, current guidelines recommend a comprehensive anatomo-functional assessment to decide on the most appropriate treatment, with patients at low-risk treated conservatively, while high-risk patients are generally referred for more aggressive therapies.[1] Specifically, revascularization strategies should be guided by the presence of haemodynamically significant coronary stenosis, while non-significant coronary stenoses may be treated conservatively.[4,5]

Recently, hybrid cardiac imaging has emerged as a non-invasive way of assessing CAD by integration of myocardial perfusion images with individual coronary anatomy.[6] Small studies have suggested superior diagnostic accuracy compared with the separate imaging modalities,[7] whereas others have reported incremental prognostic value.[8] While the technique is finding increasing acceptance in clinical practice, questions remain over the clinical role of hybrid imaging. Furthermore, the impact of the technique has never been tested in a multicentre, multi-device, real-world setting.

This study sought to assess the clinical role of hybrid cardiac imaging in a multicentre study using different equipment and practice, and to explore its value for the diagnosis of haemodynamically significant CAD.

# Methods

## Study design

The EVINCI (EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischaemic Heart Disease) study is a 'European Commission 7th Framework Program for Research and Innovation'-sponsored multimodality imaging project in 14 centres from 9 European countries.[9] The characteristics of the study population have been already described in detail[9] and are summarized in *Table 1*. Briefly, between March 2009 and June 2012, patients with symptoms suggestive of CAD and intermediate pre-test probability (20–90%)[10,11] underwent a study of coronary anatomy by computed tomography coronary angiography (CTCA) and at least one coronary functional imaging test by myocardial perfusion scintigraphy (MPS), single-photon emission computed tomography (SPECT) or positron emission tomography (PET), and/or wall motion imaging (stress echocardiography or cardiac magnetic resonance), with the recommendation to perform invasive coronary angiography (ICA) with fractional flow reserve (FFR) in intermediate lesions. Each patient was followed-up for 30 days and the referral for coronary revascularization recorded. Ethical approval was provided by each centre, and all subjects gave written informed consent.

## Image acquisition

Acquisition protocols were agreed on for each technique based on best available clinical practice. Individual core-labs were responsible for harmonization and quality control of imaging protocols. Details on imaging procedures and protocols can be found in the EVINCI publication.[9] All EVINCI subjects in whom core-lab analyses of CTCA, MPS, and ICA were available were selected for the present hybrid sub-study (*Figure 1*). Accordingly, patients submitted to wall motion imaging modalities were not included in the analysis, because their format precludes formation of 3D hybrid data sets with CTCA. No further exclusion criterion was considered.

## Image fusion

MPS and CTCA data sets were transferred to a dedicated hybrid core-lab blinded to clinical history and imaging findings (Cardiac Imaging, University Hospital Zurich, Switzerland). Image fusion of MPS and CTCA data sets was performed on a dedicated workstation (Advantage Workstation 4.4, GE Healthcare) using the CardIQ Fusion software package (GE Healthcare) as previously described.[12] In case of $H_2^{15}O$-PET images, parametric myocardial blood flow data sets, showing flows on a segmental level, were generated based on quantitative analysis performed using a commercially available software, PMOD 3.6 software package (PMOD Technologies Ltd, Zurich, Switzerland).

**Table 1 Patients' baseline characteristics**

| Parameter | Overall population ($n$ = 252) |
|---|---|
| Demographics, *n* (%) | |
| Age, years (mean ± SD) | 61 ± 9 |
| Male gender | 161 (64) |
| Clinical characteristics, *n* (%) | |
| Typical angina | 62 (25) |
| Atypical angina | 148 (59) |
| Non-anginal chest pain | 42 (17) |
| Pre-test probability of CAD | 59 ± 23 |
| Left ventricular ejection fraction | 59 ± 9 |
| Cardiovascular risk factors, *n* (%) | |
| Family history of CAD | 75 (30) |
| Diabetes mellitus | 68 (27) |
| Hypercholesterolemia | 161 (64) |
| Hypertension | 155 (62) |
| Smoking | 60 (24) |
| Obesity | 72 (29) |
| Invasive coronary angiography data, *n* (%) | |
| Normal coronaries or non-obstructive CAD | 158 (63) |
| Single-vessel disease | 60 (23) |
| Multi-vessel disease | 34 (14) |
| Myocardial perfusion imaging, *n* (%) | |
| Single-photon emission computed tomography | 180 (71) |
| $^{99m}$Tc-Sestamibi | 103 (57) |
| $^{99m}$Tc-Tetrofosmin | 77 (43) |
| Positron emission tomography | 72 (29) |
| $^{15}$O-Water | 63 (88) |
| $^{13}$N-Ammonia | 8 (11) |
| $^{82}$Rubidium | 1 (1) |

Data are given in absolute numbers and percentages (%), unless otherwise stated.

Hybrid analysis was performed using an optimized alignment tool, allowing projection of the MPS image on the left ventricular epicardial surface obtained from the CTCA, allowing a panoramic view of the coronary artery tree projected onto the left ventricular myocardial perfusion territories. In all patients, the image fusion procedure (including image generation and reading) was performed by two independent and blinded operators. Disagreement with regard to allocation of myocardial perfusion defects was resolved by consensus reading.

## Image interpretation and definitions

Image interpretation was performed in dedicated core-labs as follows.

### Computed tomography coronary angiography

CTCA was assessed using a modified 16-segment system[13] and considered abnormal if at least one coronary segment had a diameter stenosis >50%. Significant left main stem stenoses were assigned to both left anterior descending (LAD) and left circumflex (LCX) coronary arteries. To limit any selection bias, any non-diagnostic segment was considered abnormal.

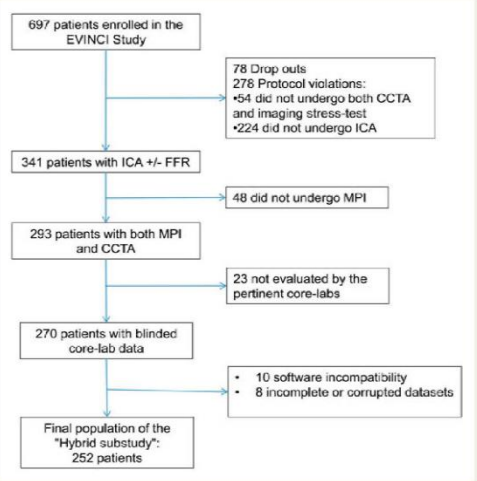



**Figure 1** Patient flow chart. CTCA, coronary CT angiography; ICA, invasive coronary angiography; FFR, fractional flow reserve; MPS, myocardial perfusion imaging.

### Myocardial perfusion scintigraphy

Perfusion in each of 17 segments[14] was visually classified as 0 = normal, 1 = mild reduction, 2 = moderate reduction, 3 = severe reduction, or 4 = absent perfusion, and the segmental scores were summed for the stress (SSS) and rest (SRS) images. $^{15}$O-$H_2O$ PET data were processed and parametric perfusion images were scored similarly. The difference between SSS and SRS was calculated as the summed difference score (SDS). On per-patient analysis, a reversible perfusion defect (ischaemia) was defined as a SDS ≥2, either from a score ≥1 in at least two contiguous segments or ≥2 in at least one segment. Myocardial scar was defined similarly as a SRS ≥2. Accordingly, MPS studies were considered pathological in the presence of significant myocardial ischaemia and/or scar.

For per-vessel analysis, a reversible perfusion defect (ischaemia) was defined as a territorial difference score ≥1, and a scar as a rest score ≥1. Each perfusion defect was assigned to one or more coronary territories according to the standardized myocardial segmentation model.[14] Similarly to CTCA analysis, any non-diagnostic segment was considered abnormal.

### Invasive coronary angiography

Coronary angiograms were subdivided using the previously mentioned segmentation model[13] and analysed using quantitative coronary angiography (QCA). A stenosis was considered haemodynamically significant if causing a >50% diameter reduction in the left main stem or >70% elsewhere, or between 30 and 70% with an FFR ≤0.80.

## Hybrid images

All hybrid MPS/CTCA images were analysed by consensus of two independent readers with regard to the presence of matched, mismatched, or normal findings. A matched finding was defined as a perfusion defect in a territory subtended by a stenotic coronary. All other combinations of pathological findings were classified as mismatched. In the absence of

**Table 2 Non-invasive imaging data**

| Parameter | Overall population ($n$ = 252) | SPECT ($n$ = 180) | PET ($n$ = 72) | $P$-value |
|---|---|---|---|---|
| Myocardial perfusion imaging data | | | | |
| Normal perfusion | 148 (59) | 111 (62) | 37 (58) | 0.175 |
| Scar | 41 (16) | 35 (19) | 6 (8) | 0.037 |
| Inducible ischaemia | 88 (35) | 54 (30) | 34 (47) | 0.013 |
| Computed tomography data | | | | 0.599 |
| One-vessel disease | 48 (19) | 38 (21) | 10 (14) | |
| Two-vessel disease | 41 (16) | 29 (16) | 12 (17) | |
| Three-vessel disease | 22 (9) | 15 (8) | 7 (10) | |
| Hybrid imaging | | | | 0.054 |
| Hybrid match | 61 (24) | 39 (22) | 22 (31) | |
| Hybrid mismatch | 88 (35) | 68 (38) | 20 (28) | |
| MPS positive and CT negative | 39 (15) | 26 (14) | 13 (18) | |
| MPS negative and CT positive | 49 (19) | 42 (23) | 7 (10) | |
| Normal hybrid | 103 (41) | 73 (41) | 30 (42) | |

Data are given as numbers and percentages, $n$ (%).

pathological findings on both CTCA and MPS, hybrid images were considered normal. Finally, all pathological MPS segments were assigned to the pertinent vascular territory by spatial co-registration according to individual coronary anatomy by both operators to determine inter-observer agreement and repeatability of hybrid-based co-registration.

### Statistical analysis

Statistical analysis was performed using the SPSS software. Continuous variables were expressed as mean ± SD, and categorical variables as percentages. Numerical values were compared using the Mann–Whitney $U$ test or Student's $t$-test, and categorical values using the $\chi^2$ test. Inter-observer agreement was assessed using Cohen's kappa statistic. Sensitivity, specificity, and accuracy were calculated for each imaging method (MPS, CTCA, and hybrid imaging) on a per-vessel and per-patient basis. The McNemar test was performed to compare the accuracy of the different imaging methods against QCA ± FFR. A value of $P < 0.05$ was considered significant.

## Results

### Patient population

A total of 252 patients underwent CTCA, MPS, and ICA and were included in the analysis (*Figure 1*). The characteristics of the study populations are shown in *Table 1*. Compared with the overall EVINCI population,[9] there were no significant differences in baseline characteristics except for a slightly higher CAD prevalence in our patient population (37 vs. 30%, $P = 0.05$) (Supplementary data online, *Table SA*).

Interestingly, as in the case of the main EVINCI population, also in the present study, traditional criteria for calculating pre-test probability[11] overestimated the prevalence of haemodynamically significant CAD, which was 37% at QCA ± FFR. FFR was performed in 58/252 patients (23% of all patients and 66% of patients with intermediate coronary stenoses) and was abnormal (≤0.80) in 19 patients.

### Imaging results: MPS and CTCA

A total of 180 (71%) patients were submitted to SPECT while 72 (29%) underwent PET (*Table 2*). Overall, 104 (41%) patients presented myocardial perfusion abnormalities in one (8%), two (41%), or three (51%) vascular territories. At core-lab analysis, MPS images were judged of non-diagnostic quality (having at least one non-diagnostic segment) in 11 patients.

On CTCA, 111 (44%) patients presented significant CAD in one (48/111, 43%), two (41/111, 37%), or three (22/111, 20%) vessels (*Table 2*) with no significant difference between patients submitted to SPECT or PET. At core-lab analysis, CT images were judged of non-diagnostic quality (having at least one non-diagnostic segment) in 8 patients.

### Hybrid imaging: feasibility and repeatability

In 18/270 (7%) patients originally submitted to CTCA and MPS, hybrid imaging could not be accomplished due to corruption of original data sets (8 patients) or software incompatibility (10 patients).

Inter-rater agreement of hybrid-based co-registration was good ($\kappa = 0.75$, 95% CI 0.70–0.80) with both observers agreeing in the classification of 92% of all pathological myocardial segments.

### Hybrid imaging: segment reclassification

A total of 4284 myocardial segments were analysed, of which 1004 (23%) were pathological. According to the standard myocardial segmentation model, 397 (39%), 269 (27%), and 338 (34%) abnormal segments were allocated to the LAD, LCX, and right coronary artery (RCA) vascular territory, respectively. After image fusion, 246 (25%) of the 1004 abnormal myocardial segments were

**Table 3 Hybrid-based reclassification of myocardial perfusion abnormalities**

| Standard coronary distribution | Myocardial segments (17 segments LV model)[a] | Perfusion abnormality, *n* | Abnormal segment reclassified, *n* (%) | To LAD, *n* (%) | To LCX, *n* (%) | To RCA, *n* (%) |
|---|---|---|---|---|---|---|
| LAD | Segment 1 | 50 | 0 (0) | – | 0 (0) | 0 (0) |
| | Segment 2 | 51 | 1 (2) | – | 0 (0) | 1 (100) |
| | Segment 7 | 56 | 0 (0) | – | 0 (0) | 0 (0) |
| | Segment 8 | 48 | 0 (0) | – | 0 (0) | 0 (0) |
| | Segment 13 | 62 | 0 (0) | – | 0 (0) | 0 (0) |
| | Segment 14 | 51 | 1 (2) | – | 0 (0) | 1 (100) |
| | Segment 17 | 79 | 6 (8) | – | 2 (33) | 4 (67) |
| LCX | Segment 5 | 72 | 25 (35) | 0 (0) | – | 25 (100) |
| | Segment 6 | 43 | 20 (47) | 19 (95) | – | 1 (5) |
| | Segment 11 | 58 | 20 (34) | 17 (85) | – | 3 (15) |
| | Segment 12 | 44 | 31 (70) | 30 (97) | – | 1 (3) |
| | Segment 16 | 52 | 35 (67) | 30 (86) | – | 5 (14) |
| RCA | Segment 3 | 55 | 13 (24) | 10 (77) | 3 (23) | – |
| | Segment 4 | 82 | 15 (18) | 0 (0) | 15 (100) | – |
| | Segment 9 | 52 | 15 (29) | 12 (80) | 3 (20) | – |
| | Segment 10 | 76 | 15 (20) | 0 (0) | 15 (100) | – |
| | Segment 15 | 73 | 49 (67) | 42 (86) | 7 (14) | – |

LV, left ventricle; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery.
[a]For exact location of perfusion segment within the LV see *Figure 2A*.

reclassified from their standard coronary distribution to another territory (*Table 3*). Segment reclassification was highest for the standard LCX (49%) and RCA (32%) segments, while it was very low for standard LAD segments (2%; $P < 0.001$ vs. both LCX and RCA). *Figure 2* shows the proportion of pathological segments reassigned by hybrid imaging.

In 45/252 (18%) patients, hybrid imaging reassigned an entire perfusion defect to another coronary territory, changing the final diagnosis in 19 cases (from a mismatched to a matched finding in 16 patients, and the opposite in 3). Interestingly, in 16 (84%) of those patients, the myocardial perfusion abnormality was correctly assigned to a territory subtended by a haemodynamically significant stenosis at QCA ± FFR. The role of hybrid analysis in the anatomo-functional characterization of patients and in identifying significant CAD is exemplified in *Figure 3*.

## 'Rule-in/rule-out' clinical algorithm

The diagnostic accuracy of hybrid imaging and of stand-alone imaging modalities in detecting significant CAD (QCA ± FFR) is reported in *Figure 4*.

Specifically, a matched finding at hybrid imaging was found in 61 patients (24%), while 103 patients (41%) had normal hybrid findings. Of the remaining 88 patients with mismatched abnormal findings (35%), 45 presented a positive CTCA in the absence of perfusion abnormalities at MPS, while 39 showed a pathological MPS despite the absence of obstructive CAD at CTCA. Revascularization rates were 70% for matched hybrid images, 36% for mismatched findings, and 10% for normal findings ($P < 0.001$) (*Figure 5*).

Interestingly, among the 41 'false-negative' hybrid studies (either normal or mismatched findings in the presence of significant CAD at QCA), the majority (80%) showed negative MPSs, despite a stenotic vessels on CTCA in 64% of the cases. FFR was performed in 17/41 patients and was positive in 13 (76%) (Supplementary data online, *Table SB*). On the other hand, the 'false-positive' hybrid studies were almost exclusively associated with the presence of intermediate coronary lesions (>30 and ≤70%) on QCA mainly in the absence of an invasive assessment of the haemodynamic relevance of stenoses by FFR (Supplementary data online, *Table SC*).

## Radiation burden of the non-invasive imaging protocol

Average radiation doses in the study population were 7.9 mSv (range 0.6–24 mSv) for CTCA, 10.4 mSv (range 3.2–17.5 mSv) for SPECT, and 1.8 mSv (range 1.7–3.5 mSv) for PET. The average radiation dose of hybrid imaging was 9.4 mSv (range 5.2–21 mSv) for PET/CTCA and 18.5 mSv (range 6–31 mSv) for SPECT/CTCA ($P < 0.001$).

# Discussion

The EVINCI hybrid sub-study is one of the largest studies to assess the clinical value of non-invasive hybrid imaging in stable CAD. Several methodological advantages, including the use of dedicated blinded core-lab image analysis, the multicentre and multivendor design, and the use of an accepted invasive gold standard (QCA ± FFR), distinguish it from previously published reports and provide greater uniformity and generalizability of its results. The main findings of the study are (i) large variability of coronary anatomy leading to systematic errors of standardized myocardial segmentation in predicting culprit coronary vessels; (ii) hybrid imaging (by 3D co-registration of CTCA and MPS) is feasible and reproducible; and (iii) a hybrid anatomo-functional protocol allows non-invasive 'rule-in/rule-out' of haemodynamically significant CAD.

Standardized myocardial segmentation models are widely used to assign myocardial territories to subtending coronary arteries.[14] However, coronary anatomy is highly variable, which may frequently lead to mistaken identification of culprit vessels by standard models.

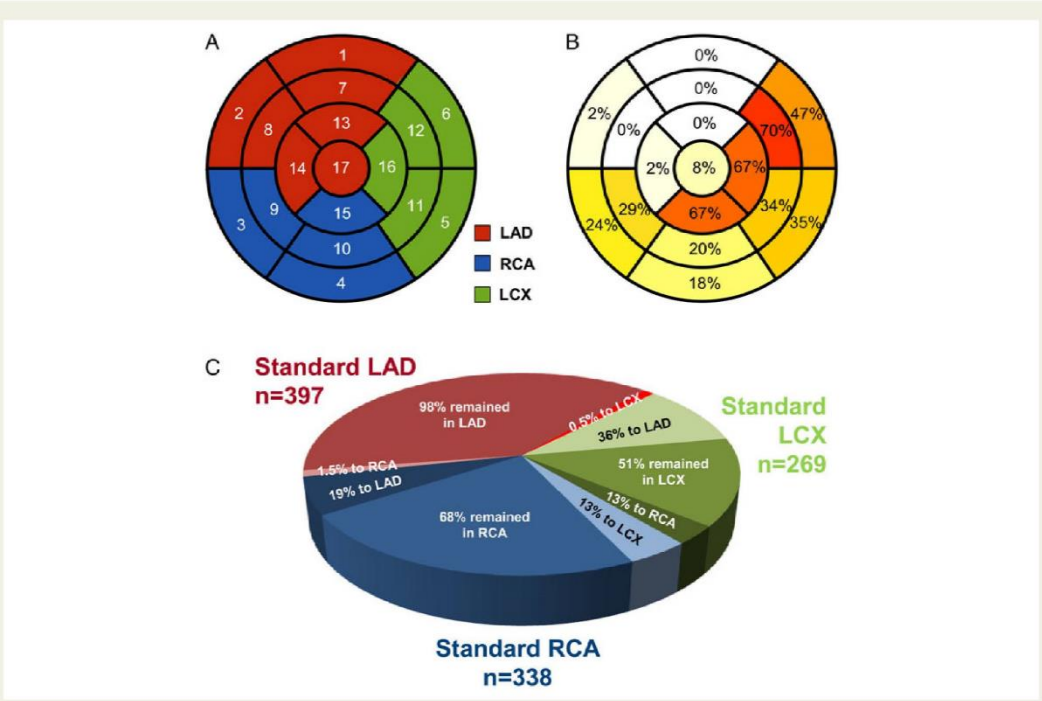



**Figure 2** (*A*) Standardized myocardial segmentation model used in this study with number codes for each segment (see *Table 3*).[14] (*B*) Reassignment rates by hybrid imaging for the 1004 pathological segments (the intensity of colours in each segment indicates the frequency of reassignment of that segment when pathological). (*C*) Pie chart indicating proportion of reassignment and reassignment fate for pathological segments in each standard coronary territory. Shades of red indicate standard LAD, of green standard LCX, and of blue standard RCA territories. Standard LCX segments were most often reassigned to LAD (36%), while standard RCA segments were equally distributed between LAD and LCX.

In this respect, it has been previously suggested that hybrid imaging may help in the individual co-localization of myocardial perfusion abnormalities and subtending coronary arteries.[15–18]

We identified systematic deviation from the standardized assignment of myocardial segments in 25% of pathological segments, localized almost exclusively in the standard LCX and RCA territories (i.e. the lateral and inferior myocardial wall). This turned out to be clinically significant in almost every fifth patient, in whom the entire perfusion defect was reassigned to another coronary artery, changing the final diagnosis in almost half of them. This result might be of particular relevance in patients considered for revascularization, where only haemodynamically significant lesions deserve treatment.[5,19]

Previous reports have shown the feasibility and reproducibility of 3D fusion of anatomical (CTCA) and functional (MPS) imaging.[12] In this study, hybrid analysis was successfully performed in 93% of the EVINCI patients originally submitted to MPS and CTCA with good inter-observer repeatability, highlighting the robustness of the technique. In fact, technical image fusion failure occurred in only 7% of patients mainly in the case of early generation SPECT devices with incomplete or corrupted data sets or software incompatibility.

Given the heterogeneity of hybrid results (combining various anatomo-functional patterns), we considered that a binary diagnostic approach disregards the complexity of CAD. Conversely, a 'rule-in/rule-out' hybrid-based approach appears more clinically meaningful, since matched positive findings allow rule-in of CAD and matched normal findings CAD rule-out (*Figure 5*). Accordingly, although in the EVINCI study the clinical management of patients, including the decision for coronary revascularization, was entirely left to the judgement of the local clinician, possibly introducing a bias in the analysis of the data, a matched positive hybrid finding was still associated with a high early revascularization rate (70%). On the other hand, in patients with a completely negative hybrid report, the revascularization rate was extremely low (≈10%), making ICA theoretically superfluous. It should be emphasized that the majority of false-negative hybrid studies were due to negative MPS downstream of a stenotic coronary vessel at CTCA, which was confirmed by a >70% lumen diameter reduction at QCA (considered as haemodynamically significant). After the FAME study,[2] published almost at the end of the EVINCI study, coronary stenoses between 70 and 90% should also be submitted to FFR since a considerable proportion of these lesions have a normal FFR. On the other hand, the false-positive hybrid imaging studies were essentially associated with the presence of intermediate coronary lesions (>30 and ≤70%) that did not undergo an invasive evaluation of their

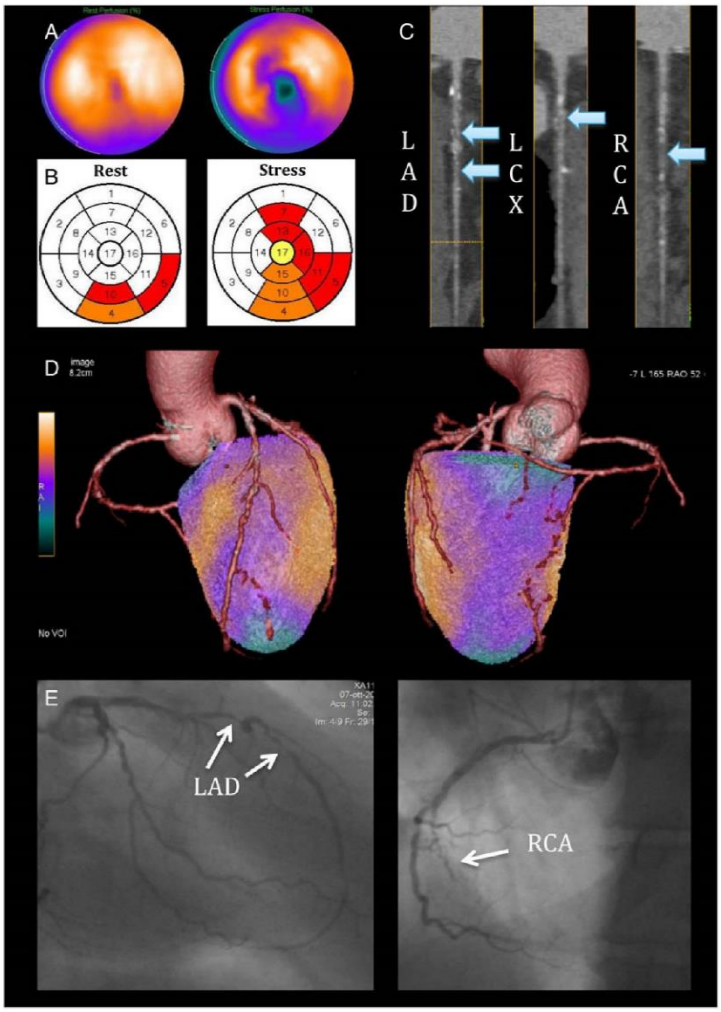
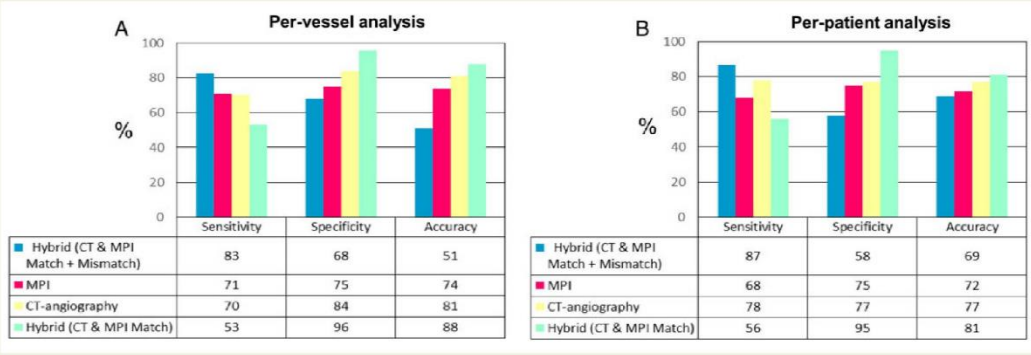



**Figure 3** A 55-year-old gentleman with atypical chest pain. (*A*) SPECT shows a reversible perfusion defect inferiorly with lateral extension, and in addition, there is a separate reversible perfusion defect involving the apical region and the mid-ventricular anteroseptal wall. (*B*) The perfusion polar maps show the SPECT core-lab interpretation (white = normal, yellow = mildly reduced, orange = moderately reduced, and red = severely reduced radiotracer uptake) with pathological segments assigned to all three coronary territories. (*C*) CTCA reveals two 70–90% mid LAD stenoses, a 50% proximal LCX stenosis, and a probable occlusion of the mid RCA (arrows). (*D*) On hybrid imaging, the entire infero-lateral perfusion defect is reassigned to the RCA, effectively changing the diagnosis from three-vessel to two-vessel disease. (*E*) Imaging findings were confirmed on QCA showing two high-grade lesions in the mid LAD, diffuse non-significant disease in the LCX, and a chronic total occlusion of the mid RCA.

haemodynamic relevance through FFR and, thus, considered as not significant. It is conceivable that, if FFR would have been more extensively performed, the number of 'false-negative' and 'false-positive' results could have been considerably reduced. Interestingly, a consistent proportion of those patients were still submitted to coronary revascularization despite the absence of an objective proof of myocardial ischaemia (either by MPS or through FFR) (Supplementary data online, *Tables SB* and *SC*), further highlighting the existing gap between evidence-based patient management[1,3,5,19] and everyday clinical conduct.[20]

Patients with mismatched findings (positive MPS/negative CTCA or negative MPS/positive CTCA) represent a heterogeneous group.




| | Sensitivity | Specificity | Accuracy |
|---|---|---|---|
| Hybrid (CT & MPI Match + Mismatch) | 83 | 68 | 51 |
| MPI | 71 | 75 | 74 |
| CT-angiography | 70 | 84 | 81 |
| Hybrid (CT & MPI Match) | 53 | 96 | 88 |

| | Sensitivity | Specificity | Accuracy |
|---|---|---|---|
| Hybrid (CT & MPI Match + Mismatch) | 87 | 58 | 69 |
| MPI | 68 | 75 | 72 |
| CT-angiography | 78 | 77 | 77 |
| Hybrid (CT & MPI Match) | 56 | 95 | 81 |

**Figure 4** Accuracy analysis of stand-alone and hybrid protocols for the diagnosis of significant CAD (by QCA + FFR) on per-vessel (*A*) and per-patient (*B*) analysis. On a per-vessel basis, when positivity was defined by the presence of at least one positive test (either matched or mismatched findings), hybrid imaging had higher sensitivity than single modalities ($P < 0.001$ vs. MPS and CTCA), at the price of lower specificity ($P < 0.001$ vs. both MPS and CTCA) and accuracy ($P < 0.001$ vs. both MPS and CTCA). When only matched findings were considered positive, hybrid imaging increased accuracy ($P < 0.001$ vs. both MPS and CTCA) driven by higher specificity ($P < 0.001$ vs. both MPS and CTCA) but with lower sensitivity ($P < 0.001$ vs. MPS and CTCA).

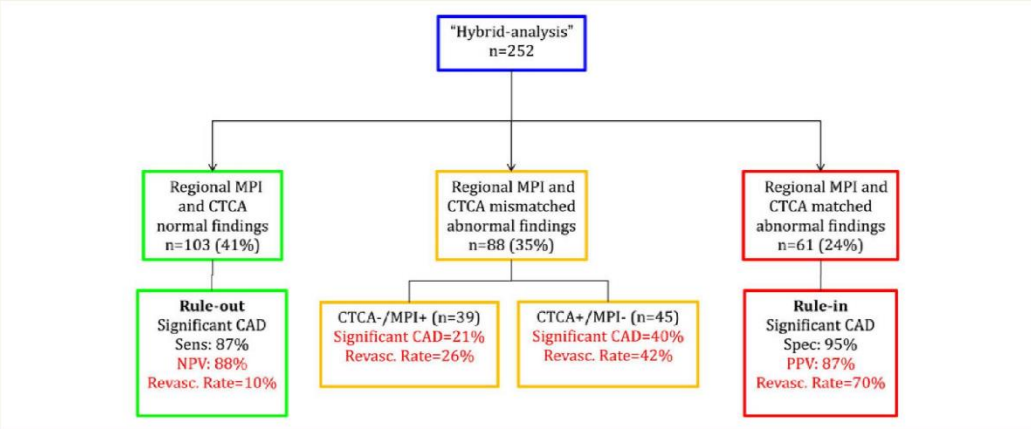



**Figure 5** Hybrid-based 'rule-in/rule-out' clinical protocol.

In the absence of coronary stenoses on CTCA, myocardial perfusion defects may represent either artefacts or microvascular/endothelial dysfunction. Accordingly, in this group, CAD prevalence and revascularization rates were low (*Figure 5*). CTCA has a very high negative predictive value as demonstrated by a vast number of studies comparing it with the angiographical gold standard of ICA.[21] The fact that we used a more comprehensive anatomo-functional gold standard (ICA + FFR) may explain to some extent the low sensitivity. Moreover, the sensitivity of CTCA by core-lab analysis in the main EVINCI trial was lower than by individual-centre analysis.[9] As a result, some lesions may have been underestimated accounting for the small number of revascularizations in this group.

Conversely, patients with significant coronary stenoses on CTCA but the absence of perfusion defects had a substantial CAD prevalence and revascularization rate (40 and 42%, respectively). This finding has several explanations. On one hand, the gold standard used in the present study was mainly anatomical (QCA), favouring agreement with CTCA rather than MPS. On the other hand, as already shown,[22] the cut-off chosen for FFR ($\leq$0.80)[5,19] may overestimate the haemodynamic significance of CAD compared with

non-invasive ischaemia testing. In line with this evidence, among the 19 patients with a pathological FFR evidenced in this study, only 21% had a matched finding on hybrid imaging. Interestingly, only 12/19 (63%) of those lesions presented a FFR $\leq$0.75, as a more stringent cut-off for positivity.[3] However, the incomplete FFR penetration observed in the present study, mainly due to protocol violations, does not allow defining whether the use of a lower cut-off value of FFR would have better correlated with hybrid findings.

Such a 'rule-in/rule-out' protocol is supported by follow-up data, indicating low event rates in patients with normal hybrid findings, high event rates for pathological matched findings, and intermediate event rates with mismatched findings.[8] Moreover, in selected cases, our integrated protocol may overcome the limitations of the more simplistic binary (i.e. either functional or anatomic) approach usually applied to CAD diagnostics, as recently reported.[23]

## Limitations

Like the overall EVINCI population, our study had a significant drop-out rate, as not every patient underwent all protocol-specified imaging studies. Additionally, data corruption and incomplete data sets accounted for further dropouts. Accordingly, 252 of the 697 patients originally enrolled in the EVINCI study were included in the present sub-study. However, those represented all the EVINCI patients that underwent MPS, CTCA, and ICA and in whom, thus, hybrid analysis could be practically performed. In fact, only a marginal portion of those patients (7%) was excluded because of technical reasons, confirming the overall robustness of 3D image fusion. Moreover, since the demographical, clinical, and angiographic characteristics of the present patients were almost superimposable to those of the main EVINCI population,[9] the presence of a significant selection bias can be excluded (Supplementary data online, *Table SA*). Second, no long-term follow-up data were obtained precluding any analysis on the impact of hybrid imaging on downstream patient management and outcomes. Third, FFR rate was only 23%, and 34% of patients with intermediate lesions were not interrogated with FFR. Incomplete FFR penetration due to frequent protocol violations highlights the sub-optimal FFR use across Europe and may have been responsible for some of the 'false-negative' hybrid findings and prevents any conclusive analysis on the 'false-positive' studies (Supplementary data online, *Tables SB* and *SC*). In our study, the respective sensitivities of CTCA and MPS were lower than anticipated from small single-centre studies (particularly for CTCA: 78%). This may be explained by selecting higher risk patients who had additional MPS performed, as well as by the inclusion of patients with intermediate stenosis (30–70%) without invasive functional evaluation, and by the exclusive use of independent core-lab data for the present analysis. In fact, the accuracies of stand-alone imaging modalities reported were almost superimposable to those of the overall EVINCI study when only core-lab data were considered.[9] Notably, on centre-based analysis, the diagnostic accuracy of the different non-invasive imaging modalities was generally improved compared with the core-lab data. Nevertheless, even when only individual-centre data were considered, hybrid imaging maintained significantly elevated specificity and overall diagnostic accuracy, at both per-patient and vessel-based analyses (Supplementary data online, *Figure S1*).

Moreover, in the accuracy analyses, MPS was considered pathological in the presence of ischaemia and/or scar. Interestingly, the presence of a matched hybrid finding showed comparable sensitivity, specificity, and accuracy if myocardial ischaemia (and not scar) was considered as the only positivity criteria (50, 96, and 79%, respectively).

Finally, the added radiation exposure from hybrid protocols must also be considered. In the present study, average radiation doses varied considerably, depending on the imaging technique (PET vs. SPECT) and on the acquisition protocol employed. Specifically, the theoretical risk related to the radiation exposure of a SPECT/CTCA hybrid protocol may appear rather high, particularly if compared with PET/CTCA imaging or other non-invasive imaging modalities.[24] However, previous results suggest that the use of modern equipment and dose-optimization protocols (e.g. prospective ECG-triggering for CTCA, stress-only for SPECT) may consistently reduce the radiation burden of hybrid imaging,[25] favouring its clinical application on a larger scale. Nevertheless, further long-term comparative studies are probably needed to conclusively define the cost-efficiency and quantitate the added radiation hazard that may be related to hybrid imaging, and to definitively assess its possible prognostic impact.

# Conclusions

Hybrid imaging allows more reliable co-localization of myocardial perfusion defects with subtending coronary arteries than standardized myocardial segmentation models accounting for variations in individual coronary anatomy. In two-thirds of patients at intermediate pre-test probability of CAD, hybrid imaging may offer a non-invasive 'rule-in/rule-out' of patients with haemodynamically significant CAD.

# Supplementary data

Supplementary data are available at *European Heart Journal – Cardiovascular Imaging* online.

**Conflict of interest:** None declared.

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**CHAPTER 4.**

## Glucose metabolism abnormalities in heart failure patients: insights and prognostic relevance

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*Heart Fail Clinics (in press)*

**Glucose metabolism abnormalities in heart failure patients: insights and prognostic relevance**

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**Synopsis**

Heart failure is a clinical syndrome characterized by left ventricular dysfunction and/or elevated intracardiac pressures, with a prevalence of about 1 – 2%. In the last decades, many metabolic disorders have been studied as linked with heart failure, in particular glucose metabolism abnormalities. Diabetes mellitus and insulin resistance are strictly related with heart failure, with a bidirectional link, where each one can influence the other. The aim of this review is to report the role of glucose metabolism abnormalities in the development of HF, defining the epidemiology, and assessing pathophysiology and prognosis of HF related to glucose metabolism disorders.

**Key words:** Heart failure; Glucose metabolism abnormalities; Diabetes mellitus; Insuline resistance; Hyperglycemia; Insulin sensitivity.

**Key points**

- Heart failure is a clinical syndrome characterized by left ventricular dysfunction and/or elevated intracardiac pressures, with a prevalence of about 1 – 2%
- There are several factors involved in HF development, that go from ischemic heart disease to metabolic disorders, passing through genetic etiology
- Although the most known cardiac nosological entity related to glucose metabolism disorders is diabetic cardiomyopathy, insulin resistance is a very frequent finding among HF patients
- the homeostasis model assessment (HOMA) index has proved to be robust and reliable tool for the assessment of insulin resistance
- Heart failure can be considered the major cause of hospitalization in patients with glucose metabolism abnormalities

## Introduction

Heart failure (HF) is a clinical syndrome caused by structural and/or functional cardiac abnormality, resulting in a reduced left ventricular function, and/or elevated intracardiac pressures.[1] The prevalence of HF is approximately 1 – 2% of the adult population, rising to ≥ 10% over 70 years of age.[1–5] Today it is not possible to define a precise etiology of HF, but there are several factors involved in HF development, that go from ischemic heart disease to metabolic disorders, passing through genetic etiology.[1] Focusing on metabolic disorders responsible of HF development, there is a wide spectrum of metabolic abnormalities that can lead to HF.

In the last years there was an increase of prevalence in many metabolic disorders, in particular in glucose metabolism abnormalities rate,[6] in the context of westernized lifestyles, high-fat diets and decreased exercise, leading to increasing levels of obesity, insulin resistance (IR), compensatory hyperinsulinemia and ultimately, type 2 diabetes mellitus (T2DM).[6] Although the most known cardiac (not ischemic) nosological entity related to glucose metabolism disorders is diabetic cardiomyopathy, IR, whether or not associated with T2DM, is a very frequent finding among HF patients, with a prevalence ranging from 33 to 70%.[7–9] There is a bidirectional link between IR and HF, where although IR can predict HF, it often develops in HF patients, with more severe symptoms and worse clinical outcome.[10–13] The degree of IR is significantly related with worsen clinical presentation and a poor prognosis in patients with HF, and it is known that metformin can prevent HF progression, improving exercise capacity.[7,10,12–14]

The scope of this appraisal is to report the role of glucose metabolism abnormalities in the development of HF, defining a classification and diagnosis of glucose metabolism defects, which can lead to T2DM, and assessing pathophysiology and prognosis of HF related to glucose metabolism abnormalities.

## Classification of glucose metabolism abnormalities

Glucose metabolism disorders are a wide spectrum of abnormalities, characterized by elevated levels of blood glucose and impaired levels of circulating insulin. The actual classification of glucose metabolism abnormalities is based on recommendations from the World Health Organization (WHO) and the American Diabetes Association (ADA)[6,15–17] (table 1).

Beyond the classical four main etiological categories of DM (table 1) identified as type 1 DM (due to destruction of pancreatic beta-cells, progressing to absolute insulin deficiency. Although is typical of young age, it can occur at any age), T2DM (characterized by a combination of IR and beta-cells failure), gestational DM, and other specific types of DM (this entity includes single genetic mutation forms, DM secondary to other diseases, drug- or chemically induced DM, and infective forms), there is group of entities called “pre-diabetic disorders”, which are strictly related to cardiovascular (CV) events and in particular to HF development, and that includes a variety of disorders to be discussed.[6,17]

***Different types of "pre-diabetic disorders"***

All the types of glucose metabolism disorders (table 1), can often be considered as precursors of blown DM (generally T2DM), but in many cases are isolated and not related to the presence of T2DM. Between these entities, there are impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), that are often referred to as "pre-diabetes", reflecting the natural history of progression from normoglycaemia to T2DM.[6] Considered the normal oscillation of fasting plasma glucose values day by day, many times these forms of glucose metabolism abnormalities could pass misdiagnosed. For this reason, IFG and IGT often can only be recognized by the results of an oral glucose tolerance test (OGTT).[6] However, the most important form of glucose metabolism disorders, that, although could lead to T2DM, is common also in non-diabetic patients, and that is strongly related bi-directionally to HF, is IR. The concept of IR was proposed first time in 1936, and is generally defined as reduced biological action of insulin, such as inhibition of hepatic glucose production and insulin-mediated glucose disposal.[18–20] There is a known correlation between obesity and IR development. In particular, patients with IR who are not obese by traditional weight criteria, may have an increased percentage of body fat distributed predominantly in the abdominal region.[17] This type of glucose metabolism abnormality frequently goes undiagnosed for many years because in the beginning there is not a clear hyperglycemia, which develops gradually, without showing the classic symptoms of diabetes.[17] Nevertheless, a great part of patients, experience CV events, including also the developing of HF, explaining the role of IR in HF pathogenesis also in

non-diabetic patients.[17] As result of normal or elevated levels of insulin in patients with IR, the higher blood glucose levels cannot be compensated by high levels of circulating insulin, due to low effect of insulin. Thus, insulin secretion is defective in a first step in these patients and insufficient to compensate for IR. In a second step, considering the possible development of T2DM, also levels of circulating insulin become insufficient, needing a supplement of them.[17]

**Diagnosis of glucose metabolism abnormalities**

***Diabetes mellitus, impaired fasting glucose and impaired glucose tolerance***

As general rule, DM is defined by an elevated level of blood glucose. Based on this assumption, the WHO criteria for diagnosis of glucose metabolism abnormalities are based on fasting plasma glucose (FPG) and 2-hour post–load plasma glucose (2hPG) (when there isn't an overt hyperglycemia (OGTT)) concentrations[6,21] (table 2). On the other hand, in addition to these parameters of glucose metabolism disorders diagnosis, ADA recommend to use also glycated hemoglobin $A_{1C}$ ($HbA_{1C}$). Therefore, for the diagnosis of DM, IFG and IGT there are several parameter to use, different between WHO and ADA. In particular, for WHO, the cut-points are the following:[6]

1. Diabetes mellitus:

- $HbA_{1C}$ can be used, with a cut-point ≥ 6,5%;
- FPG is recommended, with a cut-point ≥ 126 mg/dL;

- 2hPG is recommended, with a cut-point ≥ 200 mg/dL;

2. Impaired glucose tolerance:

- FPG is recommended, with a cut-point < 126 mg/dL;
- 2hPG is recommended, with a cut-point ≥ 140 - < 200 mg/dL;

3. Impaired fasting glucose:

- FPG is recommendend, with a cut-point of 110 – 125 mg/dL.

Regarding ADA, the cut-points are the following:[6]

1. Diabetes mellitus:

- $HbA_{1C}$ is recommended, with a cut-point ≥ 6,5%;
- FPG is recommended, with a cut-point ≥ 126 mg/dL;
- 2hPG is recommended, with a cut-point ≥ 200 mg/dL;

2. Impaired glucose tolerance:

- FPG is recommended, with a cut-point < 126 mg/dL;

3. Impaired fasting glucose:

- FPG is recommendend, with a cut-point of 110 – 125 mg/dL.

***Insulin resistance***

Regarding IR, the homeostasis model assessment (HOMA) index has proved to be a robust and reliable tool for the assessment of IR (table 2). This method is based on a homeostatic mathematic model considering FPG and fasting plasma insulin. Several studies[18,20,22–28] have assessed a normal range value for HOMA-index that is considered now between 0,23 and 2,5.

***Strategy for early detection of impaired glucose metabolism***

As known, DM does not cause specific symptoms for many years, which could explain the great number of T2DM undiagnosed over several years. Overall, this could be the reason of several CV disorders DM related which occur before the diagnosis of DM. On the other hand, as known, IR (a frequent precursor of T2DM) is always asymptomatic and is not associated with hyperglycemia, for this reason, it is more difficult to detect with routinely exams IR[6]. However, screening of hyperglycemia (or of IR), should be targeted to high-risk individuals of CV disease and HF.[6] So, according to current guidelines[6], the approaches for early detection of glucose metabolism abnormalities are:

- Measuring PG or $HbA_{1C}$;
- Using demographic and clinical characteristics to determine the likelihood of impaired glucose metabolism (for the eventual evaluation of HOMA-index);
- Evaluate the presence of possible risk factors of glucose metabolism disorders, CV events and HF (for the eventual evaluation of HOMA-index).

**Pathophysiological insights of heart failure related to glucose metabolism disorders**

As previously described, IR is a direct precursor of T2DM, and the consequent compensatory hyperinsulinemia peculiar of IR, with consequent elevated levels of PG, associated with clustering of CV risk, can lead to several CV disease.[6] As known, T2DM patients are generally obese (or with higher levels of fat abdominally distributed), and the

release of free fatty acid (FFA) from adipose tissue, directly impairs insulin sensitivity[6,29] (figure 1). So the "primum movens" is IR, with several consequent mechanism involved in HF presentation and progression. However, well known is the bidirectional link between IR and HF,[11] where several studies indicate that DM and IR are not only causative factors of HF,[30–32] but patients with HF and DM or IR showed a more aggressive form of left ventricular (LV) dysfunction, with a higher mortality rate.[33] Insulin resistance is the entity with higher prevalence in HF patients (up to 60%),[34] with a complex pathophysiological interaction between these two conditions, since IR may be the cause and consequence of HF at the same time.[30] Similarly, DM has a prevalence of 10 – 40% in patients with HF,[35] showing a quite high prevalence, but lower than IR. This could explain the more important and potential role of IR in HF development (and vice versa), compared to DM, that was widely discussed in the years.[8,9,30]

***Pathophysiology of heart failure in IR and DM***

Well known is the role of IR and DM in several functional, metabolic and structural alterations that involve myocardial tissue and that can lead to HF (figure 1). In the initial stage of HF, there is a change in substrate utilization, where glucose becomes the primary substrate oxidized.[9] Hyperglycemia is responsible for several cellular pathway abnormalities, going from increased polyol, modification of proteins, and formation of advanced glycation endoproducts, to increased protein kinase C expression, phenomenon leading to overproduction of superoxide and consequent oxidative stress[30,36] (figure 1). On the other hand, the increase of FFA myocardial uptake, typical of diabetes and obesity,

leads to long-chain FFA oxidation and to a disproportionate oxidative request to mitochondria with uncoupling of mitochondrial oxidative phosphorylation[30,37] (figure 1). In addition, the impaired expression of contractile proteins, is responsible for depressed myofibrillar ATP activities and abnormalities of the sarcoplasmic reticular and sarcolemmal calcium transport process, with consequent calcium overload and impaired diastolic function.[30,38]

***Hyperactivation of adrenergic system***

Another consideration to do is on the impairment of cardiac sympathetic innervation, commonly observed in HF patients affected by DM and/or IR. Paolillo et al,[34] in a recent study, showed in HF patients with DM, or without DM, but with IR, a more impaired cardiac sympathetic innervation, compared with non-diabetic and non-IR patients, indicating a chronic adrenergic hyperactivity, that correlates with high levels of hyperglycemia and HF development and worsening. In the same year, Rengo et al,[39] reported that levels of GRK2, a protein kinase involved in the desensitization of cardiac beta-receptors, are significantly more elevated in HF patients with DM compared with non-diabetic patients with HF, meaning a stronger adrenergic activation, known to be involved in progression and worsening of HF. This is a peculiarity of DM and IR patients with HF, where it is well known that because of adrenergic overactivity, HF is complicated by DM and IR.[8]

**Epidemiology of heart failure in glucose metabolism abnormalities and vice versa**

Since now, it was widely discussed the bidirectional link between HF and glucose metabolism disorders. Well known is the prevalence of HF in the general population, that is about 1 – 2%,[1–5] rising to 12 – 30% in diabetic patients.[40,41] Glucose metabolism abnormalities (in particular DM and IR) are independent risk factors for the development of HF. In the Framingham study, the relative risk of HF in patients with T2DM was doubled for men and six times as high in women[6,42]. These data were confirmed by the National Health and Nutrition Examination Survey, where T2DM was showed to be an independent predictor of HF (HR 1.85, 95% CI 1.51 – 2.28).[43]

On the other hand, the prevalence of DM in the general population is about 6 – 8 %,[6] but as described by MacDonald et al,[44] rises to 12 – 30% in HF patients. However, HF patients are older than general population. This could be considered a bias selection, although is widely known (also in the general population) not only the role of glucose metabolism disorders in development of HF, but also the major prevalence of glucose metabolism abnormalities (in particular DM and IR, as previously described) in HF patients. Confirming these data, the TOSCA registry,[7] a recent Italian registry, made on 526 patients (81% male, age 62.5 ± 12.2 years) has shown a prevalence of IR in HF patients of 30 – 35%, in line with previous studies, demonstrating the great impact of glucose metabolism abnormalities on HF.

## Prognosis of heart failure related to glucose metabolism abnormalities

### *Great trials*

Today, HF can be considered the major cause of hospitalization in patients with glucose metabolism abnormalities. These data were confirmed by the Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events and Ramipril (DIABHYCAR) trial,[45] with a mortality 12-fold higher in patients with HF and glucose metabolism disorders, compared to patients without HF (36% vs 3%). On the other hand, in the BEta blocker STroke (BEST) trial, impaired glucose metabolism increased the risk of hospitalization in HF patients, with T2DM as independent predictor of mortality, mostly for HF.[46] More recently, the Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure (MERIT-HF),[47] showed HF patients with glucose metabolism alterations, to be more hospitalized than patients free from DM.

### *Previous studies*

A previous study of Suskin et al,[12] studied 663 patients with abnormalities in glucose metabolism (DM or IR) and HF, assessing prognostic role of glucose metabolism, in particular, evaluating functional class, six minute walking performance and LV function. In this cohort of patients, they found a greater proportion of diabetic patients compared to non-diabetic patients in NYHA class III/IV (161 vs 77, $p = 0.011$), with a higher value of insulin in non-diabetic patients in NYHA class III/IV compared to NYHA class I/II ($19.6 \pm 2.3$ vs $10.2 \pm 0.6$ mU, $p < 0.005$). In addition, among non-diabetic patients, significantly more NYHA class III/IV patients had elevated HOMA-index levels (44% vs 28%, $p <$

0.005) compared to NYHA class I/II patients. These data were confirmed also for six minute walking distance, that was significantly shorter in diabetic patients compared to non-diabetic patients (369 ± 7 vs 385 ± 4 meters, p = 0.03). Furthermore, patients with impaired HOMA-index had a significantly shorter six minute walking distance than those with normal values (372 ± 7 vs 391 ± 5 meters, p = 0.02).

A more recent study of Doehner et al,[13] evaluated insulin sensitivity in 105 male patients with HF. After a mean follow up of 44 ± 4 months, patients with an insulin sensitivity below the median value had a worse survival (61% at two years) compared to patients with an insulin sensitivity above the median value (83% at two years) (RR 0.38, 95% CI 0.21 – 0.67, p = 0.001). Furthermore, insulin sensitivity resulted independent predictor of mortality in the study cohort.

***Therapeutic possibilities***

Considering the impact on prognosis of glucose metabolism abnormalities, it is possible affirm that DM and IR can be considered as potential target of HF. Although is known the role of several new pharmacological agents in the reduction of mortality in HF diabetic patients,[48] poor is known about the potential treatment of IR and its impact on HF prognosis. Considering this assumption, Wong et al,[14] randomized in a double-blind, placebo-controlled study 62 non-diabetic HF patients, to receive either four months of metformin or matching placebo. Compared with placebo, metformin decreased HOMA-index, improving also the secondary endpoint of the slope of the ratio of minute ventilation

to carbon dioxide production ($VE/VCO_2$ slope). These data confirm the hypothesis that treatment of IR should be protective in patients with HF.

## Summary

There is a strong correlation between glucose metabolism abnormalities and HF, with a bidirectional link between them, where glucose metabolism abnormalities at every step can affect HF. On the other hand, DM and IR are more prevalent in HF patients, and patients with both HF and DM have a worst prognosis, due to a combination of effects of both the components. Well known is the role of some new drugs in reducing the mortality in HF patients with DM, but more studies are warranted to know the real effect of treatment of IR in patients with HF.

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**Tables**

**Table 1.** Different types of glucose metabolism disorders

| **CLASSIFICATION OF GLUCOSE METABOLISM ABNORMALITIES** |
|---|
| Type 1 diabetes mellitus |
| Type 2 diabetes mellitus |
| Other specific types of diabetes mellitus |
| Genetic mutation forms |
| Secondary to other diseases |
| Diseases of the exocrine pancreas |
| Endocrinopathies |
| Drug or chemically induced |
| Infective forms |
| Gestational diabetes mellitus |
| **Pre-diabetic disorders** |
| Impaired fasting glucose |
| Impaired glucose tolerance |
| Insulin resistance |

**Table 2.** Diagnostic evaluation of glucose metabolism disorders (according to WHO and ADA)

**Diabetes mellitus**

Glycated hemoglobin $A_{1C}$ ($HbA_{1C}$)

Fasting plasma glucose

2-hour post–load plasma glucose (OGTT)

**Impaired glucose tolerance**

Fasting plasma glucose

2-hour post–load plasma glucose (OGTT)

**Impaired fasting glucose**

Fasting plasma glucose

**Insuline resistance**

Homeostasis model assessment (HOMA) index

**Figures**

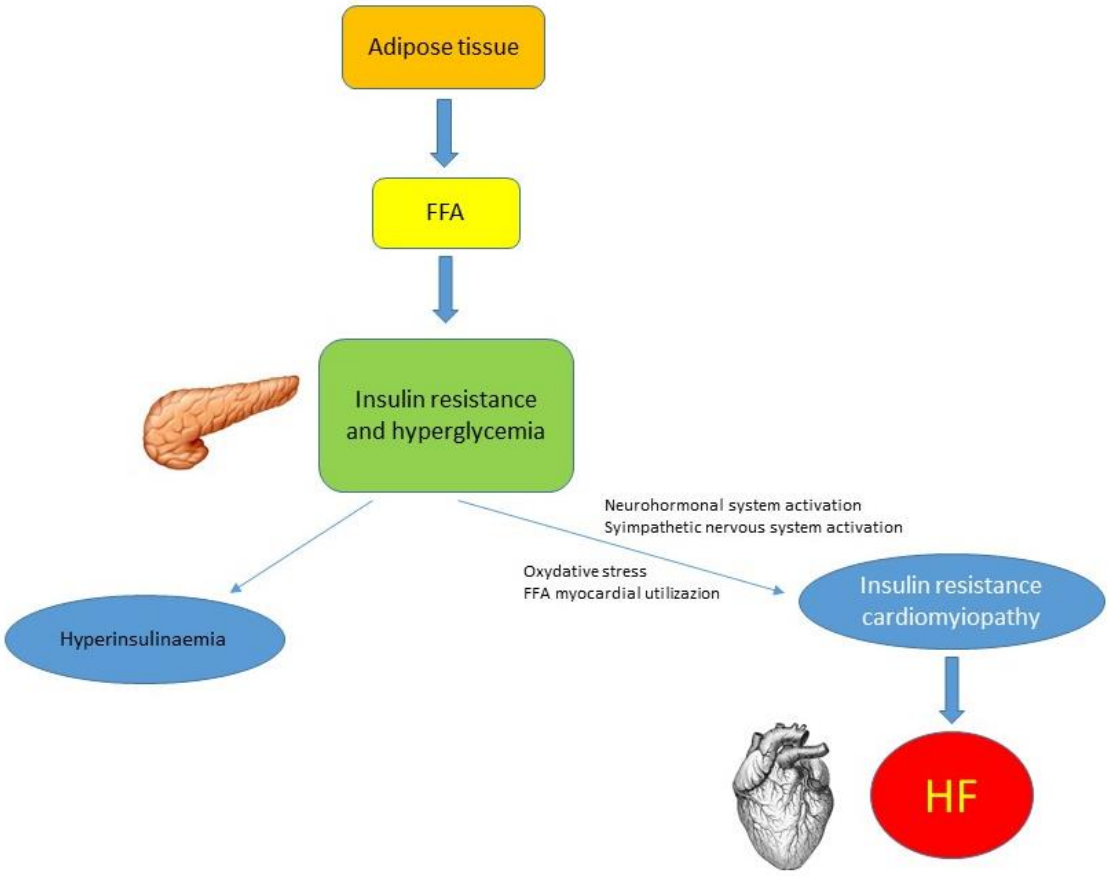


**Figure 1.** Pathophysiology of HF development related to insulin resistance.

FFA = free fatty acid; HF = heart failure.

## CHAPTER 5.

## Efficacy and safety of glucagon-like peptide-1 agonists on macrovasular and microvascular events in type 2 diabetes mellitus: a meta-analysis

Gargiulo P, Savarese G, D'Amore C, De Martino F, Lund LH, **Marsico F**, Dellegrottaglie S, Marciano C, Trimarco B, Perrone Filardi P

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SYSTEMATIC REVIEWS AND META-ANALYSES

# Efficacy and safety of glucagon-like peptide-1 agonists on macrovascular and microvascular events in type 2 diabetes mellitus: A meta-analysis

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**KEYWORDS**
Diabetes mellitus;
Glucagon-like peptide-1 agonists;
Microvascular events;
Macrovascular events;
Retinopathy;
Nephropathy;
Cardiovascular events

**Abstract** *Aims:* Glucagon-like peptide-1 (GLP-1) agonists improve glycaemic control in type 2 diabetes mellitus (DM). Outcome trials investigating macro and microvascular effects of GLP-1 agonists reported conflicting results. The aim of this study was to assess, in a meta-analysis, the effects of GLP-1 agonists on mortality, major nonfatal cardiovascular (CV) events, renal and retinal events.

*Data synthesis:* MEDLINE, Cochrane, ISI Web of Science, SCOPUS and ClinicalTrial.gov databases were searched for articles published until June 2017. Randomized trials enrolling more than 200 patients, comparing GLP-1 versus placebo or active treatments in patients with DM, and assessing outcomes among all-cause death, CV death, MI, stroke, HF, diabetic retinopathy and nephropathy were included. 77 randomized trials enrolling 60,434 patients were included. Compared to control, treatment with GLP-1 significantly reduced the risk of all-cause death (RR: 0.888; CI: 0.804–0.979; p = 0.018) and the risk of CV death (RR: 0.858; CI: 0.757–0.973; p = 0.017). GLP-1 agonists did not affect the risk of MI (RR: 0.917; CI: 0.830–1.014; p = 0.092) as well as the risk of stroke (RR: 0.882; CI: 0.759–1.023; p = 0.097), HF (RR: 0.967; CI: 0.803–1.165; p = 0.725), retinopathy (RR: 1.000; CI: 0.807–1.238; p = 0.997) and nephropathy (RR: 0.866; CI: 0.625–1.199; p = 0.385).

*Conclusions:* Treatment with GLP-1 agonists in DM patients is associated with a significant reduction of all cause and CV mortality.



*Acronyms:* GLP-1, Glucagon-like peptide-1; DM, Type 2 diabetes mellitus; CV, Cardiovascular; MI, Myocardial Infarction; HF, Heart Failure; DPP-4, Enzyme Dipeptidyl Peptidase 4; RRs, Relative risks; CIs, Confidence Intervals; HbA1c, Glycated Haemoglobin.

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## Introduction

Diabetes mellitus (DM) represents a major risk factor for cardiovascular (CV) disease, particularly atherosclerotic CV disease and heart failure (HF). Among glucose-lowering drugs, metformin in the United Kingdom Prospective Diabetes Study [1] and empagliflozin in the EMPA-REG OUTCOME trial [2,3] significantly reduced CV morbidity and mortality as well as progression of nephropathy [4]. More recently, glucagon-like peptide-1 (GLP-1) agonists have been investigated in CV outcome trials.

GLP-1 is an incretin hormone secreted by L cells in the distal small intestine in response to oral nutritional intake that increases insulin release [5]. GLP-1 agonists are resistant to degradation by enzyme dipeptidyl peptidase 4 (DPP-4) showing more prolonged effect in comparison with native GLP-1.

GLP-1 agonists are currently approved for the treatment of type 2 DM and liraglutide also for chronic weight management [6]. In addition to the primary effect on glucose metabolism, they have been shown to reduce blood pressure [7], body weight and triglycerides, with modest effect on LDL and total cholesterol levels [8,9] and to increase heart rate [7]. Outcome trials investigating CV effects of GLP-1 agonists reported conflicting results. The ELIXA trial [10] examined whether addition of lixisenatide to usual care, compared to placebo, reduce CV events in patients with type 2 DM and recent acute myocardial infarction (MI) and did not report a significant difference in the primary composite endpoint of CV death, nonfatal MI, nonfatal stroke, or hospitalization for unstable angina. In contrast, the LEADER trial [11], enrolling 9340 diabetic patients at high CV risk, randomized to liraglutide or placebo, demonstrated a significant reduction of the primary endpoint of CV death, nonfatal MI or nonfatal stroke in patients with type 2 DM treated with liraglutide compared to placebo. More recently, in the SUSTAIN-6 trial [12], semaglutide, compared to placebo, significantly reduced the composite endpoint of CV death, nonfatal MI or nonfatal stroke in high risk type 2 DM patients. However, in this study rates of retinopathy complications were significantly higher, indicating a potential detrimental effect on microvascular endpoints.

A previous meta-analysis [13], including short-term and low risk patients, demonstrated CV safety of GLP-1 agonists and a significant reduction of major CV events compared to placebo. However, this study did not include recent randomized trials purposely designed to assess CV outcomes and did not report renal and retinal endpoints.

Thus, the aim of this study was to assess, in a meta-analysis, the effects of GLP-1 agonists on mortality, major nonfatal CV events as well renal and retinal events.

## Methods

### *Data sources and search strategy*

The meta-analysis was designed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [14]. PRISMA checklist was reported in Supplemental Materials. MEDLINE, Cochrane, ISI Web of Science, SCOPUS and ClinicalTrial.gov databases were searched for articles published until November 2016 combining the following terms [("Albiglutide" OR "Exenatide" OR "Liraglutide" OR "Lixisenatide" OR "Semaglutide" OR "Taspoglutide" OR "Glucagon like peptide 1 agonist" OR "Glucagon like peptide 1 receptor agonist" OR "GLP-1 agonist") AND "randomized"]. No language restrictions were applied.

### *Study selection*

Study inclusion criteria were: randomized allocation to GLP-1 agonists vs. placebo or other glucose-lowering drugs; enrolment of more than 200 patients [15]; assess at least one of following major outcomes: all-cause death, CV death, MI, stroke, HF.

### *Data extraction*

Articles were screened for fulfilment of inclusion criteria by five independent reviewers (CDA, FDM, FM, SD, CM). Reviewers compared selected trials and discrepancies were resolved by two other authors (GS, PG). Corresponding authors were asked to provide full-text articles, if they were not available. From each study, information about methods, year of publication, number of patients in treatment and control arms, duration of follow-up, age, gender, duration of DM were collected and entered into STATA (version 14.0, StataCorps, College Station, Texas) by two authors (CDA, FDM) and checked by another author (PG).

The outcomes abstracted were all-cause death, CV death, MI, stroke, HF, diabetic retinopathy and diabetic nephropathy. MI included fatal and no fatal MI, acute MI, acute coronary syndrome. HF included cardiac failure, congestive cardiac failure, decompensated HF, congestive HF, new onset HF and HF requiring hospitalization. Stroke included cerebral infarction, cerebrovascular accident, cerebellar infarction, ischaemic stroke and brain infarction. Diabetic retinopathy included diabetic retinopathy, diabetic retinal complications and blurred vison related to DM. Diabetic nephropathy included diabetic nephropathy, renal failure and renal injury.

### *Data synthesis and analysis*

Relative Risks (RR) of the effect of randomized treatments were calculated using the metan routine (STATA Statacorp, version 14.0) to account the probability of events occurring in treatment group versus control group [16]. Relative risks (RRs) and 95% Confidence Intervals (CIs) for each outcome were calculated separately for each trial, with grouped data using the intention-to-treat principle [17]. Pooled RRs were logarithmically transformed and weighted for the inverse of variance. Overall estimates of effect were calculated with a random effects model. The assumption of homogeneity between the treatment effects in different

trials was tested by Q statistic and further quantified by I2 statistic. A significant heterogeneity was defined by a $p < 0.05$ at Q statistic; $I^2$ ranging from 0% to 40% might indicate not important heterogeneity, from 30% to 60% might represent moderate heterogeneity, from 50% to 90% might indicate substantial heterogeneity and from 75% to 100% might represent considerable heterogeneity [17]. The significance level for all outcome and heterogeneity analyses was set at $p \leq 0.05$.

### Sensitivity analysis

To investigate the effects of individual GLP-1 agonists on the outcomes, and whether the effects of GLP-1 agonists differed when compared to placebo or to active treatments, meta-analyses were performed stratifying trials according to the type of GLP-1 agonists, and according to the comparator (placebo or active treatment) used. We also performed a sensitivity analysis imputing 1 event for each study group in trials that had outcomes with 0 events in order to avoid any distortion due the different sizes of the treatment and control groups.

### Meta-regression analysis

Weighted random-effects meta-regression analysis was performed with the metareg command (STATA Statacorp, version 14.0) to test the relationship between glycated haemoglobin (HbA1c) changes and log RR of clinical events. The weight used for each trial was the inverse of the sum of the within-trial variance and the residual between trial variance. For these analyses, the achieved differences between absolute HbA1c unit changes in active treatment and control groups were considered ($\Delta$HbA1c = [(HbA1c-tf − HbA1c-tb) − (HbA1c-cf − HbA1c-cb)]) where HbA1c-f was end-study in treatments (t) and controls (c), HbA1c-b was baseline in treatments (t) and controls (c). For all meta-regression analyses, the residual maximum likelihood (REML) method was employed to explain residual heterogeneity not explained by potential effect modifiers, including an additive between-study variance component Tau2 [16]. The significance level for all meta-regression analyses was set at $p \leq 0.05$ (two sided).

### Publication bias

To evaluate potential publication bias, funnel plots were produced and a weighted linear regression was used, with the natural log of the odds ratio as the dependent variable and the inverse of the total sample size as the independent variable. This is a modified Macaskill's test, which gives more balanced type I error rates in the tail probability areas in comparison with other publication bias tests [18]. The significance level for the publication bias analysis was set at $p < 0.05$.

## Results

### Characteristics of included trials (Supplemental Table S1)

Of 975 articles identified in the initial search, 450 were retrieved for more detailed evaluation and, finally, 77 randomized trials (Table S1) were included in the analyses (Fig. 1). Of 60,434 patients, a total of 33,642 were randomized to GLP-1 agonists and 26,792 to control arms. Age ranged from 50 to 74 years, 45% of patients enrolled were women and follow-up ranged from 12 to 195 weeks.

### Outcome analysis (Fig. 2)

All-cause death occurred in 2.9% of patients treated with GLP-1 agonists compared to 3.8% of those enrolled in control arms. A significant 11.2% reduction in risk of all-cause death was observed in GLP-1 agonists treated patients (RR: 0.888; 95% CI: 0.804–0.979; comparison p: 0.018; pQ: 0.997; I2: 0%). The reduction in risk of all-cause death was statistically significant in trials comparing GLP-1 agonists vs. placebo (RR: 0.888; 95% CI: 0.804–0.982; comparison p: 0.020; pQ: 0.930; I2: 0%), but not in those comparing GLP-1 agonists vs. active drugs (RR: 0.873; 95% CI: 0.521–1.463; comparison p: 0.606; pQ: 0.985; I2: 0%) (Supplemental Fig. 1).

Similarly, CV death occurred in 2.7% of patients randomized to GLP-1 agonists compared to 3.5% of those taking control drugs, corresponding to a 14.2% significant reduction in risk in GLP-1 agonists vs. control arms (RR: 0.858; 95% CI: 0.757–0.973; p comparison: 0.017; pQ: 0.882; I2: 0%). The reduction in the risk of CV death was statistically significant in trials comparing GLP-1 agonists vs. placebo (RR: 0.863; 95% CI: 0.760–0.980; comparison p: 0.023; pQ: 0.753; I2: 0%), but not in those comparing GLP-1 agonists vs. active drugs (RR: 0.635; 95% CI: 0.259–1.560; p comparison: 0.322; pQ: 0.764; I2: 0%) (Supplemental Fig. S2).

MI occurred in 2.7% of patients randomized to GLP-1 agonists vs. 3.6% in control arms. No significant effect of GLP-1 agonists was observed on the risk of MI (RR: 0.917; 95% CI: 0.830–1.014; p comparison: 0.092; pQ: 0.983; I2: 0%). This result was consistent in trials comparing GLP-1 agonists vs. placebo (RR: 0.915; 95% CI: 0.825–1.015; p comparison: 0.093; pQ: 0.953; I2: 0%), and in those comparing GLP-1 agonists vs. active treatments (RR: 0.952; 95% CI: 0.642–1.414; p comparison: 0.809; pQ: 0.867; I2: 0%), suggesting a neutral effect of GLP-1 agonists on MI (Supplemental Fig. S3).

Rates of stroke were 1.3% in GLP-1 agonists arm vs. 1.7% in control group, indicating no statistical difference in risk (RR: 0.882; 95% CI: 0.759–1.023; p comparison: 0.097; pQ: 0.998; I2: 0%). This result was consistent in trials comparing GLP-1 agonists vs. placebo (RR: 0.869; 95% CI: 0.743–1.017; comparison p: 0.080; pQ: 0.948; I2: 0%), and in those comparing GLP-1 agonists to active agents (RR: 1.006; 95% CI: 0.621–1.627; p comparison: 0.982; pQ: 0.986; I2: 0%) (Supplemental Fig. S4).

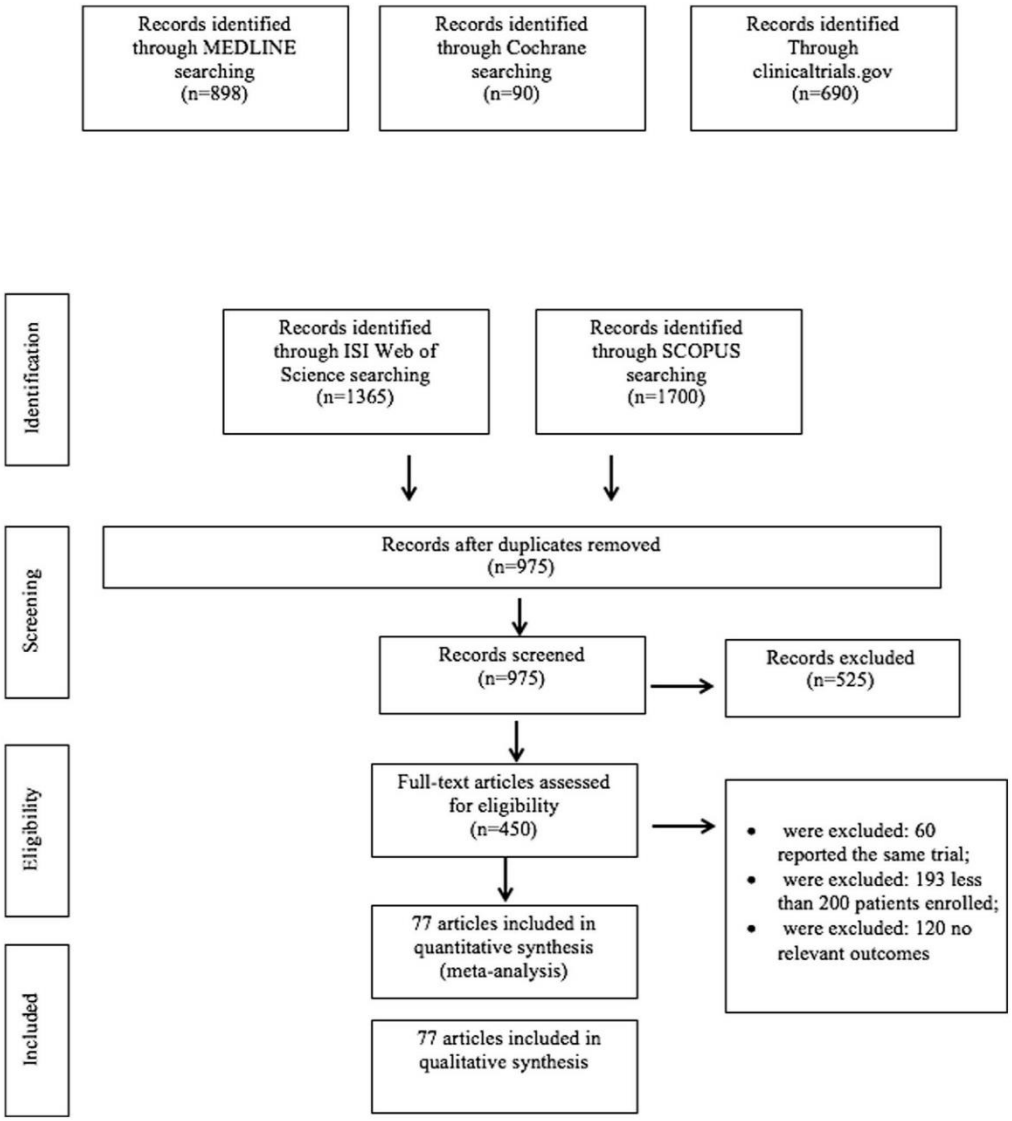


**Figure 1** Flow chart of study selection.

HF occurred in 1.3% of patients randomized to GLP-1 agonists vs. 1.7% of those in the placebo or active drugs arm. GLP-1 agonists did not affect the risk of HF (RR: 0.967; 95% CI: 0.803–1.165; p comparison: 0.725; pQ: 0.985; I2: 0%). This neutral effect was consistent in trials comparing GLP-1 agonists vs. placebo (RR: 0.982; 95% CI: 0.806–1.195; comparison p: 0.857; pQ: 0.861; I2: 0%) and in those comparing GLP-1 agonists vs. active drugs (RR: 0.853; 95% CI: 0.481–1.515; p comparison: 0.588; pQ: 0.954; I2: 0%), indicating a neutral effect of GLP-1 agonists on HF (Supplemental Fig. S5).

1.3% of patients randomized to GLP-1 agonists and to placebo or active drug arms developed diabetic retinopathy. Therefore, no statistical difference in risk was reported between treatment and control arms (RR: 1.000; 95% CI: 0.807–1.238; comparison p: 0.997; pQ: 0.318; I2: 10.0%). This result was consistent in trials comparing GLP-1 agonists vs. placebo (RR: 1.153; 95% CI: 0.754–1.763; p comparison: 0.512; pQ: 0.139; I2: 32.5%), and in those vs. active comparators (RR: 0.871; 95% CI: 0.676–1.124; comparison p: 0.288; pQ: 0.765; I2: 0%) (Supplemental Fig. S6).

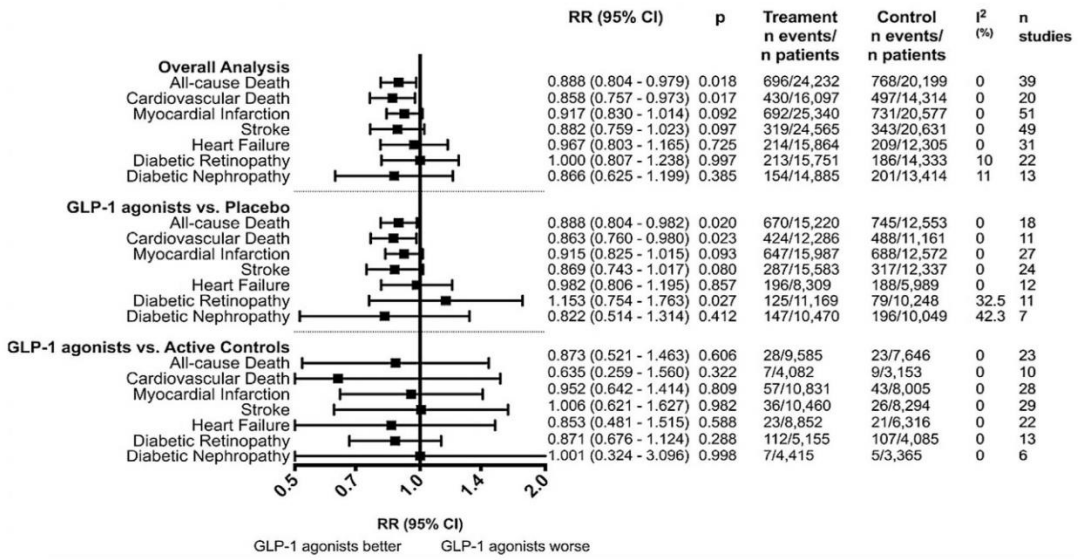


**Figure 2** Forrest plot showing the outcome analyses in Glucagon like peptide 1 (GLP-1) agonist vs. control arm.

Finally, diabetic nephropathy occurred in 1.0% of patients enrolled in the GLP-1 agonists vs. 1.5% in those in control arms. GLP-1 agonists did not affect the risk of diabetic nephropathy (RR: 0.866; 95% CI: 0.625–1.199; p comparison 0.385; pQ: 0.335; I2: 11.0%). This evidence was consistent in trials comparing GLP-1 agonists vs. placebo (RR: 0.822; 95% CI: 0.514–1.314; p comparison: 0.412; pQ: 0.109; I2: 42.3%), and in those comparing GLP-1 agonists vs. active treatments (RR: 1.001; 95% CI: 0.324–3.096; p comparison: 0.998; pQ: 0.952; I2: 0%) (Supplemental Fig. S7).

### *Sensitivity analysis*

When outcome meta-analyses were separately repeated for individual drugs, liraglutide significantly reduced the risk of all-cause (RR: 0.853; 95% CI: 0.749–0.971; p comparison: 0.016; pQ: 0.627; I2: 0%) and CV death (RR: 0.784; 95% CI: 0.660–0.931; p comparison: 0.005: pQ: 0.401; I2: 0%), with a trend toward reduction of the risk of MI (RR: 0.872; 95% CI: 0.754–1.009; p comparison: 0.066; pQ: 0.995; I2: 0%), but did not affect the risk of stroke, new HF onset, retinopathy and nephropathy. Only one trial [12] reported data on effects of semaglutide treatment on risk of stroke and nephropathy, showing a significant reduction in risk (RR: 0.614; 95% CI: 0.382–0.987; p comparison: 0.044: RR: 0.016; 95% CI: 0.001–0.259; comparison p: 0.004: respectively). On the other hand, semaglutide significantly increased the risk of retinopathy (RR: 1.685; 95% CI: 1.077–2.637; p comparison: 0.022; pQ: 0.465; I2: 0%), with no effect on the other outcomes (Fig. 3).

When the analyses were repeated imputing 1 event for each study group in trials that had outcomes with 0 events, the results of the main analysis were confirmed (Supplemental Table S2).

### *Meta-regression analysis (Table 1)*

Mean reduction of HbA1c was 1.0 ± 0.4 in GLP1-agonists arm and 0.6 ± 0.5 in control arm. Meta-regression analysis showed no significant association between HbA1c reduction difference between GLP-1 agonists and control for all outcomes, except for diabetic retinopathy (Supplemental Figs. S8–S14). These results were confirmed also after correction for age, gender and length of follow-up. When meta-regression analysis was performed separately for comparator (placebo or active comparator) no significant association between HbA1c reduction difference between GLP-1 agonists and placebo was demonstrated for all outcomes; however, no significant association between HbA1c reduction difference between GLP-1 agonists and active comparator was demonstrated for all outcomes except for HF.

### *Publication bias*

No bias was identified for any of the outcomes (Supplemental Figs. S15–S21).

## Discussion

The main findings of the present analysis show that GLP-1 agonists have a safe CV profile and favourably affect all-cause death and CV death.

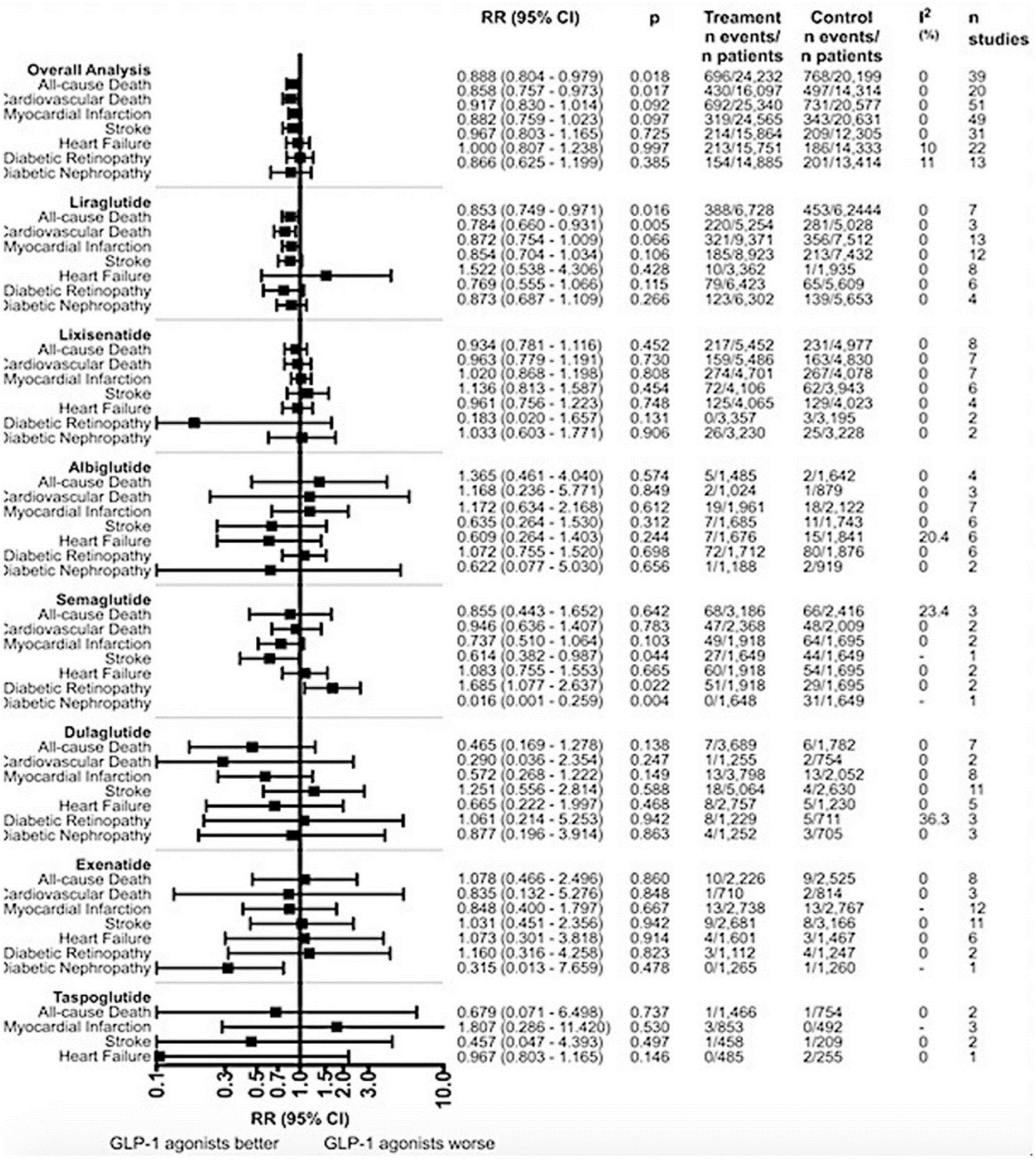


**Figure 3** Forrest plot showing the results of sensitivity outcome meta-analyses for individual drugs.

From the current analysis, treatment with GLP-1 agonists is associated with a significant reduction of all-cause mortality of CV mortality. In addition, the differences in efficacy in HbA1c lowering between GLP-1 agonists and control did not impact on differences in all-cause death and CV death between treatment and control arm, as shown by meta-regression analysis.

The favourable and glucose-lowering independent effect on CV death could be explained in light of evidence from pre-clinical studies documenting direct cardioprotective

**Table 1** Meta-regression analysis between differences in glycated haemoglobin changes versus baseline between Glucagon like peptide 1 agonists and control arm and relative risks of clinical events.

| Outcome | RR | 95% CI | p-value |
|---|---|---|---|
| **Overall** | | | |
| All-cause death | 0.859 | 0.556–1.329 | 0.486 |
| Cardiovascular death | 0.764 | 0.412–1.414 | 0.371 |
| Myocardial infarction | 1.091 | 0.617–1.929 | 0.761 |
| Stroke | 1.480 | 0.757–2.892 | 0.246 |
| Heart failure | 0.543 | 0.247–1.198 | 0.126 |
| Retinal complications | 0.568 | 0.337–0.957 | 0.035 |
| Nephropathy | 1.420 | 0.349–5.785 | 0.593 |
| **Overall adjusted for age, gender, length of follow-up** | | | |
| All-cause death | 1.031 | 0.506–2.104 | 0.930 |
| Cardiovascular death | 0.714 | 0.273–1.866 | 0.468 |
| Myocardial infarction | 1.036 | 0.638–1.683 | 0.883 |
| Stroke | 1.420 | 0.743–2.713 | 0.282 |
| Heart failure | 0.345 | 0.080–1.486 | 0.147 |
| Retinal complications | 0.408 | 0.229–0.727 | 0.004 |
| Nephropathy | 2.074 | 0.384–11.199 | 0.347 |
| **Comparator: Placebo** | | | |
| All-cause death | 1.225 | 0.146–10.299 | 0.845 |
| Cardiovascular death | 0.721 | 0.371–1.400 | 0.294 |
| Myocardial infarction | 1.159 | 0.574–2.341 | 0.668 |
| Stroke | 1.654 | 0.693–3.948 | 0.244 |
| Heart failure | 0.674 | 0.234–1.943 | 0.430 |
| Retinal complications | 0.577 | 0.126–2.643 | 0.435 |
| Nephropathy | 1.434 | 0.162–12.676 | 0.688 |
| **Comparator: Active comparator** | | | |
| All-cause death | 0.846 | 0.531–1.347 | 0.458 |
| Cardiovascular death | 1.730 | 0.093–32.058 | 0.676 |
| Myocardial infarction | 0.717 | 0.128–4.008 | 0.695 |
| Stroke | 0.886 | 0.138–5.672 | 0.895 |
| Heart failure | 0.058 | 0.04–0.747 | 0.031 |
| Retinal complications | 0.644 | 0.251–1.652 | 0.331 |
| Nephropathy | 1.752 | 0.004–733.336 | 0.809 |

effects of GLP-1 agonists. GLP-1 receptors are present in many tissues (pancreas, gut, liver, kidneys, central nervous system, muscle, adipose cells and CV system) as well as on cardiac myocytes, vascular endothelium and arterial smooth muscle cells [5], suggesting that GLP-1 agonists may have a significant role on vascular physiology. Although genome-wide association studies have shown contrasting results about association between glucagon gene and GLP-1 receptor genes and CV disease [19], GLP-1 agonists have been reported to exert cardioprotective direct effects, including protection against ischaemic–reperfusion injury [20], reduction of atherosclerotic lesions [21], improvement of endothelial function [22] and improvement of ventricular systolic function [23]. In addition, studies in rodent models of transient coronary ischaemia have demonstrated that treatment with GLP-1 receptor agonists leads to a reduction in infarct size and to improvement of left ventricular systolic function [24].

No statistical difference in risk of development diabetic retinopathy was reported between GLP-1 agonists and control arms. However, meta-regression analysis reported that an increased risk of worsening diabetic retinopathy associated with more efficacy in reducing HbA1c by GLP-1 agonists. Although an association between rapid glucose lowering and worsening of retinopathy has been reported in patients with type 1 DM [25], that might justify this observation, the effects of GLP-1 agonists on retinopathy deserve further investigation.

## Limitations

Most trials included in the present meta-analysis were designed for the assessment of the effects of GLP-1 agonists on glycaemic control, and not on CV and microvascular events. Consequently, in several of these studies no standardized diagnostic criteria for CV events were specified in trial protocols; furthermore, no formal adjudication of events was performed in most studies. Thus, the possibility of misclassification of macrovascular and microvascular events cannot be ruled out. However, this would affect both arms of each trial and unlikely bias the results. In addition, as common in similar analysis, renal outcomes differed among studies and include several endpoints such as progression of micro- or macroalbuminurea, decrease of glomerular filtration rate, doubling of serum creatinine, need of dialysis or transplantation. Similarly, retinopathy outcomes have not really been assessed in a pro-active way with appropriate technology in most studies. Indeed, the definition of renal endpoints is particularly problematic in meta-analyses of trials designed for other purposes, in which renal impairment is reported as adverse event. As consequence, renal complication not directly related to diabetic nephropathy could be included in the "diabetic nephropathy" endpoint. To limit potential overestimation of prevalence of renal and retinal complications, we included only events directly related to diabetic involvement of kidney and retina.

Finally, although 73 trials were included, three trials, ELIXA [10], LEADER [11] and SUSTAIN 6 [12], contributed >90% of all outcomes in most categories, limiting potentially the generalisability of the findings. However, as in other similar meta-analysis [26], lumping of large studies in a meta-analysis results in a substantial increase of the statistical power, allowing analysis of single endpoints that could not be assessed in single trial.

In addition, the protocol of this meta-analysis has not been previously disclosed (i.e. publication on PROSPERO website), which could be a methodological limitation of our work.

## Conclusions

Treatment with GLP-1 agonists in type 2 DM patients is associated with a significant reduction of all cause and CV mortality. Although no significant effect on retinopathy were observed in trial comparing GLP-1 agonists with other active treatments or placebo, the significant increased risk associated with more severe reduction of HbA1c need to be assessed in future studies.

## Author contribution

PG, GS, PPF: Conceived and designed the research.

GS: Performed statistical analysis.
CDA, FDM, FM, CM, SD: Acquired the data.
PG, GS, PPF: Wrote the manuscript.
LHL, BT, PPF: Revised the manuscript.

Prof. Pasquale Perrone-Filardi takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.numecd.2017.09.006.

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## CHAPTER 6.

## Vitamin D deficiency and clinical outcome in patients with chronic heart failure: a review

D'Amore C, **Marsico F**, Parente A, Paolillo S, De Martino F, Gargiulo P, Ferrazzano F, De Roberto AM, La Mura L, Marciano C, Dellegrottaglie S, Trimarco B, Perrone Filardi P

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REVIEW

# Vitamin D deficiency and clinical outcome in patients with chronic heart failure: A review

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**KEYWORDS**
Heart failure;
Vitamin D deficiency;
Vitamin D supplementation;
Cardiovascular outcomes

**Abstract** *Aim:* The aim of this review was to summarize evidence on the role of Vitamin D deficiency in heart failure (HF), from pathophysiological mechanisms to clinical effects of Vitamin D supplementation.
*Data synthesis:* Chronic HF secondary to left ventricular (LV) systolic dysfunction is a growing health problem, still associated with poor clinical outcome. In recent years, experimental and epidemiological evidence focused on the role of Vitamin D in HF. Cross sectional studies demonstrated that prevalence of HF is increased in patients with Vitamin D deficiency or parathyroid hormone (PTH) plasma level increase, whereas longitudinal studies showed enhanced risk of developing new HF in patients with Vitamin D deficiency. In addition, in patients with established HF, low plasma levels of Vitamin D are associated with worsening clinical outcome. Yet, clinical studies did not definitively demonstrate a benefit of Vitamin D supplementation for preventing HF or ameliorating clinical outcome in patients with established HF.
*Conclusions:* Despite convincing experimental and epidemiological data, treatment with Vitamin D supplementation did not show clear evidence of benefit for preventing HF or influencing its clinical course. Ongoing clinical studies will hopefully shed lights on the effects of Vitamin D supplementation on clinical endpoints along the spectrum of HF.


*Abbreviations:* AF, atrial fibrillation; CV, cardiovascular; EF, ejection fraction; HF, Heart failure; LV, left ventricular; MCS, mechanical circulatory support; MI, myocardial infarction; MMP, metallopreotease; NYHA, New York Heart Association; NT pro-ANP, NT pro-atrial natriuretic peptide; NT pro-BNP, N-terminal pro-brain natriuretic peptide; PTH, parathyroid hormone; RAAS, renin-angiotensin-aldosterone system; SCD, sudden cardiac death; TRPV6, transient receptor potential channel vanilloid type; TIMPs, tissue inhibitors of metalloproteinases; VDR, Vitamin D receptor; 1,25(OH)2D, 1,25-dihydroxivitamin D; 25(OH)D, 25-hydroxyvitamin D; 6-MWT, 6-min walking test; 6-MWD, 6-min walk distance.

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## Introduction

Heart failure (HF) is a complex syndrome secondary to inherited or acquired structural or functional heart abnormalities, and remains a leading cause of mortality and morbidity worldwide [1]. Approximately 10 millions of patients in Europe are affected by chronic HF [1] and, despite substantial advances in therapeutic options over the last years, no substantial changes in prognosis have been observed, with survival rate at 5 years after diagnosis of 35–50% [2,3]. Several mechanisms are involved in the pathogenesis of HF, including haemodynamic abnormalities, neurohormonal activation, enhanced inflammation and micronutrients availability [4], that explains the suboptimal impact of current therapies on clinical outcome.

Vitamin D is an important micronutrient with a significant role in autocrine and paracrine regulation of cellular functions and in growth and differentiation of several organs, including the heart. In fact, Vitamin D deficiency is associated with increased incidence of hypertension, myocardial infarction (MI), HF and stroke [5].

The aim of this review is to summarize evidence regarding Vitamin D deficiency in the pathogenesis and clinical course of HF with reduced systolic function and to report the effects of Vitamin D supplementation in patients with HF.

The search strategy to realize this review article, was a MEDLINE research, made with the following terms: "Vitamin D" or "Vitamin D supplementation" and "heart failure". All initially retrieved articles were subsequently individually analysed and discussed by the Authors group to establish adherence and relevance for the present review.

## Vitamin D metabolism (Physiology)

Vitamin D is a secosteroid that exists in two major forms: Vitamin $D_2$ (or ergocalciferol) and Vitamin $D_3$ (or cholecalciferol). Vitamin D can be derived from sunlight (UV-B)-induced production in the skin (80%) and from dietary intake [6]. The formation of active Vitamin $D_3$ metabolite requires two steps, the first in the liver to form 25-hydroxyvitamin $D_3$ (25(OH)D or calcifediol) and the second in the kidney to convert calcifediol in 1,25-dihydroxivitamin D (1,25(OH)2D or calcitriol) [7]. The 25(OH)D is primarily dependent on Vitamin D supply, with serum levels higher than calcitriol and with longer half-life (~3 weeks) compared to Vitamin D and calcitriol (both with few hours half life). Therefore, 25(OH)D concentrations should be measured to assess Vitamin D status [6,8].

Vitamin D exerts its action binding Vitamin D receptor (VDR), expressed on at least 36 different tissues including cardiac muscle, vascular smooth muscles, endothelium and lymphocytes [9]. VDR forms a heterodimer with the retinoic acid receptor, and this heterodimeric complex acts on gene transcription of Vitamin D response element [10], that consists of a large number of target genes [10]. Recent studies also showed that Vitamin D metabolites might act through non-genomic pathways, using an alternative binding site on VDR [9,10].

From plasma measurement of 25(OH)D, in 2011 the Institute of Medicine classified Vitamin D status as deficiency (values below 12 ng/ml), inadequacy (values from 12 to 19.9 ng/ml), adequacy (values from 20 to 50 ng/ml) and potentially harmful (values above 50 ng/ml). Interestingly, a U-shape relationship emerged between Vitamin D levels and all-cause mortality, cardiovascular (CV) diseases, selected cancer, falls and fracture. This emerging relationship has a remarkable impact in the management of patients with Vitamin D deficiency, since no additional clinical benefit is observed for 25(OH)D concentration above 30 ng/ml, and excess risk has been observed for levels above 50 mg/dl [11].

### *Vitamin D and the CV system (Mechanisms)*

Several potential biological mechanisms link Vitamin D and its metabolites to CV diseases (Fig. 1). Vitamin D acts as a negative regulator of renin-angiotensin-aldosterone system (RAAS) and several studies showed a relationship between low Vitamin D levels and increased RAAS activity [12–14]. Consistently, VDR knockout mice show increased RAAS activity, leading to hypertension, cardiac hypertrophy, increased water intake and sodium retention [12]. In addition, Vitamin D deficiency stimulates renin expression in normal mice, whereas 1,25(OH)2D injection leads to renin suppression [12]. Vitamin D may act directly on growth and differentiation of cardiomyocytes inhibiting their proliferation. This anti-proliferative property may be due to the suppression of proto-oncogene cmic and of natriuretic peptide. VDRs are also expressed on cardiac fibroblasts, and VDR knockout mice show collagen deposition [15].

Vitamin D modulates myocardial extracellular matrix turnovers. In fact, VDR knockout mice show increased metallopreotease (MMP) activity due to a reduced production of tissue inhibitors of metalloproteinases 1 and 3 (TIMP1 and TIMP 3) (MMP inhibitors). Thus, the proteolytic action of MMP promotes the destruction of myocardial tissue leading to ventricular remodelling [16,17].

Calcium deposition in atherosclerotic plaques and vascular calcification are also promoted by Vitamin D [18,19]. This observation is consistent with the inverse correlation between Vitamin D levels and coronary artery calcification [20]. Furthermore, it is documented that endothelial cells express VDR, so that Vitamin D increases nitric oxide synthase activity in vitro [21], enhances vascular endothelial growth factor production [22,23] and reduces endothelial platelet aggregation [24].

By a non-genomic pathway, the functional form of Vitamin D acts on calcium channels in cardiac myocytes inducing a rapid influx of calcium [25]. Experimental animal studies showed that calcitriol, through the phosphorylation of protein kinase C, promotes myocytes relaxation, thus participating in the homoeostasis of diastolic function [26], and enhances myocyte contractility through adenylate cyclase and cyclic adenosine monophosphate (cAMP) pathways [25].

The effects of Vitamin D on CV system are additionally mediated through elevated parathyroid hormone (PTH)

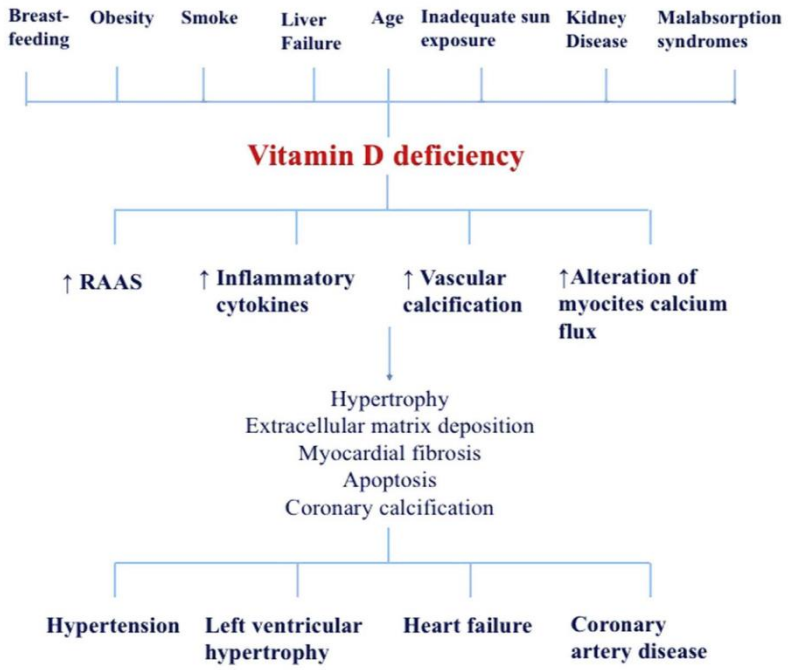


**Figure 1** Mechanisms involved in the onset and/or progression of heart failure in patients with Vitamin D deficiency; Abbreviation: RAAS: renin-angiotensine-aldosterone system.

levels. It is well established that Vitamin D is included in the calciotropic hormone system together with PTH [27]. The active Vitamin D form enhances the production and activity of the TRPV6 (Transient Receptor Potential channel Vanilloid type) ion channel and calbindin calcium binding protein in the intestinal epithelium to promote calcium absorption. In addition, 1,25(OH)2D increases renal calcium reabsorption as well as calcium reabsorption from the skeleton together with PTH [28]. Besides, VDRs are present in the parathyroid gland and 1,25(OH) suppresses production of PTH and prevents proliferation of parathyroid glands [29]. Consequently, Vitamin D deficiency is associated with elevated PTH concentration, that exerts a trophic effect on cardiomyocytes with an increase in total cellular mass and arterial stiffness [30], that is associated with development of left ventricular (LV) hypertrophy in patients with elevated PTH levels [31].

In summary, large evidence from experimental data supports a plausible mechanistic association between Vitamin D deficiency and CV damage.

## Vitamin D deficiency and HF

### *Prevalence of vitamin D deficiency in HF (Cross sectional and case–control studies)*

It is estimated that 1 billion people worldwide have Vitamin D deficiency or insufficiency, and 40–80% of the elderly population exhibits Vitamin D deficiency [32]. Risk factors for Vitamin D deficiency include sunscreen usage, dark skin, breast fed infants, ageing, inflammatory bowel disease, fat malabsorption disease, obesity and sedentary lifestyle [33].

Several cross-sectional studies showed an association between HF and 25(OH)D levels (Table 1). Shane et al. [34] showed low serum levels of 25(OH)D ($\leq$9 pg/ml) and of 1,25(OH)2D ($\leq$15 pg/ml) in 17% and 26%, respectively, of 101 HF patients being evaluated for heart transplant. Then, Zitterman et al. [35], in a case control study, also reported a statistically significant reduction of 25(OH)D and calcitriol levels in patients with HF compared to a control group.

Similar epidemiological data were obtained from the National Health and Nutrition Examination Survey (NHANES) 2001 to 2004 [28]. In this study in 8351 US adults, who had 25(OH)D levels measured, the prevalence of hypovitaminosis D (using previous definition of Vitamin D deficiency for plasma levels $<$30 ng/ml) was 74%, and a stepwise increase of CV disease was observed from the lowest to the highest serum 25(OH)D tertile (from 3,25%, to 2.4% and 1,5%). Notably, hypovitaminosis D reached 89% prevalence in patients with concomitant coronary heart disease and HF (OR 3.52, 95% CI 1.58–7.84).

Also, the Intermountain Healthcare system study, including 41,504 subjects from a general population with at least one Vitamin D measurement, showed that 36% of the population had Vitamin D levels in the range of normality, 47% had a mild-moderate reduction (16–30 ng/

**Table 1** Prevalence of Vitamin D deficiency in heart failure (Cross sectional and case–control studies).

| Author | Study design | Publication year | Patients enrolled (n) | Inclusion criteria | Definition of hypovitaminosis | Heart failure and hypovitaminosis D |
|---|---|---|---|---|---|---|
| Shane E [34] | Cross-sectional | 1997 | 101 | NYHA class III–IV; Consideration for cardiac transplant | 25(OH)D ≤ 9 pg/ml 1,25(OH)2D ≤ 15 ng/ml | 17% prevalence of hypovitaminosis D in HF patients with 25(OH)D ≤ 9 pg/ml 26% prevalence of hypovitaminosis D in HF patients with 25(OH)D ≤ 15 ng/ml |
| Zittermann A [35] | Case control | 2003 | 54 | NYHA class ≥II | | Not reported. Significant reduction of 25(OH)D and calcitriol serum levels in patients with HF (ANOVA p value < 0.001) |
| Kim DH [28] | Cross-sectional | 2008 | 8351 | Adult subject with measured 25(OH)D levels | 25(OH)D < 30 ng/ml | 89% of hypovitaminosis D in HF patients with concomitant coronary artery disease |
| Anderson JL [5] | Prospective cohort | 2010 | 41,504 | General healthcare population with measured 25(OH)D levels | 25(OH)D ≤ 30 ng/ml | 97% of HF in patients with low Vitamin D levels. 1.31 HR for new HF development in patients with low and very low Vitamin d levels. |

Abbreviations: HF: Heart failure; NYHA: New York Heart Association; 1,25(OH)2D: 1,25-dihydroxivitamin D; 25(OH)D: 25-hydroxyvitamin D.

ml) and 17% had very low levels (≤15 ng/ml). An increased prevalence of HF (90% relative and 9% absolute) was observed in very low versus normal Vitamin D categories ($p < 0.0001$), and during follow-up (1.3 ± 1.2 years) new onset HF developed in 594 (2,5%) subjects older than 50 years. Vitamin D plasma levels inversely correlated to the risk of developing HF, and adjusted hazards for HF were 2.01 and 1.30 for very low levels and low levels of Vitamin D, respectively [5].

Thus, prevalence of Vitamin D deficiency is increased in patients with HF and a correlation between reduced levels of Vitamin D and HF prevalence has been observed in clinical studies.

### *Low vitamin D and risk of HF (Observational longitudinal studies)*

The association between Vitamin D levels and HF has been also confirmed in longitudinal studies (Table 2).

The prospective Ludwigshafen Risk and Cardiovascular Health (LURIC) [36] study enrolled 3299 patients undergoing coronary angiography and evaluated the association between 25(OH)D levels and HF and sudden cardiac death (SCD) during a mean follow-up of 7.7 years. 25(OH)D and 1,25(OH)2D were independently and inversely correlated with N-terminal pro-brain natriuretic peptide (NT pro-BNP) levels ($r = -0.190$ and $-0.255$, respectively; $p < 0.001$ for both) and were inversely associated with LV ejection fraction (EF) ($p < 0.001$ for both) and higher New York Heart Association (NYHA) class ($p = 0.05$ for 1,25(OH)2D). Using multivariable adjustment for confounding factors, the inverse correlation of NT pro-BNP and LVEF with 25(OH)D and calcitriol levels was unchanged. In addition, during a median follow-up of 7.7 years, 116 patients died due to HF (110 in low Vitamin D groups and 6 in Vitamin D optimal range group) and 188 due to SCD (182 in low Vitamin D groups and 6 patients with optimal range of Vitamin D). Thus, low levels of 25(OH)D and of 1,25(OH)2D were independent risk factors for mortality due to HF (HR 2.84; 95% CI: 1.20–6.74) and for SCDs (HR 5.05; 95% CI: 2.13–11.97).

The Cardiovascular Health Study [30] measured Vitamin D and PTH levels in 2312 healthy subjects and reported that patients with Vitamin D deficiency and high PTH levels showed higher risk of MI and HF compared to controls, during 14 years follow-up. After adjustment, each 10 ng/ml lower 25(OH)D concentration was associated with a 9% greater (95% CI: 2%–17%; $p = 0.012$) relative hazard of all-cause mortality and a 25% greater (95% CI: 8%–44%; $p = 0.002$) relative hazard of MI. In addition, a serum 25(OH)D concentrations <15 ng/ml was associated with a 29% greater (95% CI: 5%–55%) risk of all-cause mortality. Instead, serum PTH concentration (≥65 pg/ml), that reflects inadequate Vitamin D stores and activity [33,37], was associated with a 30% (95% CI: 6%–61%) greater risk of incident HF.

Consistent with these observations, a recent study [38] prospectively followed up, for a mean of 13 years, 3731 men aged 60–79 years with no prevalent HF or primary hyperpathyroidism and with measured Vitamin D and PTH concentrations. In this study, elevated PTH levels (>55.6 pg/ml) were significantly associated with higher risk of developing HF after adjustment for lifestyle characteristics and comorbidities (HR 1.66; 95% CI: 1.30–2.1;

**Table 2** Low Vitamin D and risk of heart failure (Observational longitudinal studies).

| Author | Study design | Publication year | Patients enrolled (n) | Inclusion criteria | Mean age | Outcomes | Follow-up | Results |
|---|---|---|---|---|---|---|---|---|
| Pilz S [36] | Prospective cohort | 2008 | 3299 | Patient referred to coronary angiography | 63 | Association of 25(OH) with HF measures. Hazard Ratio for death due to HF and SCD according to Vitamin D status. | 7.7 years | 2.84 HR (95% CI 1.20–6.74) for HF death in patients with hypovitaminosis D.<br>5.05 HR (95% CI 2.13–11.97) for SCD |
| Kestenbaum B [30] | Prospective cohort | 2011 | 2312 | Healthy subjects aged ≥65 years. | 75 | Association of 25(OH)D and PTH concentration, separately and in combination, with incident CV events and mortality | 14 years | 29% greater (95% CI: 5%–55% greater) risk of all-cause mortality in patients with 25(OH)D < 15 ng/ml.<br>30% (95% CI: 6%–61%) greater risk of incident HF in patients with PTH ≥65 pg/ml. |
| Wannamethee SG [38] | Prospective cohort | 2014 | 3713 | General population aged 60–79 years with and without established CV disease. | 68 | Association of PTH, 25(OH)D and markers of mineral metabolism with risk of incident HF | 13 years | 1.66 HR (95% CI: 1.30–2.1) for new HF development in patients with PTH >55.6 pg/ml<br>No association of 25(OH)D or mineral metabolism (calcium or phosphate) with HF risk (HR 1.07; 95% CI: 0.67–1.71) |
| Bansal N [39] | Prospective cohort | 2014 | 6469 | Racial and ethnically different population free of prevalent clinical CV disease. | 62 | Associations of serum PTH and 25(OH)d with incident HF and LV mass | 8.46 years | 50% (95% CI: 3%–20%) greater risk of incident HF in patient with PTH > 65 pg/ml<br>No association between 25(OH)D and HF |

Abbreviations: CV: cardiovascular; HF: Heart failure; LV: left ventricular; PTH: parathyroid hormone; SCD: sudden cardiac death; 1,25(OH)2D: 1,25-dihydroxivitamin D; 25(OH)D: 25-hydroxyvitamin D.

$p < 0.0001$). Instead, no association was documented between 25(OH)D or mineral metabolism (calcium or phosphate) and HF risk (HR 1.07; 95% CI: 0.67–1.71). Finally, a recent large prospective study [39], including racial and ethnically different populations, also confirmed a significantly increased risk of HF in patient with increased PTH levels (>65 pg/ml) (95% CI: 3%–20%), whereas no association between 25(OH)D and HF was observed in a multivariable model.

The inconsistency in evidence linking Vitamin D deficiency and PTH to HF development might be explained considering that elevated PTH levels usually identify patients with low Vitamin D levels, confounding the relationship between these conditions and HF. In fact, progressive renal function decrease, physical inactivity as well as reduced calcium absorption are either causes or consequences of hypovitaminosis D that are associated with increased PTH levels [40].

In summary, in patients with no evidence of HF, reduced levels of Vitamin D or elevated levels of PTH are independently associated with an increased risk of developing HF.

### *Impact of vitamin D deficiency on prognosis in HF patients*

Several studies (Table 3) have assessed whether, in patients with HF, Vitamin D deficiency is associated with worst prognosis.

In the study of Liu et al. [41] in 548 patients with HF, followed up for 18 months, lower Vitamin D levels were associated with higher HF hospitalization and all-cause mortality, and the combined endpoint of all-cause mortality and HF rehospitalisation increased significantly across decreasing 25(OH)D tertiles. After adjustment in a multivariable Cox regression analysis, low 25(OH)D concentration remained independently associated with increased risk of the combined endpoint (HR 1.09 per 10 nmol/L decrease; 95% CI: 1.00–1.16; $p = 0.040$) and all-cause mortality (HR 1.10 per 10 nmol/L decrease; 95% CI: 1.00–1.22; $p = 0.049$). Gotsman et al. [42] selected adult members from a Health Maintenance Organization with available measurements of Vitamin D and showed that 1,25(OH)2D median levels in HF were lower than in non HF subjects (36.9 nmol/L versus 40.7 nmol/L; $p < 0.00001$) and, at Cox regression analysis, Vitamin D deficiency was a significant independent predictor of mortality in HF patients.

Since calcitriol and PTH are physiologically interrelated, Gruson et al. [43] prospectively analysed the relation between 1,25(OH)2D levels and 1,25(OH)2D to PTH ratio and CV events, including CV mortality and cardiac transplantation in 170 chronic HF patients during a mean follow-up of 4.1 years. In this study, median serum levels of 1,25(OH)2D inversely correlated to HF severity, and the combined endpoint of CV death and cardiac transplantation, occurring in 106 of 170 patients (62%), was independently predicted by 1,25(OH)2D and 1,25(OH)2D to PTH ratio in the Cox proportional hazard modelling.

**Table 3** Impact of Vitamin D deficiency on prognosis in heart failure patients.

| Author | Study design | Publication year | Patients enrolled (n) | Inclusion criteria | Outcome | Follow-up duration | Results |
|---|---|---|---|---|---|---|---|
| Liu LC [41] | Prospective | 2011 | 548 | Chronic HF; NYHA class II–IV | 1°: composite of all-cause mortality + HF rehospitalization. 2°: all-cause of mortality and HF re-hospitalization. | 18 month | 1.09 HR (95% CI 1.00–1.16) for the primary outcome per 10 nmol/L decrease of 25(OH)D levels. 1.10 HR (95% CI 1.00–1.22) for all-cause mortality per 25(OH)D 10 nmol/L decrease, No association was observed with re-hospitalization due to worsening HF. |
| Gotsman I [42] | | 2012 | 49,834 | Age ≥45 years Measured Vitamin D | Effects on mortality of Vitamin D levels and Vitamin D supplementation in HF patients | 518 days | 1.52 HR (95% CI 1.21–1.92) for mortality in patients with Vitamin D deficiency. 0.68 HR (95% CI 0.54–0.85) for mortality in patients in therapy with Vitamin D supplementation. |
| Gruson D [43] | Prospective | 2015 | 170 | Chronic HF; LVEF ≤35% | Cardiovascular death and heart transplantation | 4.1 years | The endpoint occurring in 106 of 170 patients (62%) and was predicted by 1,25(OH)2D and 1,25(OH)2D to PTH ratio in the Cox proportional hazard modelling. |

Abbreviations: HF: Heart failure; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; PTH: parathyroid hormone; 1,25(OH)2D: 1,25-dihydroxivitamin D; 25(OH)D: 25-hydroxyvitamin D.

Thus, there is convincing evidence that in patients with established HF low levels of Vitamin D are associated with poorer prognosis.

### *Vitamin D supplementation and incidence of HF (Primary prevention trials)*

There are only 2 studies that investigated whether Vitamin D supplementation prevents new HF [44,45] (Table 4). The first one [44] evaluated whether Vitamin D plus calcium (CaD) supplementation was associated with lower incidence of HF in post-menopausal women enrolled in the Women's Health Initiative without HF. In this study 35,983 post-menopausal women were randomized to receive 1000 mg/day of calcium plus 400 IU/day of Vitamin $D_3$ or placebo and were followed up for 7.1 years. CaD supplementation was not associated with reduced HF risk compared to placebo in the overall population (HR 0.95, 95% CI 0.82–1.09; $p = 0.46$). However, stratifying patients by baseline risk of HF, CaD supplementation was associated with a statistically significant 37% lower risk of HF (HR 0.63, CI 95% 0.46 to 0.87; $p = 0.005$) in the low-risk subgroup.

The second study [45] randomized 5108 adult subjects from a general population to placebo or high monthly dose of Vitamin D supplementation and studied the cumulative incidence of CVDs for a median follow-up of 3.3 years. Baseline 25(OH)D concentration was 25.3 ng/ml and was similar between Vitamin D and placebo groups. The primary outcome of CVD and death occurred in 11.8% in Vitamin D group and 11.5% in the placebo group (HR 1.02 95% CI, 0.87–1.20; $p = 0.81$) and no significant differences in any of the secondary CV outcomes, including HF, were observed. However, this study reported only the effects of a monthly administration of Vitamin D that might be different from daily or weekly dose administration.

Thus, only scant and not definitive data are available on the prevention of HF in patients with Vitamin D deficiency, yet these limited data support the need for more tailored studies in patients with Vitamin D deficiency at different risk of developing HF.

### *Vitamin D supplementation and prognosis of HF (Secondary prevention trial)*

It remains unclear whether Vitamin D supplementation favourably impacts on CV mortality and morbidity in HF patients, since only one randomized controlled trials on hard clinical endpoints is available (Table 5, Table 1s in supplementary material). Currently, 2 non-randomized [42,46] and 9 randomized (Table 6) [47–53,55,56] studies on surrogate endpoints have been reported.

#### Non-randomized studies

Gotsman et al. [42] followed up for 518 days 3009 HF patients and 46,825 control subjects. The percentage of patients with Vitamin D deficiency (25(OH)D < 25 ng/ml) was higher in HF patients compared to controls (28% vs 22%, $p < 0.00001$), and treatment with Vitamin D supplements was independently associated with reduced mortality in patients with Vitamin D deficiency (HR 0.68; 95% CI 0.52–0.85; $p < 0.0001$). Amin et al. [46] in a prospective study of 100 patients with HF and NYHA class I through III, with insufficient or deficient 25(OH)D serum levels (<30 ng/ml and <20 ng/ml, respectively), reported a significant reduction of BNP levels after 4 months of Vitamin D supplementation, together with improvement in NYHA class and 6-min walking distance (6MWD).

#### Randomized studies

In a study enrolling 123 patients with HF randomized to Vitamin D supplementation or placebo, followed up for 15 months, Schleithoff et al. [47] reported that Vitamin D reduced the levels of inflammatory cytokines including tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10). Yet, no changes were observed between treatment arms on functional parameters or survival. In this study, however, evidence of baseline Vitamin D deficiency was not a criterion for enrolment. In a randomized study enrolling 105 patients with HF and Vitamin D deficiency followed up for 20 weeks, Witham et al. [48] did not observe significant differences in the primary endpoint of 6MWD and quality of life, despite a significant decrease of

**Table 4** Vitamin D supplementation and incidence of HF (primary prevention trials).

| Author | Publication year | Study design | Patients enrolled (n) | Inclusion criteria | Outcomes | Follow-up | Vitamin D levels for enrolment | Dose | Results |
|---|---|---|---|---|---|---|---|---|---|
| Donneyong MM [44] | 2015 | Randomized, double-blind, placebo-controlled | 35 983 | Post-menopausal women with cardiovascular risk and without HF | HF cases | 7.1 years | Not required | 400 IU Vitamin D+400 calcium daily | No difference between groups. |
| Scragg R [45] | 2017 | Randomize, double-blind, placebo-controlled | 47,905 | General population | Number of participants with incident CVD and death | 3.3 years | Not required | 100,000 IU monthly | No difference between treatment and placebo group in CV events occurrence (303 vs 293) |

Abbreviations: CV: cardiovascular; CVD: cardiovascular disease; HF: heart failure.

**Table 5** Vitamin D studies in heart failure patients with available results.

| ClinicalTrials.gov identifier | Study status | Study design | Aim of the study | Inclusion criteria | Primary endpoint/outcome | Secondary endpoint/ outcome | Results |
|---|---|---|---|---|---|---|---|
| NCT01326650 (EVITA) | Completed | Randomized, double blind | Investigate whether Vitamin D supplementation reduces mortality and increases event-free survival in end-stage CHF patients | Age 18 to 80 yrs; NYHA class ≥ II. | All-cause of mortality. | Hospitalization, resuscitation, MCS implant, high urgent listing for heart transplantation and hypercalcaemia | Vitamin D supplementation not reduce mortality, but increase MCS implants |
| NCT01092130 (VitD CHF trial) | Completed | Randomized, open label | Investigate the effect of the administration of Vitamin D in patients with CHF | Age ≥18 yrs; NYHA class II–IV; Treatment with ACE-I or ARB and BB therapy for at least 4 weeks. | PRA after 6 weeks of treatment. | Safety endpoints, the effect of Vitamin D administration on additional marker of renin-angiotensin system activity, Vitamin D cascade, NT-proBNP, kidney function, extracellular matrix markers | Significant decrease in PRA and plasma renin concentration in treated group. No significant changes in serum concentration of natriuretic peptide, fibrosis markers (exception galectina-3), PTH, PRC and kidney function between the two groups |
| NCT01230307 | Completed | Randomized, double blind | Investigate how rapid Vitamin D supplementation affects biomarkers and submaximal exercise capacity in systolic HF patients with low Vitamin D status | LVEF <40%; 25(OH)D = 10–25 ng/ml; Optimized medical therapy. | How rapid vitamin D supplementation affects biomarkers (CRP, IL-6, TNF-a, PPT I, PP III, MMP-2, MM-9, TIMP-1. | Exercise capacity (6-MWT); Quality of life measured by KCCQ; Vitamin D genomics. | Outcomes were not analysed because enrolment was less than 30% of original goal |
| NCT01619891 (VINDICATE Study) | Completed | Randomized, double blind | Detect whether Vitamin D has pathophysiologically important effects | CHF; LVEF ≤45%; NYHA class II–III; 25(OH)D < 20 ng/ml. | 6-MWD. | Changes of LV cardiac function by cardiac magnetic resonance; Peak exercise capacity; Biochemical changes. | No changes in Δ6MWD. Significant variation in cardiac function (LVEF) and reduction in LVEDD, LVESD, LVEDV and LVEDD |
| NCT01005303 | Completed | Randomized, double blind | Investigate if supplementation with micronutrients (including high-dose Vitamin D) will improve the function of the heart in patients with HF | NYHA class II–III; ACE-I and BB therapy; Stable at least 6 month; LVEF ≤45%. | LVEF | Cardiac volume; Levels of inflammation; Levels of oxidative stress Biomarker of cardiac function; Quality of life; Physical function. | There was no significant difference in mean LVEF and in any of the secondary endpoints |

Abbreviations: CHF: chronic heart failure; CPX: cardiopulmonary exercise test; CRP: C-reactive protein; IL-6: interleukin-6; CV: cardiovascular; KCCQ: Kansas City Cardiomyopathy Questionnaire; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVEDV: left ventricular end-diastolic volume; LVESD: left ventricular end-systolic diameter; LVESV: left ventricular end-systolic volume; MCS: mechanical circulatory support; MI: myocardial infarction; MMP-2: matrix metalloproteinase 2; NYHA: New York Heart Association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PPT I: propeptide procollagen type I; PP III: plasma procollagen III; PRA: plasma renin activity; TIMP-1: tissue inhibitor of matrix metalloproteinases-1 TNF-a: tumour necrosis factor-a; 6-MWD 6-min walking test; 6-MWD: 6 min walking distance.

**Table 6** Randomized study of Vitamin D therapy in heart failure patients.

| Author | Year | Study design | Inclusion criteria | Vitamin D/Control patients (n) | Vitamin D Dose | Follow-up Duration | Primary Endpoints | Secondary endpoint | Results |
|---|---|---|---|---|---|---|---|---|---|
| Schleithoff SS [47] | 2006 | Randomized, double-blind, placebo-controlled | CHF; HYHA class ≥II | 61/62 | 2000 IU/d | 9 months 15 months (for survival rate) | Survival rates and biochemical variables | LVEF<br>LVEDD<br>$VO_2$max<br>Blood Pressure | Significant reduction in serum concentration of TNF-α (−2.0 vs 2.7 pg/ml; p = 0.006) and IL-10 (0.24 vs −0.20 pg/ml; p = 0.042).<br>No significant variation for other parameters. |
| Witham MD [48] | 2010 | Randomized, double-blind, placebo-controlled | Age ≥70 y; CHF; NYHA class II–III; LV systolic dysfunction; 25(OH)D < 20 ng/ml. | 53/52 | 100,000 IU at baseline and 10 wk | 20 weeks | 6MWD | TGUG, daily physical activity levels, quality of life and cardiovascular and inflammatory markers | Significant decrease in BNP level in treatment group in confront of placebo (−22 vs +78 pg/ml; p = 0.04).<br>No significant variation for other parameters. |
| Shedeed SA [49] | 2012 | Randomized, double-blind, placebo-controlled | Infants with CHF; LVEF <40% | 42/38 | 1000 IU/d | 12 weeks | Renin-angiotensin system cytokines, clinical, biochemical and echocardiographic parameters | | Significant improvement in HF score, LVEDD, LVESD, LVEF, serum IL-10 and a decrease in PTH, IL-6 and TNF-α in Vit D group compared with control group. |
| Boxer RS [50] | 2013 | Randomized, double-blind, placebo-controlled | Age ≥50 y; NYHA class II–III; 25(OH)D ≤ 37.5 ng/ml | 31/33 | 50,000 IU/wk | 6 months | Peak $VO_2$ | TGUG<br>6MWD<br>isokinetic muscle testing | No effect |
| Boxer RS [51] | 2014 | Randomized, double-blind, placebo-controlled | Age ≥50 y; NYHA class II–III; 25(OH)D ≤ 37.5 ng/ml | 31/33 | 50 000 IU/wk | 6 months | Effect on Hormone and Biomarker | Echocardiographic parameters, health status | Significant decrease of serum aldosterone in Vit D group (37% vs 14%; p = 0.02).<br>No significant variation for other parameters. |
| Schroten NF (VitD-CHF trial) [52] | 2013 | Randomized, open-label | Age ≥18 y; CHF; LVEF <45% | 50/51 | 2000 IU/d | 6 weeks | Plasma renin activity | NT-proBNP, fibrosis markers, PTH, PRC, kidney function | Significant decrease in PRA and plasma renin concentration in treated group.<br>No significant variation for other parameters. |

(*continued on next page*)

**Table 6** (*continued*)

| Author | Year | Study design | Inclusion criteria | Vitamin D/Control patients (n) | Vitamin D Dose | Follow-up Duration | Primary Endpoints | Secondary endpoint | Results |
|---|---|---|---|---|---|---|---|---|---|
| Dalbeni A [53] | 2014 | Randomized, double-blind, placebo-controlled | Age >40y; CHF; LVEF <55%; NYHA class > II; 25(OH)D < 30 ng/ml | 18/18 | 4000 IU/d | 6 months | LVEF | echocardiographic and laboratory parameters | Significant increase in LVEF in Vit D group (6.71% vs −4.3%; $p < 0.001$). Significant increase in PIP serum concentration in control group than placebo (1140. 9 vs −145 mcg/L; $p < 0.05$). No significant variation for other parameters. |
| Witte KK (VINDICATE Study) [55] | 2016 | Randomized, double-blind, placebo-controlled | CHF; LVEF ≤45%; NYHA class II–III; 25(OH)D < 20 ng/ml | 80/83 | 4000 IU/d | 12 months | 6MWD | Change in structure and cardiac function | No changes in Δ6MWD. Significant variation in treated group than placebo in: LVEF (+7.65 vs + 1.36%; $p < 0.0001$) LVEDD (−2.45 vs 0.08 mm; $p = 0.002$), LVESD (−2.72 vs −0.99 mm; $p = 0.043$), LVEDV (−16.47 vs −3.83 ml) and LVESD (−18.77 vs −8.49 ml) |
| Zitterman A (EVITA) [56] | 2017 | Randomized, placebo-controlled | Adult patient (18-79ys): NYHA class ≥II; 25(OH) < 75 nmol/L | 201/199 | 4000 IU/d | 3 years | All-cause of mortality | Hospitalization, resuscitation, MCS implantation | Increase MCS implantation in patient that received Vitamin d supplementation (15.4% vs 9%, with HR of 1.96, 95% CI 1.04–3.66; $p = 0.031$). No significant variation for other parameters. |

Abbreviations: BNP: B-type natriuretic peptide; CHF: chronic heart failure; CRP: C-reactive protein; IL: interleukin; IU: international units; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVEDV: left ventricular end-diastolic volume; LVESD: left ventricular end-systolic diameter; LVESV: left ventricular end-systolic volume; MCS: mechanical circulatory support; NYHA: New York Heart Association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PIP: carboxyterminal propeptide of procollagen type I; PTH: parathyroid hormone; 6MWD: 6-min walk distance; TGUG: Timed get Up and Go; TNF-α: tumour necrosis factor-α; Vit D: vitamin D; VO2: oxygen volume; 25(OH)D: 25(OH) vitamin.

natriuretic peptides. However, this study enrolled only elderly (≥70 years) patients for a relatively short follow-up. In contrast, Shedeed et al. [49], in a randomized study in 80 paediatric patients with HF and LVEF <40% randomized to Vitamin D or placebo and followed up for 12 weeks, reported a significant improvement of functional LV parameters (reduced LV volumes and increased EF) together with improved inflammatory profile (reduced IL-6 and TNF alfa-α). In a randomized study involving 64 patients with HF, Boxer et al. [50] did not observe significant differences in the primary outcome of peak oxygen consumption after 6 months of treatment. In this study, however, patients were enrolled independently of LVEF (mean 39 ± 13% in treated patients) and Vitamin D basal levels, that might represent a limitation. The same Author [51] subsequently investigated the effects of Vitamin D supplementation on RAAS activation and functional parameters in a randomized study of 64 patients with HF and Vitamin D deficiency followed up for 6 months. In this study, Vitamin D supplementation significantly reduced aldosterone levels but did not impact LV function or health status. Consistent with the previous study, also Schroten et al. [52], in the VitD-CHF trial, that enrolled 101 patients with HF and EF <45% followed up for 6 weeks in an open label randomized study, observed a significant reduction of renin activity (representing the primary endpoint of the study) in Vitamin D treated patients. However, no changes of natriuretic peptides or functional parameters were reported. In a small study in 36 patients with HF with reduced vitamin D levels (<30 ng/ml) and LVEF <55%, followed up for 6 months, Dalbeni et al. [53] reported a significant increase of LVEF in vitamin D treated patients (6.7% vs −4.3%, $p < 0.001$). A meta-analysis of these 7 randomized studies [54] reported that vitamin D supplementation in HF patients was associated with a significant decrease of TNF-α, C-reactive protein and PTH, but with no significant changes of LVEF, NT pro-BNP and 6MWD. However, as it appears from the description of the 7 included studies, large heterogeneity was present in this analysis, due to large variability in the age of populations, enrolling criteria for Vitamin D and LVEF at baseline, as well as outcome definition and study duration.

More recently the VINDICATE (VitamIN D treatIng patients with Chronic heArT failurE) placebo-controlled randomized trial [55] investigated the effects Vitamin D supplementation on 6MWD (primary endpoint) in 163 patients with systolic HF and low vitamin D levels (25(OH) D < 20 ng/ml), followed up for 1 year. Vitamin D supplementation did not significantly improve 6MWD in HF patients, although the study resulted underpowered to detect a significant difference. However, Vitamin D supplementation was associated with a significant improvement of LVEF (+6.07%, 95% CI: 3.20–8.95; $p < 0.0001$) and of LV end diastolic diameter (−2.49 mm, 95% CI: −4.09 to −0.90; $p = 0.002$) and end systolic diameter (−2.09 mm, 95% CI: −4.11 to −0.06; $p = 0.043$). Thus, although the study did not meet its primary endpoint, the favourable effects on LV remodelling, i.e. on parameters that are associated with clinical outcomes in HF patients, are hypothesis-generating for future clinical studies.

Finally, the EVITA trial (Effect of Vitamin D on all-cause mortality in heart failure) [56], randomized 400 HF patients with 25(OH)D levels <30 ng/ml to receive 4000 IU Vitamin D daily or placebo for 3 years, with primary endpoint of all-cause mortality and secondary endpoint of hospitalization, resuscitation and mechanical circulatory support (MCS) implantation. Although mortality did not significantly differ in treated and placebo group (19.6% vs 17.9% with HR of 1.09, 95% CI 0.68–1.71; $p = 0.726$), there was a significant greater need for MCS implantation in treated patients compared to placebo (15.4% vs 9%, HR 1.96, 95% CI 1.04–3.66; $p = 0.031$).

In summary, there is no definitive evidence supporting a favourable role of Vitamin D supplementation in patients with HF. Yet, completed and published studies so far, appear inadequately powered to provide a clear understanding of the benefit, neutrality or even potential harm of Vitamin D supplementation in HF.

## Conclusions

Although an epidemiological association between Vitamin D deficiency and risk of CV events, including HF, is demonstrated pathophysiological mechanisms are still not fully understood. Interventional studies reported inconsistent results on the clinical effects of Vitamin D supplementation in patients with or at risk of HF, and, therefore, additional evidence from ongoing randomized studies is needed to assess whether add-on supplementation therapy with Vitamin D has a role in the prevention and/or management of HF.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2017.07.009.

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# PART 2

**Cardiovascular risk in children: role of HIV in cardiovascular disease**

## CHAPTER 7.

## Left ventricular function, epicardial adipose tissue and carotid intima-media thickness in children and young adults with vertical HIV infection

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*Under submission*

**Left ventricular function, epicardial adipose tissue and carotid intima-media thickness in children and young adults with vertical HIV infection**

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**Abstract**

BACKGROUND: Life expectancy of HIV patients has increased considerably as a result of antiretroviral therapy, and cardiovascular (CV) disease has emerged as an important late concern. HIV infection may affect systolic function either in adults or children, however data on diastolic function and markers of CV risk, such as epicardial adipose tissue (EAT) and intima-media thickness (IMT), are lacking. Aim of the present study is to evaluate left ventricular function, EAT and IMT in children and young adults with vertically-acquired HIV infection.

METHODS and RESULTS: We enrolled 29 subjects on antiretroviral therapy (ART) (13, 45% male; median age of 14.0 and IQR 8.7), and 29 age-matched controls. All patients and controls underwent echocardiographic evaluation, with study of the systo-diastolic function and measurement of the EAT, and a carotid ultrasound study for IMT measurement. Comparing HIV-infected patients to healthy controls, we found a statistical significant increase of EAT and IMT (EAT: 3,16 ± 1,05 vs 1,24 ± 0,61 mm; $p < 0,0001$. IMT: 0,77 ± 0,15 vs 0,51 ± 0,11 mm; $p < 0,0001$), and a significant reduction of ejection fraction, evaluated with biplane Simpson method (52,3 ± 17,49% vs 66 ± 4,24%; $p = 0,029$). These results are not related with age, gender, degree of lipodystrophy, dyslipidemia, hyperinsulinism and ART duration or the use of single antiretroviral classes.

CONCLUSIONS: Vertically-infected HIV children and young adults show an increased thickness of the EAT and IMT, expression of potentially increased CV risk. They also show an impaired systolic function.

## Introduction

Human immunodeficiency virus (HIV) infection is a major cause of morbidity and mortality worldwide. In developed countries, where life expectancy has increased considerably as a result of antiretroviral therapy (ART), cardiovascular diseases (CVD) have emerged as an important late comorbidity in HIV patients [1,2].

Results from the Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection (P2C2 HIV) study have shown that subclinical cardiac abnormalities develop early in HIV1-infected children, and that cardiac alterations are frequent, persistent, and often progressive [3-6]. Common abnormalities include dilated cardiomyopathy (decreased left ventricular [LV] contractility and LV dilation) and inappropriate LV hypertrophy (high LV mass with decreased height and weight) [3].

Echocardiographic abnormalities can be found in up to 44% of patients infected with HIV [7]. These findings include pericardial effusion, LV dysfunction, dilated cardiomyopathy, infective endocarditis, pulmonary arterial hypertension, and cardiac masses such as lymphoma and Kaposi's sarcoma of the heart. In addition, ART has been associated with the development of ischemic heart disease and LV diastolic abnormalities [8].

Although most data come from adult populations, evidence is also available for an association between HIV infection and systolic and diastolic dysfunction in children [9,10], and it has been demonstrated that children undergoing ART show decreased incidence of CVD [11,12].

In recent years new markers of increased CV risk have emerged. Epicardial adipose tissue (EAT), that has the same embryogenic origin of the visceral fat [13], has been reported to be associated with CV risk and metabolic syndrome [14,15]. Increased EAT thickness in HIV-infected adults compared to healthy controls has been reported [16,17], with an association between EAT and lipodystrophy [18]. However, data on children and young adults with vertically acquired HIV infection are lacking.

Similarly, the increase in intima-media thickness (IMT) is associated with higher CV risk, and a higher IMT has been reported in HIV-infected children, compared to non-infected patients [19]. The IMT thickness appears higher in naïve children than in HIV-patients undergoing ART therapy [20].

The aim of this study was to assess EAT, IMT and left ventricle function in young patients with vertically-acquired HIV infection, to correlate these parameters to metabolic profile and anti-retroviral treatment.

**Methods**

This cohort study was carried out between January 1$^{st}$, and November 30$^{th}$ 2017 at the Regional Reference Center for Pediatric HIV/AIDS of the University of Naples Federico II. The Referral Center covers a territory of about 5 million inhabitants in the most populous region of the Southern Italy, and manages about 30 HIV-infected children and adolescents with 1-2 new diagnosis/year of HIV infection in the last years.

All patients currently in follow-up were enrolled in the study, and a group of age-matched subjects was enrolled as controls. The study was conducted according to the principles of the Helsinki declaration and the study protocol was approved by the Ethical Committee of the University Federico II of Naples (protocol number 153/16). All patients and caregivers, according to age, signed an informed consent after receiving specific information from the study coordinators.

***Anamnestic evaluation***

All patients underwent anamnestic and lifestyle evaluation (physical activity, smoking, drinking, drug addiction, eating habits), clinical and viro-immunological assessment with measurement of HIV viral load and $CD4^+$ count. In addition, current ART, history of ART regimens and duration of single antiretroviral classes were reviewed.

***Evaluation of the metabolic and CV risk***

All patients underwent clinostatic and orthostatic blood pressure measurement, followed by the evaluation on blood sample of lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides) and glucose profile (fasting glucose, basal insulin, homeostatic model assessment (HOMA) index). We also evaluated the presence of metabolic syndrome, considering the International Diabetes Federation (IDF) diagnostic criteria and the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria [21]. Lipodystrophy was

classified as absent, mild (mild lipodystrophy of face and arms) and severe (severe lipodystrophy of face and arms, with involvement of abdomen and legs).

***Echocardiography and carotid assessment***

For all echocardiographic measurements, the final values were obtained after averaging over two cardiac cycles. The evaluation of LV ejection fraction was made with Simpson biplane mode, tracing tele diastolic and tele systolic volume both in four and two chamber apical view (n.v. 52-70%).

The evaluation of LV diastolic function was made evaluating the E/A ratio and the E/E' ratio. The E/A ratio was evaluated in a four chambers apical view, using pulsed Doppler, applied on the coaptation point of the mitral flaps. The E wave is the early component of the left ventricle diastolic filling, meanwhile the A wave corresponds to the atrial systole. The E/A ratio was considered normal for a value higher than 1. Values lower than 1 (first type of diastolic dysfunction) or higher than 2 (third or fourth type of diastolic dysfunction) were considered abnormal. The E' wave was obtained applying a tissue Doppler on the mitral annulus (medial and lateral). A E' mean value lower than 8 indicate a diastolic dysfunction, but, considering the E/E' ratio, the normal value is lower than 8. A value between 8 and 13 can be considered as first type of diastolic dysfunction, meanwhile a value higher than 13 can be considered as a third or fourth type of diastolic dysfunction.

The EAT was measured in a parasternal long-axis view, on the top of the right ventricle free wall, considering the maximum thickness, as end-systolic measurement

(**Figure 1**). In the evaluation of the CV profile we also included a carotid ultrasonography, with a Doppler linear probe, for the evaluation of the IMT. Specifically, IMT was measured at common carotid level 1 cm before carotid bifurcation in both right and left carotid arteries, using an average all the 2 measurements. All measurements were performed by FM. Inter-observer variability for EAT, LV ejection fraction and IMT were calculated in 10 randomly selected exams. A second observer (CDA), who was blinded to the initial analysis, repeated all measurements for quantification of inter-observer variability.

***Statistical analysis***

Data were collected in a Microsoft Excel database and statistical analysis were performed by using IBM SPSS Statistics v25.0. Categorical variables were reported as percentages. Continuous variables were reported as mean ± standard deviation (SD), and variables with skewed distributions were presented as median and interquartile range (IQR). Normal distribution was assessed by

Kolmogorov- Smirnov test. Student t test for paired samples was used to compare mean

for baseline characteristics and bio humoral parameters between patients and controls. Student t test for unpaired data was used to compare differences in LV systolic function, LV diastolic function, EAT and IMT between HIV-infected patients and controls. Correlation between cardiovascular risk factors and type of therapy with EAT levels and IMT was assessed by Pearson's (or Spearman's) correlation and univariate regression. Multiple linear regression analysis was used to adjust for potential confounders. Inter-

observer variability was evaluated by calculating the interclass correlation coefficient, with a cut-off value > 0.75 as indication of good agreement, as proposed by Burdock et al [22]. Statistical significance has been accepted at $p \leq 0.05$.

## Results

### *Study population*

We enrolled 29 consecutive HIV-infected patients on ART (13, 45% male; median age of 14.0 and IQR 8.7 (baseline characteristics reported in **Table 1**), and 29 age-matched healthy controls, without differences for demographic, lifestyle and metabolic characteristics with patients (**Table 1)**. According to the CDC classification of HIV-infected children and adolescents [23], most patients were in class 3 (21, 72%), 3 patients in class 2 (10%) and 5 patients in class 1 (17%).

The majority of patients (72%) had undetectable viral load defined as HIV RNA < 40 copies/ml and the mean $CD4^+$ count value was 1032 cells/μL at enrollment (**Table 1**). At the time of enrollment, all patients were on anti-retroviral treatment with nucleoside reverse transcriptase inhibitors backbone in association with a protease inhibitor in 23 (79%) patients (15 patients on lopinavir/ritonavir, 8 patients on darunavir/ritonavir) or an integrase strand transfer inhibitor in 6 (21%) patients (5 dolutegravir and 1 raltegravir). The median duration of overall ART in the study population was 120 months (IQR 156); specifically, patients were treated with at least one nucleoside reverse transcriptase inhibitors for 98 months (IQR 130.5), with protease inhibitors for 96 months (IQR 130.8), with non-nucleoside reverse transcriptase inhibitors for a median of 18 months (IQR 23)

and with an integrase strand transfer inhibitor for a median of 15.5 months (IQR 23.5). None of patients in our cohort had hypertension (mean SBP 115 ± 12, and DBP 68 ± 8 mmHg) or diabetes (mean fasting glucose value 72 ± 15 mg/dl), but 6 patients (21%) showed an HOMA-index > 2,5, indicating an insulin-resistance state. Dyslipidemia was found only in 2 (7%) patients, with a mean value of LDL cholesterol of 95 ± 26 mg/dl. Only 2 patients had an altered body mass index (mean value 20 ± 4 $kg/m^2$), and 1 patient met ICD or NCEP-ATP III criteria for metabolic syndrome (**Table 1**).

A variable degree of lipodystrophy was reported in about a third of patients (**Table 1**).

***Cardiovascular evaluation***

Compared with healthy controls, HIV-infected children showed a statistical significant increase in EAT (3,16 ± 1,05 vs 1,24 ± 0,61 mm; p < 0,0001, **Figure 2A)** and IMT (0,77 ± 0,15 vs 0,51 ± 0,11 mm; p < 0,0001, **Figure 2B**). Although absolute values of left ventricular ejection fraction were within the normal values in both populations, the mean

left ventricular ejection fraction was significantly reduced in HIV-infected patients in comparison to healthy control subjects (58,5 ± 6,66 vs 66 ± 4,24 %; p = 0,029) (**Figure 2C**). We didn't find statistical significant differences between patients and controls regarding diastolic function.

Patients' age and gender, HIV class and level of CD4, the duration of ART (including each antiretroviral drug class), the use of first generation protease inhibitors, the use of specific antiretroviral drug class, the presence or degree of lipodystrophy, the presence of

dyslipidemia, the degree of insulin resistance were investigated as possible factors affecting the increase of EAT and/or IMT, however for none of them a statistical significant correlation was demonstrated. Children with HOMA index >2.5 seemed to have a trend toward increase in EAT, showing higher value (3.25 ± 0.87) than children with HOMA<2.5 (2.92 ± 0.85), but this difference was not statistically significant (p=0.34). Similarly, children with HOMA > 2.5 had slightly higher values of IMT (0.76 ± 0.16 vs 0.64 ± 0.31), although this difference did not reach statistical significance (p=0.27).

The interclass correlation coefficient for inter-observer variability for LV ejection fraction, EAT and IMT were 0.925, 0.987 and 0.960 respectively.

**Discussion**

Cardiac dysfunction has been previously reported in adults and children with HIV infection, although it's not completely clear whether those alterations are directly related to HIV or to antiretroviral treatment. We confirmed a decrease of the absolute value of ejection fraction and increased values of IMT in a population of children and adolescents with vertically-acquired HIV infection. Moreover, this is the first study documenting a significant increase of EAT in children and adolescents with HIV infection. Systolic function reduction is consistent with previous studies, that also documented a reduction in ejection fraction in subjects with HIV infection. Lipshultz et al [9], showed in a cohort of 93 HIV children, a reduction of LV fractional shortening after 8 months of follow-up, compared with healthy control subjects. More recently, the same group [10], confirmed

these data showing a reduction in cardiac function in HIV children with higher current viral load. The CHAART-2 study [24] showed the effectiveness of ART in improving LV fractional shortening and LV contractility by comparing a cohort of 74 HIV children on ART with 140 HIV-infected controls unexposed to ART. In particular, they found a significant difference (expressed as Z-score of normal values) in LV fractional shortening (0.26 vs -1.19 respectively, $p = 0.02$) and LV contractility (0.09 vs -0.88, $p = 0.002$), between cases and controls respectively. According with our data, LV function appeared different from controls but not significantly impaired, however, they also reported a relevant impact of ART on LV function, not confirmed in our study.

In addition, as previously reported in other cohort of HIV-infected children and adults, we showed an increase of IMT in our population. Although other studies, even in large population, confirmed this finding in HIV-infected patient aged below 30 years [25], the clinical relevance and the correlation of this finding with HIV state of infection and ART are still matter for discussion. Charakida et al [19] reported a significant greater IMT in HIV infected children than in healthy control subjects, and demonstrated a potential role of ART, showing higher IMT in those patients who received protease inhibitors. In contrast, Idris et al [20] found higher IMT values in naïve children with vertically acquired HIV infection compared to those on ART and healthy children.

In the last two years, other studies confirmed an increase in the IMT in HIV-infected adults [26] and children or adolescents [27] patients. In contrast with our results, Augustemak de Lima et al also reported alteration in CV profile and a possible role of ART on IMT in underage patients [27].

This is the first study investigating the EAT in HIV-affected children and adolescents. Currently, there are only few studies that evaluated EAT in HIV adult patients, showing an increase of epicardial fat in these patients. Zona et al [28], in a cohort of 240 HIV adult patients, showed an increase of EAT, more evident in men than in women. Differently from our study, they found a correlation between the higher EAT and $CD4^+$ levels. Other studies correlated EAT to body-weight in HIV patients [16], showing a correlation between EAT and metabolic syndrome. Guaraldi et al [18] found an increased EAT that correlated with metabolic syndrome in 876 adult patients and a correlation between EAT and lipodystrophy. Finally, more recently, another study of Brener et al [17], studied 579 HIV-infected adult patients and 353 controls, showing a thicker EAT in

HIV patients treated with ART. However, differently from our study, Brener et al [17] found a greater EAT in patients with greater BMI, in hypertensive patients, in diabetic patients and in dyslipidemic patients, suggesting a potential contribution of CV risk factors on EAT. In our study, there were no correlation between CV risk factors and increased EAT or IMT. Moreover, we didn't find any correlation between HIV class and level of CD4, the duration of ART, the use of old generation protease inhibitors, the use of any different antiretroviral drug class, the presence and degree of lipodystrophy therapy and EAT or IMT. These results suggest a potential role of the disease defining an increased CV risk in these patients.

**Limitations**

The relatively small number of patients is a limitation of this study, although the cohort of patients includes all subject currently seen at the reference center and the matching with controls may ensure a statistical reproducibility of results. Lack of follow up makes the current findings hypothesis generating and warranting further evaluation.

**Conclusions**

The findings of the present study demonstrated that in patients with HIV treated with ART, systolic function is reduced and IMT and EAT are increased compared to healthy control. Taken together these findings suggest subclinical cardiovascular damage in HIV patients despite the absence of conventional CV risk factors.

**Acknowledgements**

Dr. Fabio Marsico has been supported by a research grant provided by the Cardiovascular Pathophysiology and Therapeutics PhD program.

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**Table**

| General characteristics | Patients (n = 29) | Controls (n = 29) | p value |
|---|---|---|---|
| Male (N, %) | 13 (45) | 13 (45) | |
| Age at enrollment (median, IQR) | 14.0 (8.7) | 13.6 (8.2) | 0.932 |
| Age at diagnosis (median, IQR) | 1 (6) | NA | |
| Smokers (N, %) | 7 (24.1) | 5 (17.2) | 0.265 |
| Regular alcohol consumer (N, %) | 1 (3.4) | 0 (0) | |
| Drug consumer (N, %) | 0 (0) | 0 (0) | |
| **Viro-immunological characteristics** | | | |
| Patients in class 1 (N, %) | 5 (17.2) | NA | |
| Patients in class 2 (N, %) | 3 (10.4) | NA | |
| Patients in class 3 (N, %) | 21 (72.4) | NA | |
| Patients with undetectable HIV load (N, %) | 21 (72.4) | NA | |
| HIV viral load (mean ± SD) | 6176 ± 11686 | NA | |
| $CD4^+$ cell count (mean ± SD) | 1032 ± 875 | NA | |
| Percentage of $CD4^+$ (mean ± SD) | 33.9 ± 10.6 | NA | |
| Duration of ART (median, IQR) | 120 (156) | NA | |
| Duration of PI treatment (median, IQR) | 96 (130) | NA | |
| Duration of NRTI treatment (median, IQR) | 98 (130) | NA | |
| Duration of NNRTI treatment (median, IQR) | 18 (23) | NA | |
| Duration of II treatment (median, IQR) | 15.5 (23.5) | NA | |
| **Metabolic and cardiovascular parameters** | | | |
| BMI (mean ± SD) | 20 ± 4 | 19 ± 6 | 0.153 |
| BMI < 5 percentile (n, %) | 3 | NA | |
| BMI 5-25 percentile (n, %) | 11 | NA | |
| BMI 26-75 percentile (n, %) | 8 | NA | |
| BMI 76-95 percentile (n, %) | 2 | NA | |
| BMI > 95 percentile (n, %) | 3 | NA | |
| Total cholesterol in mg/dl (mean ± SD) | 157 ± 36 | 156 ± 46 | 0.959 |
| HDL cholesterol in mg/dl (mean ± SD) | 42 ± 12 | 54 ± 21 | 0.063 |
| LDL cholesterol in mg/dl (mean ± SD) | 95 ± 26 | 101 ± 29 | 0.407 |
| Triglycerides in mg/dl (mean ± SD) | 124 ± 50 | 101 ± 65 | 0.058 |

| | | | |
|---|---|---|---|
| Metabolic Syndrome (N, %) | 1 (3) | 1 (3) | |
| No lipodystrophy (N, %) | 21 (72.4) | NA | |
| Mild lipodystrophy (N, %) | 5 (17.2) | NA | |
| Severe lipodystrophy (N, %) | 3 (10.4) | NA | |
| Fasting glucose in mg/dl (mean ± SD) | 72 ± 15 | 82 ± 28 | 0.151 |
| HOMA index (mean ± SD) | 1,9 ± 3,9 | NA | |
| Systolic blood pressure (mean ± SD) | 115 ± 12 | 117 ± 16 | 0.639 |
| Diastolic blood pressure (mean ± SD) | 68 ± 8 | 65 ± 11 | 0.725 |

**Table 1.** Patients baseline characteristics

IQR: interquartile range; SD: standard deviation; HOMA: homeostasis model assessment.

**Figures**

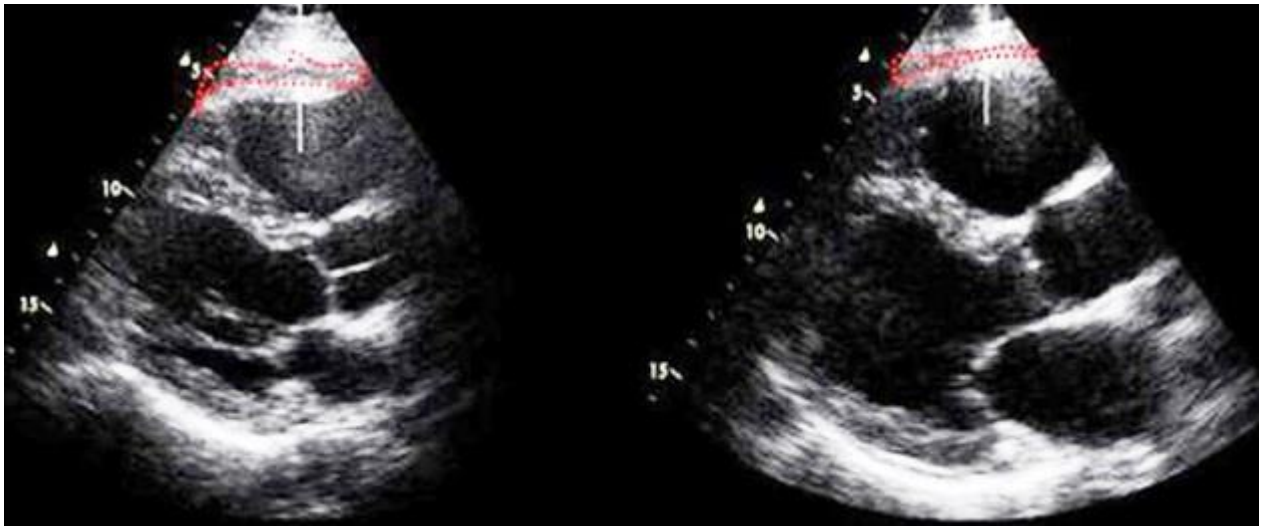


**Figure 1.** Echocardiographic assessment of epicardial adipose tissue. The left side is an end-systolic image, where EAT is more represented. The right side is an end-diastolic image, with the minimum thickness of EAT.

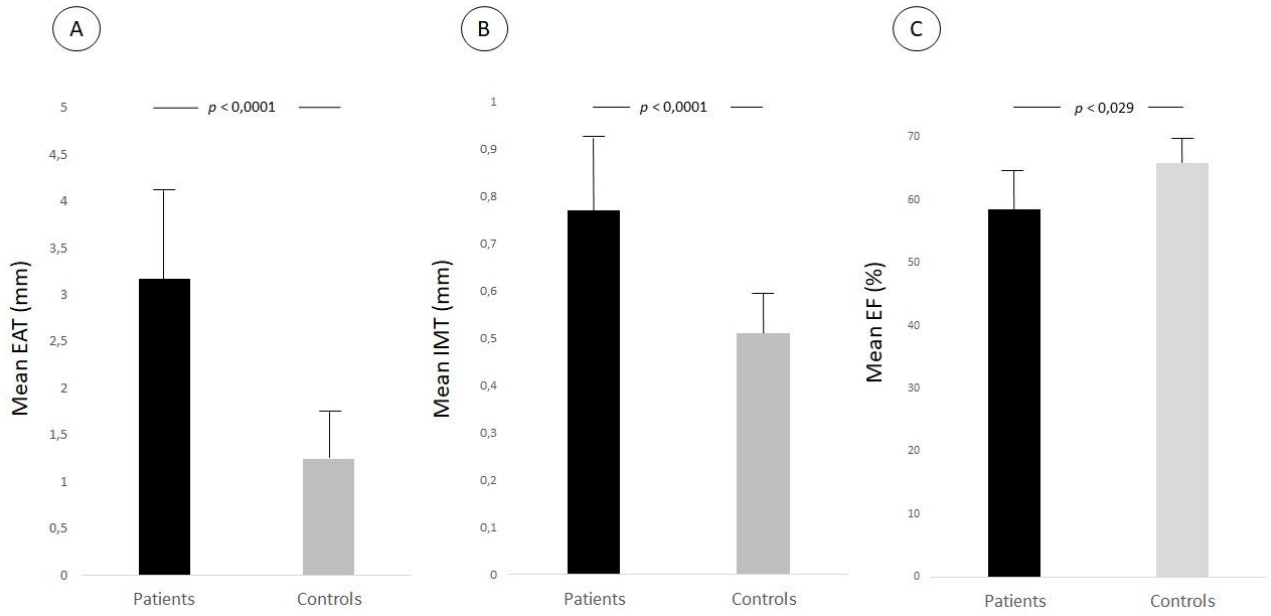


**Figure 2.** Difference of EAT, IMT and left ventricular ejection fraction between patients and controls

A: difference of EAT between patients and controls. B: difference of IMT between patients and controls. C: difference of left ventricular ejection fraction between patients and controls.

EAT: epicardial adipose tissue. IMT: intima-media thickness.

# PART 3

**Congenital heart disease: from childhood to adulthood**

## CHAPTER 8.

## Hypertrophic cardiomyopathy in mitochondrial disorders: description of an uncommon clinical case

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# Hypertrophic cardiomyopathy in mitochondrial disorders: description of an uncommon clinical case

## Introduction

The mitochondria are complex organelles responsible for several functions in every cell, including the assembly of ATP molecules that are the final product of the respiratory chain via oxidative phosphorylation,[1,2] as well as heat production, apoptosis, and transmission of maternal genetic traits.[3,4]

The respiratory chain, embedded in the inner mitochondrial membrane, is composed of five enzyme complexes (I, II, III, IV, and V) and mitochondrial respiratory chain proteins are under the genetic control of both nuclear and mitochondrial genes. Mutations involving these genes may cause defects in oxidative phosphorylation.[5] Such mitochondrial disorders (MIDs) can be caused by defects in either mitochondrial or nuclear DNA, but mitochondrial DNA (mtDNA) mutations are the commonest cause of mitochondrial disease in adults, identified in up to 70% of patients.[6]

Several clinically distinct subgroups of MIDs exist, and the most frequently identified biochemical abnormalities are deficiencies in NADH-coenzyme Q (CoQ) reductase (complex I).[7]

The heart is mainly dependent on aerobic respiration for its energy requirements,[8,9] so it is one of the organs most frequently compromised in MIDs.[10,11]

The most frequent cardiac manifestation of MIDs is hypertrophic cardiomyopathy (HCM), more frequently reported in association with mtRNA gene mutations, and in deficiencies in NADH-CoQ reductase (complex I respiratory chain). Yet, MIDs can also, more rarely, cause dilated cardiomyopathy, restrictive cardiomyopathy, or left ventricular non-compaction.[6] Conduction system disease occurs commonly in patients with mtDNA disease, often progressing to high-grade atrioventricular block.[6] Finally, ventricular pre-excitation, Wolff–Parkinson–White syndrome, and supraventricular and ventricular tachyarrhythmias are also more common in patients with mtDNA disease than in the general population.[6]

The pathogenesis linking mtDNA to cardiac involvement remains poorly understood. One hypothesis calls for a deficit of complex I activity described in cardiomyocytes of end-stage mtDNA-related cardiomyopathy, as well as in non-cardiac tissues of patients with mtDNA disease. In contrast to skeletal muscle, in cardiac muscle, proliferation of intermyofibrillar mitochondria due to oxidative phosphorylation dysfunction interferes with sarcomeric function, promoting adverse cardiac remodelling. In addition, in normal heart, activation of complex I and fatty acid oxidation increase oxygen consumption, whereas in mtDNA cardiomyopathy, cardiac metabolism shift from fatty acid oxidation to glucose oxidation reduces oxygen consumption and promotes left ventricular (LV) hypertrophy.[6,12–15]

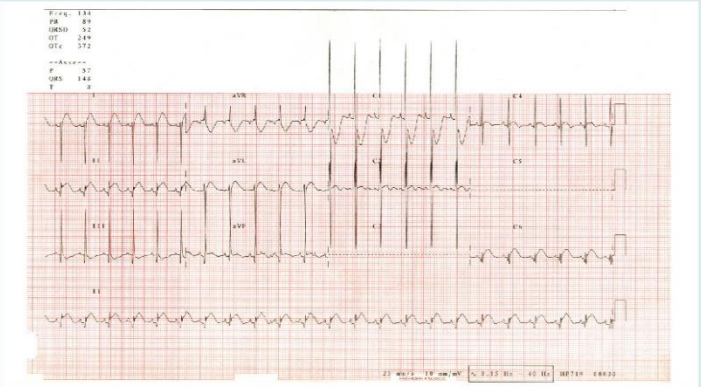

**Figure 1** Twelve lead electrocardiogram.

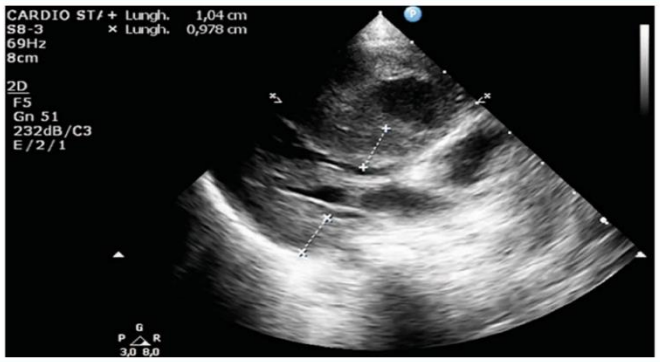


**Figure 2** Echocardiographic long axis view.

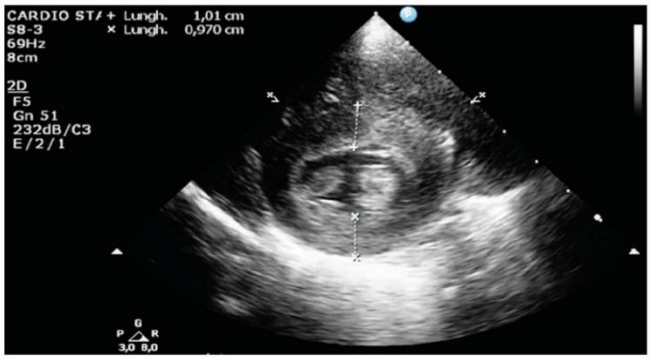


**Figure 3** Echocardiographic short axis view.

## Case report

We report the case of an infant born from a Caesarean section, after 37 weeks of pregnancy, with no perinatal complications, but preventively admitted to the neonatal intensive care unit at Federico II University of Naples (Italy) because of mild prematurity and a history of premature son death due to uncertain cause.

On admission body temperature, blood pressure, heart rate, breathing rate and blood oxygen saturation were normal. At clinical examination no cyanosis or clubbing were present and no heart murmurs, wheezing, rhonchi and crackles, or hepatosplenomegaly were present, with normal peripheral pulses. On the second day, blood gas analysis documented metabolic acidosis, with a pH of 7.180, a $pCO_2$ of 34.5 mmHg, a $pO_2$ of 64.3 mmHg, and $HCO_3^-$ of 12.6 mmol/L, with low base-excess levels and high lactate levels (16.98 mmol/L), that were treated with $NaHCO_3$ administration.

The patient underwent cerebral magnetic resonance, which showed high levels of lactate in the white matter of the centrum semiovale, and genetic testing. A blood sample documented a lactic acid value of 1908 mmol/mol of creatinine (normal range 1–25), and elevated pyruvic dehydrogenase complex activity, commonly observed in patients with lactic acidosis. Thus, therapy including vitamin D3, biotin, thiamin, riboflavin, coenzyme Q10, carnitine, and $NaHCO_3$ was initiated.

During the hospital stay, the patient also developed electrocardiographic abnormalities, with high voltage in all leads, and a diffuse abnormal repolarization pattern, typical of HCM (*Figure 1*), followed on the third week by echocardiographic features of HCM, with massive biventricular hypertrophy. Interventricular septum and LV posterior wall were both 10 mm thick (Z-score 4.83 and 6.70, respectively), causing an obliteration of the cardiac cavity, with a septum to cavity ratio of 0.77, documenting advanced HCM[16] (*Figures 2* and *3*), for which propranolol was initiated.

After 4 weeks, multi-organ involvement emerged, including loss of amino acids and electrolytes in the urine, neurological involvement with pyramidal signs and axial hypotonia, and involvement of the respiratory muscles, for which non-invasive respiratory support (continuous positive airway pressure, CPAP), and subsequent invasive mechanical ventilation were necessary. However, the clinical course was complicated by two episodes of cardiac arrest treated with cardiopulmonary resuscitation. Regrettably, multi-organ involvement prevented cardiac transplantation, leading to fatal ventricular arrhythmia at 3 months after birth.

Based on the clinical suspicion of a mitochondrial defect, the patient had undergone muscular biopsy, which confirmed the diagnosis of MID, with substantial improvement of the activity of the respiratory chain complex I (NADH CoQ1) (0.6 nmol/min/mg, normal value 17–34 nmol/min/mg), normal activity of complexes II, III, IV, and V, and increased pyruvic dehydrogenase complex activity (7.3 nmol/min/mg, normal value 0.50–2.50 nmol/min/mg).

Thus, genetic screening was performed for the patient and his parents.

Genetic analysis of the patient documented two different nuclear DNA mutations, inherited in heterozygosis, involving the acyl-CoA dehydrogenase 9 (*ACAD9*) gene, on chromosome 3q21.3. In particular, the mother had the mutation c.555-2A > G in intron 5 of the *ACAD9* gene, present in heterozygosis, and the father had the mutation c.1168G > A in exon 12 of the *ACAD9* gene, present in heterozygosis. The child inherited the two variants in heterozygosis of the *ACAD9* gene: in the splicing accepting site of intron 5, the change c.555-2A > G; in exon 12, the change c.1168G > A, causing the substitution p.Ala390Thr. This genotype could be associated with the phenotype of the patient.

## Discussion

The present case was characterized by a compound heterozygosity mutation in the *ACAD9* gene not previously described. The *ACAD9* gene encodes a protein involved in the assembly of complex I of the respiratory chain, and it is known that mutations in this gene could be associated with HCM. ACAD9 deficiency is an autosomal recessive multisystemic disorder, often characterized by infantile onset of acute metabolic acidosis, HCM, and muscle weakness associated with a deficiency of mitochondrial complex I activity in muscle, liver, and fibroblast cells.[17] Haack *et al.*[17] reported four patients carrying mutations in *ACAD9*. The first patient had an early onset, with cardiorespiratory depression, HCM, encephalopathy, and lactic acidosis, and died at 46 days of age. The other patients had an onset of HCM and lactic acidosis, with longer survival. The authors identified compound heterozygosity for two mutations in the *ACAD9* gene, different from the mutations of the current case. In addition, Haack *et al.*[18] also reported a family in which three patients had HCM, hypotonia, lactic acidosis, and exercise intolerance associated with complex I deficiency associated with a homozygous mutation in the *ACAD9* gene. Leslie *et al.*,[19] in an autopsy study of a fatal neonatal lethal lactic acidosis due to mutations in *ACAD9* that reduced complex I activity, identified compound heterozygous variants in the *ACAD9* gene: c.187G > T and c.941 T > C, also different from our case. Collet *et al.*,[20] in a retrospective analysis of 20 children with cardiac hypertrophy and isolated complex I deficiency, identified compound heterozygosity for missense, splice site, or frame shift *ACAD9* variants in eight patients (40%). Age at onset ranged from neonatal period to 9 years and 5/8 died in infancy. Heart

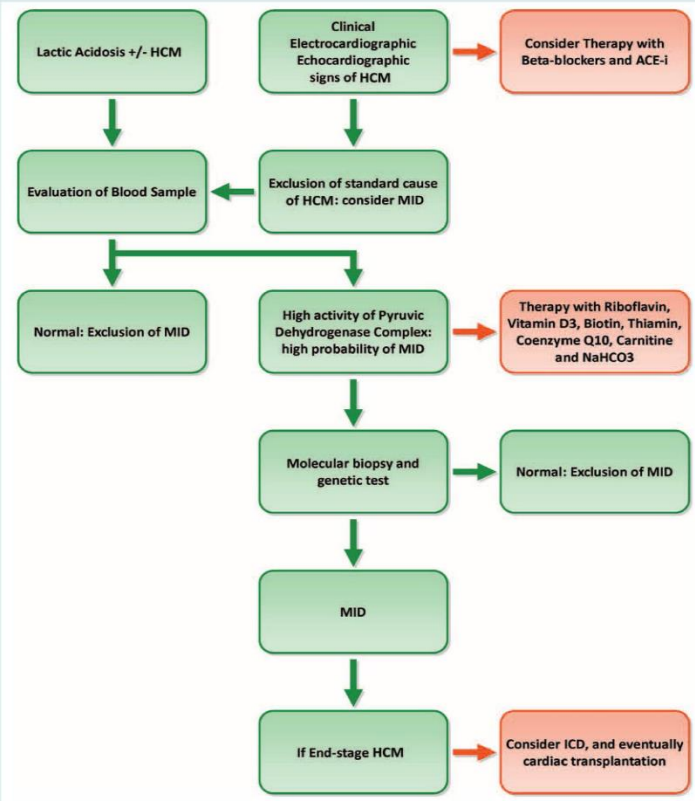


**Figure 4** Diagnostic and therapeutic flow chart. HCM, hypertrophic cardiomyopathy; ACE-I, angiotensin converting enzyme inhibitors; MID, mitochondrial disorder; ICD, implantable cardioverter defibrillator.

transplantation was possible in 3/8 patients. Finally, Dewulf *et al.*[21] reported nine patients from three unrelated families with a wide spectrum of cardiac involvement among families and patients within the same families. All patients exhibited elevated lactate levels, and *ACAD9* mutations were identified in all patients.

The pathomechanism that links genotype to phenotype of ACAD9 deficiency was investigated by Schiff *et al.*[22] who assessed, using *in vitro* functional expression assays in *Escherichia coli*, the ACAD enzymatic dehydrogenase activity of 16 pathogenic *ACAD9* mutations from 24 patients with complex I deficiency. In this study, a significant inverse correlation between residual ACAD enzymatic dehydrogenase activity and phenotypic severity ($P = 0.034$) was observed. These results indicate that ACAD9 regulates fatty acid oxidation in cells where it is strongly expressed and suggest that fatty acid oxidation defect contributes to the severity of phenotype in ACAD9-deficient patients.

The first therapeutic approach in MIDs is symptomatic, treating lactic acidosis when present with carnitine, vitamin D3, $NaHCO_3$, and riboflavin supplementation (*Figure 4*).[23]

Conventional pharmacological and device therapy, according to general guidelines, is indicated in HCM and dilated cardiomyopathy phenotypes (*Figure 4*),[24,25] pacemaker implantation in patients with an early stage of conduction system dysfunction is recommended,[6,26] whereas heart transplantation is the last reasonable chance for patients at end-stage disease. In patients with one of these phenotypes, it should be considered the diagnosis of a MID. In fact, as shown in our case, it is important to evaluate in a newborn with lactic acidosis and HCM the activity of the pyruvic dehydrogenase complex, muscular biopsy, and genetic testing (*Figure 4*).[6,17–20] However, heart transplantation could only be considered in patients without multi-organ engagement, and has been performed successfully in patients with mtDNA disease.[27]

**Conflict of interest:** F.M. was supported by a research grant provided by the Cardiovascular Pathophysiology and Therapeutics PhD programme of the University of Naples Federico II, Italy. The other authors declare no conflict of interests.

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**CHAPTER 9.**

# Cardiac imaging for prognostication in patients with pulmonary hypertension: the accuracy of magnetic resonance imaging versus echocardiography and baseline values versus interval changes

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*Draft*

**Cardiac imaging for prognostication in patients with pulmonary hypertension: the accuracy of magnetic resonance imaging versus echocardiography and baseline values versus interval changes**

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**Abstract**

**Background:** Pulmonary hypertension (PH) is a progressive disease with high mortality rate. Although is known the role of imaging RV size and function in predicting prognosis, it is unknown the role of the interval changes of these parameters on prognosis.

**Purpose:** The aim of this study is to evaluate the impact of CMR and echocardiographic parameters of RV size and function on prognosis in patients with PH, at baseline and during follow-up, considering also the comparison of echocardiographic parameters of circumferential RV function with CMR-derived RV function.

**Methods:** PH patients with both CMR and TTE exams within 1 month were followed over a mean period of 57±24 months. In 20 of these patients, after 47±16 months, a second CMR and echocardiographic exam were performed. As primary endpoint, a composite of death for any cause, hospitalization for worsening of PH, start with i.v. prostanoid therapy, long term oxygen therapy, atrial septostomy or lung transplantation was defined. The secondary endpoint was defined as disease progression, i.e. a composite endpoint including a reduction in 6 minute walking test distance of more than 15%, accompanied by worsening in NYHA class or escalation of pulmonary vasodilator therapy, or increase in NT-Pro-BNP value by more than 50%.

**Results:** Thirty-six patients (15 females/21 males), mean age 44±16 years were included in the study. The primary endpoint was reached by 14 patients, while progression of the disease was present in 30 patients. None of the baseline parameters was predictive of outcome, nor of disease progression. In the 20 patients with follow-up exams, an interval

increase in RV volumes assessed by CMR was highly predictive of an adverse outcome, with a more likely adverse event during the subsequent follow-up period of 14±12 months after the second imaging exam. None of the echocardiographic interval changes were predictive of outcome. A correlation of FAC with CMR-derived RV-EF ($r = 0.469$, $p = 0.003$) was found.

**Conclusion:** An increase in RV end-diastolic volumes on CMR is associated with a worse outcome. A weak correlation of fractional area change with CMR-derived RV function was found.

## Introduction

Pulmonary arterial hypertension (PAH) is a progressive vascular disease leading to right ventricular failure. Despite the advent of disease targeted therapies, contemporary registries still observe mortality rates of 20-30% at 3 years and prognostication remains an important aspect in the management of PAH patients (1, 2). A first equitation to estimate survival at the time of diagnosis was proposed in 1991 based on the National Institute of Health registry data. Prognosis in PAH was derived from hemodynamic measurements, incorporating right atrial pressure, mean pulmonary artery pressure and cardiac index (3). In a more contemporary equitation and in the current practice guidelines, hemodynamic measures are complemented by clinical variables related to exercise capacity (e.g. 6 minutes walking distance, peak oxygen consumption, functional class), gender, PAH subgroup, neurohumoral activation and imaging parameters (e.g. the presence of pericardial effusion and right atrial size) in order to further improve prognostication (2, 4).

As the clinical outcome in PAH depends on ability of the right ventricle to cope with the increased vascular load, imaging studies assessing right ventricular (RV) function may be particularly helpful in refining PH prognosis. Transthoracic echocardiography provides an easily available non-invasive tool to monitor pulmonary arterial pressure and RV size and function (5). Several echocardiographic indices of RV function have been evaluated for prognostication in PH, such as the amount of systolic tricuspid annular motion (TAM), its systolic velocity on tissue Doppler imaging, the RV endsystolic remodeling index (RVESRI), the fractional area change (FAC) of the right ventricle or its

global strain (6-11). Recently, cardiac magnetic resonance imaging (CMR) has emerged as reference method for quantifying RV volumes, ejection fraction, mass and pulmonary flow (12). CMR-derived stroke volume as well as right and left ventricular volumes at baseline were also shown to be predictive of PAH prognosis (13), and interval changes in RV ejection fraction (EF) were superior to measuring pulmonary vascular resistance in predicting outcome (14).

So far, no head-to-head comparison of the prognostic ability of both imaging techniques in unselected PAH patients has been reported. Transthoracic echocardiography is widely available, inexpensive and safe, but has limitations in terms of obtaining adequate images and accurate measurement of RV volumes. CMR provides high-resolution three-dimensional images and avoids any geometrical assumptions to quantify RV volumes and function. However, it is expensive, time-consuming, and requires specific technical and analytical expertise. CMR is contraindicated in certain patients with electronic devices and breath holding may be challenging (15). All in all, both imaging techniques have their advantages and drawbacks.

In the present pilot study, we aimed to compare the prognostic accuracy of standard echocardiographic surrogates of RV function with CMR-derived RV EF and volumes. In a second step, we analyzed the predictive value of baseline measures versus interval changes. We hypothesized that CMR-derived RV EF is superior to echocardiographic measures of RV function in predicting outcome and that interval changes are more predictive of outcome than baseline measures.

## Methods

### *Study patients*

We evaluated 36 patients with pulmonary hypertension of different etiologies. All patients underwent a CMR exam and a transthoracic echocardiogram within 1 month. In 20 patients, a second CMR and echocardiographic exam were performed after 47 ± 16 months (range 24-75 months) of follow-up. After the second CMR exam, these 20 patients were under clinical follow-up for another 14 ± 12 months. We routinely scan all patients with pathologies affecting the right ventricle (i.e. with congenital cardiac defects, RV dysplasia or pulmonary hypertension) with CMR in a 3 - 5 years interval or earlier if clinically necessary. In all patients, NYHA functional class, resting oxygen saturation, 6-MWD, serum NT-proBNP and concomitant medication at the time of the first and second CMR exam were documented.

### *Transthoracic echocardiography*

Transthoracic Doppler echocardiography was performed using an Acuson Sequoia C 512 (Acuson Corporation, Siemens, Mountain View, CA, USA) or Philips IE 33 ultrasound systems (Philips, Amsterdam, The Netherlands) with a 3.5 MHz transducer including second harmonic imaging. All echocardiography images and clips were stored off-line (Argus version 4.01, Syngo MR B13, Siemens Medical Solutions, Erlangen, Germany). Values were obtained after averaging over two cardiac cycles. Patients were examined in supine left lateral position.

The following surrogates of right ventricular function were obtained in the RV four chamber view: 1) TAM: defined as the distance between the basal end-diastolic to systolic motion of the tricuspid annulus measured on M-mode pictures of the tricuspid lateral annulus (16); 2) FAC: calculated as the difference between end-diastolic and end-systolic area of the right ventricle divided by end-diastolic area (16); 3) RVESRI: defined as the lateral wall to septal wall height ratio. The lateral wall length was measured from the lateral tricuspid annulus to the insertion point of the right ventricle on the interventricular septum. The septal height was measured as a straight line from the septal tricuspid annulus to the RV insertion on the interventricular septum (see also figure 1) (11); 4) Peak systolic tricuspid annular motion velocity (S'): S' was measured with pulsed-wave tissue Doppler Imaging at the lateral free wall (17). In addition, the TAM to systolic pulmonary artery pressure (SPAP) ratio was calculated. SPAP was calculated by continuous Doppler imaging of the tricuspid valve insufficiency, measuring the peak systolic tricuspid transvalvular velocity plus right atrial pressure. Right atrial pressure was defined by inferior vena cava respiratory variability in its diameter, where a value of 5 mmHg was added if the inferior vena cava had a diameter < 17 mm and a normal inspiration excursion, a value of 10 mmHg if inferior vena cava had a diameter > 17 mm or an impaired inspiration excursion, or a value of 15 mmHg if inferior vena cava had a diameter > 17 mm and an impaired inspiration excursion (18). All measurements were performed by FM. Inter-observer and intra-observer variability for all the echocardiographic parameters of RV function were calculated in 10 randomly selected exams. A second observer (EG), who

was blinded to both the initial analysis and the CMR data repeated all measurements for quantification of inter-observer variability.

***Cardiac magnetic resonance imaging***

Detailed information about the methodology of the CMR studies has been described elsewhere (19). Study patients were examined using a 1.5 T (Magnetom Symphony) or a 3 T (Magnetom Trio, both from Siemens Medical Solutions, Erlangen, Germany) whole-body clinical MRI system with cardiac synchronization by ECG electrodes. Ventricles were imaged from the base towards the apex during short end-expiratory breath-holds using contiguous short-axis slices in 8 mm increments. A cine steady-state free procession technique with retrospective gating was used. CMR analysis was done off-line on a dedicated workstation using commercially available software (ArgusVersion 4.01, Syngo MR B13, Siemens Medical Solutions, Erlangen, Germany) by two experienced observers (KW and AW). End-diastolic and end-systolic contours were manually traced for each slice. RV volumes were determined according to the modified Simpson's rule (disk summation, no geometrical assumption). The stroke volume to endsystolic volume ratio was calculated as simplified evaluation of the Ees/Ea ratio (20).

***Study endpoints***

The primary clinical endpoint of this study was defined as adverse event, i.e. a composite clinical endpoint consisting of death for any cause, hospitalization for worsening of PAH, start with i.v. prostanoid therapy, start with long term oxygen therapy, atrial septostomy or lung transplantation, whichever occurred first. This endpoint was used

in the GRIPHON trial (21). The secondary endpoint was defined as disease progression, i.e. a composite endpoint including a reduction in 6-MWD of more than 15%, accompanied by worsening in NYHA class or escalation of pulmonary vasodilator therapy, or increase in NT-Pro-BNP value by more than 50%, whichever occurred first.

***Statistical analysis***

Statistical analysis was performed with IBM SPSS Statistics v21.0. Categorical variables were reported as percentages. Continuous variables were reported as mean ± standard deviation (SD), and non-normally distributed continuous variables were presented as median and its interquartile range (IQR). Comparisons of interval changes in the echocardiographic and CMR parameters at baseline and follow-up were analyzed with a paired t-test. Comparisons between groups were performed using an un-paired t-test or the Mann-Whitney test for non-normally distributed variables. The correlation of the echocardiographic surrogates of RV function with CMR-derived RV EF was analyzed with a linear regression model. Receiver operating characteristic (ROC) analysis was performed with the echocardiographic parameters as test variables and RV EF by CMR as the state variable. Cox proportional hazard regression models were used to define the association of baseline imaging parameters or interval changes with the primary and secondary endpoints. Parameters with a p value $< 0.1$ were entered in a second step in a multivariate Cox proportion model. For calculating predictors of outcome from baseline parameters the maximal time of observation was used for the analysis. For calculating the predictive value of interval changes, the time from the last CMR to the last follow-up was used. In a time-

to-event analyses, endpoints were estimated with the use of the Kaplan-Meier method and were analyzed with the use of the log-rank test. Measurements of reproducibility were evaluated by calculating the interclass correlation coefficient (ICC)for observer agreement. A cut-off value > 0.75 indicated good agreement (22). In all analyses, the null hypothesis was rejected for p values < 0.05.

## Results

### *Patients' characteristics*

Thirty-six patients (mean age 44 ± 16 years) were included in the study. Baseline characteristics of the patients are reported in table 1. Twenty-eight patients had pulmonary arterial hypertension (PAH) (n = 5 idiopathic PAH, n = 20 shunt-induced PAH, n = 3 PAH in association with connective tissue disease), 2 patients had combined pre- and postcapillary PH due to left heart disease, and 6 patients had not operable chronic thromboembolic PH (CTEPH). At the time of the first combined imaging exams, 30 (83%) patients were under treatment with disease targeted therapies: n = 23 (64%) patients were on vasodilator monotherapy, one of them on Calcium antagonists, n = 5 (14%) patients were under a dual therapy, and n = 2 (6%) patients received a triple therapy. The absence of therapy in the remaining 6 patients was due to their functional class (n = 3 with Eisenmenger syndrome and functional class I-II), intolerance to therapy (n = 1) and etiology of PH (n = 2 with PH due to left heart disease). During the follow up period, in 23 patients (60%) a vasodilator was either newly introduced or added to the previous therapy. In 5 out of these 23 patients, i.v. prostanoid therapy was started.

After a mean follow-up period of 57 ± 24 months, 14 patients (39%) reached the composite primary endpoint defined as adverse event: n=7 patients had to be hospitalized for worsening of PH, n=3 patients started with i.v. prostanoid therapy, 2 patients died for any cause, and 2 patients started long term oxygen therapy. In a Kaplan Meier analysis, the event free survival (Figure 2) at 2 years, 4 years, 6 years and 8 years was 91%, 83%, 54% and 20%, respectively. In addition, 30 patients reached the secondary endpoint, defined as disease progression: n=12 patients had an increase in nt-proBNP values by more than 50%, n=8 patients experienced an escalation of pulmonary vasodilator therapy, n=7 patients had a worsening in NYHA class, and n=3 patients had a reduction in 6-MWD of more than 15% as first event. In a Kaplan Meier analysis, the survival without disease progression at 2 years, 4 years, 6 years and 8 years was 91%, 61%, 16%, and 4% respectively (Figure 3).

***Comparison between echocardiographic parameters of RV function and CMR-derived RV ejection fraction at baseline.***

There was only a weak correlation between echocardiographic parameters of RV function and CMR-derived RV EF. In a univariate linear regression analysis, a significant correlation was found between FAC and CMR-derived RV EF ($r = 0.469$, $p = 0.003$, 95% CI 0.190-0.855), but the correlation coefficient was low (Figure 4). No significant correlation was found for RVESRI and CMR-derived RV EF ($r = 0.311$, $p = 0.057$, 95% CI -29.407-0.435) (Figure 4). For the echocardiographic parameters of longitudinal RV contraction (S' and TAM), there was again a significant but very weak correlation

observed with CMR-derived RV EF (r = 0.378, p = 0.005, 95% CI of 0.12-0.56 for S', and r = 0.341 with a p = 0.007, 95% CI of 0.09-0.5 for TAM) (Figure 4).

Figure 5 demonstrates the areas under the curve (AUC) of the corresponding ROC analysis with RV EF by CMR ≤ 40% as the state variable. The AUC was highest for FAC (AUC 0.791, 95% CI 0.672-0.910). A FAC value of 30% had a sensitivity of 86% and specificity of 58% to detect a RV-EF ≤ 40%. The others corresponding values for sensitivity and specificity for different cut-offs on the ROC curves are listed in the figure legend.

ICC for intra-observer variability for FAC, TAM, S', and RVESRI were as follows: 0.973, 0.999, 0.999, and 0.926, respectively. Inter-observer variability for the same parameters, also evaluated by ICC was 0.909, 0.934, 0.981, and 0.770, respectively.

***Predictive value of baseline parameters on endpoints***

At baseline, there was no difference in age, gender, resting blood pressure, 6 minutes walking distance, and NT-proBNP levels in patients with and without an adverse event during follow-up. Patients with an adverse event had a lower BMI (23 ± 3 kg/m$^2$ vs 27 ± 5 kg/m$^2$, p = 0.008) and lower oxygen saturation (88 ± 8% vs 93 ± 3%, p = 0.019), but higher resting heart rate (87 ± 11 bpm vs 76 ± 12 bpm, p = 0.008) at baseline compared to patients without event. Of note, the functional class at baseline was predictive of outcome (p = 0.002).

Imaging parameters at baseline in both patient groups are presented in table 2. A

higher degree of tricuspid regurgitation and a higher SPAP at baseline were more often encountered in patients who reached the primary endpoint. As a consequence, the TAM/SPAP ratio was lower in patients with a worse outcome during follow-up. However, none of the echocardiographic surrogates of RV function and none of the CMR parameters of RV size and function were found to differ at baseline among patients who reached the primary endpoint or not. With respect to disease progression during follow-up, only RV cardiac output evaluated by CMR was lower in patients with vs. without disease progression (table 2). By univariate Cox regression analysis, none of the baseline parameters who different significantly between patients with and without adverse advent was predictive of an adverse event during follow-up nor of disease progression.

***Predictive value of interval changes parameters on endpoints***

Twenty patients had a combined echocardiographic and CMR follow up exam (4 females / 16 males, mean age 43 ± 15 years) after a period of 47 ± 16 months. In the follow up group at the time of the second imaging exam, n = 17 (85%) patients were on specific pulmonary vasodilator therapies (n = 1 of them on Calcium antagonists, and n = 5 patients (25%) were on combination therapy with 2 or 3 vasodilators). A total of n = 3 (15%) patients were still without disease targeted therapies because of their preserved functional class (NYHA I/II) in the setting of shunt-induced pulmonary hypertension. Four (20%) patients were in functional NYHA class III, n = 15 (75%) patients in NYHA class II and n = 1 patient (5%) in NYHA class I.

Out of these n= 20 patients, n = 7 (35%) patients reached the primary endpoint after a mean follow up period of 14 ± 12 months: n = 5 patients died for any cause and n = 2 patients had to be hospitalized for worsening of PAH.

At the time of the second imaging exam, there was no difference in age, gender, resting blood pressure, and 6 minutes walking distance in patients with and without an adverse event. Patients with an adverse event had however a lower BMI (22 ± 2 kg/m$^2$ vs 27 ± 5 kg/m$^2$, p = 0.03), a lower oxygen saturation at rest (86 ± 8% vs 94 ± 2%, p = 0.004), but a higher resting heart rate (87 ± 6 bpm vs 72 ± 8 bpm, p < 0.001), worse functional class (NYHA III/II vs NYHA II/I, p = 0.032) and a higher Nt-proBNP value (2224 ± 2133 ng/L vs 436 ± 433 ng/L, p = 0.015) compared to patients without events.

Imaging interval changes between the baseline and follow-up exam in both patient groups are presented in table 3. Patients with a positive adverse event during follow-up showed from the first to second imaging exam a reduction of FAC, an increase of RVESRI, and an increase in RV end diastolic volume. In a multivariate Cox regression analysis, only an increase in RV end diastolic volume was predictive of a subsequent adverse event. (table 4, table 5). In a log-rank analysis of the Kaplan-Meier survival estimates, patients who survived to the second follow-up exam but had an increase in RV end-diastolic volumes were more likely to have an adverse event during the subsequent follow-up period of 14 ± 12 months after the second imaging exam (log rank p = 0.006) (Figure 6).

## Discussion

In this study with a small sample size, we could show that there is only a weak correlation between echocardiographic markers of right ventricular function and CMR-based assessment of RV ejection fraction in PAH patients. In addition, baseline evaluation of right ventricular function by neither echocardiography nor CMR is predictive of long-term outcome. However, interval changes over long period (4 years in our study) are predictive of further adverse events. Changes in RV volumes were more robust than changes in RV function for prognostication.

### *Comparison between echocardiographic and CMR parameters of RV function*

Surrogates of RV systolic function are pre- and afterload dependent (23). In a chronically pressure loaded RV, the contribution of longitudinal shortening to ejection becomes less important than the contribution of circumferential contraction (24-25). In theory, echocardiographic surrogates relying exclusively on longitudinal shortening may be less accurate for quantification of systolic RV function in PAH patients compared to surrogates incorporating longitudinal and circumferential shortening. Our findings are in line with this hypothesis, the best – but still rather weak – correlation between echocardiography and CMR evaluation of RV function was found for FAC and RV-EF. Echocardiographic markers of longitudinal RV function (TAM or S') had a lower AUC in the ROC curve than FAC.

Some previous studies have shown a stronger correlation between

echocardiographic surrogates of RV function and CMR-derived RV-EF. In a recent study, Hoette et al. (23) compared TAM and FAC with RV-EF in a cohort of 54 patients with PH. They found a stronger correlation for both echocardiographic markers of RV function (r = 0.81 and r = 0.63 respectively) compared to our study (r = 0.47 for FAC and r = 0.34 for TAM). As another example, also da Costa et al. (26) found a good correlation of echocardiographic surrogates of RV function with CMR-derived RV-EF (r = 0.61 for FAC, r = 0.59 for TAM, and r = 0.27 for S') in a cohort of 66 PH patients. They identified global free wall RV strain as most reliable surrogate of CMR-derived RV-EF and as powerful predictor of long-term outcomes. All these studies, as the present, are limited by a rather small sample size, although the study of da Costa et al. has a larger cohort. Interobserver variations in measurement may contribute to discrepant findings. In addition, we studied echocardiographic pictures taken in the imaging laboratory during routine exams by not study-specific trained sonographers. This may additionally hamper the accuracy of RV function measurements but reflects more likely daily life experience.

***Baseline measures of RV function – echo and CMR – and their predictive power***

In our study, none of the clinical and echocardiographic, nor CMR parameters at baseline resulted predictors of endpoints. These data clash with some previous studies, where is well described a potential predictive role of some clinical, echocardiographic and CMR parameters on endpoints in PH patients. A previous study of Ghio et al. (27), evaluated 59 idiopathic PH patients. They found a prognostic role of some echocardiographic surrogates of RV function, in particular FAC and TAM in predicting a

worse survival (HR 0.004, 95% CI 0.002-0.62, and HR 0.91, 95% CI 0.83-0.99, respectively). These results are different from our study, where we did not found any baseline echocardiographic predictors of endpoints, maybe due to the different sample size and the different etiology of PH. On the other hand, in our study was evaluated the prognostic role of CMR parameter of RV function and dimension, not evaluated by Ghio et al. A more recent meta-analysis of 38 studies (28) evaluated the potential prognostic role of baseline echocardiographic surrogates of RV function in patients with heart failure with preserved ejection fraction, of whom 70% had PH. They found a prognostic role (predicting mortality) of FAC (HR 1.16, 95% CI 1.08-1.24) and TAM (HR 1.26, 95% CI 1.16-1.38). These results are again different from ours, where we did not found any predictive value for baseline echocardiographic parameters. Differently from this meta-analysis, we considered only PH patients, considering also a potential prognostic role of CMR parameters of RV function. Amsellam et al. (11), evaluating 228 PH patients, studied the prognostic role of RVESRI, together with some clinical parameters, also studied in our study. At multivariable analysis, they found a prognostic role of higher functional class, higher value of NT-proBNP and of higher value of RVESRI. Differently from them, clinical, nor imaging parameters at baseline affected prognosis and outcomes. In the paper of Amsellam et al., the mean RVESRI for the whole study population was $1.47 \pm 0.19$; a value of $> 1.35$ was indicative of adverse RV remodeling with impaired RV function and a value of $> 1.70$ indicated severely adverse RV remodeling with a further decline in RV function. In our study, the range value for RVESRI was 1.67-1.75, but

without impact on adverse events. The different impact of baseline value of RVESRI on prognosis between the study of Amsellam et al. and our study could be due to the smaller sample size of our research, considering also the similarity in primary endpoints evaluated in both the studies, although the study design was a bit different.

Another new echocardiographic parameter was studied by Guazzi et al. (29), considering the TAM/SPAP ratio as independent predictor of worse outcomes. In their study they defined 3 tertiles of TAM/SPAP ratio (< 0.35, 0.35-0.57, > 0.57), considering the lowest value associated with lower pulmonary artery compliance and higher pulmonary vascular resistence. Different from this study, in our study, TAM/SPAP ratio at baseline was not predictive of outcomes, nor its interval change, although it resulted lower in patients with a worse outcome during follow-up, maybe due to the smaller cohort compared with those of Guazzi et al.

The previously cited study of da Costa et al. (26), in a cohort of 66 PH patients and 25 healthy controls, evaluated the prognostic role of some clinical parameters and of some echocardiographic parameters of RV function, and in particular RV strain, in patients with PH. In their study, after a mean follow up period of 3.3 years, they argued that not only a higher functional class, but also all the studied echocardiographic parameters could play a role in the prognostic evaluation of PH patients. Differently from them, in our study we did not evaluate 3D strain, without finding in clinical and echocardiographic parameters at baseline a prognostic role in predicting outcomes. On the other hand, we evaluated also the prognostic role of CMR-derived parameters of RV size and function, evaluating also interval changes of all the studied parameters.

Moceri et al. (10), studied, by 3D echocardiography, 104 PH patients and 34 healthy controls, finding a prognostic role in predicting survival of 3D RV-EF, and RVEDV baseline values. In particular, they found a higher mean value of RVEDV in patients than in controls (91 ± 41 ml vs 51 ± 22 ml, $p < 0.001$), with a prognostic role of RVEDV in predicting survival (HR 1.02, 95% CI 1.01-1.03, $p < 0.001$). In line with our study, there is a prognostic role in RVEDV, but with several differences: first, the study of Moceri et al. is a 3D echocardiographic study, while we evaluated RV volumes by CMR. Then, in our study, we found a predictive role on outcomes for the indexed value of RVEDV, and finally, baseline value of RVEDVi did not predict adverse events.

In a previous study, Yamada et al. (30), evaluated 121 CMR in PH patients with a mean follow up of 45 months. They defined as endpoints death and hospitalization for right heart failure. With a mean value of 123 ± 43 ml/$m^2$, baseline value of RVEDVi was defined as predictor of mortality and hospitalization for right heart failure. These finding are very consistent with our study in considering RVEDVi as prognostic marker, with similar values of it. However, differently from Yamada et al., our study evaluated the role of interval change of RVEDVi in predicting outcomes, and consequently, the survival rate for the increase of RVEDVi.

Finally, Swift et al. (31), in a large cohort of 576 PH patients, after a follow up period of 42 months, evaluated the specific role of baseline parameters of CMR in predicting prognosis. They found at univariate Cox regression analysis, that RV volumes (both RVEDV and RVEDVi) predicted mortality. However, in the multivariate analysis,

only increased RVESVi (and not the end-diastolic volume) was associated with a worst outcome. These results are quite similar with ours, where we defined a worst survival in patients with an increase in RVEDVi after a follow up period of 55 months, data not confirmed for RVESV. However, this study shows an important role of RV volumes, evaluated by CMR, in defining prognosis in patients with PH.

***Interval change measures of RV function – echo and CMR – and their predictive power***

As widely discussed, we showed a prognostic role of RVEDVi increase from baseline to last follow up, confirming a worst prognosis in PH patients with higher value of RVEDVi after a second image exam. These results confirm the results of few previous studies, showing an important role of RV size changes during follow up in PH patients. In a previous CMR study, van Wolferen et al. (13) evaluated 64 PH patients, with a 1-year follow up in 54 patients, studying the prognostic role of several CMR parameters of RV function as predictor of mortality. Consistent with our study, they found a prognostic role of RVEDVi, with a poor prognosis in patients with a higher RVEDVi. In this study, they defined a mean value of RVEDVi of 85 ± 25 ml/$m^2$, defining as independent predictor of prognosis not only RVEDVi at baseline, but also its change during follow up. They also showed a better survival in patients with a median value of RVEDVi at baseline < 84 ml/$m^2$. The results of van Wolferen et al., are very consistent with ours in defining a prognostic role of RV volumes, and in particular of RVEDVi. In our research, we defined a worst survival in patients who increased RVEDVi than in patients who did not. However, there are some differences between the 2 studies: the values of RVEDVi, that are higher in

our study (123 ± 64 ml/$m^2$), and in our study, only interval change of RVEDVi resulted predictor of prognosis. Furthermore, in our study, we found an increase in RVEDV from baseline to follow up, while van Wolferen et al., found a decrease of it after 1-year of follow up, maybe due to a shorter follow up time compared with our study. Finally, in our study we focused also on some echocardiographic parameters of RV function, without finding any prognostic role of them.

More consistently with our results, a meta-analysis of 8 studies (32), evaluated the impact of CMR parameters of RV size and function in predicting clinical deterioration in PH patients. They found a prognostic impact on mortality of RV-EF and of RVEDVi and RVESVi, defining a mortality rate per 5 ml/$m^2$ of increase of RVEDVi. These results are very consistent with ours, where we defined a prognostic role of RVEDVi increase of at least 22 ml/$m^2$, defined as median interval change responsible of bad prognosis.

Finally, the previous cited study of Amsellam et al. (11), described a further worsening of prognosis in patients with an increase of RVESRI after 1-year follow. In our study, a value of RVESRI > 1.70, with an increase of at least 0.3 after follow up period, was associated with the presence of an adverse event. Nevertheless, these data were not confirmed by Cox regression analysis, where only the increase in RVEDVi resulted predictor of adverse event.

***Clinical implications***

The finding that an increase in RVEDVi is related with a worst survival is in line with some cited studies. It also confirms our hypothesis of superiority of CMR over

echocardiography in predict prognosis in PH patients. What is unexpected, is the prognostic role of only one CMR parameter, and of none of the echocardiographic parameters, or clinical parameters, such as oxygen saturation, NT-proBNP values and NYHA class. Although they have a clinical value in PH patients, in our study, also resulting lower (oxygen saturation) or higher (NT-proBNP and NYHA class) in patients with an adverse event, they did not result predictors of outcomes. Indeed, what we should expect, was a role of RV-EF as prognostic marker of outcomes (33), in addition to the prognostic role of the interval change between baseline and follow up of RVEDVi, defined as an increase of at least 22 $ml/m^2$ from baseline to last follow up evaluation. However, also RV-EF did not predict outcomes both at baseline and after follow up.

**Limitations**

This was a single center study, with a small sample size, with also a small number of patients making the second CMR, so these data should be confirmed by other centers to validate the results, and a larger cohort is warranted. Considering the prognostic role of echocardiographic surrogate of RV function, we did not evaluate 3D echocardiography and RV echocardiographic strain, that in several studies have shown a prognostic role in PH patients (9-11,26).

**Conclusions**

In a cohort of 36 patients, followed up for a mean period of 57 ± 24 months, with a

number of 20 patients making a second CMR study, the increase of RVEDVi, evaluated by CMR, has a prognostic role, with a worst survival compared with patients who did not increase RVEDVi after treatment during follow up. None of the baseline imaging parameters of RV function, nor echocardiographic or other CMR interval changes (such as RV-EF) are predictive of outcomes. This study showed also a weak correlation between FAC, S' and TAM and CMR-derived RV-EF, not found for RVESRI, defining so a weak diagnostic role of echocardiographic surrogates of RV function in PH patients.

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**Table 1.**

**Patients' baseline characteristics**

| | All patients | Patients with a second CMR |
|---|---|---|
| | n = 36 | n = 20 |
| **Age, years** | 44 ± 16 | 43 ± 15 |
| **Women (%)** | 15 (42%) | 4 (20%) |
| **BMI, kg/m$^2$** | 25 ± 4 | 24 ± 5 |
| **Systolic blood pressure, mmHg** | 120 ± 14 | 122 ± 15 |
| **Diastolic blood pressure, mmHg** | 73 ± 10 | 74 ± 8 |
| **Heart rate, bmp** | 80 ± 12 | 85 ± 11 |
| **Oxy sat, %** | 91 ± 7 | 88 ± 9 |
| **Six minute walk distance, m** | 450 ± 132 | 400 ± 138 |
| **NT pro-BNP, ng/L** | 399, 191-977 | 493, 226-1269 |
| **NYHA class I (%)** | 2 (6%) | 0 (0%) |
| **NYHA class II (%)** | 26 (72%) | 15 (75%) |
| **NYHA class III (%)** | 8 (22%) | 5 (25%) |

Values are expressed as mean ± SD or number (percentage), or median and interquartile range (IQR). BMI: body mass index, BNP: brain natriuretic peptide, IQR: interquartile range.

**Table 2.**

**Univariate analysis for baseline values as predictor of adverse events and disease progression**

| | Adverse event | | | Disease progression | | |
|---|---|---|---|---|---|---|
| | Positive (n=14) | Negative (n=22) | P value | Positive (n=30) | Negative (n=6) | P value |
| **ECHOCARDIOGRAPHY** | | | | | | |
| **SPAP (mmHg)** | 86±20 | 51±21 | < 0.001 | 64±27 | 55±18 | 0.528 |
| **Tricuspid regurgitation grade** | 2.3±0.8 | 1.4±0.6 | 0.001 | 1.9±0.8 | 1.2±0.4 | 0.087 |
| **FAC (%)** | 35±14 | 36±9 | 0.920 | 35±11 | 38±12 | 0.575 |
| **TAM (mm)** | 19±6 | 19±5 | 0.931 | 19±6 | 16±4 | 0.256 |
| **S' (cm/s)** | 11±3.5 | 10±2.7 | 0.564 | 11±3 | 10±2 | 0.744 |
| **RVESRI** | 1.70±0.36 | 1.68±0.22 | 0.799 | 1.67±0.30 | 1.75±0.21 | 0.604 |
| **LV-EF** | 57.9±11.7 | 60.7±9.4 | 0.429 | 60.7±10.1 | 54±9.9 | 0.148 |
| **TAM/SPAP** | 0.2±0.14 | 0.4±0.2 | 0.012 | 0.34±0.23 | 0.34±0.1 | 0.967 |
| **Cardiac Magnetic Resonance** | | | | | | |
| **RVEDVi (ml)** | 131±72 | 118±60 | 0.564 | 118±61 | 147±75 | 0.322 |
| **RVESVi (ml)** | 75±56 | 64±40 | 0.518 | 66±45 | 82±56 | 0.434 |
| **CO (l/min)** | 8±2 | 7±3 | 0.464 | 7±2 | 10±4 | 0.042 |
| **RV-EF (%)** | 49.9±14.9 | 47.8±11.5 | 0.646 | 48.9±13.5 | 47.3±9.1 | 0.792 |
| **LV-EF (%)** | 53.9±12.5 | 58.8±10 | 0.209 | 57.1±11.5 | 55.5±10.2 | 0.749 |
| **RV-SV/RVESV** | 0.99±0 | 1±0.5 | 0.88 | 1±0.5 | 0.94±0. | 0.7 |

| | | | |
|---|---|---|---|
| .5 | 0 | 33 | 05 |

Values are expressed as mean ± SD or number (percentage), or median and interquartile range (IQR). RA-RV: right atrial-right ventricular, SPAP: systolic pulmonary arterial pressure, FAC: fractional area change, TAM: tricuspid annular motion, S': tricuspid annular systolic velocity, RVESRI: right ventricle end systolic remodeling index, LV-EF: left ventricle ejection fraction, CMR: cardiac magneti resonance imaging, RVEDVi: right ventricle end-diastolic volume indexed, RVESVi: right ventricle end-systolic volume indexed, CO: cardiac output, RV-EF: right ventricle ejection fraction, SV: stroke volume.

**Table 3.**

**Univariate analysis for interval change values as predictor of adverse events and disease progression**

| | **Adverse event** | | | **Disease progression** | | |
|---|---|---|---|---|---|---|
| | Positive (n=7) | Negative (n=13) | P value | Positive (n=16) | Negative (n=4) | P value |
| **ECHOCARDIOGRAPHY** | | | | | | |
| **Delta RA-RV gradient** | 0, -6.2-26.2 | -1, -10-0 | 0.335 | 0, -10-0 | 12.5, -26.2-28.7 | 0.505 |
| **Delta SPAP (mmHg)** | 0, 0-28.7 | 0, -20-0 | 0.088 | 0, -3.7-0 | 0, 0-25 | 0.999 |
| **Delta FAC (%)** | 8, -1-19 | -8, -13.7-1.7 | 0.006 | -2.5, -8-7.7 | -3.5, -15-7.2 | 0.750 |
| **Delta TAM (mm)** | 4, 0-5 | 0, -3.5-2 | 0.056 | 1, -2.7-3.7 | -0.5, -2.5-2.2 | 0.617 |
| **Delta S' (cm/s)** | 0, -1-0 | -1, -2-0 | 0.285 | 0, -2-0 | -1, -2-0 | 0.912 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Delta RVESRI** | -0.3, -0.3-0.2 | 0.1, -0.1-0.4 | 0.003 | -0.1, -0.3-0.2 | 0.1, -0.1-0.4 | 0.178 |
| **Delta LV-EF (%)** | 4, -8-5 | -5, -8-2 | 0.479 | -2, -7-4 | -6, -8-0 | 0.471 |
| **Cardiac Magnetic Resonance** | | | | | | |
| **Delta RVEDVi (ml/m$^2$)** | -22, -46-5 | 2, -9-48 | 0.037 | -8, 26-13 | 47, -4-129 | 0.099 |
| **Delta RVESVi (ml/m$^2$)** | -12, -39-8.7 | -1, -6-43 | 0.106 | -5, -16-17 | -33, -1-105 | 0.124 |
| **Delta CO (l/min)** | -0.2, -0.9-0.7 | -0.1, -0.4-0.9 | 0.639 | -0.2, -0.6-0.4 | 6.1, 0-7 | 0.152 |
| **Delta RV-EF (%)** | -1, -3-17 | -2, -7-3 | 0.311 | -0.5, -5-8 | -5, -14-1 | 0.122 |
| **Delta LV-EF (%)** | -3, -6-10 | 1, -4-8 | 0.757 | 2, -3-10 | -9,-12-1 | 0.029 |

Values are expressed as mean ± SD or number (percentage), or median and interquartile range (IQR). Delta values were obtained subtracting follow up values from baseline values. IQR: interquartile range, RA-RV: right atrial-right ventricular, SPAP: systolic pulmonary arterial pressure, FAC: fractional area change, TAM: tricuspid annular motion, S': tricuspid annular systolic velocity, RVESRI: right ventricle end systolic remodeling index, LV-EF: left ventricle ejection fraction, CMR: cardiac magneti resonance imaging, RVEDVi: right ventricle end-diastolic volume indexed, RVESVi: right ventricle end-systolic volume, CO: cardiac output, RV-EF: right ventricle ejection fraction.

**Table 4.**

**Univariate Cox regression model for interval changes parameters**

| | | Adverse event | |
|---|---|---|---|
| **Variable** | **HR** | **95% CI** | **P value** |
| **FAC** | 1.064 | 0.994-1.138 | 0.075 |
| **RVESRI** | 0.116 | 0.011-1.247 | 0.075 |
| **RVEDVi** | **0.943** | **0.905-0.983** | **0.005** |

HR: hazard ratio, CI: confidence interval, FAC: fractional area change, RVESRI: right ventricle end systolic remodeling index, RVEDVi: right ventricle end-diastolic volume indexed.

**Table 5.**

**Multivariate Cox regression model for interval changes parameters**

| | | Adverse event | |
|---|---|---|---|
| **Variable** | **HR** | **95% CI** | **P value** |
| FAC | 0.995 | 0.919-1.077 | 0.893 |
| RVESRI | 0.265 | 0.007-10.549 | 0.480 |
| **RVEDVi** | **0.945** | **0.900-0.993** | **0.024** |

HR: hazard ratio, CI: confidence interval, FAC: fractional area change, RVESRI: right ventricle end systolic remodeling index, RVEDVi: right ventricle end-diastolic volume indexed.

**Figures**

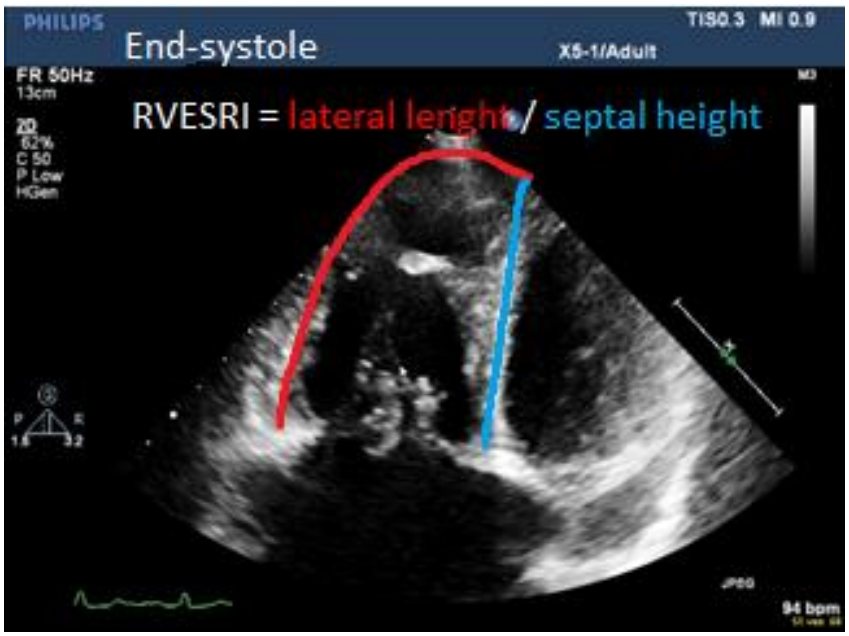


**Figure 1.** Representative echocardiographic images of the assessment of right ventricular systolic function: right ventricular end-systolic area for the assessment of RVESRI. RVESRI represents the ratio of end-systolic lateral length / septal height.

RVESRI: right ventricle end systolic remodeling index.

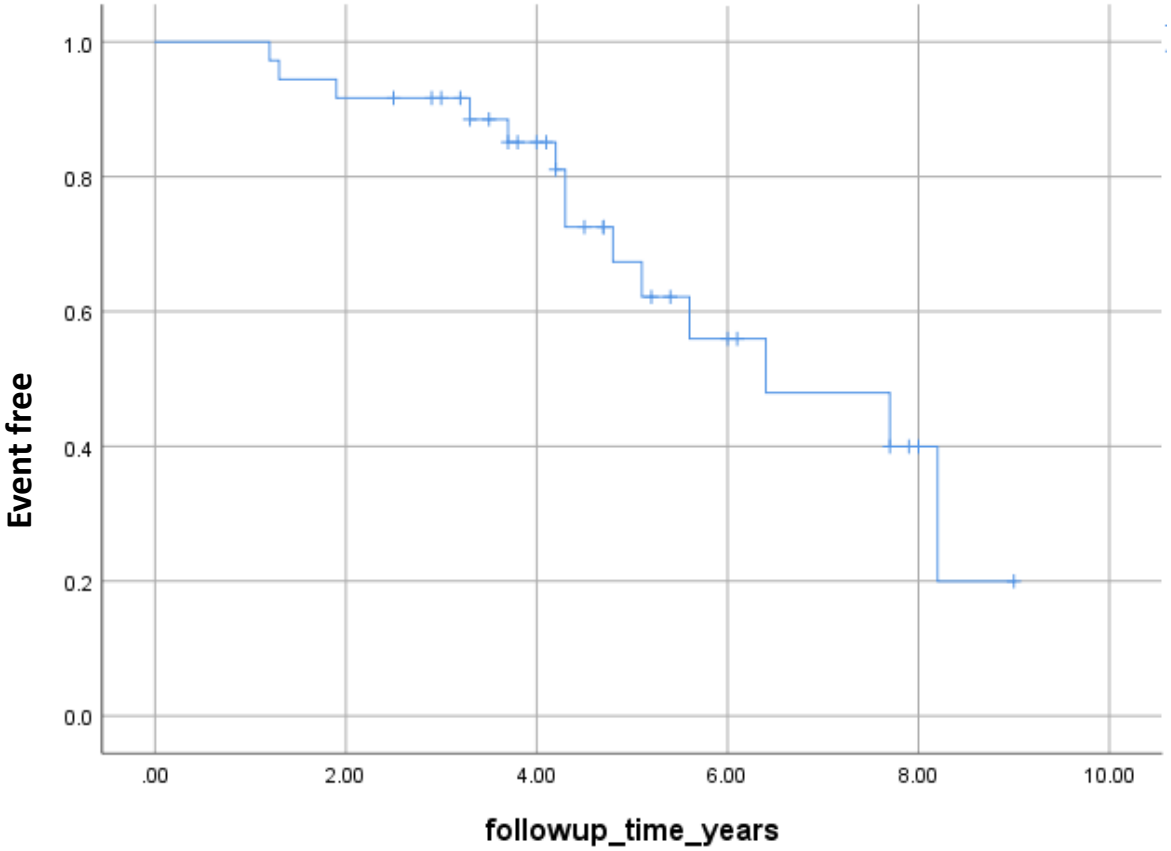


**Figure 2.** Kaplan Meier curve for event free survival rate for the composite primary endpoint defined as adverse event.

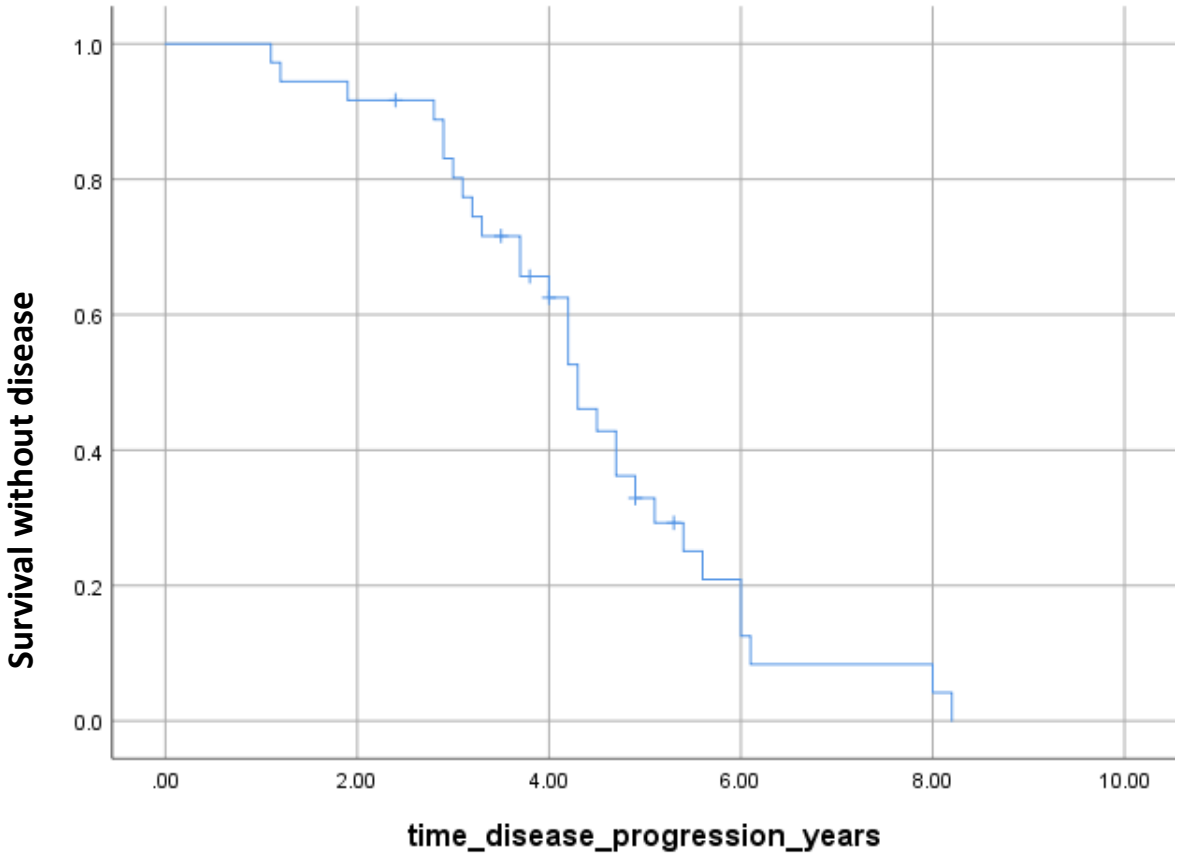


**Figure 3.** Kaplan Meier curve for event free disease progression rate.

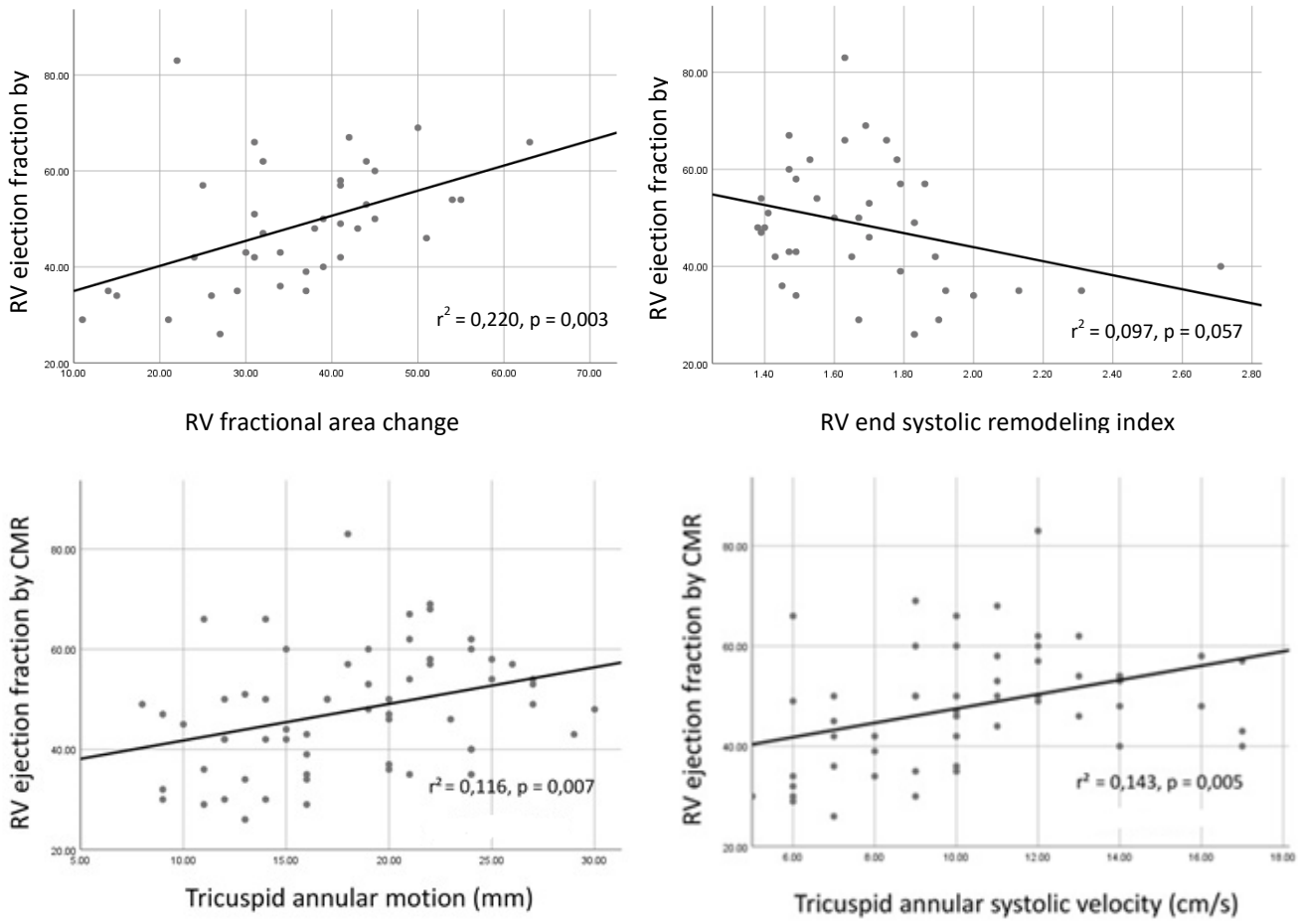


**Figure 4.** Correlation coefficient depicted by linear regression graphic, showing the correlation between RV-EF evaluated by CMR and the four echocardiographic parameters of systolic RV function.

RV: right ventricular, CMR: cardiac magnetic resonance.

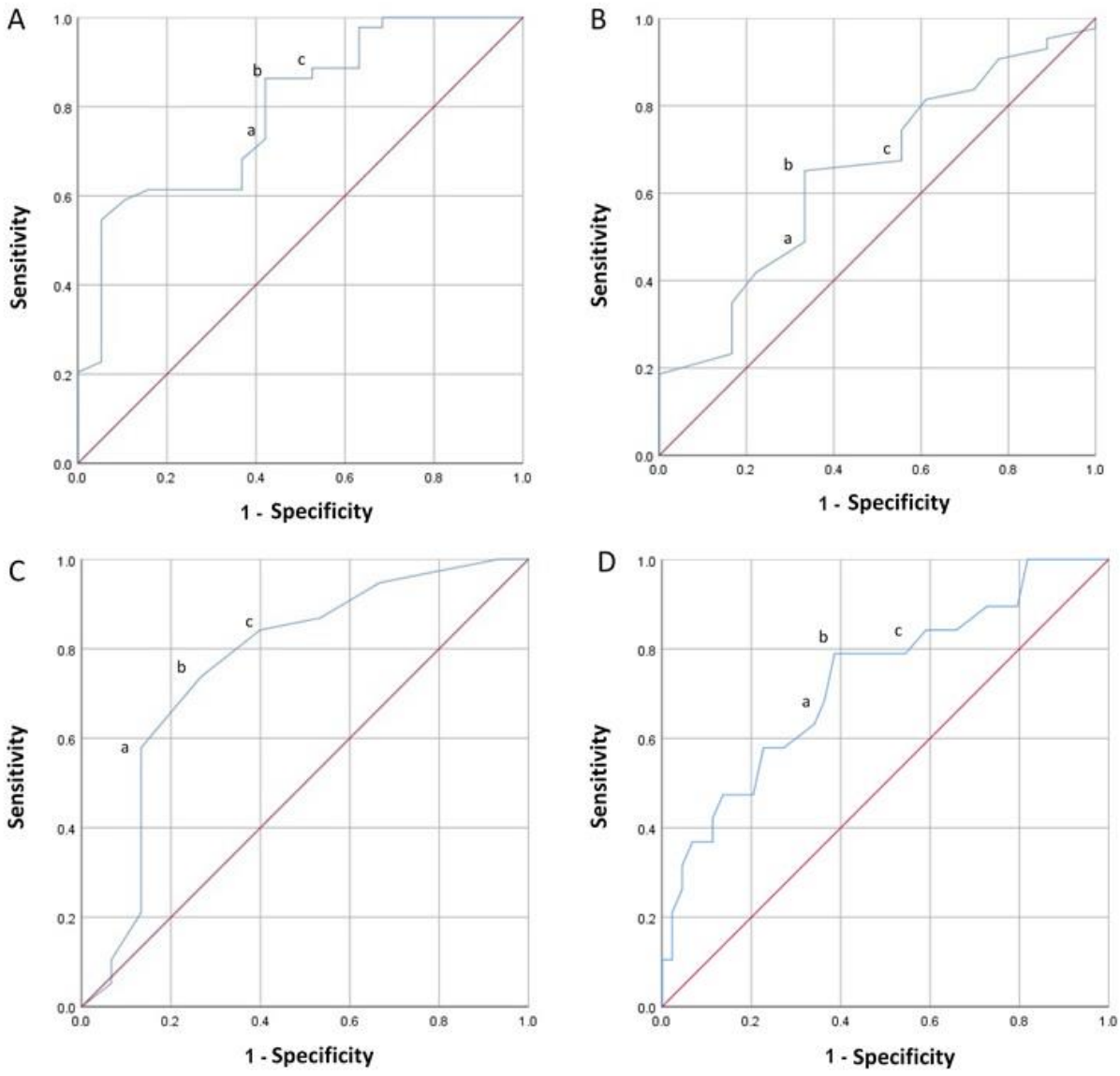


**Figure 5.**

Receiver operating characteristic analysis curves for the distinction by the four Doppler echocardiographic parameters examined in moderate to severe impaired RV-EF. (A) FAC; (B) TAM; (C) S'; (D) RVESRI. For every single parameter we considered three different cut-off values. For FAC, cut-off value of 33%, sensitivity 73% and specificity 58% (a); cut-off value of 30%, sensitivity 86% and specificity 58% (b); cut-off value of 28%, sensitivity 86% and specificity 47% (c). For TAM, cut-off value of 19 mm, sensitivity 49% and specificity 67% (a); cut-off value of 17 mm, sensitivity 65%, specificity 67% (b); cut-off value of 15 mm, sensitivity 67% and specificity 44% (c). For S', cut-off value of 10

cm/s, sensitivity 58% and specificity 87% (a); cut-off value of 9 cm/s, sensitivity 74% and specificity 73% (b); cut-off value of 8 cm/s, sensitivity 84% and specificity 60% (c). For RVESRI, cut-off value of 1.70, sensitivity 63% and specificity 66% (a); cut-off value of 1.66, sensitivity 79% and specificity 61% (b); cut-off value of 1.61, sensitivity 79% and specificity 46% (c).

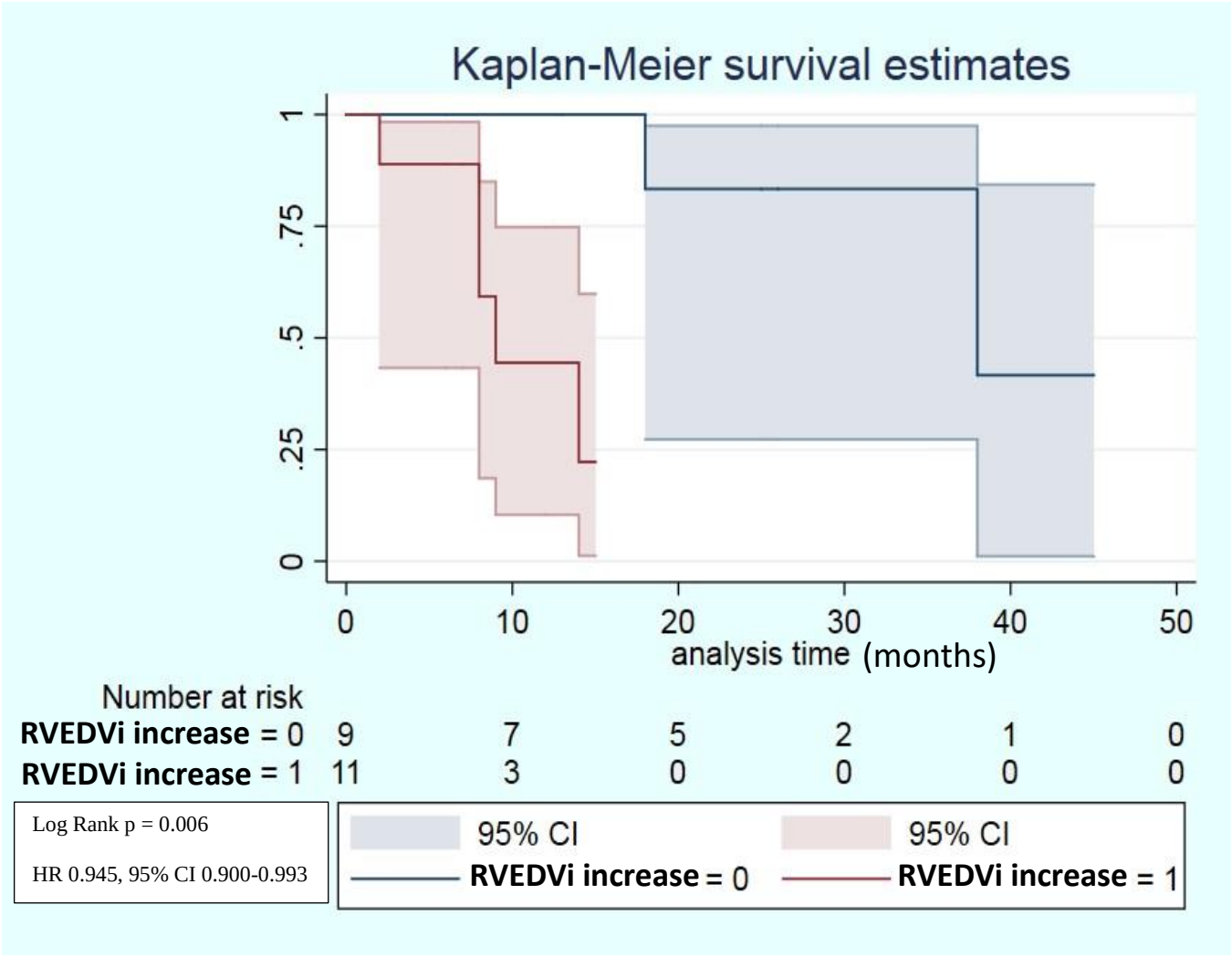


**Figure 6.** Cumulative survival rate for the 2 groups of RV end-diastolic volume indexed increment.

RVEDVi: right ventricle end-diastolic volume indexed.

**CHAPTER 10.1.**

# Predictors of cardiovascular outcomes in adult patients with repaired coarctation

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*Draft*

**Predictors of cardiovascular outcomes in adult patients with repaired coarctation**

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**Abstract**

**Introduction:** Coarctation of the aorta (CoA) is a congenital narrowing of the proximal descending aorta in the region of the ligamentum arteriosum. Despite the improvement in operative techniques, the prevalence of cardiovascular events and aortic re-interventions is still high. The aim of this study is to assess the incidence of adverse cardiovascular events in patients with repaired CoA, defining the predictors of outcomes in these patients.

**Methods:** We retrospectively evaluated adult patients with repaired CoA by different techniques who underwent clinical and imaging follow-up evaluation. Primary endpoint was defined as the composite of death, heart failure, presence of atrial fibrillation, cerebrovascular ischemic events, coronary revascularization, aortic dissection, re-CoA requiring re-intervention and pseudoaneurysm formation.

**Results:** Overall, 280 patients (112 females, 40%), age 33,6 ± 13,2 years were included in the study. After a follow-up period of 25,5 ± 12,7 years after CoA repair, composite clinical outcomes occurred in 29 patients (10%), where the more prevalent was the presence of atrial fibrillation (20; 7%). Composite outcome including re-CoA requiring an intervention and pseudoaneurysm formation, occurred in 116 patients (41%) after a mean follow-up period of 19,4 ± 13,6 years. 102 patients (36%) needed a second intervention for re-CoA. A pseudoaneurysm at the CoA site was diagnosed in 27 patients (10%) after a mean follow-up period of 24,9 ± 12,6 years. At multivariate Cox regression analysis, stenting procedure (HR 487.216, 95% CI 37.451-6338.445, $p < 0.001$) and older age at the

time at last follow up (HR 0.736, 95% CI 0.636-0.851, $p < 0.001$) were predictors of composite endpoint.

**Conclusions:** stenting procedure, related to older age of CoA intervention and the presence of hypoplastic aortic arch play an important role in predict outcomes.

## Introduction

Aortic coarctation (CoA) is a congenital disease of the proximal descending aorta defined as a hemodynamic significant aortic obstruction, with an incidence of about 1 in 2,500 live births, and a 2:1 predominance in males, accounting for 5–8% of children with congenital heart defects (1-3). The diagnosis of CoA is sometimes missed in infancy and childhood, and some subjects with a CoA are not detected until adult life, with a result of an high mortality rate in untreated patients (about 80% due to several complications, including aortic rupture, heart failure, and intracranial hemorrage) (1-2,4). In many cases, CoA is associated with a bicuspid aortic valve or a hypoplastic aortic arch and isthmus, but it can also be associated with Turner syndrome, or other congenital cardiac lesions like ventricular septal defect or large persistent arterial ductus arteriosus (PDA), or other more complex congenital heart disease (5). Today, to treat these patients from childhood to adulthood, surgical techniques comprise a large spectrum of different techniques (end-to-end anastomosis, subclavian flap plasty, patch augmentation plasty, local implantation of an interposition graft or surgical creation of an extra-anatomic aortic ascending-to-descending bypass (EAADB)), where percutaneous stent implantation is the preferred treatment in adult patients with focal native coarctation or focal restenosis (6-11).

Late hypertension in patients after CoA repair is most oftenly associated with elevated sympathetic activity and endothelial dysfunction which play a role in the elevated risk for cardiovascular events in repaired CoA patients (12,13). Despite the well-known

improvements in operative techniques and associated with a more strict surveillance and management of residual arterial hypertension, comorbid cardiovascular diseases and CoA related complications, both, morbidity and mortality remain high, and is reported to be highest in patients with corrective interventions after 20 years of age (2,14).

Furthermore, residual hypertension may lead to hypertensive cardiopathy with increased left ventricular (LV) wall thickness and mass, diastolic dysfunction, increased left atrial size and increased risk for atrial fibrillation (15).

There is a reported risk for cerebral hemorrage due to aneurym of the cerebral arteries (3-5%), that generally involve the circle of Willis (berry aneurysms), where hypertension is not a necessary precondition for cerebral complication, and a reported risk of coronary artery disease (25-37%), that can be considered a common cause of early and late death in operated CoA patients (5).

In addition, it is well known that aortic stiffness is increased in CoA patients, especially after late CoA repair (13). In the literature, it is reported that impaired aortic stiffness leads to a worse prognosis in CoA patients blood-pressure independenatly and could therefore be the force for increased mortality and morbidity. (16-19).

The aim of this study was to assess the incidence of adverse cardiovascular events in adult patients with repaired CoA, and to define which echocardiographic and clinical variables, results by cardiac magnetic resonance (CMR) and computed tomography (CT) as well as different repair techniques predict adverse long-term clinical outcome. We also

focused on analysing the need for re-intervention for aotic re-stonosis and the presence of any pseudoaneurysm formation beeing small and under surveillance or a target for re-intervention.

## Methods

### *Study patients*

We retrospectively evaluated 280 adult patients with repaired CoA which underwent clinical follow-up in three Swiss centers for Adult Congenital Heart Disease (ACHD). Overall, 122 patients from Zurich, 119 patients from Bern and 39 patients from Basel and included into the Swiss Adult Congenital HEart disease Registry (SACHER) (20) were used for our analysis. In our analysis, time and type of CoA repair technique, presence of other concomitant defects (i.e. bicuspid aortic valve, hypoplastic and/or Gothic arch, Turner syndrome), presence of other congenital defects (i.e. ventricular septal defects, and other congenital heart diseases) were accounted. Data from the most recent clinical evaluation with right cubital and leg office blood pressure measurements (left arm measurements in patients with a right-sided aortic arch), ambulatory 24-hour blood pressure measurement of the right arm (left arm measurements in patients with a right-sided aortic arch) maximal exercise test with cubital blood pressure measurement (left arm measurements in patients with a right-sided aortic arch) and transthoracic echocardiography, type and dosage of the current anti-hypertensive medication were

recorded and included into our analysis. Two-hundred and twenty-five patients (80%) underwent a CMR with MR-aortography. In 32 patients (11%) an aortic angiography by CT-scan was performed due to presence of CMR-contra-indications.

***Adverse events***

A composite endpoint of death, heart failure, history or presence of atrial fibrillation, history of cerebrovascular ischemic and/or hemorrhagic events, coronary revascularization, aortic dissection, significant re-coarctation requiring re-intervention and pseudoaneurysm formation was defined. We divided our outcomes into clinical outcomes (death, heart failure, history or presence of atrial fibrillation, cerebrovascular ischemic and/or hemorrhagic events, coronary revascularization and aortic dissection) and CoA-related outcomes (re-coarctation requiring re-intervention and focal pseudoaneurysm formation at the repair site). Re-coarctation requiring re-intervention was defined as the presence of a blood pressure gradient between upper and lower extremities > 20 mmHg, a diastolic "run-off" at Doppler echocardiography and a ≥ 50% aortic narrowing relative to the aortic diameter at the diaphragm level (by CMR or CT-scan) (21). The presence of a pseudoaneurysm was defined as dilation of the aorta due to disruption of the wall layers, which is only contained by the periaortic connective tissue (3,22).

***Doppler echocardiography***

Transthoracic Doppler echocardiography was performed by echocardiographers with expertise in ACHD using a 3.5 MHz transducer including second harmonic imaging. In all exams left atrium and left ventricular dimensions were measured, calculating left ventricular mass. Ascending aortic dimensions were measured for evaluation of aortic distension in the parasternal long axis view and calculated as (end-systolic diameter – end-diastolic diameter)/(end-diastolic diameter) and aortic distensibility, defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure). Ascending aorta was measured using the inner-edge-to-outer-edge method, at a distance of 4-6 centimeters from the aortic anulus. For evaluation of left ventricular systolic function, Simpson formula was used. Diaphragmatic aorta dimension and pulsed Doppler flow velocity signal were also obtained. Aortic arch diameter and aortic diameter at CoA site were measured using the inner-edge-to-inner-edge method, and aortic flow in the site of minumum diameter of CoA site (descending aorta) by both pulsed and continuous wave Doppler was measured, to search for presence or absence of a diastolic tail in the flow velocity curve. Pulse wave velocity (PWV) was evaluated by (center-line derived lenght of the aorta from aortic valve closing point to diaphragmatic aorta, assessed by CMR or CT))/(time delay between diaphragmatic foot wave and aortic valve foot wave. Diaphragmatic and aortic valve foot wave were obtained by pulsed wave Doppler, positioned to distal diaphragmatic aorta and closing point of aortic valve respectively). Aortic distension was acquired in 258 patients, aortic distensibility in 256 patients and PWV in only 142 patients, and all these parameters

were measured offline by FM. All other measurements were calculated online and data were collected retrospectively.

***Cardiac magnetic resonance imaging (CMR) and computed tomography (CT)***

Study patients were examined using a 1.5 T or a 3 T whole-body clinical MRI system with cardiac synchronization by ECG electrodes. In 27 patients only a MR-angiography for the evaluation of aortic dimensions was performed, without the evaluation of ventricular volumes, function and mass. For all the other patients, ventricles were imaged from the base towards the apex during end-expiratory breath-holds by a stack of retrospectively ECG-triggered SSFP-cine sequence acquisitions. End-diastolic and end-systolic contours were manually traced for each slice offline to determine left ventricular volumes and mass according to the modified Simpson's rule (disk summation). MR aortography using time-adjusted contrast medium injection was used to assess aortic arch and descending aortic geometry, diameter and presence or absence of pseudoaneurysm or restenosis at CoA repair site. Reconstructed aortic 3D volumes were used for offline centerline assessment of the aortic length between the ascending (sinotubular junction site) and diaphragmal aorta for PWV calculations. Retrospectively gated steady-state free procession cine CMR images of the aortic arch, along with the ascending, transverse, descending and diaphragmatic aorta were routinely undertaken. Throughplane phase contrast flow measurements were acquired during breath-hold and in retrospectively ECG-gating technique in the ascending, diaphragmal and descending aorta at the CoA repair site and in

patients with a hypoplastic arch. Aortic distension of the ascending aorta was calculated as (maximum systolic area – maximum diastolic area)/(maximum diastolic area). Aortic distensibility of the ascending aorta was defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure) after offline manually tracings of the maximal and minimal aortic areas within the acquired 25 frames. PWV was calculated as (length of the aorta, from sinotubular junction to diaphgramatic aorta, derived from the MR-aortography)/(time delay between diaphragmatic foot wave and ascending aorta foot wave, derived from flow velocity measurements in the ascending and descending aorta). Measurements of aortic distension, aortic distensibility and PWV were calculated offline by FM. Unfortunately, ascending aortic distension and aortic distensibility were acquired only in 100 patients due to missing cine data in the other patients. PWV was performed in only 81 patients due to missing flow acquisitions in the rest of the patients.

CT scan was performed in 32 patients who had contraindications to perform CMR. Patients were examined while supine, taking images extending from the base of the neck to the diaphragm, using retrospective ECG-gated cardiac CT scanning. A 64-slices CT-scan was used, with a slice thickness of 1 mm and a reconstruction interval of 0.75 mm. A 3D reconstruction of the complete aorta was obtained, allowing measurement of the diameter of aorta from ascending to diaphragmatic portion. The length of the aorta from the ascending to the diaphragmal aorta derived from center-line measurements were used for the calculation of PWR by echo Doppler flow profiles.

### *Statistical analysis*

Categorical variables are reported as absolute numbers and percentages. Continuous variables are reported as mean ± standard deviation (SD), and non-normally distributed continuous variables as median and interquartile range (IQR). Chi-square test and Fisher's exact test were used to compare pseudoaneurysm formation in patients among intervention types. Univariate Cox proportional hazard regression model was used to assess the association between clinical, echocardiographic, CMR, and CT-scan variables and adverse outcomes. Variables with $p < 0.10$ at univariate analysis were included as covariates into the multivariate Cox regression models to test which variables were independently associated with the outcomes. Statistical significance in the multivariate analysis was defined for a p value $< 0.05$ and hazard ratios and their 95% confidence intervals are presented. In time-to-event analyses, outcomes were estimated with the use of the Kaplan-Meier method and were analyzed by a log-rank test. Statistical analysis were performed with IBM SPSS Statistics v25.0 (Armonk, New York, United States of America).

## Results

### *Clinical and imaging results*

Two-hundred and eighty patients (112 females, 40%), mean age of 33,6 ± 13,2, were included into the study. Baseline characteristics of the patients are reported in table 1. In our cohort, the more prevalent type of first intervention was end-to-end anastomosis (64%), followed by stenting procedure (14%) (figure 1). One-hundred and eighty-seven

patients had a concomitant bicuspid aortic valve (67%), and a hypoplastic aortic arch (defined as a diameter of less than 50% of the diameter of the ascending aorta) (10,23) was present in 132 patients (47%). One-hundred and thirty-four patients were under anti-hypertensive treatment (48%). Thirty-eight patients were under anti-hypertensive mono-therapy, 54 were treated with dual therapy, 31 with triple-therapy and 11 patients needed a combination with four or more antihypertensive agents.

24-hour ambulatory blood pressure monitoring was performed in 141 patients, where nocturnal impaired blood pressure response in the whole population was found in 45 patients (16%), and nocturnal impaired blood pressure response in normotensive patients was found only in 14 patients (5%). Exercise test was performed in 210 patients, of whom 167 reached the peak of the exercise (defined at least 85% of the maximal expected heart rate). The mean blood pressure profile at exercise peak was 205 ± 34 mmHg for systolic blood pressure and 80 ± 15 mmHg for diastolic blood pressure. An echocardiographic exam was performed in 278 patients. Dilated left atrium was found in 54 patients (19%). We found left ventricular hypertrophy in 59 patients (21%), defined as a left ventricular mass > 95 $g/m^2$ in females and 115 $g/m^2$ in males. Twelve patients (4%) had a left ventricular dysfunction, defined as a left ventricular ejection fraction evaluated by Simpson biplane ≤ 50%. The mean value of CoA site diameter evaluated by echocardiography was 13 ± 3 mm, and only 8 patients (3%) had an echo-diastolic tail. Mean value of PWV evaluated by echocardiography was 10.9 ± 6.4 m/s. In the 225 CMR, 44 patients had a dilated left atrium (20%), 50 patients had a left ventricular hypertrophy (22%), and 9

patients (4%) had a left ventricular dysfunction. The mean value of CoA site diameter evaluated by CMR was 14 ± 3 mm, with only 8 patients (4%) with a diastolic tail at CoA site. Collaterals at CMR were found in 16 patients (7%). Mean value of PWV evaluated by CMR was 10.9 ± 8.8 m/s. Finally, the mean value of aorta diameter evaluated by CT-scan was 17 ± 7 mm.

***Cardiovascular events***

After a mean follow up period of 25,5 ± 12,7 years after first intervention for CoA repair, composite adverse events occurred in 29 patients (10%). Atrial fibrillation was the most prevalent adverse outcome (20; 7%) and was diagnosed in the older patients (mean age 53,9 ± 16.1 years) (table 2) (figure 2). Results of the univariate and multivariate Cox proportional hazard regression model are reported in tables 4-8. By multivariate Cox regression analysis, aortic stenting procedure, age of repair > 1 year, and older age at last follow up were independent predictors of the composite primary endpoint. Older age at first CoA repair (> 12 years) (but not an age of repair > 1 year) is an independent predictor of composite adverse clinical outcomes and of the presence of atrial fibrillation (> 7,7 years). Impaired aortic distension evaluated by transthoracic echocardiography on ascending aorta, is associated with adverse composite clinical outcomes. None of the other echocardiographic and CMR parameters, nor aortic stiffness retrospectively evaluated by CMR, or residual hypertension statistically affected outcomes by multivariate analysis.

***Re-coarctation and pseudoaneurysm formation***

CoA-related adverse outcomes (significant re-CoA requiring re-intervention or pseudoaneurysm at the CoA-repair site), occurred in 116 patients (41%) (table 3) (figure 3). Significant re-CoA requiring re-interventions was diagnosed in 102 patients (36%) after a mean follow-up time of 19,4 ± 13,6 years after first repair. From these 102 patients, 55 were treated by aortic stenting (54%); in 18 patients a surgically creation of an EAADB was performed (18%); 17 patients were operated by an aortic patch plasty (17%); in 6 patients an interposition graft was implanted (6%); 5 patients were treated by an end-to-end anastomosis (5%); and 1 patient was repaired by subclavian flap plasty technique (1%). Finally, 25 patients (9%) received a third operation due to severe re-re-CoA. From these, 13 patients were treated by stenting (52%), 4 patients by EAADB (16%), 3 patients by end-to-end anastomosis (12%), 3 patients by aortic patch plasty (12%) and 2 patients received an interposition graft (8%).

A pseudoaneurysm was detected by CMR or CT in overall 27 patients (10%) after a mean follow-up time of 24,9 ± 12,6 years after first CoA repair. In 11 patients that had performed an end-to-end anastomosis (6%) was detected a pseudoaneurysm. Seven patients operated by aortic patch plasty procedure (32%) experienced a pseudoaneurysm and in 5 patients who had received a stenting procedure (13%) was found a pseudoaneurysm. The prevalence of pseudoaneurysm formation was not statistically different between patients treated with an aortic patch plasty procedure or stenting procedure, although pseudoaneurysm was more prevalent in patients operated by aortic

patch plasty procedure (p = 0.283). The mean size of pseudoaneurysm was 21 ± 18 mm. Pseudoaneurysm size progression was reported in 5 patients. Of 27 pseudoaneurysms, 9 (33%) patients went to a re-intervention due to large and progressive size of the pseudoaneurysm. Of these 9 patients, 5 were treated with surgically exclusion of the pseudoaneurysm and creation of an extra-anatomic ascending-to-descending aortic bypass (56%), whereas 3 patients were treated by insertion of a local interposition graft (33%) and 1 patient was treated by aortic stenting procedure (11%).

By multivariate analysis, stenting procedure as the first CoA-repair technique also is an independent predictor for re-CoA requiring re-intervention, and pseudoaneurysm formation of any size. Older age at first CoA repair (> 8 years) (but not an age of repair > 1 year) predicts the occurrence of significant re-CoA. The presence of a hypoplastic aortic arch is statistically associated with the higher risk of pseudoaneurysm formation.

Kaplan-Meier curves show and confirm a higher prevalence of composite primary endpoint (figure 4), of severe re-CoA (figure 5) and of pseudoaneurysm formation (figure 6) in patients treated by stenting procedure. Finally, by Kaplan-Meier curves also a higher prevalence of pseudoaneurysm formation (figure 7) in patients with a hypoplastic aortic arch was shown and confirmed.

**Discussion**

This multicentric study, made on 280 adult patients with repaired CoA, showed a high

incidence of severe re-CoA requiring re-intervention (36%), with a lower incidence of pseudoaneurysm formation (10%), and a very low incidence of clinical outcomes, of which the most frequent was atrial fibrillation (7%). Several clinical, surgical and imaging variables resulted predictive of outcomes at univariate analysis, moreover, at multivariate analysis, stenting procedure showed to play an important role in affect composite primary endpoint, severe re-CoA requiring re-intervention and pseudoaneurysm formation.

***Clinical outcomes***

Our results showed a lower mortality rate compared with some previous studies (11-12). In fact, in our study, we found a mortality rate of 0,4%. Brown et al (2), in 819 patients with isolated CoA, described an overall early mortality rate of 2,4%, with a survival rates of 93,3%, 86,4%, and 73,5% at 10, 20 and 30 years, respectively. Larger cohort of Brown at al could explain the higher mortality rate of their study. In a less recent study, Hager et al (24), in a cohort of 191 repaired CoA, found a mortality rate of 2,6%. However, in their study, death registered were related to intervention complications. More recently, Bambul Heck et al (14), studied 143 patients with isolated CoA, finding a mortality rate of about 6%. In this study, patients enrolled were older than in our study, and this could explain the quite higher mortality rate. Always comparing our results with the study of Bambul Heck et al (14), in our study, we found a major total number of clinical outcomes, in particular of atrial fibrillation (there are no data about this outcome in literature), but also of cerebrovascular events (9 vs. 2) and aortic dissection (3 vs. 1). Maybe these results could be due to the larger population of our study. Also for coronary

revascularization (with a rate of 1,4% in our study) there are no studies in literature. Instead is well known the association between left-sided congenital heart diseases and heart failure (in particular also for CoA) (25).

***Re-coarctation and pseudoaneurysm outcomes***

Regarding CoA-related outcomes, in our study we found a higher restenosis rate in CoA site compared to previous studies, although there are few recent data in literature. In particular, Hager et al (24), described a restenosis rate of 17%, lower when compared to 36% found in our study. Considering type of intervention and related restenosis in our population, restenosis was present in 71 patients operated by end-to-end anastomosis, in 11 patients after stenting procedure, in 11 patients that had received aortic patch plasty, in 7 patients with subclavian flap repair and 1 patient with ascending-to-descending bypass or interposition graft. In our study, by multivariate analysis, stenting procedure and an older age (> 8 years) at first CoA repair resulted predictive of restenosis. Chen et al (26), in a population of 247 adult operated CoA patients, found a restenosis rate of 31%, more prevalent in patients operated when younger and that had received an end-to-end anastomosis. In line with our study, there was an overall higher prevalence of restenosis in end-to-end anastomosis patients, but differently from their study, in our study, stenting procedure, and an older age at first CoA repair resulted as predictor of restenosis. The study of Chen et al is in line with the paper of Brown et al (2), where they found a higher rate of re-CoA requiring re-intervention in patients younger at time of first intervention and underwent to end-to-end anastomosis. It is well known that younger age at first CoA repair

should be considered as risk factor for re-CoA, maybe due to a severe CoA in patients diagnosed at young age. However, differently from these 2 studies, in our study, the higher rate of re-intervention was found in patients with an age > 8 years at first CoA repair. It is known that children that receive stenting for CoA have a restenosis rate of 18% (27), and this is quite in line with our study, where we had 15 stenting procedure performed < 18 years. This first consideration could explain the difference of our study from the previous 2 cited studies, where Chen et al performed stenting procedure only in adult patients, and Brown et al did not evaluate stenting procedure. On the other hand, when stenting procedure is performed during adulthood, there is a progressive increase of restenosis year by year of follow-up (27), and also this consideration is consistent with our study, where we had a longer follow-up compared to the study of Chen et al.

Moreover, maybe due to a mean older age of our patients, as a new finding compared with previous studies, stenting procedure resulted predictor of restenosis, where the study of Brown et al did not evaluate interventional procedure for CoA repair.

In our study, we found an incidence of pseudoaneurysm formation of 10%, which was more prevalent in end-to-end anastomosis, followed by aortic patch plasty and stenting procedure (although without a statistical significant difference). Considering previous studies, Chen et al (26), found a pseudoaneurysm formation rate of 13%, more frequent after aortic patch plasty. These results are in line with our study, but differently from them, maybe due also in this situation to the older age of repair, in our study, stenting procedure was found as predictor of pseudoaneurysm formation, together with the presence of

hypoplastic aortic arch, that in the study of Chen et al did not influence pseudoaneurysm formation. In another study, Cramer et al (28), studied 63 patients undergone to aortic patch plasty operation, finding a very high incidence of pseudoaneurysm formation (47%). This study suggest the great impact of aortic patch plasty intervention on pseudoaneurysm formation, but differently from our study, Cramer et al did not evaluate patients that had received other types of intervention, with a consequent small sample size compared to our study. On the other hand, Hoffman et al (29), found pseudoaneurysm formation in 28 of 399 patients operated for CoA. Differently from other studies, they found a higher prevalence of pseudoaneurysm in patients treated by stenting procedure than in patients receiving surgery (13% vs. 4% respectively, $p < 0.05$), with a predictive value of stenting procedure on pseudoaneurysm formation at Cox analysis (HR 6.00, 95% CI 2.60-13.84, $p < 0.001$). These data are in line with our study, where we found a predictive value of stenting procedure on pseudoaneurysm formation.

**Limitations**

This is a retrospective study, with a relative small sample size, with a small number of outcomes, which could affect the statistical results of our analysis. On the other hand, the different time of intervention for each operation, in particular for stenting procedure, that generally is performed in adult age, could affect some results, explaining a statistical significance for it compared to other interventions, such as aortic patch plasty, in which, although was found a major incidence of pseudoaneurysm formation (however not

statistically different), resulted not statistically related to it. In addition, considering aortic stiffness, it was evaluated in a poor number of patients by CMR, and also by echo, PWV was evaluable only in about half of the entire population. This could be a reason of loss of power for aortic stiffness in predictive value on outcomes. Finally, the different time of follow up, with a quite strict follow up after stenting procedure, compared to other surgical procedures, could affect our results.

## Conclusions

In a cohort of 280 patients from 3 Swiss ACHD centers, was found a high incidence of severe re-coarctation, requiring an intervention, with a lower incidence of pseudoaneurysm formation, with a consequent and unexpected role of stenting procedure, related to older age of CoA intervention and the presence of hypoplastic aortic arch in predict outcomes.

## Acknowledgements

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**Table 1.** Patients' characteristics

| | Patients (n = 280) |
|---|---|
| Age (years) (range) | 33,6 ± 13,2 (17,1-84,5) |
| Female | 112 (40%) |
| BSA ($m^2$) | 1,84 ± 0,23 |
| BMI ($Kg/m^2$) | 25,4 ± 10,2 |
| Age at first intervention (years) (range) | 3,6 (0,1-59,7) |
| Systolic blood pressure (mmHg) | 129 ± 14 right arm, or left arm if right arch |
| Diastolic blood pressure (mmHg) | 73 ± 11 right arm, or left arm if right arch |
| Leg blood pressure (mmHg) | 124 ± 16 |
| Type of primary coarctation intervention | |
| End-to-end anastomosis | 181 (64%) |
| Stenting | 39 (14%) |
| Aortic patch plasty | 22 (8%) |
| Subclavian flap-repair | 22 (8%) |
| Ascending-to-descending bypass | 11 (4%) |
| Interposition graft | 5 (2%) |
| Bicuspid aortic valve | 187 (67%) |
| Hypoplastic aortic arch | 132 (47%) |
| Gothic arch | 37 (13%) |
| Ventricular septal defect | 57 (20%) |
| Other congenital heart disease | 52 (19%) |
| Congenital valvular aortic stenosis | 16 (6%) |
| Atrial septal defect | 13 (5%) |
| Taussig-Bing Heart | 6 (2%) |
| Transposition of the great arteries | 3 (1%) |
| Pulmonary stenosis | 3 (1%) |
| Aorto-pulmonary window | 2 (0.7%) |
| Severe mitral valve prolapse | 2 (0.7%) |
| Mitral stenosis | 2 (0.7%) |
| Anomalous coronary artery origin | 1 (0.4%) |
| Double orifice mitral valve | 1 (0.4%) |
| Ebstein syndrome | 1 (0.4%) |
| Tetralogy of Fallot | 1 (0.4%) |
| Double inlet left ventricle | 1 (0.4%) |
| Turner syndrome | 10 (4%) |
| Anti-hypertensive treatment | 134 (48%) |
| Beta-blockers | 67 (50%) |
| ACE-i | 51 (38%) |

| | |
|---|---|
| ARBs | 66 (49%) |
| Calcium-antagonists | 56 (42%) |
| Diuretics | 42 (31%) |
| Alpha-blockers | 1 (0,7%) |

BSA: body surface area; BMI: body mass index; ACE-i: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blockers.

**Table 2.** Clinical outcomes

| | Patients (n = 280) |
|---|---|
| Clinical Outcomes | 29 (10%) |
| Death | 1 (0,4%) |
| Atrial fibrillation | 20 (7%) |
| Cerebrovascular events | 9 (3%) |
| Coronary revascularization | 4 (1,4%) |
| Heart failure | 5 (1,8%) |
| Aortic dissection | 3 (1%) |

**Table 3.** Coarctation-related outcomes

| | Patients (n = 280) |
|---|---|
| Coarctation-related outcomes | 116 (41%) |
| Severe re-coarctation needing re-intervention | 102 (36%) |
| Pseudoaneurysm formation | 27 (10%) |

Categorical variables reported as absolute numbers and percentages. Continuous variables reported as mean ± standard deviation, and non-normally distributed continuous variables presented as median and interquartile range (IQR).

**Table 4.** Cox analysis of composite endpoint

| Univariate analysis composite primary endpoint | | | |
|---|---|---|---|
| Variables | HR | 95%CI | P value |
| **Age** | 0.872 | 0.844-0.900 | **<0.001** |
| **Sex** | 0.657 | 0.457-0.943 | **0.023** |
| **Age at repair (>1 vs < 1 year)** | 0.621 | 0.430-0.897 | **0.011** |
| **Stenting procedure** | 29.798 | 14.600-60.814 | **<0.001** |
| **Presence of bicuspid aortic valve** | 0.695 | 0.486-0.993 | **0.046** |
| **Presence of hypoplastic aortic arch** | 1.626 | 1.151-2.297 | **0.006** |
| **Dilated echo LA** | 0.574 | 0.379-0.872 | **0.009** |
| **Echo LV mass i** | 0.994 | 0.989-1.000 | **0.039** |
| **Echo CW peak at CoA site** | 1.005 | 1.001-1.008 | **0.005** |
| **Presence of echo diastolic dysfunction** | 0.390 | 0.108-0.731 | **0.003** |
| **Echo aortic distension** | 207.632 | 11.558-3729.853 | **<0.001** |
| **Dilated CMR LA** | 0.508 | 0.305-0.847 | **0.009** |
| **CMR aortic distension** | 4.277 | 1.170-15.636 | **0.028** |
| Multivariate analysis primary endpoint | | | |
| Variables | HR | 95%CI | P value |
| **Age** | 0.636 | 0.510-0.793 | **<0.001** |
| **Age at repair (>1 vs < 1 year)** | 6.002 | 1.442-24.975 | **0.014** |
| **Stenting procedure** | 1180.009 | 50.271-27698.551 | **<0.001** |

HR: hazard ratio; CI: confidence intervals; LV: left ventricular; LA: left atrium; CW: continue wave; CMR: cardiac magnetic resonance.

**Table 5.** Cox analysis for clinical outcomes

| Univariate analysis clinical outcomes | | | |
|---|---|---|---|
| Variables | HR | 95%CI | P value |
| **Age of first CoA repair** | 1.052 | 1.018-1.087 | **0.002** |
| **Interposition graft procedure** | 8.922 | 1.916-41.553 | **0.005** |
| **Dilated echo LA** | 2.277 | 1.029-5.036 | **0.042** |
| **Echo aortic distension** | 6052.482 | 6.344-5774652.373 | **0.013** |
| Multivariate analysis clinical outcomes | | | |
| Variables | HR | 95%CI | P value |
| **Age of first CoA repair** | 1.061 | 1.013-1.112 | **0.013** |
| **Echo aortic distension** | 78792.756 | 44.080-140843099.7 | **0.003** |

HR: hazard ration; CI: confidence intervals; CoA: coarctation; LA: left atrium.

**Table 6.** Cox analysis for atrial fibrillation

| Univariate analysis atrial fibrillation | | | |
|---|---|---|---|
| Variables | HR | 95%CI | P value |
| **Interposition graft procedure** | 16.237 | 3.107-84.857 | **0.001** |
| **Age of first CoA repair** | 1.068 | 1.030-1.107 | **<0.001** |
| **Echo LV mass i** | 1.015 | 1.001-1.029 | **0.034** |
| Multivariate analysis atrial fibrillation | | | |
| Variables | HR | 95%CI | P value |
| **Age of first CoA repair** | 1.075 | 1.012-1.142 | **0.020** |

HR: hazard ratio; CI: confidence intervals; CoA: coarctation; LV: left ventricular.

**Table 7.** Cox analysis for re-coarctation

| Univariate analysis re-coarctation | | | |
|---|---|---|---|
| Variables | HR | 95%CI | P value |
| **Age** | 0.973 | 0.956-0.990 | **0.002** |
| **Sex** | 0.607 | 0.398-0.928 | **0.021** |
| **Stenting procedure** | 2.123 | 1.097-4.106 | **0.025** |
| **Age of first CoA repair** | 0.952 | 0.921-0.983 | **0.003** |
| **Age at repair (>1 vs < 1 year)** | 0.501 | 0.337-0.745 | **0.001** |
| **Presence of hypoplasic aortic arch** | 1.678 | 1.130-2.490 | **0.010** |
| **Echo CW peak at CoA site** | 1.005 | 1.001-1.008 | **0.011** |
| Multivariate analysis re-coarctation | | | |
| Variables | HR | 95%CI | P value |
| **Stenting procedure** | 7.930 | 2.768-22.713 | **<0.001** |
| **Age of first CoA repair** | 0.934 | 0.876-0.996 | **0.037** |

HR: hazard ratio; CI: confidence intervals; CoA: coarctation; CW: continue wave.

**Table 8.** Cox analysis for pseudoaneurysm formation

| Univariate analysis pseudoaneurysm formation | | | |
|---|---|---|---|
| Variables | HR | 95%CI | P value |
| **Age of first CoA repair** | 1.040 | 1.007-1.074 | **0.018** |
| **End-to-end anastomosis procedure** | 0.257 | 0.119-0.556 | **0.001** |
| **Stenting procedure** | 14.573 | 4.000-53.097 | **<0.001** |
| **Interposition graft procedure** | 6.286 | 1.443-27.376 | **0.014** |

| | | | |
|---|---|---|---|
| **Presence of hypoplastic aortic arch** | 3.674 | 1.605-8.413 | **0.002** |
| Multivariate analysis pseudoaneurysm formation | | | |
| Variables | HR | 95%CI | P value |
| **Stenting procedure** | 6.401 | 1.511-27.119 | **0.012** |
| **Presence of hypoplastic aortic arch** | 3.816 | 1.561-9.325 | **0.003** |

HR: hazard ratio; CI: confidence intervals; CoA: coarctation.

## Figures

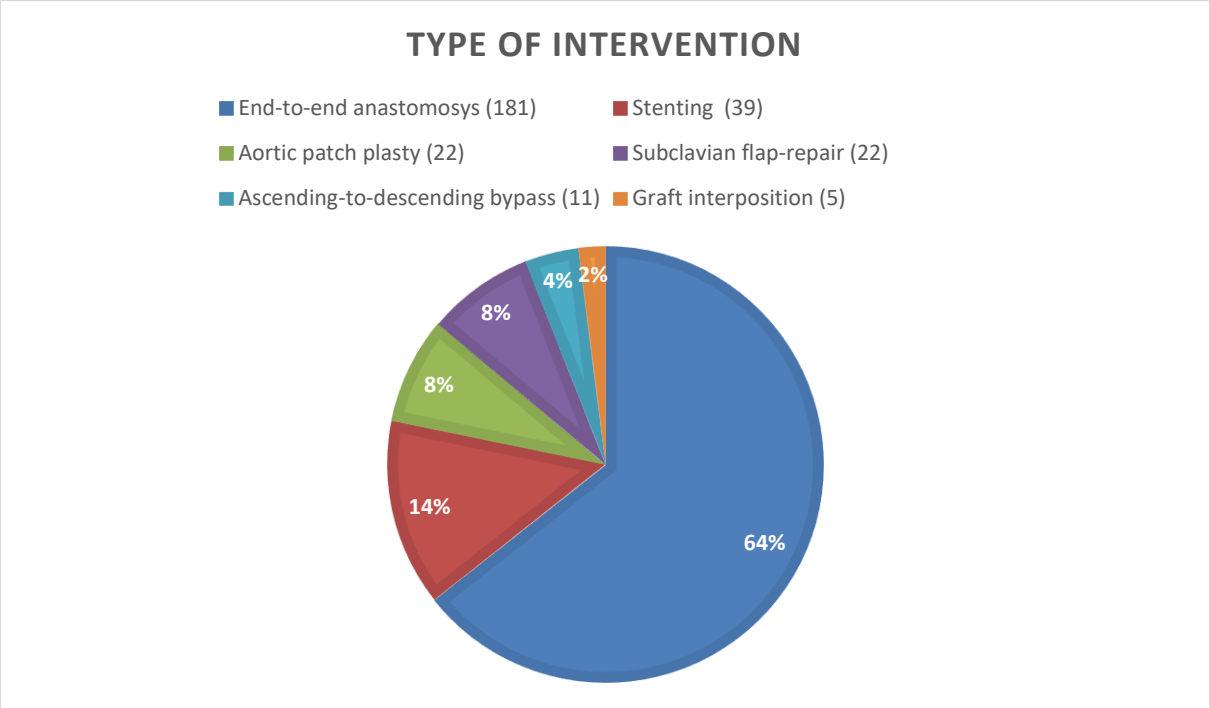


**Figure 1.** Distribution of type of CoA intervention

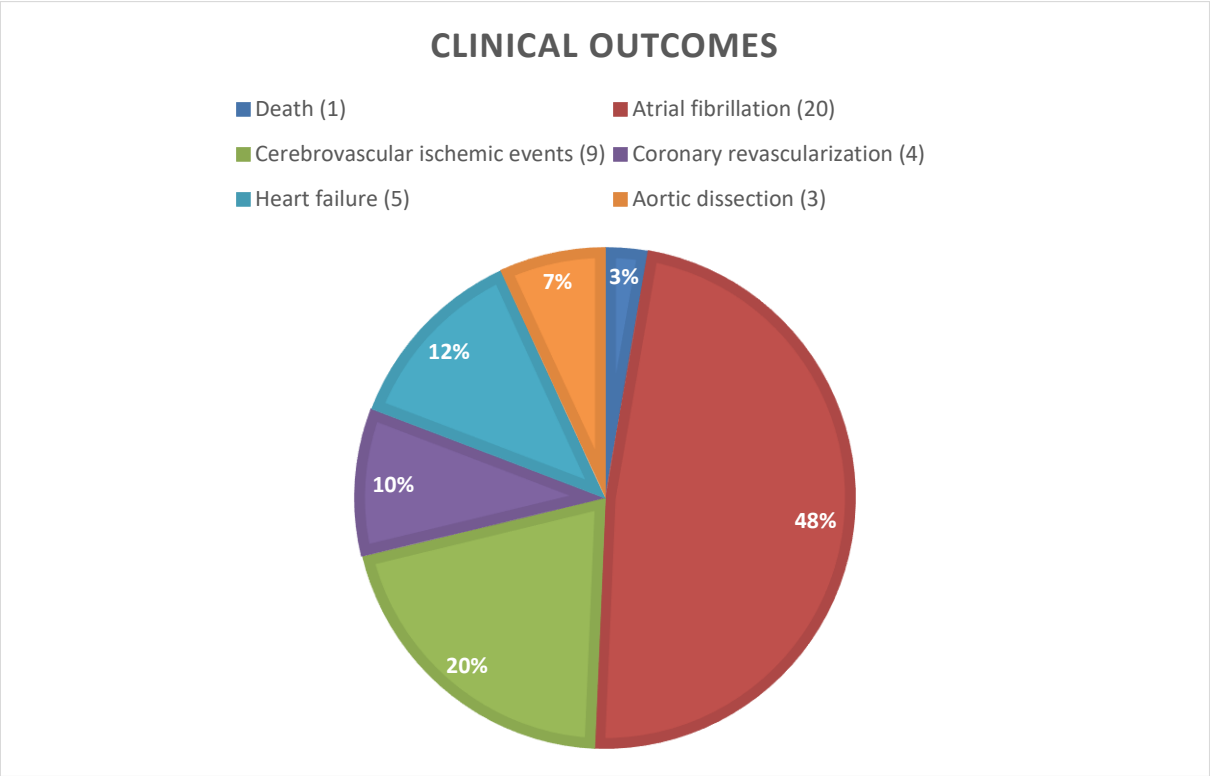


**Figure 2.** Distribution of clinical outcomes

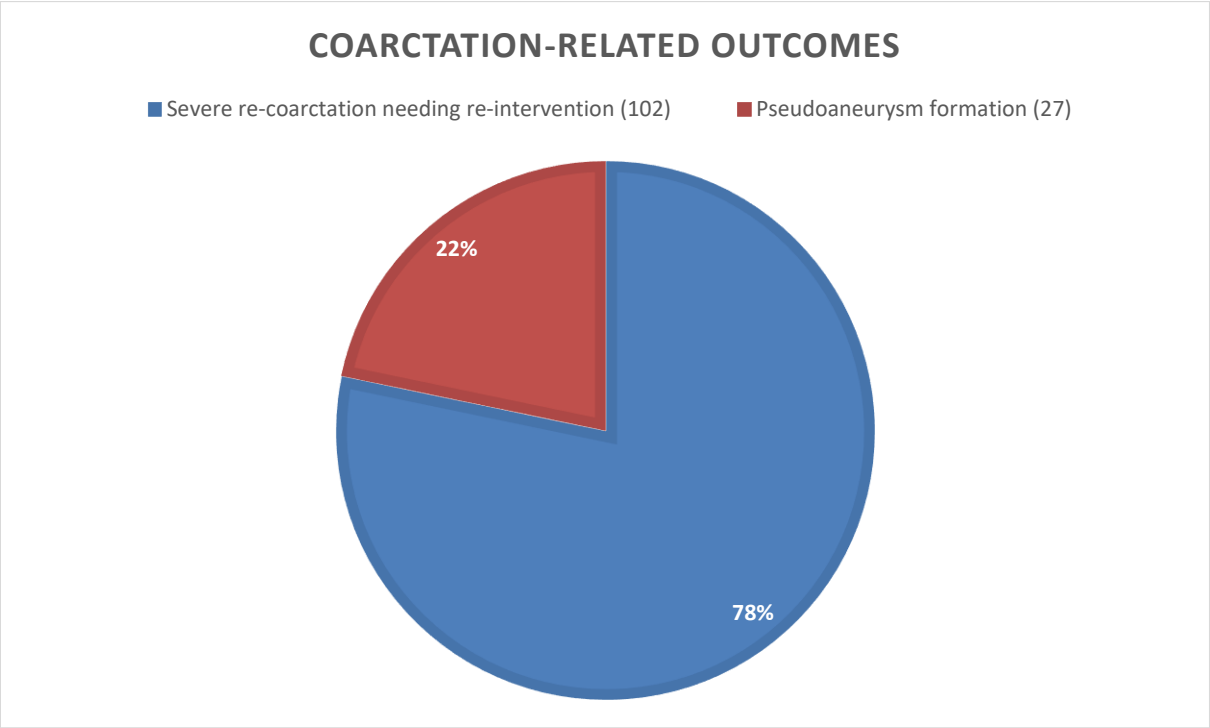


**Figure 3.** Distribution of coarctation-related outcomes

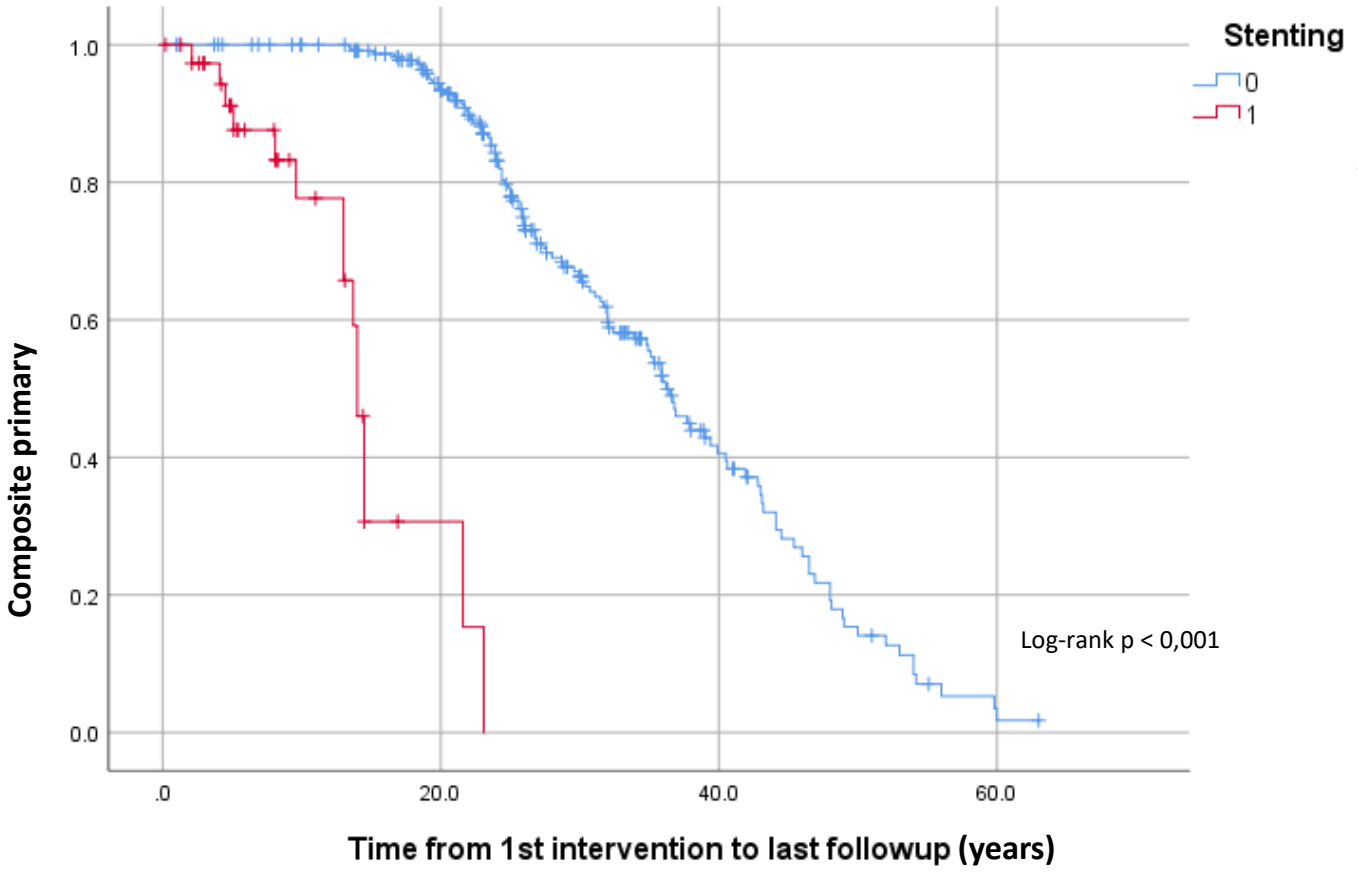


**Figure 4.** Kaplan-Meier curve for stenting procedure and composite primary endpoint.

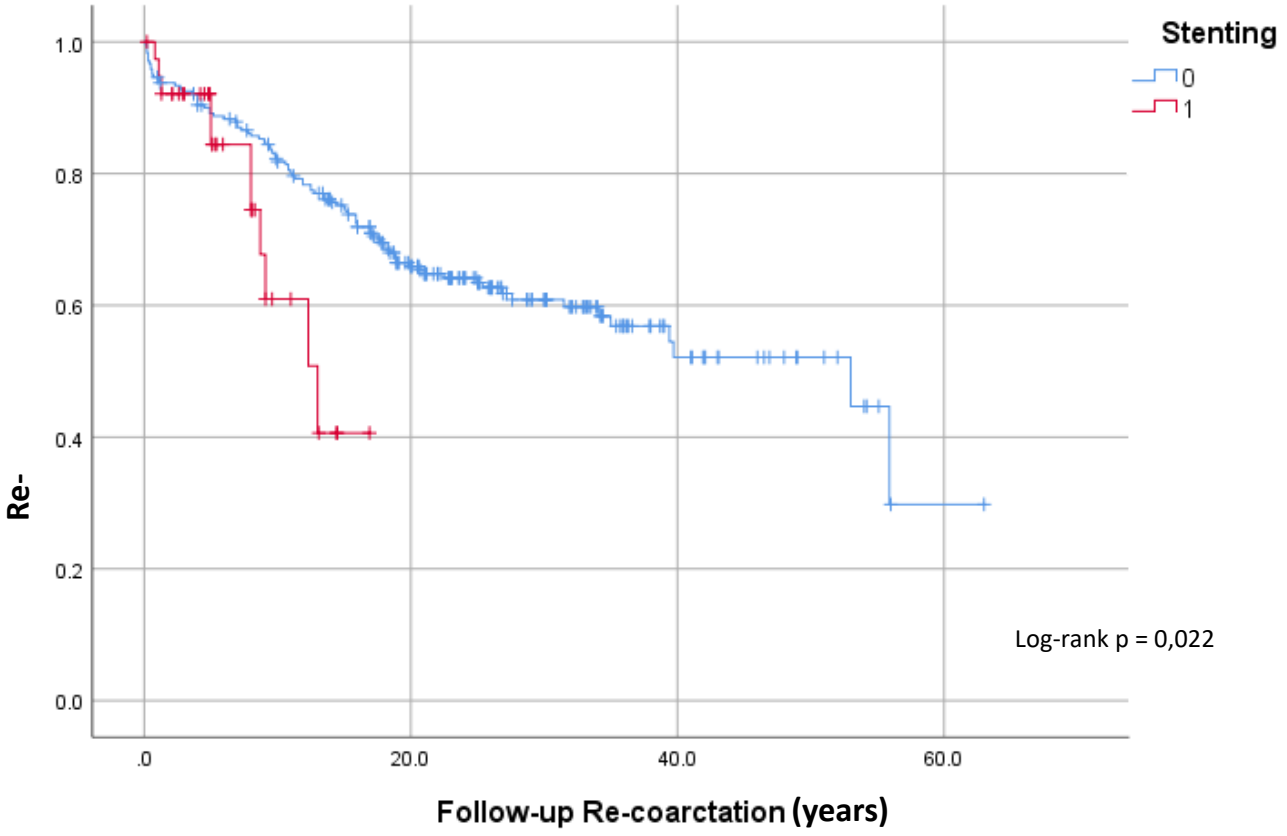


**Figure 5.** Kaplan-Meier curve for stenting procedure and severe re-coarctation

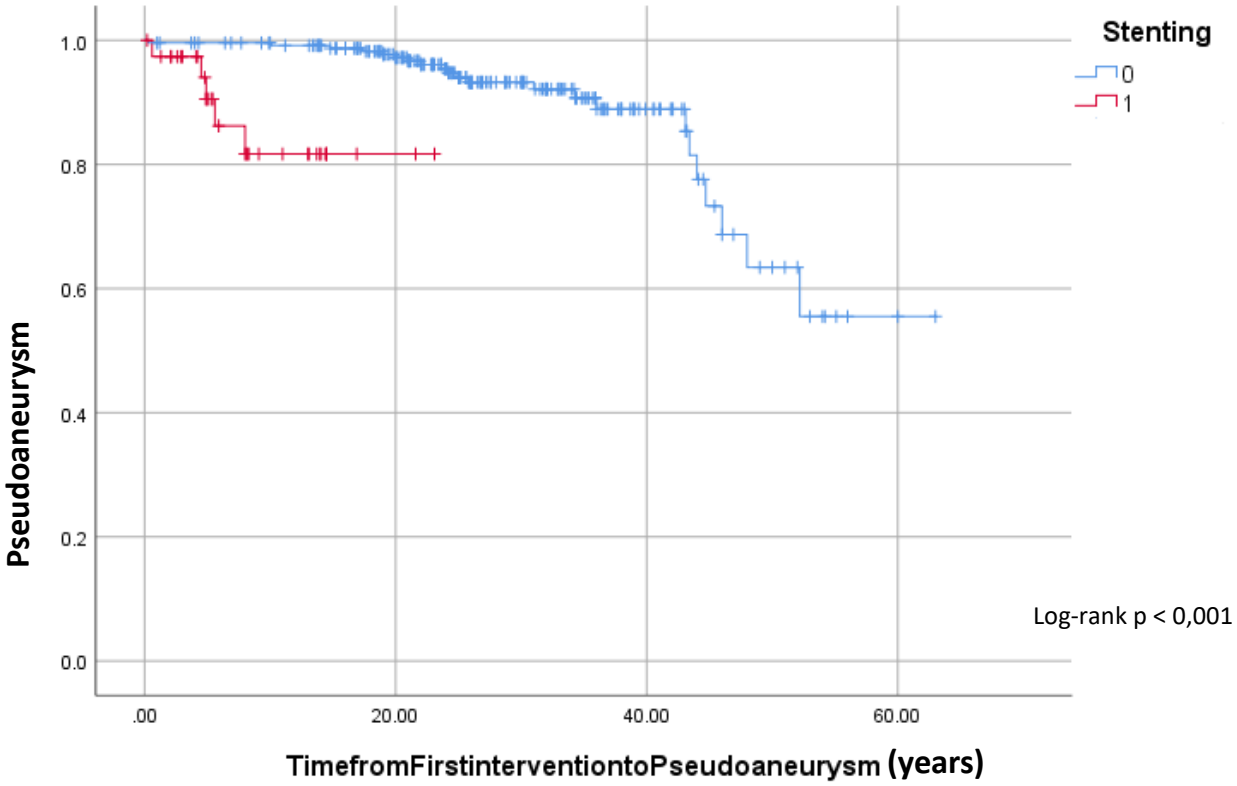


**Figure 6.** Kaplan-Meier curve for stenting procedure and pseudoaneurysm formation

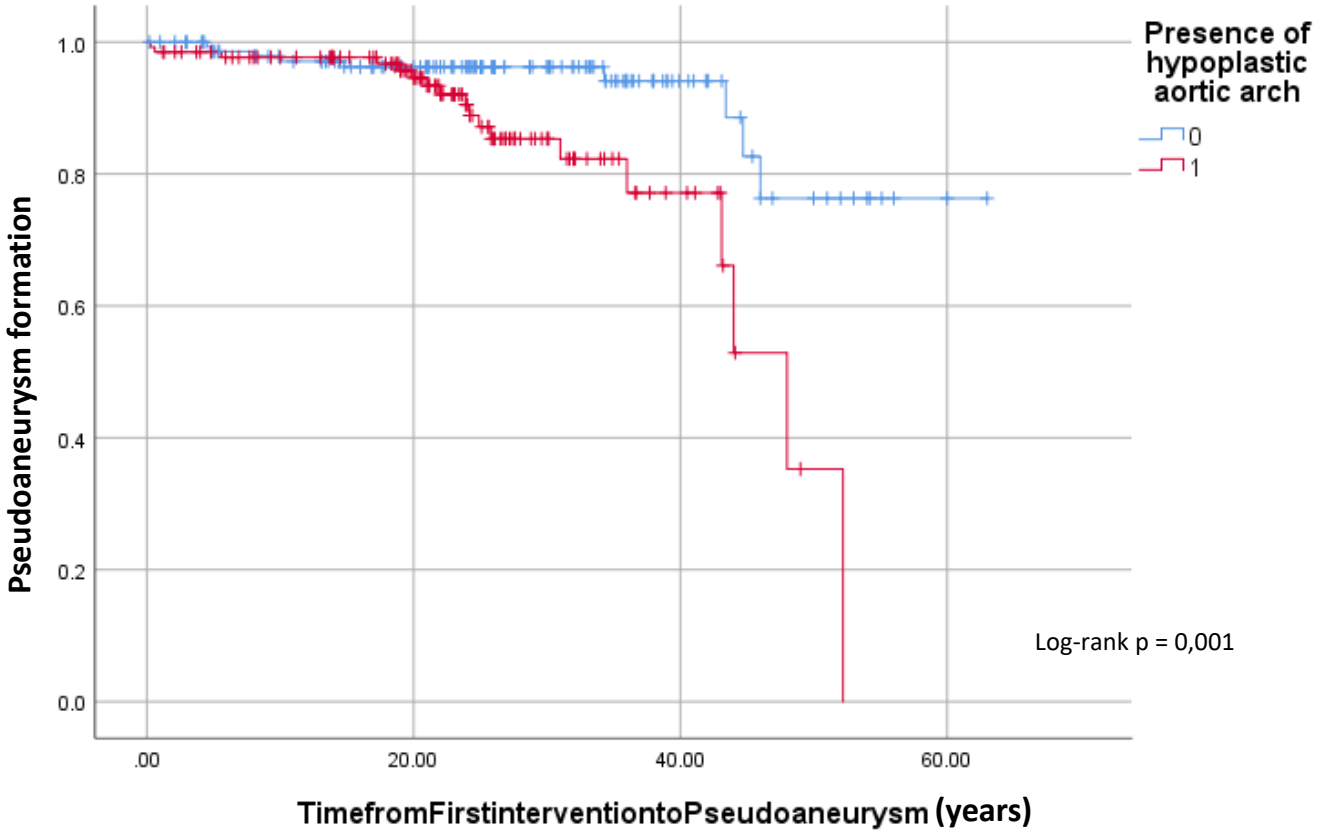


**Figure 7.** Kaplan-Meier curve for presence of hypoplastic aortic arch and pseudoaneurysm formation

**CHAPTER 10.2.**

# Blood pressure profile evaluated by ambulatory measurement and ambulatory blood pressure monitoring in repaired CoA adult patients

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*Draft*

**Blood pressure profile evaluated by ambulatory measurement and ambulatory blood pressure monitoring in repaired CoA adult patients**

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**Abstract**

**Introduction:** Aortic coarctation (CoA) is a congenital disease, characterized by narrowing and obstruction of descending aorta, with a high prevalence of post-interventional residual arterial hypertension. The aim of this study is to assess the blood pressure profile in operated CoA patients.

**Methods:** We retrospectively evaluated 280 adult patients with repaired CoA with different techniques. All patients underwent to clinical and imaging evaluation, with great attention to blood pressure profile, considering also ambulatory blood pressure monitoring (ABPM) for the evaluation of nocturnal blood pressure profile.

**Results:** Two-hundred and eighty patients (112 females, 40%), mean age of 33,6 ± 13,2, were included in the study. After a mean follow-up period of 20,1 ± 13,1 years, residual arterial hypertension was found in 137 patients (49%), nocturnal impaired blood pressure response in the whole population was found in 45 patients (16%). At multivariate Cox regression analysis, male patients, (HR 0.409, 95% CI 0.240-0.698, $p = 0.001$), patients with an older age (HR 0.874, 95% CI 0.837-0.913, $p < 0.001$), patients 8 years of age or older at the age of first CoA repair (HR 1.184, 95% CI 1.133-1.238, $p < 0.001$) resulted at higher risk of residual arterial hypertension.

**Conclusions:** In patients with repaired CoA there is a great prevalence of residual arterial hypertension, related to older age at time of first intervention and at follow-up, with an also quite great prevalence of impaired nocturnal blood pressure response, evaluated by ABPM.

## Introduction

Aortic coarctation (CoA) is a congenital disease of the proximal descending aorta, characterized by a hemodynamic significant aortic narrowing and obstruction, frequently associated with diffuse hypoplasia of the aortic arch, the presence of gothic arch, the presence of bicuspid aortic valve or other congenital cardiac lesions, and is often related to Turner syndrome (1). The greatest part of CoA patients are treated in infancy or childhood by different surgical options, but percutaneous stent implantation is nowadays the preferred treatment in adult patients with a delay of diagnosis, or in not complicated forms that arrive to adulthood, but also for focal restenosis (2-5).

The improvements in operation techniques, just few reflected the reduction in outcomes incidence in CoA patients, where is still present a high prevalence of cardiovascular events, but also of re-intervention for severe re-coarctation (13%), and for pseudoaneurysm formation (9%) (1,6,7). High morbidity and mortality found in several studies (6,7), is strongly related to the high incidence of severe arterial hypertension present in CoA patients. In fact, the elevated risk for cardiovascular events can be explained by persistent elevated sympathetic activity, endothelial dysfunction, and late hypertension in patients after CoA repair (8,9). Based on this assumption, the major complication and determinant of mortality after repair of CoA is the high incidence of late arterial hypertension, which may be present in 20-60% of patients with successful repair and without restenosis (6-8,10).

On the other hand, there is an important role played by aortic stiffness on morbidity and mortality, that independently from the presence of arterial hypertension, can have an impact on blood pressure (11-15), becoming both cause and effect of late hypertension, and has been shown to be increased in CoA patients late after repair (9).

The aim of this study was to assess the prevalence of residual hypertension, considering also the prevalence of non-dipper patients evaluated by ambulatory blood pressure monitoring (ABPM), defining the predictors of hypertension and of impaired nocturnal blood pressure response in these patients.

## Methods

### *Study patients*

We retrospectively evaluated 280 adult patients with repaired CoA by 3 different centers (122 patients from Zurich, 119 from Bern and 39 from Basel), belonging to the Swiss Adult Congenital HEart disease Registry (SACHER) (16). All patients underwent to a clinical evaluation, including right cubital and leg office blood pressure measurements (left arm measurements in patients with a right-sided aortic arch), considering as presence of hypertension, according to actual guidelines (17-18) a value ≥ 140/90 mmHg after an ambulatory measurement, and ABPM of the right arm (left arm measurements in patients with a right-sided aortic arch). ABPM was performed in 141 patients (50,4%), evaluating

the nocturnal blood pressure response in the whole population and in normotensive patients. Dipper subjects were defined for a physiological nocturnal reduction in blood pressure values from 10% to 20% (18). Maximal exercise test with cubital blood pressure measurement (left arm measurements in patients with a right-sided aortic arch) and transthoracic echocardiography, type and dosage of the current anti-hypertensive medication were recorded and included into our analysis. Two-hundred and twenty-five patients (80%) underwent to a CMR (in 55 patients (20%). CMR was not performed due to the presence of a pacemaker or for claustrophoby, and in 32 patients (11,4%) a computed tomography (CT) angiography of the aorta, for the detection of aortic dimension, was performed.

***Doppler echocardiography***

Transthoracic Doppler echocardiography was performed by expert echocardiographers in ACHD using a 3.5 MHz transducer including second harmonic imaging. In all exams left atrium and left ventricular dimensions were measured, calculating left ventricular mass. Ascending aortic dimensions were measured, with the inner-edge-to-outer-edge method, for evaluation of aortic distension in the parasternal long axis view and calculated as (end-systolic diameter – end-diastolic diameter)/(end-diastolic diameter) and aortic distensibility, defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure). For evaluation of left ventricular systolic function, Simpson formula was used. Diaphragmatic aorta dimension and pulsed Doppler flow velocity signal were also obtained. Aortic flow in the CoA site by both pulsed and continuous wave Doppler was

measured, to search for presence or absence of a diastolic tail in the flow velocity curve. Aortic arch diameter and aortic diameter at CoA site were measured using the inner-edge-to-inner-edge method, and. Pulse wave velocity (PWV) was evaluated by (center-line derived lenght of the aorta from aortic leaflet point to diaphragmatic aorta, assessed by CMR or CT))/(time delay between diaphragmatic foot wave and aortic valve foot wave. Diaphragmatic and aortic valve foot wave were obtained by pulsed wave Doppler, positioned to distal diaphragmatic aorta and closing point of aortic valve respectively). Aortic stiffness parameters were measured offline by FM. All other measurements were calculated online and data were collected retrospectively.

***Cardiac magnetic resonance imaging (CMR) and computed tomography (CT)***

Study patients were examined using a 1.5 T or a 3 T whole-body clinical MRI system with cardiac synchronization by ECG electrodes. In 27 patients only a MR-angiography for the evaluation of aortic dimensions was performed. For all the other patients, ventricles were imaged from the base towards the apex during end-expiratory breath-holds by a stack of retrospectively ECG-triggered SSFP-cine sequence acquisitions. End-diastolic and end-systolic contours were manually traced for each slice offline to determine left ventricular volumes and mass according to the modified Simpson's rule (disk summation). MR aortography using time-adjusted contrast medium injection was used to assess aortic arch and descending aortic geometry, diameter and presence or absence of pseudoaneurysm or restenosis at CoA repair site. Reconstructed aortic 3D volumes were used for offline centerline assessment of the aortic length between the

ascending (sinotubular junction site) and diaphragmal aorta for PWV calculations. Retrospectively gated steady-state free procession cine CMR images of the aortic arch, along with the ascending, transverse, descending and diaphragmatic aorta were routinely undertaken. Throughplane phase contrast flow measurements were acquired during breath-hold and in retrospectively ECG-gating technique in the ascending, diaphragmal and descending aorta at the CoA repair site and in patients with a hypoplastic arch. Aortic distension of the ascending aorta was calculated as (maximum systolic area – maximum diastolic area)/(maximum diastolic area). Aortic distensibility of the ascending aorta was defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure). PWV was calculated as (length of the aorta, from sinotubular junction to diaphgramatic aorta, derived from the MR-aortography)/(time delay between diaphragmatic foot wave and ascending aorta foot wave, derived from flow velocity measurements in the ascending and descending aorta). Measurements of aortic distension, aortic distensibility and PWV were calculated offline by FM.

CT scan was performed in 32 patients who had contraindications to perform CMR. Patients were examined while supine, taking images extending from the base of the neck to the diaphragm, using retrospective ECG-gated cardiac CT scanning. A 64-slices CT-scan was used, with a slice thickness of 1 mm and a reconstruction interval of 0.75 mm. A 3D reconstruction of the complete aorta was performed to obtain the complete length of the aorta, allowing measurement of the diameter of aorta from ascending to diaphragmatic portion.

### *Statistical analysis*

Categorical variables were reported as percentages. Continuous variables were reported as mean ± standard deviation (SD), and non-normally distributed continuous variables were presented as median and interquartile range (IQR). For comparison of continuous variables among the different groups of dippers and non-dippers, a factorial analysis of variance was used. Univariate Cox proportional hazard regression model was used to assess the association between clinical, echocardiographic and CMR variables and the outcomes. Due to a small number of non-dippers subjects, only variables with $p < 0.05$ at univariate analysis were included as covariates in the multivariate Cox regression

models to test which variables were independently associated with the events. For the events with a low number of events, two multivariate models were applied, considering clinical, demographic or imaging variables statistical significant at previous univariate analysis. Also for the multivariate analysis, statistical significance was defined for a p value $< 0.05$. Hazard ratios and their 95% confidence intervals were presented. Statistical analysis was performed with IBM SPSS Statistics v25.0 (Armonk, New York, United States of America).

## Results

Two-hundred and eighty patients (112 females, 40%), mean age of 33,6 ± 13,2, were included in the study. Baseline characteristics of the patients are reported in table 1. In this

cohort, the more prevalent type of first intervention was the end-to-end anastomosis (64%), followed by stenting procedure (14%) (figure 1). One-hundred and eighty-seven patients had a concomitant bicuspid aortic valve (67%) and hypoplastic aortic arch (defined as a diameter of less than 50% of the diameter of the ascending aorta) (7,17) was present in 132 patients (47%). One-hundred and thirty-four patients were under anti-hypertensive treatment (48%) (a single agent was given to 38 patients, dual therapy was given to 54 patients, a triple therapy to 31 patients and 11 patients gave 4 anti-hypertensive agents). After a mean follow-up period of 20,1 ± 13,1 years, residual arterial hypertension was found in 137 patients (49%), nocturnal impaired blood pressure response in the whole population was found in 45 patients (16%), and nocturnal impaired blood pressure

response in normotensive patients was found only in 14 patients (5%) (table 2, figure 2). Comparing in the whole population the nocturnal blood pressure response, there were no differences between dippers and non-dippers subjects for demographic, clinical, echocardiographic, cardiac magnetic resonance and aortic stiffness parameters (table 3).

Results of the univariate and multivariate Cox proportional hazard regression model are reported in tables 4-6. By multivariate Cox regression analysis, male patients, (HR 0.409, 95% CI 0.240-0.698, $p = 0.001$), patients with an older age (HR 0.874, 95% CI 0.837-0.913, $p < 0.001$), patients of 8 years of age or older at the age of first CoA repair (HR 1.184, 95% CI 1.133-1.238, $p < 0.001$), and patients with a higher continue wave Doppler at CoA site (HR 1.005, 95% CI 1.001-1.010, $p = 0.016$), resulted at higher risk of residual arterial hypertension. Considering ABPM profile, in non-dippers hypertensive and

normotensive, older age (HR 0.942, 95% CI 0.910-0.975, p = 0.001 for the whole population; HR 0.871, 95% CI 0.790-0.961, p = 0.006 for normotensive patients) and the surgical creation of an extra-anatomic aortic ascending-to-descending bypass (EAADB) (HR 7.446, 95% CI 1.412-39.281, p = 0.018 for the whole population; HR 14.169, 95% CI 1.885-106.486, p = 0.010 for normotensive patients) impacted on the abnormal nocturnal blood pressure response. In addition, also stenting procedure, in the whole population of non-dippers, predicted an abnormal nocturnal blood pressure response (HR 8.377, 95% CI 2.505-28.012, p = 0.001). None of the other echocardiographic and CMR parameters, nor aortic stiffness, resulted predictors of residual arterial hypertension, or of impaired nocturnal blood pressure response at multivariate analysis.

**Discussion**

This multicentric study, conducted on 280 patients from 3 Swiss centers, showed a high prevalence of residual hypertension (49%) in a cohort of adult patients with repaired CoA. Furthermore, there is also a quite high prevalence (16%) of impaired nocturnal blood pressure response, evaluated by ABPM. Moreover, evaluating predictor parameters of impaired blood pressure profile in this cohort, several clinical, surgical and imaging (considering also aortic stiffness) parameters play a role in predicting hypertensive profile. However, adjusting these parameters in a multivariate model, only male patients, patients older at follow-up, or older at the age of CoA repair have significantly more hypertension, meanwhile, an older age, and two types of operation (stenting procedure, or EAADB) resulted predictors of impaired nocturnal blood pressure profile.

These results are quite in line with previous studies, where was found a high prevalence of residual hypertension, most of all in older patients and in patients older at time of intervention. In some previous studies (19,20), was described a lower prevalence of residual hypertension (32% and 30% respectively). Toro-Salazar et al (21), in a previous study, evaluated a smaller cohort of repaired CoA patients (92 patients), finding also them a lower prevalence of hypertension (35%), with an increased hypertensive risk in patients older than 10 years at time of operation. These results are similar with the results of our study, where we found a higher hypertensive risk in patients older than 8 years at time of operation, with a quite similar prevalence of hypertension, but with a larger cohort in our study. On the other hand, Hager et al (10), differently from our study, found a more prevalent residual hypertension (55%) in a cohort of 245 adult operated CoA patients without restenosis, with a similar high prevalence in patients older at intervention and at follow-up, and male patients. The higher prevalence found in their study should be due to the selection of hypertensive patients, where they defined hypertension on ambulatory blood pressure as > 133/78 mmHg. Also Brown et al (6), in a more recent study, evaluated a larger cohort (819 patients), finding a prevalence of hypertension from 40% up to 70%, based on the follow-up time. In this study, they defined as predictor of residual hypertension, only the older age at time of CoA repair. These results differ from ours considering the prevalence of residual hypertension, that is higher (maybe due to the larger cohort evaluated by Brown et al), but they defined like us, a role of older age at time of operation, but without finding any other predictors. A systematic review of Canniffe et al (22), made on 26 studies, defined a prevalence of residual hypertension in operated CoA

patients of 32,5% (range 25-68%), considering as factor influencing the presence of residual hypertension age at time of surgery, age at follow-up, and type of intervention. The different prevalence found in our study should be explained by the different blood pressure cut-off used by the other authors included in the systematic review of Canniffe et al. On the other hand, similarly to our study, they found a role of older age in predict residual arterial hypertension, but differently from us, they defined a role of type of intervention, that in our analysis resulted only at the univariate analysis for end-to-end anastomosis, stenting procedure, and subclavian flap repair procedure, but that weren't confirmed at the multivariate model. Finally, in a more recent study, Rinnström, et al (23), in a cohort of 653 operated CoA patients, found a similar prevalence of residual arterial hypertension of our study (52%), with an association (as in our study) with male sex, older age at follow-up and older age at time of intervention.

On the other hand, considering the nocturnal blood pressure response, evaluated by ABPM, there are no data in literature, in particular regarding non-dipper profile, both in hypertensive and normotensive patients. In our study, we found a prevalence of 16% of general non-dippers, and a prevalence of 5% of normotensive non-dippers, with a correlation of them with older age, stenting procedure and EAADB for all non-dippers, adding also aortic distension and distensibility at univariate analysis for normotensive non-dippers, not confirmed by multivariate model. These results are very impressive, and more studies are warranted to confirm these data, also to define an additional role of ABPM in the follow-up of repaired CoA patients, to find concealed hypertension in these patients.

## Limitations

This is a retrospective study, with a relative small sample size, and a small number of ABPM, that was performed only in a little part of normotensive patients.

## Conclusions

In a cohort of 280 operated CoA patients, from 3 different centers, was found a high prevalence of residual hypertension, with a role of older age at follow-up and of an age > 8 years at time of intervention in define the presence of hypertension. Moreover, this is the first study evaluating the nocturnal blood pressure profile by ABPM, finding a 16% prevalence of non-dippers in operated CoA patients.

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**Table 1.** Baseline characteristics of patients

| | Patients (n = 280) |
|---|---|
| Age (years) (range) | 33,6 ± 13,2 (17,1-84,5) |
| Female | 112 (40%) |
| BSA ($m^2$) | 1,84 ± 0,23 |
| BMI ($Kg/m^2$) | 25,4 ± 10,2 |
| Age at first intervention (years) (range) | 3,6 (0,1-59,7) |
| Systolic blood pressure (mmHg) | 129 ± 14 |
| Diastolic blood pressure (mmHg) | 73 ± 11 |
| Leg blood pressure (mmHg) | 124 ± 16 |
| Type of intervention | |
| End-to-end anastomosys | 181 (65%) |
| Stenting | 39 (14%) |
| Aortic patch plasty | 22 (8%) |
| Subclavian flap-repair | 22 (8%) |
| Ascending-to-descending bypass | 11 (4%) |
| Graft interposition | 5 (2%) |
| Bicuspid aortic valve | 187 (67%) |
| Hypoplastic aortic arch | 132 (47%) |
| Gothic arch | 37 (13%) |
| Ventricular septal defect | 57 (20%) |
| Other congenital heart disease | 52 (19%) |
| Turner syndrome | 10 (4%) |
| Anti-hypertensive treatment | 134 (48%) |
| Beta-blockers | 67 (50%) |
| ACE-i | 51 (38%) |
| ARBs | 66 (49%) |
| Calcium-antagonists | 56 (42%) |
| Diuretics | 42 (31%) |
| Alfa-blockers | 1 (0,7%) |

BSA: body surface area; BMI: body mass index; ACE-i: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blockers.

Categorical variables reported as absolute numbers and percentages. Continuous variables reported as mean ± standard deviation, and non-normally distributed continuous variables presented as median and range.

**Table 2.** blood pressure profile prevalence

| | Patients (n = 280) |
|---|---|
| Blood pressure profile | 150 (54%) |
| Residual arterial hypertension | 137 (49%) |
| Sex (F/M) | 40/97 |
| Non-dippers | 45 (16%) |
| Normotensive non-dippers | 14 (5%) |

F: female; M: male.

Categorical variables reported as absolute numbers and percentages. Continuous variables reported as mean ± standard deviation, and non-normally distributed continuous variables presented as median and interquartile range (IQR).

**Table 3.** Differences between Dipper subjects and non-Dipper subjects

| | Non Dipper subjects (n=45) | Dipper subjects (n=96) | p value |
|---|---|---|---|
| Age (years) | 34,15 ± 13,32 | 32,8 ± 13,92 | 0.587 |
| BMI ($kg/m^2$) | 27,16 ± 5,42 | 24,96 ± 3,93 | 0.007 |
| Systolic blood pressure (mmHg) | 134,42 ± 15,09 | 133,31 ± 12,7 | 0,648 |
| Diastolic blood pressure (mmHg) | 73,89 ± 12,28 | 72,52 ± 11,82 | 0,527 |
| Leg systolic blood pressure (mmHg) | 129,52 ± 18,28 | 124,46 ± 13,79 | 0.107 |
| Echo LVEDd (mm) | 49,4 ± 5,06 | 48,65 ± 6,36 | 0.496 |
| Echo LVESd (mm) | 31,54 ± 4,19 | 30,2 ± 5,3 | 0.155 |
| Echo LV mass ($g/m^2$) | 102,72 ± 31,2 | 102,16 ± 36,32 | 0.935 |
| Echo LVEF (%) | 60,75 ± 6,82 | 62,74 ± 6,36 | 0.097 |
| Echo aortic distension | 0,141 ± 0,049 | 0,146 ± 0,057 | 0.618 |
| Echo aortic distensibility ($mmHg^{-1}x10^{-3}$) | 0,0026 ± 0,0014 | 0,0026 ± 0,0015 | 0.857 |
| Echo PWV (m/s) | 8,88 ± 3 | 10,46 ± 6,04 | 0.293 |
| CMR LVEDV (ml) | 165,54 ± 39,98 | 156,39 ± 39,32 | 0.294 |
| CMR LVESV (ml) | 63,71 ± 19,58 | 57,52 ± 16,79 | 0.161 |
| CMR LVEF (%) | 61,65 ± 5,8 | 62,85 ± 7,21 | 0.411 |
| CMR LV mass ($g/m^2$) | 67,46 ± 18,92 | 75,03 ± 22,64 | 0.131 |

| | | | |
|---|---|---|---|
| CMR aortic distension | 0,54 ± 0,31 | 0,44 ± 0,2 | 0.218 |
| CMR aortic distensibility ($mmHg^{-1}x10^{-3}$) | 0,0093 ± 0,0058 | 0,0074 ± 0,0038 | 0.185 |
| CMR PWV (m/s) | 7,18 ± 2,63 | 10,32 ± 7,54 | 0.226 |

BMI: body mass index; LVEDd: left ventricle end-diastolic diameter; LVESd: left ventricle end-systolic diameter; LV: left ventricular; LVEF: left ventricle ejection fraction; PWV: pulse wave velocity; CMR: cardiac magnetic resonance; LVEDV: left ventricle end-diastolic volume; LVESV: left ventricle end-systolic volume.

**Table 4.** Cox analysis for residual arterial hypertension

| Univariate analysis residual arterial hypertension | | | |
|---|---|---|---|
| Variables | HR | 95%CI | p value |
| **Age** | 0.965 | 0.949-0.981 | **<0.001** |
| **Sex** | 0.486 | 0.335-0.704 | **<0.001** |
| **Age of first CoA repair** | 1.033 | 1.018-1.047 | **<0.001** |
| **End-to-end anastomosis procedure** | 0.680 | 0.478-0.967 | **0.032** |
| **Stenting procedure** | 6.557 | 3.724-11.545 | **<0.001** |
| **Subclavian flap repair procedure** | 0.361 | 0.147-0.885 | **0.026** |
| **Presence of hypoplastic aortic arch** | 1.727 | 1.219-2.446 | **0.002** |
| **Echo CW peak at CoA site** | 1.007 | 1.003-1.010 | **<0.001** |
| **CMR aortic arch diameter** | 0.936 | 0.894-0.980 | **0.005** |
| **CMR diastolic tail at CoA site** | 3.939 | 1.582-9.807 | **0.003** |
| **CMR diastolic tail at diaphragmatic** | 3.716 | 1.604-8.608 | **0.002** |

| | | | |
|---|---|---|---|
| **aorta** | | | |
| Multivariate analysis residual arterial hypertension | | | |
| Variables | HR | 95%CI | p value |
| **Age** | 0.874 | 0.837-0.913 | **<0.001** |
| **Sex** | 0.409 | 0.240-0.698 | **0.001** |
| **Age of first CoA repair** | 1.184 | 1.133-1.238 | **<0.001** |
| **Echo CW peak at CoA site** | 1.005 | 1.001-1.010 | **0.016** |

HR: hazard ratio; CI: confidence intervals; CoA: aortic coarctation; CW: continue wave; CMR: cardiac magnetic resonance.

**Table 5.** Cox analysis for non-dipper ABPM profile

| Univariate analysis non-dipper ABPM profile | | | |
|---|---|---|---|
| Variables | HR | 95%CI | p value |
| **Age** | 0.950 | 0.920-0.980 | **0.001** |
| **End-to-end anastomosis procedure** | 0.531 | 0.292-0.966 | **0.038** |
| **Stenting procedure** | 11.490 | 4.290-30.775 | **<0.001** |
| **Ascending-to-descending bypass procedure** | 5.485 | 1.265-23.777 | **0.023** |
| Multivariate analysis non-dipper ABPM profile | | | |
| Variables | HR | 95%CI | p value |
| **Age** | 0.942 | 0.910-0.975 | **0.001** |
| **Stenting procedure** | 8.377 | 2.505-28.012 | **0.001** |
| **Ascending-to-descending bypass procedure** | 7.446 | 1.412-39.281 | **0.018** |

ABPM: ambulatory blood pressure monitoring; HR: hazard ratio; CI: confidence intervals.

**Table 6.** Cox analysis for normotensive non-dipper ABPM profile

| Univariate analysis normotensive non-dipper ABPM profile | | | |
|---|---|---|---|
| Variables | HR | 95%CI | p value |
| **Age** | 0.902 | 0.833-0.978 | **0.012** |
| **End-to-end anastomosis procedure** | 0.271 | 0.093-0.788 | **0.016** |
| **Stenting procedure** | 8.285 | 1.809-37.948 | **0.006** |
| **Ascending-to-descending bypass procedure** | 13.906 | 2.875-67.251 | **0.001** |
| **Echo aortic distension** | 101218.837 | 8.922-1148304968 | **0.016** |
| **Echo aortic distensibility** | >100 | 1->1000 | **0.026** |
| **CMR aortic distensibility** | <100 | 1->1000 | **0.036** |
| Multivariate analysis normotensive non-dipper ABPM profile | | | |
| Variables | HR | 95%CI | p value |
| **Age** | 0.871 | 0.790-0.961 | **0.006** |
| **Ascending-to-descending bypass procedure** | 14.169 | 1.885-106.486 | **0.010** |

ABPM: ambulatory blood pressure monitoring; HR: hazard ratio; CI: confidence intervals; CMR: cardiac magnetic resonance.

## Figures

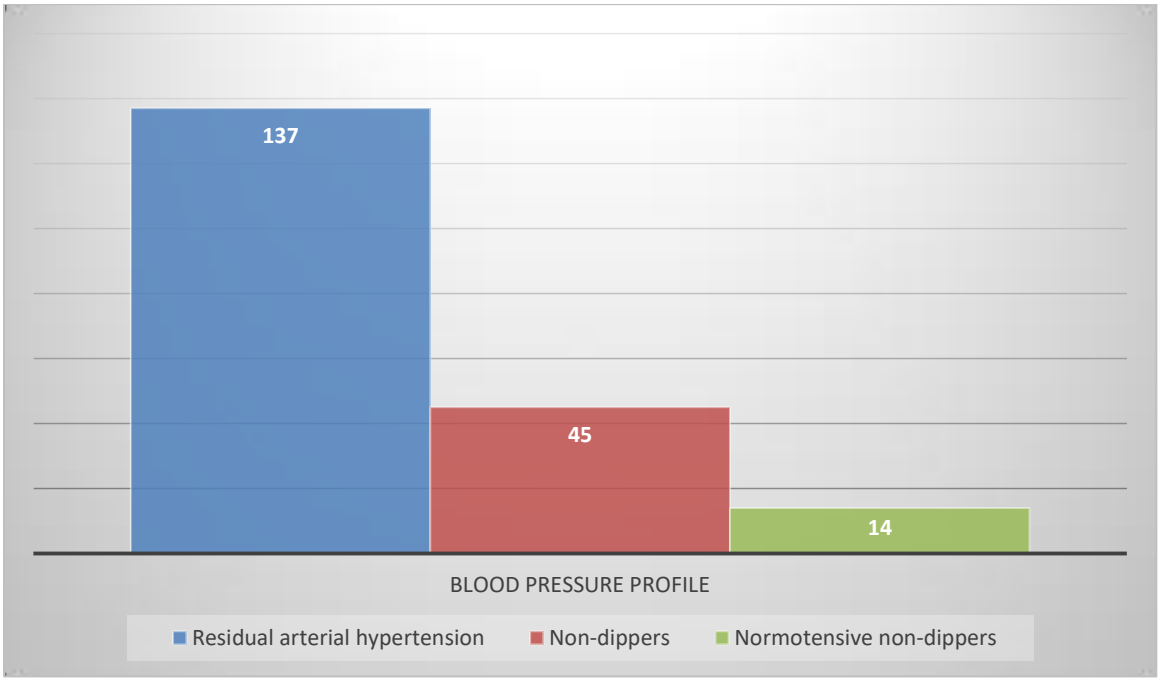


**Figure 1.** Blood pressure profile prevalences

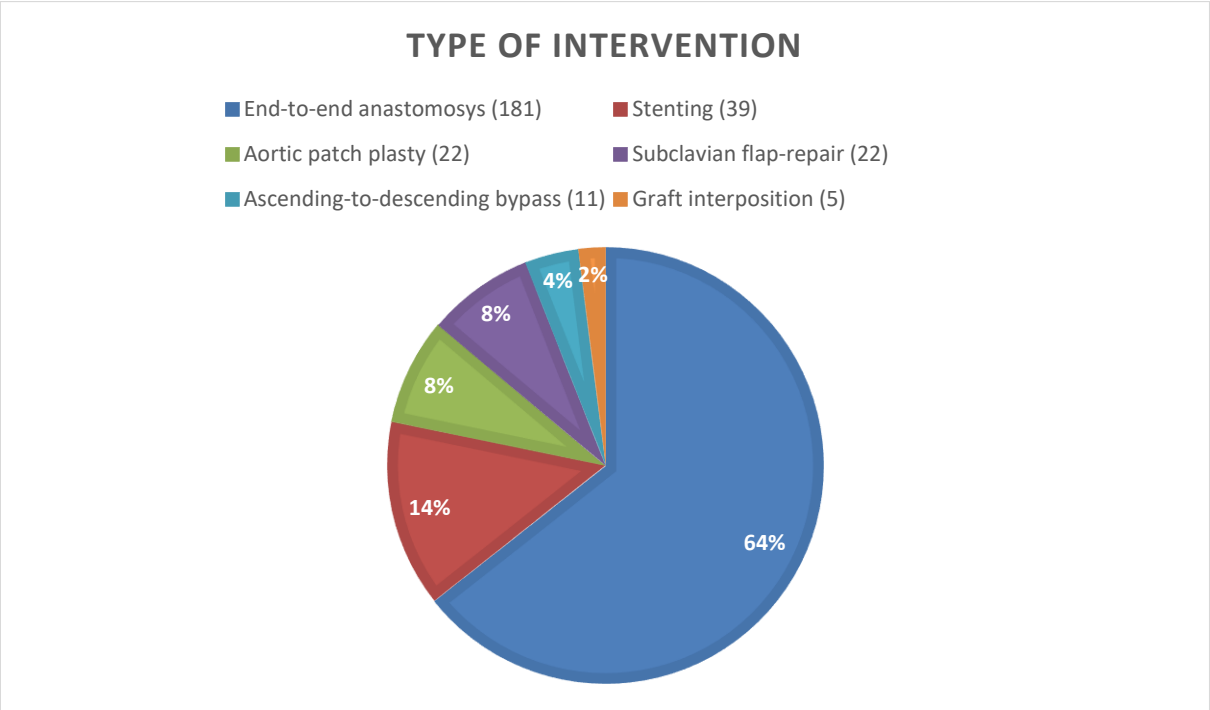


**Figure 2.** Distribution of type of CoA intervention

**CHAPTER 10.3.**

# Comparison of echocardiographic and cardiac magnetic resonance aortic stiffness assessment in adult patients with repaired CoA

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*Draft*

**Comparison of echocardiographic and cardiac magnetic resonance aortic stiffness assessment in adult patients with repaired CoA**

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**Abstract**

**Introduction:** Aortic and arterial stiffness are directly related to increased morbidity and mortality and are associated with varies cardiovascular risk factors, and have been shown to be increased in CoA patients late after repair. The aim of this study is to assess aortic stiffness by echocardiography and CMR and to compare these two techniques.

**Methods:** We retrospectively evaluated 119 adult patients with repaired CoA. All patients underwent to an echocardiographic exam, and of 119 patients, 109 underwent to a cardiac magnetic resonance (CMR) exam for the assessment of aortic distension, aortic distensibility and pulse wave velocity (PWV).

**Results:** Seventy-four patients (62%) were male, mean age 31,7 ± 13,4 years. Impaired aortic stiffness was found in 55 patients (46%). Comparing the 3 parameters of aortic stiffness by echocardiography and CMR, only for PWV resulted a correlation between the 2 techniques ($p < 0.001$, 95% CI -1.390-0.233).

**Conclusions:** There is a good correlation between echocardiography and CMR for the detection of PWV in adult patients with repaired CoA. This correlation was not found for aortic distension and aortic distensibility.

## Introduction

Patients with aortic coarctation (CoA), even after successful intervention, remain at high cardiovascular risk, most related to residual arterial hypertension. Different pathogenic mechanisms can play a role in define abnormal blood pressure response in these patients, bringing to an elevated risk for cardiovascular events (1). One of these is arterial stiffness, one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall (2).

Aortic and arterial stiffness are directly related to increased morbidity and mortality, both due to themselves, but also for their association with varies cardiovascular risk factors (3-5). Nevertheless, aortic and arterial stiffness play an important role in predict cardiovascular morbidity and mortality blood-pressure independently (6). Aortic stiffness by means of impaired ascending aorta and aortic arch distension and distensibility, but not impaired descending aorta distension and distensibility, has been shown to be increased in CoA patients late after repair (7-8). It has often been associated with aortic arch anomalies like gothic aortic arch (1,7). Assessment of aortic stiffness can be made by measurement of parameters such as aortic distension, aortic distensibility and pulse-wave velocity (PWV), and has been validated in the last 10 years (9-11), with the definition of normal reference values, also in children and young adults (2,12). These parameters can be analyzed in the ascending and diaphragmal aorta by echocardiography and CMR data with through-plane phase contrast flow velocity measurements.

The aim of this study was to assess aortic stiffness (aortic distension, aortic distensibility and PWV) by echocardiography and CMR and to compare these two techniques, to define also a cost-effectiveness strategy to follow in adult patients with repaired CoA.

## Methods

### *Study patients*

We retrospectively evaluated 119 adult patients with repaired CoA from a single center belonging to the Swiss Adult Congenital HEart disease Registry (SACHER) (13). Data from the most recent clinical evaluation with right cubital and leg office blood pressure measurements (left arm measurements in patients with a right-sided aortic arch), ambulatory 24-hour blood pressure measurement of the right arm (left arm measurements in patients with a right-sided aortic arch), and type and dosage of the current anti-hypertensive medication were recorded and included into our analysis. All patients underwent to an echocardiographic exam, and of 119 patients, 109 underwent to a CMR exam (10 patients did not perform CMR due to the presence of a pacemaker or for claustrophoby). In 5 patients (4%) an aortic angiography by computed tomography (CT)-scan was performed.

### *Echocardiographic assessment of aortic stiffness*

Transthoracic Doppler echocardiography was performed using an Acuson Sequoia C 512 (Acuson Corporation, Siemens, Mountain View, CA, USA) or Philips IE 33 ultrasound systems (Philips, Amsterdam, The Netherlands) with a 3.5 MHz transducer including second harmonic imaging. All echocardiography images and clips were stored off-line (Philips IntelliSpace Cardiovascular, Version 2.3, Best, Netherlands). In parasternal long-axis view, were defined aortic dimensions, in particular ascending aorta, for the evaluation of aortic distension, defined as (systolic diameter – diastolic diameter)/(diastolic diamenter) and aortic distensibility, defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure). Ascending aorta was measured using the inner-edge-to-outer-edge method. Aortic arch diameter and aortic diameter at CoA site were measured using the inner-edge-to-inner-edge method. Pulse wave velocity (PWV) was evaluated by (center-line derived lenght of the aorta from aortic valve closing point to diaphragmatic aorta, assessed by CMR or CT))/(time delay between diaphragmatic foot wave and aortic valve foot wave. Diaphragmatic and aortic valve foot wave were obtained by pulsed wave Doppler, positioned to distal diaphragmatic aorta and closing point of aortic valve respectively). For all measurements, final values were obtained after averaging over three cardiac cycles. Intra-observer variability for all the echocardiographic parameters of RV function was calculated in 30 randomly selected exams.

***CMR assessment of aortic stiffness***

Study patients were examined using a 1.5 T (Magnetom Symphony) or a 3 T (Magnetom Trio, both from Siemens Medical Solutions, Erlangen, Germany) whole-body clinical MRI system with cardiac synchronization by ECG electrodes. MR aortography using time-adjusted contrast medium injection was used to assess aortic arch. Retrospectively gated steady-state free procession cine CMR images of the aortic arch, along with the ascending, transverse, descending and diaphragmatic aorta were routinely undertaken. Through plane phase contrast flow measurements were acquired during breath-hold and in retrospectively ECG-gating technique in the ascending, diaphragmal and descending aorta at the CoA repair site and in patients with a hypoplastic arch. Aortic distension of the ascending aorta was calculated as (maximum systolic area – maximum diastolic area)/(maximum diastolic area). Aortic distensibility of the ascending aorta was defined as (aortic distension)/(systolic blood pressure – diastolic blood pressure). PWV was calculated as (length of the aorta, from sinotubular junction to diaphgramatic aorta, derived from the MR-aortography)/(time delay between diaphragmatic foot wave and ascending aorta foot wave, derived from flow velocity measurements in the ascending and descending aorta). Measurements of aortic distension, aortic distensibility and PWV were calculated offline by FM.

***CT scan for the evaluation of aorta***

CT scan was performed in 5 patients who had contraindications to perform CMR. Patients were examined while supine, taking images extending from the base of the neck to the diaphragm, using retrospective ECG-gated cardiac CT scanning. A 64-slices CT-scan

was used, with a slice thickness of 1 mm and a reconstruction interval of 0.75 mm. A 3D reconstruction of the complete aorta was performed, obtaining the entire length of the aorta from the ascending to the diaphragmal aorta, used for the calculation of PWV by echo Doppler flow profiles.

### *Statistical analysis*

Categorical variables were reported as percentages. Continuous variables were reported as mean ± standard deviation (SD), and non-normally distributed continuous variables were presented as median and interquartile range (IQR). T-test for independent samples was used to compare PWV in Gothic arch and aortic arch with normal shape. Comparisons between echocardiographic and CMR aortic stiffness (aortic distension, aortic distensibility and PWV) were performed using an un-paired t-test or the Mann-Whitney test for non-normally distributed variables. Measurement of reproducibility was evaluated calculating the interclass correlation coefficient (ICC), for the assessment of observer agreement, with a cut-off value proposed by Burdock et al. (14) up than 0.75 to signify good agreement. In all analyses, the null hypothesis was rejected for p values < 0.05. Statistical analysis was performed with IBM SPSS Statistics v25.0 (Armonk, New York, United States of America).

## Results

Seventy-four patients (62%) were male, mean age 31,7 ± 13,4 years. Baseline characteristics of patients are shown in table 1. In this cohort, the more prevalent type of first intervention was the end-to-end anastomosis (72; 61%), followed by stenting procedure (18; 15%) and aortic patch plasty procedure (16; 13%) (figure 1). Seventy-seven patients had a concomitant bicuspid aortic valve (65%) and hypoplastic aortic arch

(defined as a diameter of less than 50% of the diameter of the ascending aorta) (15,16) was present in 75 patients (63%), and Gothic arch was present in 25 patients (21%). Sixty-six patients were under anti-hypertensive treatment (55%).

Aortic distension and distensibility were detected by echocardiography and CMR in 100 patients (84%), meanwhile, PWV was detected in 80 patients (67%). Considering a mean normal value based on different values of aortic stiffness per aorta section studied, impaired aortic stiffness was found in 55 patients (46%). Comparing PWV between patients with Gothic arch (14 patients), and patients with normal aortic arch shape (66 patients), there was no difference between the groups (normal shape PWV 11 ± 9.3 vs Gothic arch PWV 9.6 ± 5.7, $p = 0.446$). Comparing the 3 parameters of aortic stiffness by echocardiography and CMR, only for PWV resulted a correlation between the 2 techniques ($p < 0.001$, 95% CI -1.390-0.233) (figure 2), instead for aortic distension and distensibility, the correlation was not significant ($p = 0.985$, 95% CI -0.359/-0.261; $p = 0.142$, 95% CI -0.006/-0.004, respectively) (table 2).

ICC for intra-observer variability for aortic distension, aortic distensibility and PWV evaluated by echocardiography were as follows: 0.973, 0.963, 0.865, respectively.

**Discussion**

This study made on 119 patients showed a high rate of impaired aortic stiffness in a population of adult with repaired CoA. PWV is defined as gold standard for the assessment of aortic stiffness (17), and can be easily measured by echocardiography and CMR. In Our study, for PWV, but not for aortic distension and aortic distensibility, a correlation between echocardiography and CMR measurement was found.

Several previous studies have shown a high prevalence of impaired aortic stiffness in patients with repaired CoA. In particular, three studies (1,7,18) described an impaired aortic stiffness in repaired CoA, in particular with a high rate in presence of Gothic arch. Ou et al (7), in a cohort of 63 repaired CoA, with three types of aortic arch (Gothic, Crenel, and Romanesque) and 63 controls, evaluating aortic distensibility by CMR, found a worst aortic stiffness in CoA patients, and also in Gothic arch when compared with the other arch morphologies. The same author (18), studied a population of 55 repaired CoA (20 Gothic arches and 35 Romanesque arches) and 20 control subjects. In this study was evaluated not only aortic distensibility, but also PWV by CMR. They found a significantly lower aortic distensibility and a higher PWV in Gothic arch when compared with control subjects and Romanesque arch. This difference was not found between Romanesque arch and healthy controls. Also Donazzan et al (1), in a population of 26 repaired CoA, compared aortic distensibility by CMR in 6 patients with Gothic arch morphology and 20 patients with normal arch shape, finding a significantly lower aortic distensibility in Gothic arch. These

three studies show a role of aortic arch morphology in define an impaired aortic stiffness, with particular attention on Gothic arch. In our study, differently from them, there wasn't a difference between Gothic arch and normal shape, not confirming the role of aortic arch

morphology in define an impaired aortic stiffness. However, in our study there isn't a control population, so the only difference is in repaired CoA patients. Based on this assumption, other studies (19,20) have described an impaired aortic stiffness in patients with repaired CoA, independently from aortic arch shape. Ou et al (19), compared a population of 40 normotensive subjects after CoA repair and 20 control subjects, describing in repaired CoA patients a significantly reduced aortic stiffness and an increased PWV, both evaluated by CMR. In the same year, Vitarelli et al (20), compared 26 adult normotensive patients who had successful CoA repair in infancy and 24 control subjects. In this study, aortic stiffness was evaluated by echocardiography. They found an increased aortic stiffness in patients, compared to control subjects. These two studies confirm our hypothesis of impaired aortic stiffness in adult patients after CoA repair (in our population we had a 46% prevalence of impaired aortic stiffness), but differently from us, they compare aortic stiffness of repaired CoA with healthy subjects.

Finally, our study is the first in comparing aortic stiffness measurement between echocardiography and CMR in adult patients with repaired CoA. Previous studies (16,21,22) have evaluated different ways to detect aortic stiffness, also comparing CMR with less expansive methods. Giannattasio et al (21), measured aortic distensibility and PWV by tonometry and CMR in a population of 28 normotensive subjects. In this study

was found a significant correlation between aortic distensibility evaluated by tonometry and CMR, while no correlation was found for PWV. Our results are opposite with those of Giannattasio et al, in fact we found a correlation only for PWV. Differently from them, we have a larger population, and all our patients are operated for CoA. More recently, Feistritzer et al (17), Compared in 40 volunteers the measurement of PWV by oscillometric method and CMR. In line with our study, they found a correlation between the 2 methodology of measurement. However, in our study we evaluated also aortic distension and aortic distensibility, and our study was performed on repaired CoA. Finally, the same author (22), evaluated PWV by oscillometric method and CMR in 60 reperfused myocardial infarction, finding also in this case a good correlation between oscillometric method and CMR for the measurement of PWV. Although also these results are in line with our results, there is a different type of population, where our study is the only one considering comparison between CMR and an ultrasound method to evaluate aortic stiffness in patients with repaired CoA.

**Limitations**

This study is a retrospective single center study, made on a relative small population. Moreover, aortic stiffness, and in particular PWV was not performed in the whole population.

## Conclusions

This is the first study evaluating the comparison between aortic stiffness measured by ultrasound and CMR in a cohort of repaired CoA patients. In a population of 119 patients, was found a good correlation between CMR and echocardiography in measuring PWV, but not for aortic distension and aortic distensibility. However, a prospective study is warranted to confirm these data.

## Acknowledgements

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**Table 1.** Baseline characteristics of patients

| | Patients (n = 119) |
|---|---|
| Age (years) (range) | 31,7 ± 13,4 (17,1-84,5) |
| BSA ($m^2$) | 1,8 ± 0,2 |
| BMI ($Kg/m^2$) | 24,8 ± 3,8 |
| Age at first intervention (years) (range) | 4,2 (0,1-59,1) |
| Type of intervention | |
| End-to-end anastomosys | 72 (61%) |
| Stenting | 18 (15%) |
| Aortic patch plasty | 16 (13%) |
| Subclavian flap-repair | 7 (6%) |
| Ascending-to-descending baypass | 4 (3%) |
| Graft interposition | 2 (2%) |
| Bicuspid aortic valve | 77 (65%) |
| Hypoplastic aortic arch | 75 (63%) |
| Gothic arch | 25 (21%) |
| Ventricular septal defect | 27 (23%) |
| Other congenital heart disease | 15 (13%) |
| Atrial septal defect | 3 (20%) |
| Transposition of the great arteries | 3 (20%) |
| Aorto-pulmonary window | 2 (13%) |
| Congenital valvular aortic stenosis | 2 (13%) |
| Double orifice mitral valve | 1 (7%) |
| Severe mitral valve prolapse | 1 (7%) |
| Ebstein syndrome | 1 (7%) |
| Tetralogy of Fallot | 1 (7%) |
| Double inlet left ventricle | 1 (7%) |
| Turner syndrome | 2 (2%) |
| Anti-hypertensive treatment | 66 (55%) |

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

Categorical variables reported as absolute numbers and percentages. Continuous variables reported as mean ± standard deviation, and non-normally distributed continuous variables presented as median and range.

**Table 2.** Correlation between PWV, aortic distension and aortic distensibility evaluated by echocardiography and cardiac magnetic resonance.

| | Echocardiographic assessment (mean ± SD) | Cardiac magnetic resonance assessment (mean ± SD) | P value |
|---|---|---|---|
| PWV (m/s) | 10,28 ± 7,15 | 10,86 ± 8,82 | < **0.001** |
| Aortic Distension | 0,145 ± 0,064 | 0,455 ± 0,232 | 0.985 |
| Aortic Distensibility ($mmHg^{-1}x10^{-3}$) | 0,003 ± 0,0014 | 0,008 ± 0,005 | 0.142 |

SD: standard deviation; PWV: pulse wave velocity.

## Figures

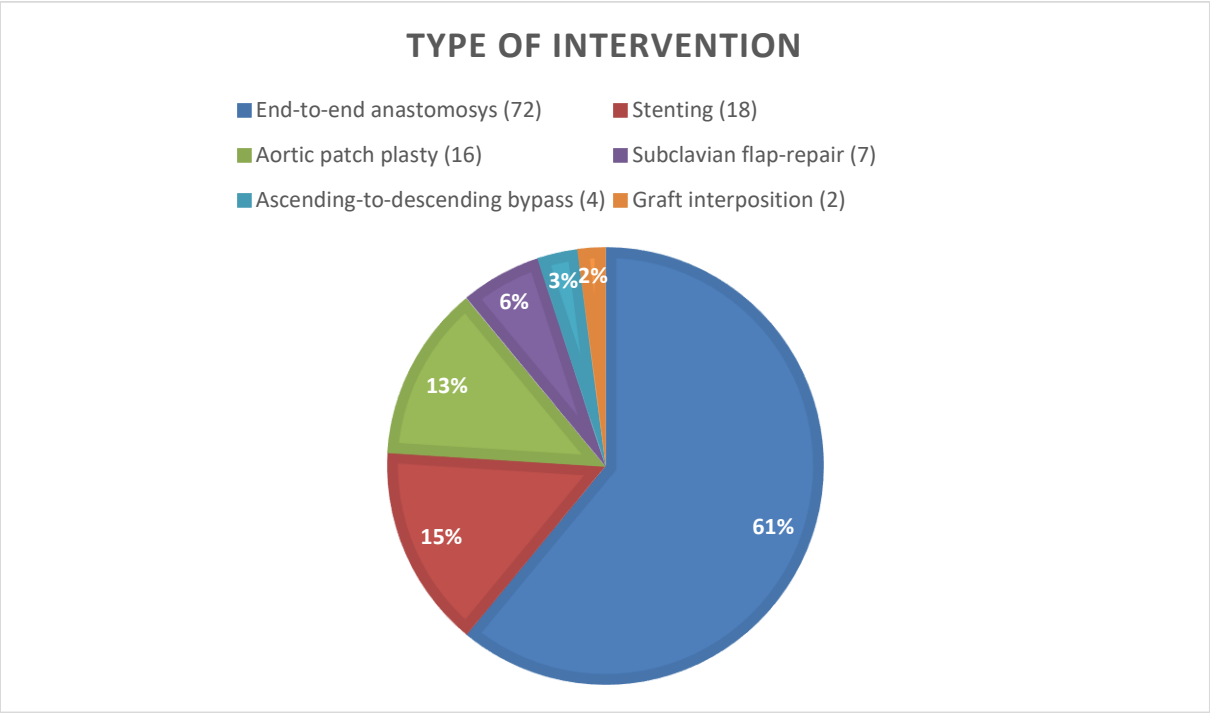


**Figure 1.** Distribution of type of CoA intervention

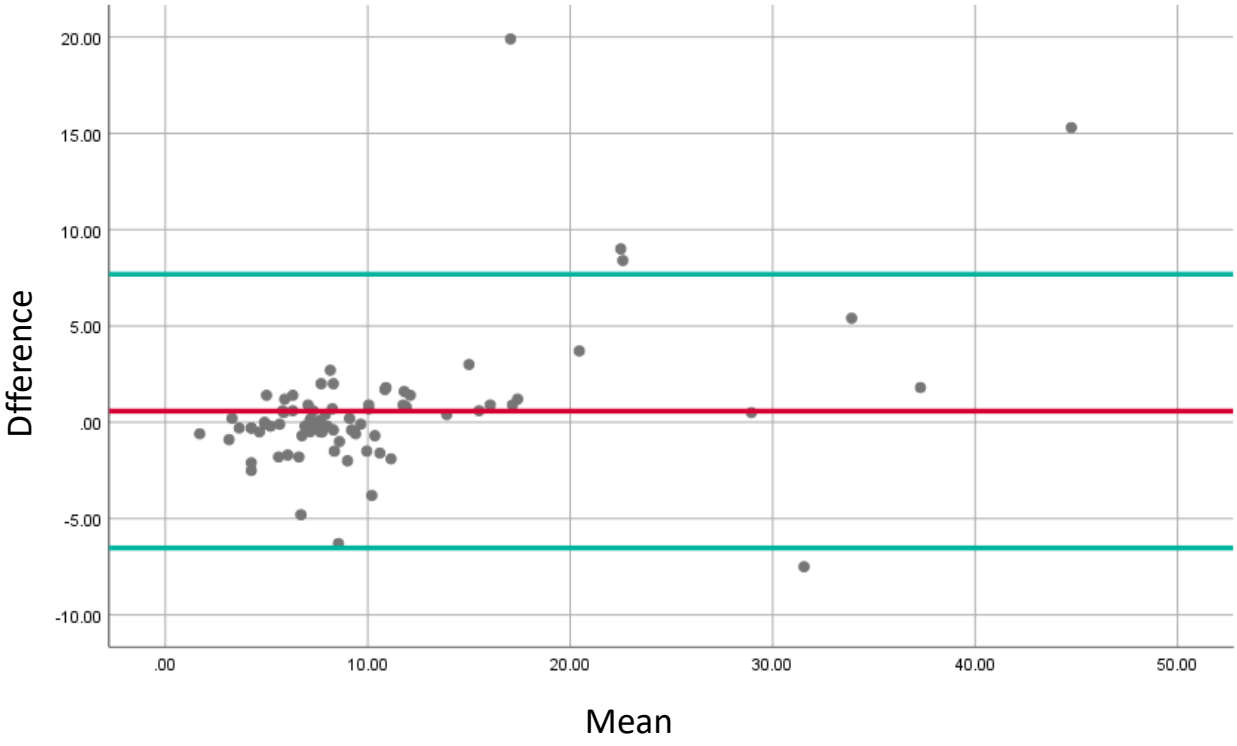


**Figure 2.** Bland Altman plot for pulse wave velocity

## CHAPTER 11.

### Discussion and conclusion

Since many years, well known is the role of some non-conventional risk factors, which can influence CV risk. In particular, there is a iatrogenic effect of some drugs, that increase CV risk, leading to an atherosclerotic burden in several patients (Chapter 2). In particular, patients with rheumatic disease, patients with chronic pain, are exposed (many times since childhood) to a major CV risk using NSAIDs chronically. For this reason, current guidelines recommend caution on the use of these agents, in particular in patients with a previous history of CV disease. However, there is a range of safety for some of these drugs, in particular naproxen have shown a lower risk for CV side effects.

A great part of CV risk is subclinical, especially when it starts during childhood. This could lead to the use of non-invasive imaging techniques, to early detect coronary artery disease (Chapter 3). And now, it is recognized the role of non-invasive imaging, especially in low-to-intermediate pre-test probability of coronary artery disease. On the other hand, in the definition of early detection of clinical and subclinical coronary artery disease, but also of CV risk, it is well known the role of IMT and EAT, that reflect the CV burden of risk in several patients. In more detail, while IMT is recognized also by guidelines as a real CV risk factor (although called “non-conventional”), regarding EAT, that is an emerging CV risk factor, poor is known about its real role, although today it is considered as visceral fat. These two non-conventional risk factors, largely studied in some particular cohort of

patients (especially obese patients), today also in HIV patients are often evaluated (Chapter 7). Increased IMT in HIV patients is widely recognized, meanwhile, poor was known about EAT in HIV patients. In Chapter 6, it is described for the first time an increase of EAT in HIV children and young adults, compared to healthy subjects. These results, together with the increase of IMT in this population, could explain an increased CV risk in HIV patients, and in particular, in HIV children and young adults vertically infected.

Regarding non-conventional CV risk factors again, there are also some metabolic deficit involved in CV disease. In particular, one of the most recently studied is Vitamin D deficiency (Chapter 6). Vitamin D deficiency should be responsible of atherosclerotic side effects and of heart failure development. Vitamin D deficiency or insufficiency generally starts since childhood, and this can explain also the cumulative effect of this deficit, leading to CV disease. Nevertheless, what is interesting, is the not completely clear role of Vitamin D supplementation, which although in some studies is related to an improvement of CV risk and disease, in others a real beneficial effect of supplementation was not seen.

However, modern medicine does not focus only on non-conventional CV risk factors, but there is always something new also in traditional CV risk factors. In particular, there is a new class of drugs, GLP-1 agonists, which are very effective in the oral treatment of diabetes. It is well known that diabetes, at every ages, is one of the most important CV risk factors, first for atherosclerotic disease, but also for heart failure (chapter 4). However, although there is an effect of GLP-1 agonists on CV disease related to treatment of diabetes, the most interesting thing is that GLP-1 agonists reduce all cause and CV

mortality, independently from is effects on glycaemia (Chapter 5). This effect is clear on macrovascular and microvascular events, but also on heart failure.

Although the important role of conventional and non-conventional risk factors in development of CV disease from childhood to adulthood, the major part of CV disease in children are the congenital ones. Thanks to the progress in surgery techniques, a great part of CHD arrive to adulthood, so called GUCH. However, there are some forms of CHD, which are related to particular genetic disorders, which in a great parte are incompatible with life. One of the more typical manifestation of severe CHD is hypertrophic cardiomyopathy, which in several cases is related to a specific gene mutation (Chapter 8). In more detail, there is a spectrum of genetic disease, called mitochondrial disorders, which can often be the cause of hypertrophic cardiomyopathy, and in a great number of cases are fatal. Therefore, for these class of patients, the only solution could be the heart transplant when feasible.

Nevertheless, luckily, there is a wide part of CHD that can be treated by surgery, and that lead to adulthood. However, GUCH patients develop during the years several CV risk factors and CV disease. One of the most common problem related to CHD shunt-related is the development of pulmonary hypertension (PH) (Chapter 9). Therefore, in these patients, there is a worsening of prognosis year by year, which needs a prompt treatment. In particular, the involvement of right heart is very important, and in the follow up of these patients, is known the role of the interval changes, in particular for right ventricular volumes, on the worse prognosis PH related.

Another important “side effect” that can develop in GUCH patients is arterial hypertension, especially in operated CoA patients, where there is a wide spectrum of possible side effects secondary to the intervention. CoA is a congenital disease of the proximal descending aorta defined as a hemodynamic significant aortic narrowing (Chapter 10.1, 10.2, 10.3). There are several type of intervention useful to treat CoA, from childhood to adulthood, based on the era of the diagnosis. Generally, percutaneous stent implantation is the type of intervention used in adults. These patients are generally followed up after the intervention, with several exams, such as echocardiography and cardiac magnetic resonance for the assessment of ejection fraction, left ventricular volumes, diameters of the aorta and presence of diastolic tails, and evaluation of aortic stiffness; exercise stress test, with the evaluation of blood pressure profile; ambulatory blood pressure monitoring. All these exam are important to detect the presence of several outcomes, and to define the potential predictors of endpoints, that can be responsible of a bad prognosis in operated CoA patients. In particular, beyond the clinical outcomes in these patients, it is important also to consider re-CoA rate, that could require a second intervention, and pseudoaneurysm formation, that is a common complication for these patients, that in many cases can require a re-intervention. Finally, aortic stiffness can play a role in the prognosis of operated CoA patients, in fact well known is that aortic stiffness is frequently impaired in these patients, so it should be important also define the best, less expensive, and less invasive way to measure it to define prognosis.

In conclusion, it is not true that CV disease are a peculiarity of older patients. In fact, there are several conventional and non-conventional risk factors, iatrogenic factors and metabolic deficit, which starting in childhood, could lead to CV risk and CV disease in adulthood. On the other hand, it is not true that CHD are a peculiarity of children, where thanks to the progresses in cardiac surgery, a great part of these patients arrives to adulthood, needing to be treat as adult patients with CV disease.

**CURRICULUM VITAE**

WORK EXPERIENCE

Current Position (from November 2015):
PhD in Cardiovascular Pathophysiology and Therapeutics, coordinator Prof. Bruno Trimarco, with great interest in ambulatory management of pediatric cardiology and congenital heart disease, and in research about pediatric cardiology, congenital heart disease, heart failure and transthoracic echocardiography.

January 2018-October 2018
Research training period to Inselspital, Bern, under supervision of Prof. Markus Schwerzmann, with the study of grown-up congenital heart disease, in particular on the following topics: "outcomes in patients with congenital heart disease and pulmonary hypertension" and "evaluation of outcomes in adult patients with repaired coarctation of the aorta".

From May 2015:
Specialist in Adult Cardiology.
*(Areas of interest: heart failure, pulmonary hypertension, prevention, coronary artery disease, transthoracic echocardiography, cardiopulmonary exercise testing, peripheral artery disease, pediatric cardiology, congenital heart disease).*

*Title of thesis: Prevalence and severity of asymptomatic coronary and carotid artery disease in patients with abdominal aortic aneurysm and lower limb arterial disease.*

September 2014-March 2015:
Further Pediatric Cardiology Training at Pediatric Hospital "Bambino Gesù", Rome, under supervision of Dr. Gabriele Rinelli, for the study and research in pediatric cardiology and congenital heart disease.
May 2010-May 2015:

Residency in Adult Cardiology under supervision of Dr. Pasquale Perrone Filardi at Medical School of Naples, "Federico II" University.

AWARDS

May 2018-October 2018
Research Grant of the European Society of Cardiology, conducted to Inselspital, Bern, with the project entitled "Outcomes in adult patients with repaired coarctation of the aorta".

April 2016-April 2017
Scholarship Grant of the Italian Society of Cardiology and MSD ITALY-MERCK SHARP & DOHME Corporation, with the project entitled "Evaluation of left ventricle systo-diastolic function, epicardial adipose tissue and carotid intima-media thickness in HIV children and young adults"

EDUCATION AND TRAINING

July 2009:

Degree in Medicine, 110 cum laude/110, under supervision of Dr. Pasquale Perrone Filardi and Dr. Massimo Chiariello at Medical School of Naples, "Federico II" University.

*Title of Medical Doctor thesis: Evaluation of C-Reactive Protein levels in patients with suspicious Coronary Artery Disease evaluated with SPECT.*

Mother tongue(s) Italian

Other language(s)

| | UNDERSTANDING | | SPEAKING | | WRITING |
|---|---|---|---|---|---|
| | Listening | Reading | Spoken interaction | Spoken production | |
| English | C1 | C1 | C1 | C1 | C1 |
| | English | | | | |
| French | A2 | A2 | A1 | A1 | A1 |
| | French | | | | |

ADDITIONAL INFORMATION

Memberships ESC (European Society of Cardiology)
SIC (Societa' Italiana di Cardiologia)

Partecipation to clinical trials

**Protocol TRA2P-TIMI-50.** Title: Thrombin Receptor Antagonist in Secondary
Prevention of Atherothrombotic Ischaemic Events (Sub-Investigator).

**Protocol EVINCI**. Title: EValuation of INtegrated Cardiac Imaging for the Detection and Characterization of Ischemic Heart Disease (Sub-Investigator).

**Protocol ERCAF.** Title: Efficacy of the Rhythm Control in Atrial Fibrillation patients (Sub-Investigator).

**Protocol SMARTool.** Title: Simulation Modeling of coronary ARTery disease: a tool for clinical decision support (Sub-Investigator).

**Project: Prevalence and Severity of Asymptomatic Coronary and Carotid Artery Disease in Patients With Abdominal Aortic Aneurysm.** Retrospective monocentric project conducted on patients with AAA (Co-Principal Investigator). (Related article Published in 2015).

**Project: Prevalence and severity of asymptomatic coronary and carotid artery disease in patients with lower limbs arterial disease.** Retrospective monocentric project conducted on patients with PAD (Co-Principal Investigator). (Related article Published in 2013).

**Project: Left ventricular function, epicardial adipose tissue and carotid intima-media thickness in HIV children and young adults.** Prospective monocentric bi-dipartimental project on patients with HIV (Co-Principal Investigator). (Related article in press).

**Project: Cardiovascular outcome in adults with repaired coarctation.** Retrospective multicentric project on repaired CoA patients (Co-Principal Investigator). (Related articles in press).

## LIST OF PUBLICATIONS

1. Heart rate during exercise: mechanisms, behavior, and therapeutic and prognostic implications in heart failure patients with reduced ejection fraction.

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"There comes a time in your life when you have to let go all the pointless drama and the people who create it and surround yourself with people who make you laugh so hard that you forget the bad and focus solely on the good. After all, life is too short to be anything but happy"

Carl Marx

If I dig back into my memory to the first time I thought about the possibility to do a PhD, it was the end of 2014, when I was doing my training period in pediatric cardiology to the "Bambino Gesù" hospital of Rome, under the supervision of Dr. Gabriele Rinelli. During that period, although I was involved most of all in clinical practice, I "felt the necessity" to restart with research, a field that thanks to Prof. Pasquale Perrone Filardi (my mentor since 2006, yes, it wasn't wrong, since 2006) I had entertained in the previous years, also with discrete results.

At the beginning of 2015, when I went back to Naples, I decided to ask first of all to Prof. Pasquale Perrone Filardi, and consequently to Prof. Bruno Trimarco (the cardiology head of the Federico II University of Naples), if there was a real possibility to approach the PhD in our University Federico II of Naples. Shortly thereafter, I had the great news that for the first time in Naples, thanks to the work of Prof. Bruno Trimarco, in collaboration with Prof. Emanuele Barbato, Prof. De Bruyne (Aalst, Belgium), Prof. Windecker (Bern), and

Prof. Tesorio (Mercogliano, Avellino), I should have the possibility to apply for the international PhD “Cardiovascular Pathophysiology and Therapeutics – CardioPaTh”.

I started my PhD in Naples, where I spent the first two years. In these two years I have to thank first Prof. Perrone Filardi, that supported me for the research, giving me some ideas of new papers, and correcting all my draft papers, rewarding my efforts. Nevertheless, I have also to thank Prof. Trimarco, not only for the opportunity that gave me for the PhD, but also because he appreciated a lot my efforts in research and clinical practice during my two years in Naples. The last, but not the least, I have to thank all my colleagues, that collaborated with me, in particular Dr. Claudia D’Andrea, that was a real guide in the clinical practice on pediatric cardiology, and also Dr. Stefania Paolillo, that since I started my internalship in cardiology in Naples, was always a guide for me.

As a great rule of the International PhD, I spent my last year abroad. In particular, I had the pleasure to stay to the Inselspital of Bern, in the GUCH department, under supervision of Prof. Markus Schwerzmann. During this year, I followed two projects, the first under the direct supervision of Prof. Schwerzmann, based on Pulmonary Hypertension, the second one under the supervision of Dr. Kerstin Wustmann, based on outcomes in adult patients with operated coarctation (this project gave me also the opportunity to win an ESC Research Grant).

I have to admit that life in Bern is not so simple, especially if you move from a city like Naples. First, Bern is very expensive, and the ESC Research Grant was for me not only

an important award, but also an important economic support. On the other hand, social life is not so active like the one of Naples. However, I was lucky, because I met some colleagues, which helped me in the social life; they have been an important support during this experience.

Now I am at the end of this wonderful experience, which enriched not only my career, but also my human side. For this, I have to thank Prof. Schwerzmann and all the GUCH staff, which gave me further teaching in research, although I have a lot to learn yet.

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Finally, I can't forget to thank my family. My father with his suggestions, never wrong. With his experience, he had always the "right word" for every situation. My mother, who always supported and continue to support me, without "answer back". I will never forget

when I was at my first year of medicine, and I found her to browse the pages of my anatomy book, for the curiosity about what I was studying. My brother, who was a guide for a great part of my life. A landmark for all my childhood, I was his “favorite toy”. My sister in law, that always try to understand my work (she is an engeener). Moreover, my nephew, my joy, the expression of my light heartedness, that now is too little, but I hope in the future will be able to read this thesis.


***Senectus ipsa est morbus*** is a famous sentence of the Latin writer P. Terenzio Afro, belonging to his first comedy *Phormio* (160 b.C.). He meant that diseases are typical of the elderly, asserting that elderly should be the disease. Although the major part of cardiovascular disease typically present in the older age, there is a wide spectrum of cardiovascular risk factors that starting from youth, can lead to cardiovascular disease since middle age, and on the other hand, there are several cardiac disease typical of childhood. So, very important for the cardiologist, is to be able to recognize as soon as possible cardiovascular disease and risk factors since childhood and youth.