

Novel approaches to risk stratification in common cardiovascular diseases using circulating and imaging biomarkers

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Abstract

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality. The number of individuals at risk of CVD complications continues to rise, making CVD preventative strategies an ongoing public health priority. Current risk-stratification approaches have limited predictive value, which poses a significant challenge in delivering preventative therapies. Therefore, finding new biomarkers for CVD is of interest and may improve the identification of individuals who would benefit most from preventative therapies.

The main aim of this thesis was to identify novel approaches to risk stratification in common CVD states using circulating and imaging biomarkers. This thesis consists of four studies that evaluated:

- 1) The prognostic role of circulating angiotensin converting enzyme 2 (ACE2) in coronary artery disease (**Chapter 3**);
- 2) The role of circulating and myocardial ACE2 in aortic stenosis (**Chapter 4**);
- 3) The role of abnormal myocardial tissue characterisation using native T1 time, a cardiac magnetic resonance technique in aortic stenosis (**Chapter 5**); and
- 4) The role of opportunistic screening using cardiac computed tomography (CT) to identify markers of vascular disease such as coronary artery calcium and thoracic aortic dilation in atrial fibrillation (**Chapter 6**).

The first study (**Chapter 3**) was a prospective evaluation of circulating ACE2 levels in patients with obstructive coronary artery disease. ACE2 is an integral membrane protein that degrades angiotensin II and has an emerging role as a circulating biomarker of CVD. Over a median follow up of 10.5 years, circulating ACE2 activity independently predicted the occurrence of

major adverse cardiovascular events (MACE) [hazard ratio (HR) 2.4, 95% confidence interval (CI) 1.24–4.72].

The second study (**Chapter 4**) investigated the relationship between circulating ACE2 activity levels and all-cause mortality in patients with aortic stenosis. Additionally, the relationship between circulating ACE2 activity, myocardial ACE2 gene expression, and myocardial fibrosis were examined. Higher circulating ACE2 levels were associated with increased myocardial structural abnormalities such as increased LV mass index and increased LV volumes. Over a median follow-up of 5 years, elevated circulating ACE2 activity was an independent predictor of all-cause mortality with incremental prognostic value after adjustment for relevant clinical, imaging, and biochemical parameters (HR: 2.28; 95% CI: 1.03 to 5.06). In a subset of patients with myocardial tissue collection, increased circulating ACE2 was associated with reduced myocardial ACE2 gene expression and severe myocardial fibrosis.

The third study (**Chapter 5**) investigated whether diffuse myocardial fibrosis characterised by greater native T1 times on cardiac magnetic resonance provides prognostic utility to predict MACE (all-cause mortality or heart failure hospitalisation) in patients severe AS being evaluated for transcatheter aortic valve replacement. Native T1 times were higher than previously reported values in healthy controls. Patients with higher T1 times were more likely to have evidence of myocardial decompensation demonstrated by higher NT-proBNP levels and lower left ventricular ejection fraction. Over a median follow-up of 3.1 years, increased native T1 time (HR:1.68; 95% CI:1.08 - 2.62) and age > 75 years (HR:1.82;1.01–3.28) were independent predictors of MACE.

The fourth study (**Chapter 6**) investigated the prevalence of incidental vascular diseases, including thoracic aortic aneurysmal disease and coronary atherosclerosis, both important causes of cardiovascular mortality, in patients with atrial fibrillation. In a cross-sectional study of 1,000 consecutive patients with atrial fibrillation, one in five were found to have newly identified thoracic aortic aneurysmal disease on multidetector chest CT. A small proportion (1%) had significantly aneurysmal dimensions that approached surgical thresholds. Although two-thirds of the cohort had coronary calcification, 38% were not taking lipid-lowering therapy. The findings therefore represent an opportunity to substantially optimise preventative measures in AF.

Overall, the findings of the thesis suggest that the circulating and imaging biomarkers evaluated in the above studies, provide incremental diagnostic and/or prognostic information in patients with CVD. The findings need confirmation in large independent cohorts before these biomarkers can be incorporated into risk stratification algorithms.

Declaration

This is to certify that:

The thesis comprises my original work towards the Doctor of Philosophy
except where indicated in the preface;

1. Due acknowledgement has been made in the text to all other materials
used;
2. The thesis is fewer than 100,000 words, exclusive of tables, maps,
bibliographies and appendices.

Dr Jay Ramchand

Preface

This PhD thesis by publication combines multiple projects with the common theme of studying novel approaches to risk stratification in common cardiovascular diseases using circulating and imaging biomarkers.

This thesis includes three original manuscripts that have been published in peer-reviewed cardiovascular journals and one submitted manuscript under review. Each manuscript is listed as an original research chapter within the thesis. These manuscripts include multiple co-authors, as the work was generated from active collaboration between researchers. All co-authors that have actively collaborated on material contained within the thesis have signed a 'Co-author Authorisation Form' for each manuscript.

The contribution of the candidate for each chapter is outlined in the table below. The candidate performed the literature review, designed the studies with supervision, created case record forms, obtained ethics board approvals, directly participated in patient recruitment, collected/processed patient blood and tissue samples, performed data collection, master database creation, data cleaning and statistical analysis. Recruitment for the aortic stenosis cohort was performed by Dr Leighton Kearney. Pathology testing was conducted by the Department of Pathology, Austin Health. Cardiac tissue was collected with the assistance of Associate Professor George Matalanis and colleagues within the Department of Cardiac Surgery, Austin Health. Performance of circulating ACE2 activity and gene expression studies was aided by the laboratory staff of the University of Melbourne, Department of Medicine, Austin Health. The candidate was responsible for the formation of all initial manuscript drafts, coordination of feedback from all co-authors, editing each manuscript according to feedback

from co-authors and peer reviewers, completing submission and the peer review process for each manuscript (including proof review) for final publication. The School of Mathematics and Statistics, University of Melbourne provided statistical support for the publication in *JACC Cardiovasc Imaging*.

Thesis Chapter	Publication title	Publication Status	Nature and extent of student contribution
3	Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease	Published	Manuscript concept, design, creation and preparation (70%)
4	Plasma ACE2 activity predicts mortality in aortic stenosis and is associated with severe myocardial fibrosis	Published	Manuscript concept, design, creation and preparation (70%)
5	Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with chronic kidney disease and severe aortic stenosis	Under review (revision submitted 17/02/2021)	Manuscript concept, design, creation and preparation (70%)
6	Incidental thoracic aortic dilation on chest computed tomography in patients with atrial fibrillation	Published	Manuscript concept, design, creation and preparation (70%)

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Figure 2, page 10	Grace JA et al. <i>Clin Sci (Lond)</i> 2012;123:225-39	Yes
Figure 3, page 11	Grace JA et al. <i>Clin Sci (Lond)</i> 2012;123:225-39	Yes

Publications

(a) Peer-reviewed journal papers

(i) Related to thesis

The following peer-reviewed journal articles and research reports were published, accepted or submitted during PhD candidature. Those included as chapters (**) or appendices (*) in this thesis are indicated.

1. Nagaraja V, Ramchand J (co-first author), Bolen MA, Das R, Krishnaswamy A, Kapadia SR, Puri R, Harb S. What is the Role of Cardiac Magnetic Resonance Imaging in Transcatheter Management of Aortic Valve Stenosis? *Structural Heart (in press)*. *
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(ii) Other publications completed during candidature

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Abbreviations

ACE2	Angiotensin converting enzyme 2
ACS	Acute coronary syndrome
AF	Atrial fibrillation
ANG	Angiotensin
AS	Aortic stenosis
AVA	Aortic valve area
AVR	Aortic valve replacement
BNP	B-type natriuretic peptide
CAC	Coronary artery calcium
CAD	Coronary artery disease
CI	Confidence intervals
CVD	Cardiovascular disease(s)
CMR	Cardiac magnetic resonance
CRP	C-reactive protein
CT	Computed tomography
ECG	Electrocardiogram
GLS	Global longitudinal strain
HR	Hazard ratio
LGE	Late gadolinium enhancement
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
MACE	Major adverse cardiovascular events

MI	Myocardial infarction
NT-proBNP	N-terminal pro B-type natriuretic peptide
NRI	Net reclassification index
RAS	Renin-angiotensin system
TAAD	Thoracic aortic aneurysmal disease
TAVR	Transcatheter aortic valve replacement

Chapter 1: Introduction

1.1 Thesis outline

Cardiovascular disease (CVD) is a leading cause of mortality in Australia, accounting for one in four deaths (1). Primary and secondary prevention of CVD and its sequelae are therefore essential public health priorities. Commonly used risk-stratification approaches have limited predictive value, which poses a significant challenge to the timely delivery of preventative therapies to the individuals at highest risk of CVD complications (2,3). Therefore, finding new biomarkers for CVD is of interest and may improve the identification of individuals who would benefit most from preventative therapies.

With regards to circulating biomarkers, angiotensin converting enzyme 2 (ACE2) has emerged as a marker of adverse outcomes in CVD (4-6). ACE2 is a member of the counter-regulatory axis of the renin-angiotensin system (RAS), and its primary role is to degrade the vasoconstrictor and pro-hypertrophic, profibrotic peptide, angiotensin (Ang) II, thereby limiting its adverse effects. We have previously demonstrated that circulating ACE2 levels are low in healthy individuals without apparent CVD (7). Conversely, circulating ACE2 levels are elevated and predict adverse outcomes in individuals with CVD, including heart failure (4,8) and atrial fibrillation (AF) (6). Prior to the commencement of this PhD, there had been no studies that comprehensively evaluated circulating ACE2 in common CVDs such as aortic stenosis (AS) or coronary artery disease (CAD), and no human studies that assessed both circulating and myocardial ACE2.

Improvements in imaging techniques such as cardiac magnetic resonance (CMR) and computed tomography (CT) have led to the increasing use of these modalities in detecting subclinical disease (9-13). In AS, there is interest in identifying subclinical markers of LV decompensation beyond the use of LVEF and clinical symptoms alone. There is accumulating evidence that *focal* myocardial fibrosis detected on CMR is predictive of adverse cardiovascular outcomes (9,10). More recently, novel T1 mapping techniques have been used to estimate *diffuse* fibrosis (14). Estimates of myocardial T1 values correlate with the extent of fibrosis on histology (14-17), though studies relating these to clinical outcomes are limited. Additionally, the clinical significance of T1 mapping techniques in high-risk populations with AS, such as in those with chronic kidney disease (CKD) (18,19), is not fully understood.

Similar to CMR, cardiac CT has a growing list of applications to enhance risk stratification. Cardiac CT is particularly well suited to identify vascular pathologies, such as CAD and thoracic aortic aneurysmal disease (TAAD), leading causes of morbidity and mortality. In patients with AF, cardiac CT is used routinely before catheter ablation to assess pulmonary venous anatomy (20). This provides an unexploited opportunity to concurrently screen for adverse markers of vascular disease such as coronary artery calcium (CAC) and thoracic aortic dilation.

In this thesis, a sensitive circulating ACE2 assay was used to measure ACE2 and assess its prognostic role in common CVDs such as CAD and AS (**Chapters 3 and 4**). Myocardial tissue studies were also incorporated to examine the relationship between circulating ACE2 activity, myocardial ACE2 gene expression, and myocardial fibrosis (**Chapter 4**). The role of advanced cardiac imaging techniques using CMR and CT to

enhance cardiovascular risk stratification in AS and AF was evaluated. (**Chapters 5 and 6**).

1.2 Hypothesis

We hypothesised that the addition of novel circulating and imaging biomarkers would provide incremental diagnostic and/or prognostic information in common cardiovascular conditions.

1.3 Aims and objectives

1. To investigate the long-term prognostic utility of circulating ACE2 activity levels to predict cardiovascular events in patients with CAD (**Chapter 3**);
2. To evaluate the relationship between ACE2 activity levels, the severity of stenosis and myocardial remodelling in patients with AS; and to determine if circulating ACE2 levels offered incremental prognostic usefulness to predict mortality (**Chapter 4**);
3. To evaluate the prognostic role of abnormal myocardial tissue characterisation using native T1 time, a CMR technique, in AS (**Chapter 5**); and
4. To evaluate the role of opportunistic screening using cardiac CT to identify the presence of incidental vascular disease in patients with AF (**Chapter 6**).

Chapter 2: Literature Review

2.1 Biomarkers in cardiovascular disease

2.1.1 Burden of cardiovascular disease

According to the Australian Institute of Health and Welfare, an estimated 1.2 million individuals (5.6%) live with CVD (1). Despite advances in CVD treatment, mortality from CVD remains high, accounting for one in four of all deaths in Australia (1). Importantly, Medicare expenditure attributable to CVD treatment poses a significant economic burden, costing an estimated \$10.4 billion in 2015-2016 (1). As such, preventative measures to reduce the burden of CVD remain public health priorities.

2.1.2 General rationale for cardiovascular biomarkers

For preventative measures to be delivered successfully, there is a need to reliably identify individuals at the highest risk of developing adverse cardiovascular events. This process is known as risk prediction and conventionally relies on assessing risk factors or predisposing conditions such as dyslipidaemia, hypertension and diabetes (21). By and large, the process of CVD risk stratification has not substantially changed since the phrase “factors of risk” was coined by Dr William Kannel in 1961 (22). Although this traditional method of risk factor assessment remains valuable in modern-day cardiovascular medicine, it has limitations. For instance, a large proportion of those who develop CAD (up to 50%), have none or only one of these traditional risk factors (3,23). Paradoxically, such individuals are less likely to be targeted with primary prevention strategies if

traditional risk factors are considered in isolation (24). Therefore, adjunctive methods to enhance risk stratification are needed, so preventative therapies can be appropriately targeted to those most vulnerable for future cardiovascular events.

The need to supplement traditional risk factor assessment has led to the discovery of alternative biomarkers, which permit better identification of individuals at risk of CVD and to prognosticate more precisely. In 2001, the National Institutes of Health standardised the definition of a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (25). The standardised definition emphasises that biomarkers are not limited to circulating markers, which is the typical context in which it is used in cardiovascular literature. Thus, biomarkers can be measured in various biological samples (such as blood, urine or genetic material) or it may also be a characteristic on physical examination, recordings such as electrocardiography or derived from imaging tests such CMR.

There is definitive evidence that CVD development occurs over long periods of time, evolving from predisposing risk factors to subclinical disease before culminating as apparent CVD (26-29). The utility of a biomarker, therefore, depends on its representation in the trajectory of disease evolution. Thus, biomarkers can be classified as antecedent markers (before developing disease), screening (for subclinical disease), diagnostic, staging (of disease severity) or prognostic (predictive of disease trajectory) (25). Imaging biomarkers typically detect subclinical CVD but have limited value in earlier disease formation phases (Figure 2.1) (29,30). On the other hand, genetic markers may indicate the inherent risk of CVD development though it provides no indication about the

development of subclinical disease (31,32). Circulating biomarkers can reflect physiological pathways that precede subclinical and clinically manifest disease (33-35).

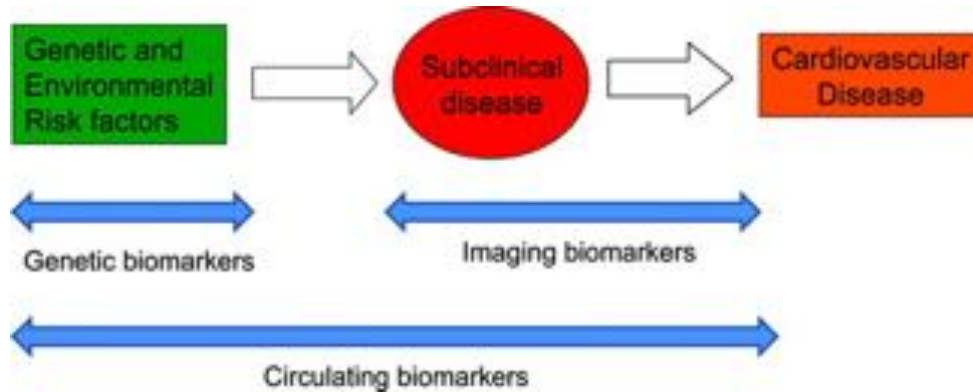


Figure 2. 1 Progression from risk factors to overt cardiovascular disease

The stages at which biomarkers are most effective are shown. Genetic markers indicate the inherent risk of cardiovascular disease development whereas imaging markers detect subclinical or established disease. Circulating biomarkers can reflect pathophysiological processes early or late in disease development. Reproduced with permission from Wang TJ. Circulation 2011; 123:551-65 (21).

2.1.3 Features of an optimal cardiovascular biomarker

The primary expectation of an optimal biomarker is to improve patient management. For example, in the evaluation of patients presenting with acute chest pain, use of biomarkers may expedite accurate diagnosis by reliably distinguishing between acute coronary syndromes (ACS) (high sensitivity troponin T) or pulmonary embolism (CT pulmonary angiogram). In patients with established CVD, biomarkers may enhance patient management through reliable prognostication. For instance, post myocardial infarction (MI), persistently low left ventricular ejection fraction (LVEF) is associated with sudden death, the risk of which can be attenuated with implantation of a cardiac defibrillator

(36,37). Regardless of its intended use, an optimal biomarker should ideally be 1) accurate, 2) reliable 3) non-invasive and easy to obtain 4) easy to measure and interpret, 5) rapid laboratory or processing turnaround time and 6) highly sensitive and specific (38-40). Other criteria to support the routine clinical use of a biomarker include incremental usefulness above existing models, cost-effectiveness and prospective validation in independent patient cohorts.

Assessing the incremental value of a biomarker over traditional risk factors is challenging. Although it is necessary to have a statistically significant association on multivariable models, this in itself is insufficient to confirm incremental usefulness (21). Statistical parameters such as hazard ratio (HR) may be significant despite overlap in biomarker distribution between normal and at-risk groups (41). Other criteria to assess for incremental usefulness are therefore recommended. These include discrimination (C statistic and/or integrated discrimination index, IDI), calibration (Hosmer-Lemeshow statistic) and reclassification (net reclassification index, NRI) (42,43).

2.2 Circulating biomarkers and cardiovascular disease

2.2.1 An overview of guideline endorsed circulating biomarkers

There are a plethora of available circulating CVD biomarkers in the literature. Some are deeply embedded in risk stratification algorithms and concurrently serve as treatment targets (24,44). These include total cholesterol, high-density lipoprotein, low-density lipoproteins, haemoglobin A1c and glucose levels. Aside from these traditional markers, the two cardinal circulating markers commonly used include the cardiac natriuretic

peptides [B-type natriuretic peptide (BNP) and N-terminal pro BNP] and cardiac troponin (36,45-48), reflecting myocyte load and injury respectively.

In acute CVD presentations such as heart failure and ACS, natriuretic peptides and troponin measurements have transformed management algorithms and are guideline-endorsed (36,45,46). In chronic CVD however, there remains a lack of circulating markers to refine risk stratification (49). Although both natriuretic peptides and troponin remain robust prognostic markers, these markers have no definite role in altering management decisions in the chronic setting. Indeed, in conditions such as chronic CAD and heart failure, management guidelines mostly offer generic recommendations with limited opportunities for personalised therapy titration beyond the first few years of diagnosis (36,50-52).

In preventative cardiology, there are no guideline-recommended circulating biomarkers beyond the aforementioned traditional metabolic markers (24,44). There has been emerging interest in studying markers of chronic inflammation, considering the major role of inflammation in the pathogenesis of atherosclerosis (53). The role of C-reactive protein (CRP), an acute phase protein released by the liver (54), as a cardiovascular biomarker has been most extensively studied. Despite being strongly associated with adverse cardiovascular events, there is accumulating evidence that at most, there are only minimal improvements in discrimination and reclassification when compared to the coronary calcium score with a net reclassification improvement of 66% (55-57). Consequently, the routine use CRP is generally not recommended with the possible exception of guiding preventative therapies in those with intermediate cardiovascular risk (24).

Thus, although powerful biomarkers such as natriuretic peptides and troponin are in our armamentarium to manage acute cardiovascular conditions, there remains a need for circulating biomarkers in chronic CVD to allow more personalised treatment. Additionally, there are no clinically used circulating markers that specifically reflect other important cardiovascular pathophysiological pathways including dysregulation of the RAS or myocardial fibrosis.

2.2.2 Where does circulating angiotensin converting enzyme 2 fit in?

There is accumulating evidence that circulating levels of ACE2, a peptide within the RAS, may serve as a biomarker of adverse cardiovascular outcomes. In the subsequent sections, the role of ACE2 as it relates to the alternative arm of the RAS and the role of circulating ACE2 as a prognostic biomarker in CVD are discussed.

2.2.3 Discovery of angiotensin converting enzyme 2

Approximately two decades ago, during efforts to search for ACE homologues, two groups independently identified ACE2 from a LV cDNA library prepared using an explanted heart from a heart transplant recipient (58) and a human lymphoma cDNA library (59). A few years later, ACE2 was found to be a functional receptor for the severe acute respiratory syndrome (SARS) coronavirus (60) responsible for the 2003 epidemic. More recently, there have been global efforts to improve our understanding of ACE2 following the discovery that SARS-CoV-2 (61,62), the betacoronavirus strain responsible

for the ongoing coronavirus pandemic (COVID-19), also uses ACE2 as a functional receptor to gain entry into human cells (63) through an interaction with its spike protein.

ACE2 is an integral cell membrane protein that can be found ubiquitously in various tissues including the cardiovascular system (64-67), kidneys (68,69), retina (70), brain (71) and lungs (60,72-74). ACE2 is a member of the counter-regulatory, protective axis of the RAS, and its primary role is to degrade the pro-hypertrophic, pro-fibrotic and vasoconstrictor peptide, Ang II (58,75). It cleaves Ang II to form the peptide Ang-(1-7), which in turn acts on the Mas receptor to exert opposing vasodilatory and anti-fibrotic effects (Figure 2.2) (76).

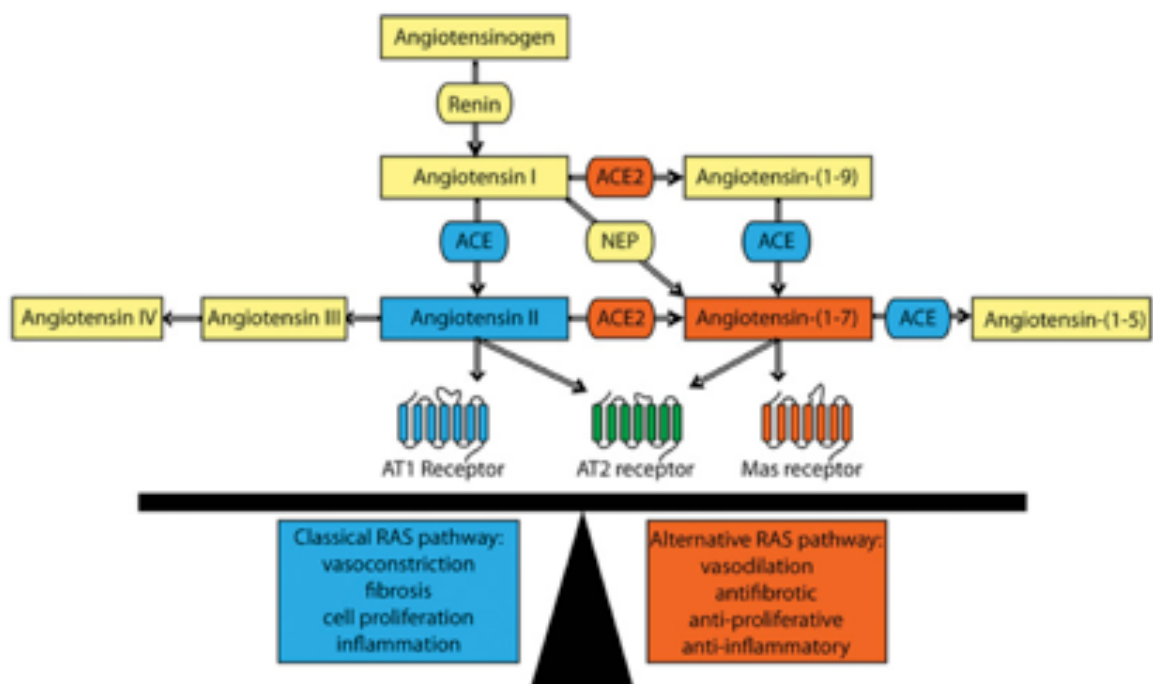


Figure 2. 2 Overview of the renin-angiotensin system

The renin-angiotensin system is an important hormonal system involved in cardiovascular physiology. The net effects of the renin-angiotensin system are governed by a balance between the angiotensin II mediated, ‘classical axis’ shown in blue and the

angiotensin 1-7 mediated 'alternative axis' shown in orange. The effects of the angiotensin II mediated pathway is pro-inflammatory and pro-fibrotic whereas the angiotensin 1-7 pathway is anti-inflammatory and anti-fibrotic. Reproduced with permission from Grace JA et al. Clin Sci (Lond) 2012;123:225-39 (76) .

Mice deficient in ACE2 have elevated Ang II levels and severe myocardial dysfunction that can be reversed by the genetic deletion of ACE (67). On the other hand, over-expression of myocardial ACE2 prevents Ang II-induced hypertension and fibrosis (77). Together, these initial studies defined the important cardioprotective role of ACE2 in maintaining normal cardiovascular function. ACE2 exists in both a membrane-bound and a soluble form (Figure 2.3); the latter results from proteolytic cleavage of the catalytically active ectodomain by the proteinase ADAM17 (a disintegrin and metalloprotease), also known as tumour necrosis factor- α converting enzyme (TACE) (78). This shedding leads to the release of ACE2 into the circulation, which may confer a loss of protection against the classic RAS at the tissue level.

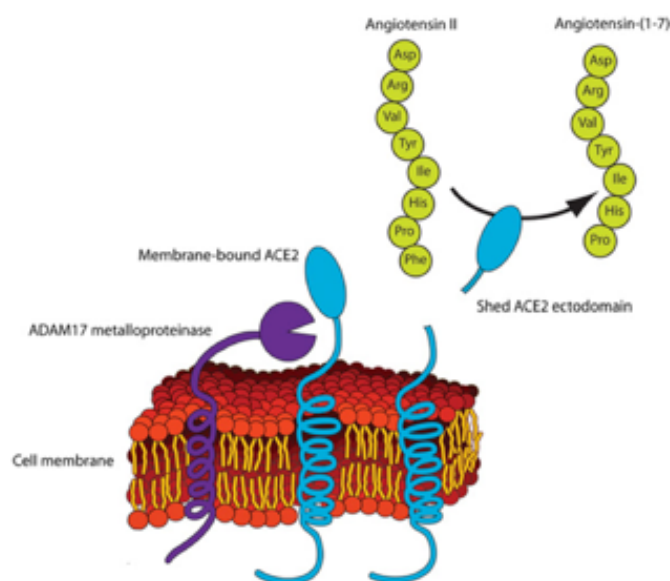


Figure 2. 3 *Cleavage of membrane-bound angiotensin converting enzyme 2 by ADAM17*

Tissue ACE2 undergoes shedding by the metalloprotease ADAM17 which releases of a soluble form of ACE2 into the circulation. The significance of this shedding process is not entirely clear though is suspected to result in the loss of protection from the 'classic' RAS axis in tissue. Reproduced with permission from Grace JA et al. Clin Sci (Lond) 2012;123:225-39 (76)

2.2.4 Measurement of circulating angiotensin converting enzyme 2

As a result of shedding from tissue membranes (78), ACE2 can be measured in the circulation (7). Most of the initial reports on circulating ACE2 activity in humans used an ACE2-specific quenched fluorescent substrate (QFS) [(7-methoxycoumarin-4-yl)acetyl (Mca)-Ala-Pro-Lys-2,4-dinitrophenyl (Dnp)]. The initial study by Vickers et. al (75) reported specific hydrolysis of this ACE2 specific QFS with recombinant human ACE2. A QFS is a short peptide that is recognised and cleaved by the peptidase of interest and is flanked by a matched fluorophore and quencher pair (79). This pairing takes advantage of the fluorescent resonance energy transfer, whereby the quencher moiety absorbs energy emitted by the fluorophore. This quenching effect occurs only when the two flanking components are close to one another (less than 50 Angstrom). Cleavage of the linking amino acid sequence by a peptidase allows the quencher to no longer remain linked with the released fluorophore Mca-Ala-Pro and results in increased fluorescence (Fig. 2) (80). The fluorescence of the samples is then measured using a fluorometer with prespecified excitation and emission wavelengths (79). The assay sample is generally set up in parallel with an ACE2-specific inhibitor to determine non-specific activity and the relative fluorescence of the inhibited sample is then subtracted from the non-inhibited sample (7,80,81). The actual amount of ACE2 enzyme activity in the assay is then

determined from a standard curve of relative fluorescent units plotted against known concentrations of the fluorophore (7,80,81). Although the use of enzymatic assays to measure ACE2 provide accurate data regarding its functional level, it does not allow for determination of serum protein levels.

Other methods previously used to measure circulating ACE2 concentration include the enzyme-linked immunosorbent assay (ELISA) and high-throughput multiplex immunoassay-based methods. In the latter method, up to 92 markers can be simultaneously measured using proprietary proximity extension assay technology (82-84). Each biomarker is matched to a pair of antibodies, coupled to unique, partially complementary oligonucleotides, and measured by quantitative real-time polymerase chain reaction yielding relative but not absolute quantification of ACE2 levels. Importantly, there are no published reports correlating relative levels detected by these assays to known concentrations of ACE2 using validated assays.

We have reported that ACE2 activity can be detected in human plasma although an endogenous inhibitor must be removed in order for activity to be measurable (7). The addition of neat plasma to recombinant soluble human ACE2 inhibited ACE2 activity in a dose-dependent fashion (7). Partial purification of this inhibitor indicated that the inhibitor was small and cationic, and removal of this using anion-exchange chromatography allowed detection of plasma ACE2 activity, measured using the aforementioned QFS-based assay. In a subsequent study by Roberts et al in patients with CKD, plasma ACE2 activity was measured with and without extraction of the endogenous inhibitor (85). The authors reported significantly lower ACE2 without

extraction of the endogenous inhibitor, confirming the physiological relevance of this inhibitor.

2.2.5 Circulating angiotensin converting enzyme 2 in those without apparent cardiovascular disease

The Leeds family study by Rice et al was the first to measure circulating ACE2 in individuals without apparent CVD or diabetes (N = 534). Circulating ACE2 activity was only detectable in 40 (7%) individuals (86) who were older, more likely to be obese with higher blood pressure, glucose and cholesterol levels. Our group then measured ACE2 activity in healthy volunteers and found that ACE2 activity levels were low and within a tight distribution (mean $\pm$ SEM of 4.44 ± 0.56 pmol/ml/min) (7). Taken together, these initial reports found low circulating ACE2 levels in healthy individuals without apparent CVD and increased levels in subjects with cardiovascular risk factors.

2.2.5.1 Sex differences in circulating angiotensin converting enzyme 2

Circulating ACE2 levels have been shown to vary according to sex, with higher levels observed in male subjects. In a large cohort of control subjects from the NEFRONA study without known CVD (control group N = 568), male gender was associated with higher plasma ACE2 activity (male subjects 61.4 ± 2.3 vs. female 42.5 ± 1.6 RFU/ μ L/h) (87). In another study performed in the general population (PURE study, N = 10,753), male gender was the strongest determinant of plasma ACE2 levels (84). In a study from our group involving patients with a history of kidney disease, male patients on haemodialysis had higher levels than female patients on haemodialysis [N = 100, 12.1 pmol/mL/ min

(6.8–19.6) compared with 4.4 pmol/mL/ min (2.5– 10.3), $P < 0.01$] (85). As will be discussed in the following section, there is also clear evidence from other studies involving patients with CVD that male gender is independently associated with higher plasma ACE2 levels (6,83,87). The underlying reason why ACE2 activity is higher in men is uncertain but requires further examination considering that the development of CVD also demonstrates gender-specific differences. It is of interest that the *ACE2* gene is located on the X chromosome and genetic *ACE2* studies have also demonstrated sex specific associations (88,89). Although one female X chromosome is randomly inactivated (X-inactivation), the location of ACE2 on Xp22 is within an area that can escape X-inactivation, which may explain phenotypic differences between sexes (89-92). In a study from our group involving Caucasians with type 2 diabetes, genetic variation in ACE2 was associated with reduced LVEF in men, but not in women (88).

2.2.6 Circulating angiotensin converting enzyme 2 and cardiovascular disease

There is a growing body of evidence to suggest that plasma ACE2 activity may serve as a marker of cardiovascular abnormalities and/or adverse outcomes in CVD including heart failure, CAD and AF as discussed in the sections below.

2.2.6.1 Heart Failure

Epelman et al. (4) were the first to assess the feasibility of measuring plasma ACE2 in human heart failure in a cohort of patients suspected of having heart failure (N = 228). Using a modified QFS-based assay, they found that plasma ACE2 activity was elevated in patients with heart failure and that levels correlated with worsening LVEF and New York Heart Association functional class. In another study, Epelman et al. delineated the

relationship between plasma ACE2 activity and imaging indices of cardiac structure and function in a cohort of 113 patients with stable chronic systolic heart failure (8). They found elevated circulating ACE2 activity correlated with depressed biventricular systolic function, increased LV diameter, elevated pulmonary artery pressure and higher NT-pro-brain natriuretic peptide (NT-proBNP) levels. Circulating ACE2 independently predicted adverse clinical events defined as a composite of death, cardiac transplant and heart failure hospitalisations (8). Patients with both NT-proBNP and circulating ACE2 activity elevation had increased mortality compared to those with an elevation of only one biomarker, suggesting the incremental value of adding ACE2 to BNP measurements. In a study by Wang et al. involving patients with idiopathic dilated cardiomyopathy, plasma ACE2 activity was higher in patients with cardiomyopathy compared to healthy controls and those with higher ACE2 levels had increased adverse events (death or cardiac transplantation) (93). In a report of patients with heart failure due to Chagas disease (94), the collective measurement of circulating ACE2 activity and BNP improved the prediction of adverse cardiac events, once again suggesting that the combined assessment of these two biomarkers levels may have a role in improving risk prediction in heart failure.

2.2.6.2 Atherosclerotic cardiovascular diseases

Several lines of evidence suggest that plasma ACE2 activity may serve as a marker of atherosclerosis. In a study of circulating ACE2 activity in subjects with stage 3-5 CKD (N = 1458) without prior cardiovascular events, higher circulating ACE2 activity at baseline was independently associated with progressive, silent atherosclerosis defined by an increase in the number of carotid and peripheral vascular territories with plaques at

two-year follow-up (95). These findings suggest that measurement of circulating ACE2 activity may help identify individuals with CKD at highest risk of atherosclerotic CVD. This is particularly important considering CVD is a leading cause of mortality in patients with CKD (96). Many other circulating cardiovascular biomarkers such as troponin and BNP are known to be increased in the setting of CKD (97), and efforts to establish the role of circulating ACE2 as a biomarker in CKD is of interest particularly considering that kidney dysfunction in itself is not known to alter circulating ACE2 activity levels (85).

In another study (5) of subjects with angiographically confirmed CAD following ST-elevation MI (N = 95), circulating ACE2 activity measured within 48 hours of the event was significantly elevated when compared to controls without known CVD (N = 22). Serum ACE2 inversely correlated with LVEF and positively correlated with infarct size on CMR though it unclear whether the increase in circulating ACE2 was a reflection of acute myocardial injury or underlying atherosclerosis (5).

In a prospective case-control study comparing patients with embolic stroke of undetermined source against a matched control group (N = 98), circulating ACE2 activity was significantly higher in the stroke group. ACE2 activity was independently associated with stroke independent of left atrial volume (98).

2.2.6.3 Arrhythmias

Recent data suggest an association between increased plasma ACE2 activity and more advanced myocardial remodelling in arrhythmic disorders. Walters et al. examined

plasma ACE2 activity in patients with AF (N = 123) (99). Elevated plasma ACE2 was associated with older age, male gender, hypertension, vascular disease, elevated LV mass, impaired diastolic function and imaging evidence of advanced atrial disease. There was a significant relationship between ACE2 activity and electrophysiologic indices of advanced atrial remodelling (99). Given that advanced atrial remodelling is associated with poorer outcomes from catheter ablation of AF, plasma ACE2 measures may thus have a role in guiding the prediction of the likelihood of successful catheter ablation.

In a small study (N = 57) of patients with severe LV dysfunction with primary prevention implantable cardioverter/defibrillator, serum ACE2 activity was significantly increased in patients who developed ventricular arrhythmias (N = 16) over one-year follow-up compared to patients who did not (N = 41) (100). Similar to above findings in human AF, elevated plasma ACE2 may hence reflect more advanced ventricular remodelling. The study findings are consistent with available experimental evidence in ACE2 transgenic mice, who have a higher incidence of sudden death (101). Detailed electrophysiology studies in these animals reveal severe, progressive conduction and rhythm disturbances with sustained ventricular tachycardia and terminal ventricular fibrillation (101).

2.2.6.4 Evidence gaps

The precise relationship between tissue and circulating levels of ACE2 is not yet understood. In experimental models, deletion or loss of ACE2 from the myocardium is associated with increased levels of tissue Ang II, which leads to cardiac fibrosis and dysfunction (67), as well as increased levels of circulating ACE2 activity (102). There are no human studies that have simultaneously assessed circulating and myocardial levels

of ACE2, and none that have comprehensively evaluated circulating ACE2 in common CVD states such as AS or CAD.

2.3 Imaging biomarkers and cardiovascular disease

Although circulating biomarkers such as BNP and troponin provide actionable information on vital pathophysiological aspects of CVD such as myocardial stretch and cardiomyocyte damage, other key mechanisms of cardiovascular pathophysiology such as calcification or fibrosis remain mostly unsatisfactorily assessed by circulating markers alone. The ability to detect some of these mechanisms to prevent CVD progression is therefore a major clinical interest and has led to ongoing developments in non-invasive cardiovascular imaging techniques. There are a plethora of available imaging markers in the literature. Some of the most important prognostic imaging markers and those relevant to this thesis are discussed below.

2.3.1 Atherosclerosis markers

2.3.1.1 Coronary artery calcium

The presence of CAC, a pathognomonic marker of atherosclerosis, has emerged as a factor strongly associated with the development of adverse cardiovascular events. CAC on CT has been demonstrated to strongly correlate with atherosclerotic plaque burden on histology (103). Initial studies of CAC were performed using chest radiography or fluoroscopy though, in recent decades, precise quantification of CAC was made possible using multidetector CT scanning (104). Contemporary CAC protocols can now be

accomplished with 10-15 minutes of total scan time with an effective radiation dose of about 1mSv, comparable to that of screening mammography (105). A standard approach to quantify CAC is the Agatston method which is derived semi-automatically using the product of CAC plaque area in each slice and the plaque density factor, summed for all coronary artery slices (106). The density factor increases proportionally with increasing attenuation as measured by Hounsfield units.

Multiple population-based cohort studies have convincingly demonstrated that the presence of coronary calcification quantified using the Agatston score predicted the occurrence of adverse cardiovascular events beyond conventional risk factors (11-13,107-110). One example is the Multi-Ethnic Study of Atherosclerosis study involving 6814 individuals from four racial groups without known CAD, followed initially for 3.8 years (13). Compared with individuals with no CAC, the hazard ratio for an adverse coronary event was 7.7 for a score between 100-300 and 9.7 for a calcium score of >300. The use of CAC led to a significant net reclassification of 55% [95% confidence interval (CI) 41-69%] among subjects at intermediate risk. Major society guidelines therefore recommend the use of CAC to guide commencement of primary prevention statin therapy in those at intermediate (7.5 – 19.9%) ten-year atherosclerotic risk (24,44,111,112). With some exceptions, it is also recommended that CAC is not generally used in those at low (<5% ten-year atherosclerotic CVD risk) or high risk (>20% ten-year atherosclerotic CVD risk) as the results are unlikely to alter management.

Although CAC is one of the most robust non-invasive biomarkers, there are a few notable limitations that relate to current methods of quantification. Importantly, as non-calcified plaque can occur, the absence of CAC should not be equated to the absence of coronary

atherosclerosis (113). Furthermore, as mentioned above, the Agatston CAC score increases with increased calcium density which would suggest an increased risk of developing adverse cardiovascular events. Importantly however, there is recent evidence that although CAC volume is positively associated with adverse cardiovascular events, CAC density actually correlated inversely with adverse cardiovascular outcomes (114-116). Thus, the Agatston method to quantify CAC may not be the most optimal metric in determining long-term cardiovascular risk and future refinements to this longstanding approach of quantifying CAC are needed.

2.3.1.2 Carotid intima-media thickness

The carotid intima-media thickness (CIMT) measures the carotid artery intima-media complex distance in various segments using high resolution ultrasound or magnetic resonance imaging (112). In a meta-analysis on individual data from 14 studies involving 45,484 individuals without known CVD, CIMT predicted the incidence of first MI or stroke (HR 1.09 per 0.10mm CIMT difference, 95% CI 1.07-1.12) (112). There was only modest net reclassification improvement (0.8% in the entire cohort and 3.6% in those at intermediate risk). Additionally, it remains unknown if therapeutic strategies in those with abnormal CIMT offer measurable cardiovascular benefits. The most recent guidelines for assessing CVD risk from the American College of Cardiology/ American Heart Association have therefore not recommended the routine use of this technique (44).

2.3.2 Cardiac chamber quantification and diastology

2.3.2.1 Left ventricular ejection fraction

The LVEF is the most commonly reported measurement of left ventricle (LV) systolic performance. It is one of the most frequently used imaging parameters in diagnosing, prognosticating and managing a number of cardiovascular disorders. Lower LVEF is a strong predictor of worse outcomes in a number of disorders including heart failure, AS and CAD (117-120). Therefore, assessment of LVEF is incorporated in management algorithms to guide various treatment decisions for example, timing of AVR in AS or implantation of a defibrillator in individuals with heart failure. Given its widespread availability, transthoracic echocardiography is typically the first-line imaging modality recommended to measure LVEF (36). CMR, radionuclide ventriculography or cardiac CT can be considered if echocardiography is suboptimal. The LVEF has many, clinically significant shortcomings due to suboptimal image quality, variation between different imaging modalities and lack of reproducibility (121). These factors diminish the reliability of LVEF to detect earlier and more subtle changes in LV systolic performance necessitating the need for complementary methods.

2.3.2.2 Myocardial deformation

LV contraction and relaxation are complex processes involving longitudinal (base to apex), radial (transverse) and circumferential (twisting) motions (122-124). The development of tissue Doppler imaging and speckle tracking echocardiography has enabled quantitative analysis of individual components of myocardial deformation to provide more sensitive information compared to the use of LVEF alone. Speckle tracking echocardiography is a more recently developed angle-independent technique for

assessing myocardial function that can be used to derive parameters of myocardial function such as velocity, strain and strain rate which can be superimposed upon real-time echocardiography images (125). Today, the component of LV function most widely assessed by myocardial deformation is myocardial strain. Myocardial strain describes the proportional change in length of a myocardial segment and can be assessed in longitudinal, radial or circumferential planes (122-124). Strain is typically measured during systole and is expressed as a percentage. Systolic contraction of longitudinal and circumferential fibres results in negative strain such that a 10 cm muscle segment that contracts to 8 cm has a strain of -20% (126). Feature tracking using CMR is another method that allows the measurement of myocardial strain without the need for dedicated sequences of complex post-acquisition analysis (123,124).

LV global longitudinal strain (GLS), an automated echocardiographic technique to assess LV longitudinal deformation is one of the most studied myocardial deformation parameters. There is accumulating evidence that LV GLS is more reproducible than LVEF and offers incremental prognostic information in unselected individuals undergoing echocardiography and in those undergoing valvular surgery (127-129). At present, the precise clinical role of LV GLS remains to be established. There are ongoing initiatives by the American Society of Echocardiography, the European Association of Cardiovascular Imaging and the ultrasound imaging industry to standardise strain measurements given confounders such as inter-vendor and inter-software variability (126).

2.3.2.3 LV size and wall thickness

Increased LV mass or left ventricular hypertrophy (LVH) is an established predictor of cardiac events in the general population and in those with CVD (130-132). The risk of adverse cardiac events is proportional to the degree of LVH severity (133-135). Importantly, regression of LVH (on imaging or ECG), is associated with cardiovascular risk reduction (136,137). Because of its widespread availability, a 12-lead ECG is typically the first-line investigation to screen for LVH, with the sensitivity when using novel electrocardiography criteria approaching 65% (138). Cardiac imaging techniques such as echocardiography or CMR can more reliably determine LV mass though are more costly so is typically considered only when there is suspicion of end-organ damage in individuals with hypertension.

Volumetric assessment for LV size determination is an essential marker of CVD risk. LV enlargement can be seen in a wide variety of conditions, including primary cardiomyopathies or can also be due to secondary causes including valvular heart diseases, and complications of CAD (50,139). The most recent American Society of Echocardiography chamber quantification guidelines recommends using LV volumetric assessment for LV size determination given that conventionally used linear measurements may not optimally reflect ventricular remodelling (140). The utility of volumetric assessment was highlighted in a recent study involving 1,100 patients with moderate to severe aortic regurgitation. The authors reported a stronger correlation of volumetric rather than linear LV size measurements with clinical outcomes (141).

2.3.2.4 Assessment of diastolic function and the left atrium

The assessment of LV diastolic function is an important step in evaluating patients with suspected heart failure from any aetiology (142). LV diastolic function can be reliably estimated non-invasively in most cases with an accuracy of 87% in a recent prospective study, using an integration of several echocardiographic parameters (143). These parameters include assessment of the left atrial size, tricuspid regurgitation velocity, the ratio of the mitral inflow velocities (E/A ratio) and the ratio between the mitral inflow E wave and the early (e') mitral annular velocity (E/e') (142). Diastolic dysfunction can be classified from mild (grade I) to severe (grade III) with increasing risk of heart failure with higher grades (144). Importantly, the diagnosis of diastolic dysfunction can be challenging even in expert hands, particularly given the overlap that exists in the indices between normal and abnormal (142). Given these challenges, the use of cardiac natriuretic peptides has been proposed in scenarios where the diagnosis of diastolic dysfunction or heart failure with preserved ejection fraction remains uncertain. This is supported by multiple lines of evidence that demonstrate that there is a graduated increase in levels of natriuretic peptides with worsening diastolic dysfunction, independent of age, sex, body mass index, LVEF and kidney function (145-147).

In addition to being a useful parameter to assess LV diastolic function, imaging of the left atrium can provide information diagnostic and prognostic information. Because of its irregular shape, left atrial size estimation should be made using volumetric techniques. Increasing LA volumes is associated with CVD including AF, CAD, cardiomyopathies and mitral valve disease (140,142,144,148,149). The assessment of LA function using speckle tracking echocardiography has been increasingly studied (150-152). Analogous to the concept of LV GLS, where systolic deformation of the base toward the apex is quantified, peak atrial longitudinal strain captures the maximum elongation of the LA

during LV systole (152). Recent evidence suggests that left atrial strain can predict the occurrence of AF in patients with heart failure and in patients post permanent pacemaker insertion (150,151). Unlike assessment of LA volume, the assessment of LA strain has not yet been translated into routine clinical practice.

2.3.2.5 Indices of right ventricular size and function.

There is increasing evidence that right ventricular enlargement and dysfunction are associated with worsening mortality and morbidity in various CVD states including pulmonary hypertension, CAD, heart failure (153-156). Echocardiographic parameters such as tricuspid annular plane systolic excursion and fractional area change, RV ejection fraction and strain imaging have been used to assess RV function (153-156). Fractional area change seems to correlate best with CMR derived RV ejection fraction and decreased values are also associated with worsened clinical outcomes in the VALIANT and SAVE substudies (149,153). The retrosternal position of the right ventricle can pose challenges for echocardiographic evaluation but can be overcome by using other imaging modalities such as CMR or CT.

2.3.3 Aortic size quantification

Complications arising from aneurysmal disease of the aorta are a leading cause of morbidity and mortality (157). As most individuals with TAAD have no symptoms, the diagnosis is often made incidentally on imaging performed for an unrelated condition. Increased aortic size as determined by echocardiography, CT or CMR is an important marker of adverse events (158). In a review of over 1,600 patients at Yale University with

TAAD, the risk of complications such as aortic rupture, dissection or death occurred in up to 6.5% of cases in ascending aortic sizes $<6\text{cm}$ and accelerate significantly to 14.1% in those with aortic diameters of $>6\text{ cm}$ (159).

2.3.4 Markers of significant valve dysfunction

Conventional haemodynamic markers of valvular dysfunction are important predictors of adverse clinical outcomes and form the basis of treatment decisions in valvular heart disease. The importance of haemodynamic parameters in AS was demonstrated in a prospective study by Otto et al. in 123 patients with AS (aortic jet velocity $>2.5\text{ m/s}$) (160). The likelihood of survival and freedom from AVR varied inversely with the aortic jet velocity, approaching 20% when the velocity was $>4\text{ m/sec}$, the value that defines the presence of severe AS (139). Another prognostically significant haemodynamic parameter in AS is the aortic valve area (AVA). In patients with asymptomatic severe AS ($\text{AVA} < 1.0\text{cm}^2$), a valve area $<0.6\text{cm}^2$ was significantly associated with increased all-cause mortality (HR 3.39, 95% CI 1.80-6.40) (161). It is important to note that the AVA may be underestimated in patients with low flow (cardiac output) resulting in valve leaflets that are not forced to open maximally. This limitation can be partly overcome by calculating the *projected* valve area, an estimate of the AVA at a standardised, average flow rate (250 ml/min). In a recent, multicentre prospective evaluation of 186 patients with low-flow, low-gradient AS who underwent dobutamine stress echocardiography, the projected valve area $\leq 1\text{cm}^2$ was a better predictor of aortic stenosis severity and mortality compared to conventionally used dobutamine stress echocardiography criteria (peak stress mean gradient $\geq 40\text{mmHg}$ and $\text{AVA} \leq 1.0\text{cm}^2$) (162).

In patients where there remains uncertainty regarding AS severity, there is increasing evidence to support the use of CT aortic valve calcium scoring (163,164). In a study of 918 patients from eight centres, CT aortic valve calcium scores accurately identified severe AS compared to echocardiography and independently predicted the combined outcome of AVR or death (HR 3.90, 95% CIs 2.19-6.78) (164).

The importance of hemodynamic parameters on imaging also extends to regurgitant valvular lesions such as aortic and mitral regurgitation (165). The two most commonly used parameters to quantify valvular regurgitation include effective regurgitant orifice area and regurgitant volume (165). In a cohort of 3,914 patients attending the Mayo Clinic for isolated mitral regurgitation, routinely measured effective regurgitant orifice area as part of clinical care independently predicted mortality (HR:1.19, 95% CI 1.13-1.24 per 10 mm²) (166). There is also an emerging role for CMR quantification of regurgitant valvular lesions. In a prospective, multicentre study of 103 patients who underwent both CMR and echocardiography prior to surgery for mitral regurgitation, there was a strong correlation between favourable post-surgical LV remodelling as assessed by CMR ($r = 0.85$; $p < 0.0001$) but not by echocardiography ($r = 0.32$; $p = 0.1$) (167). In another study involving 113 patients with moderate-to-severe aortic regurgitation, regurgitation fraction $> 33\%$ on CMR was the best marker of progression to symptoms or needing corrective surgery (area under the curve 0.93) (168). Importantly, this had better discriminatory ability than CMR ventricular volumetric measurements (area under the curve 0.88), considered the non-invasive ‘gold-standard’ method of ventricular volume assessment. Thus, CMR based hemodynamic parameters may provide a new paradigm in the evaluation of patients with native valvular regurgitation.

2.3.5 Myocardial fibrosis

Myocardial fibrosis is the common final response to chronic myocardial injury in several cardiovascular disorders (169-171) and is a marker of ventricular decompensation (14). Fibrosis is associated with abnormal LV contractility and poor outcomes such as sudden death (172,173). As some fibrosis forms are reversible (169,174), it is desirable to identify such pathological myocardial changes before it becomes irreversible, ~~as targeted treatments may be possible~~. To date, there are no circulating biomarkers that reliably correlate with histological myocardial fibrosis which has led to a major interest in detecting fibrosis using imaging techniques such as CMR. Two patterns of fibrosis can be observed using CMR: 1) focal replacement fibrosis (Figure 2.4) evident on T1-weighted late gadolinium enhancement (LGE) imaging that appears irreversible, and 2) diffuse interstitial fibrosis (Figure 2.5) that may be reversible (169,174).

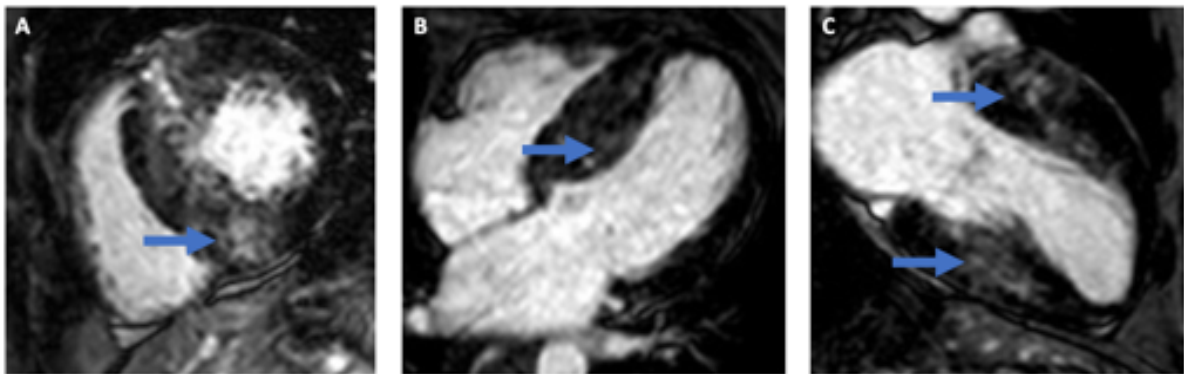


Figure 2. 4 Focal replacement fibrosis

Phase sensitive inversion recovery sequence in short axis (A), four-chamber (B) and vertical long-axis views approximately 10 minutes following the administration of intravenous gadolinium contrast. There is evidence of moderate delayed gadolinium enhancement (blue arrows) indicative of replacement fibrosis in a patchy mid-myocardial

distribution affecting the RV insertion points, basal to mid septum extending into the anterior and inferior walls.

Myocardial interstitial fibrosis changes the longitudinal relaxation properties (T1 values) of tissue. Using CMR T1 mapping techniques, these abnormal relaxation properties can be exploited to measure the extent of diffuse fibrosis (15). Myocardial T1 mapping allows quantitative tissue characterisation, yielding a value, unlike relying on visually recognisable contrast differences (17). The T1 relaxation time corresponds to the specific time decay constant when the proton recovers approximately 63% of its longitudinal magnetisation equilibrium value. Native T1 mapping produces a voxel-based map of the myocardium that estimates absolute myocardial T1 values with higher T1 values correlating with a greater extent of diffuse interstitial fibrosis (15).

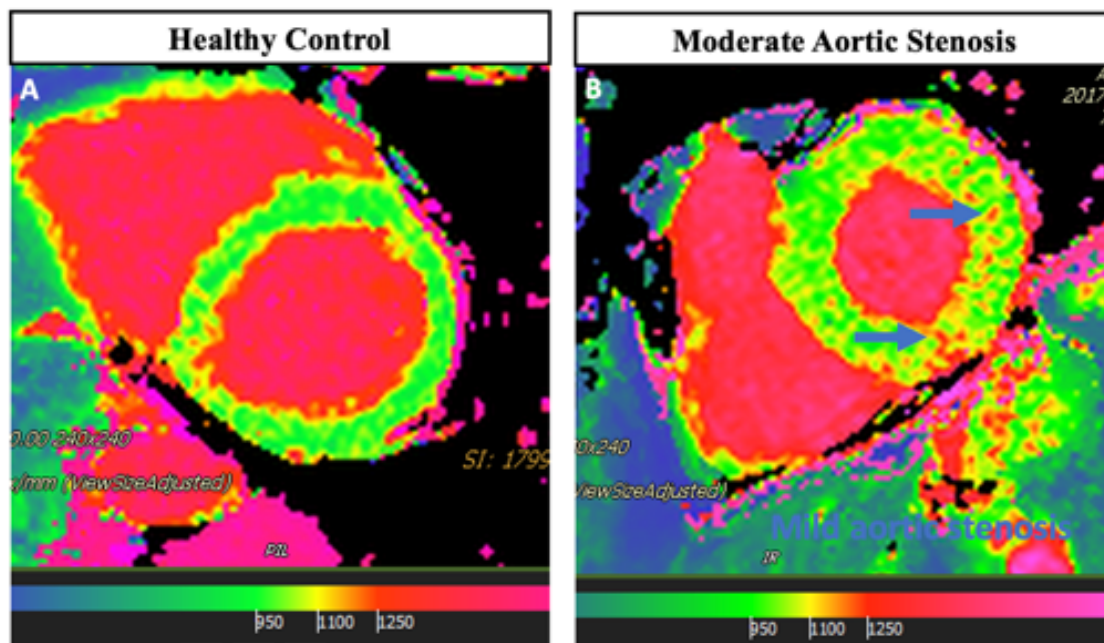


Figure 2. 5 Diffuse interstitial fibrosis

T1 maps from a healthy subject without apparent cardiovascular disease (A) and a patient with moderate aortic stenosis (B) generated using the modified Look-Locker

sequence. There is elevated myocardial T1 time in the patient with aortic stenosis appearing red in the colour scale (arrows) which is not observed in the healthy control.

There is accumulating evidence that the presence of replacement myocardial fibrosis is associated with worse outcomes. For instance, in AS, infarct and non-infarct LGE are associated with increased adverse outcomes (14,175) and does not regress after AVR (10,174). Recent observational studies have demonstrated an association between T1-based indices of interstitial fibrosis and the extent of myocardial fibrosis on histology, though studies relating these to clinical outcomes are limited (15-17), particularly in high-risk cohorts of patients with AS.

2.4 Opportunities to enhance risk stratification in common cardiovascular conditions

2.4.1 Coronary artery disease

2.4.1.1 Clinical manifestations

The European Society of Cardiology defines CAD as “a pathological process characterised by atherosclerotic plaque accumulation in the epicardial arteries, whether obstructive or non-obstructive” (51). CAD is a chronic, progressive disease that can be stable for a long time before acutely becoming unstable due to a thrombotic complication caused by atherosclerotic plaque rupture or erosion in most cases (51,176). In Australia, the burden of CAD is substantial. CAD was the leading disease in men and women, accounting for 6.9% of the total burden of disease in 2015 (1).

The clinical presentations of CAD can vary and are broadly divided into either acute coronary syndromes (ACS) or chronic coronary syndromes (CCS) with very different risk stratification and management approaches (51).

2.4.1.2 Acute coronary syndromes and risk stratification

ACS includes ST-elevation MI and non-ST elevation ACS (46,177). Patients with suspected ACS typically present with chest pain but can have other symptoms that include dyspnoea, or less specific symptoms such as fatigue. Risk stratification approaches have revolutionised diagnosis and management of ACS. An electrocardiogram (ECG) should

be performed within ten minutes of presentation to guide immediate management (46,177). Those with ST-elevation in two contiguous ECG leads are diagnosed with ST-elevation MI and require urgent reperfusion by either percutaneous coronary intervention or thrombolysis. Patients with non-ST elevation ACS on the other hand, do not typically undergo urgent reperfusion unless they have very high-risk features including cardiogenic shock, overt heart failure, refractory angina, haemodynamic stability or unstable ventricular arrhythmias (46). It is recommended that all patients with non-ST elevation ACS undergo early risk stratification using available risk scores to guide between immediate angiography, an early invasive strategy or conservative management.

A number of validated risk prediction models can be used to guide management of patients with non-ST elevation ACS. The TIMI risk score incorporates seven variables to predict the incidence of major cardiovascular events at 14 days (178). These variables include age ≥ 65 years, presence of > 3 risk factors for CAD, prior coronary stenosis of $\geq 50\%$, $\geq$ two anginal episodes in 24 hours, elevated serum biomarkers and use of aspirin in the prior seven days. The score has been validated in multiple cohorts and correlates with more severe angiographic disease (179-181). Another commonly used risk score is the GRACE risk model derived from a registry involving 94 hospitals in 14 countries (182). The GRACE model for in-hospital mortality comprises eight independent risk factors: age, Killip class, systolic blood pressure, ST-segment deviation, cardiac arrest, serum creatinine concentration, elevated cardiac biomarkers and heart rate. The GRACE score for in-hospital mortality is also predictive of adverse outcomes at six months and one year (183,184).

2.4.1.3 Chronic coronary syndromes and challenges of current risk stratification approaches

CCS encompasses a wide array of patients with suspect and/or confirmed CAD including 1) patients with stable angina symptoms; 2) patients with new-onset heart failure or LV dysfunction; 3) patients with stabilised symptoms <1 year after an ACS or revascularisation; 4) patients >1 year after initial diagnosis or revascularisation; 5) patients with angina and suspected vasoplastic or microvascular disease; 6) patients in whom CAD is detected at screening (51). Importantly, although all these scenarios are classified as CCS, each is expected to have varying risks of future adverse events.

Although patients with CCS were typically collectively labelled as having ‘stable’ CAD or ‘stable’ angina, the notion of stability is misleading, considering the continuing risks of long-term adverse events of patients with chronic coronary syndromes (185-187). As CCS encompasses a diverse spectrum of patients with different risk characteristics, personalisation of risk stratification is therefore important to establish therapeutic priorities. Unfortunately, excluding situations where acute coronary thrombosis (i.e. ACS) (188) is the predominant clinical feature, current guideline recommendations mostly involve generic treatment strategies with little opportunity for personalised risk stratification or attendant pharmacological titration of treatment (51). One possible explanation is the lack of specific biomarkers to refine currently rudimentary secondary prevention risk stratification strategies. When combined in a prognostic model, such biomarkers may help identify the patients at highest risk of complication and thus help inform preventative initiative and guidelines.

There is evidence that the excess cardiovascular risk of patients with CCS are grossly under-estimated and may contribute to treatment gaps in secondary prevention of CVD. The importance of risk stratification in contributing to the uptake of secondary prevention measures was demonstrated in an evaluation of high-risk adults (5-year risk of cardiovascular events $\geq 15\%$) with established CVD participating in the Australian Hypertension and Absolute Risk Study (N = 1,453; 63% with CAD) (189). Remarkably, only 11% of subjects self-identified as being at high risk of developing recurrent cardiovascular events. The general practitioners only correctly identified 30% of these individuals as being at high risk of future adverse events. Importantly, patients who recognised their increased risk were significantly more likely to cease smoking and had greater use of preventative therapies that included antiplatelet agents, anti-hypertensives and statins (189).

To summarise, the notion of ‘stable’ CAD is misleading, considering the continuing risks of long-term adverse events of patients with chronic coronary syndromes. Although there are a number of models that allow risk-stratification in ACS, the number of universally accepted biomarkers and prognostic models designed for those with more chronic forms of CAD remain limited.

2.4.2 Aortic stenosis

2.4.2.1 Natural history and clinical manifestations

Degenerative AS is characterised by progressive valvular thickening and calcification. Valvular narrowing is initially mild, but ultimately becomes severe (139,190). AS is the

leading cause of valvular heart disease in the Western world, and its prevalence is increasing as the population ages, affecting up to 10% of individuals between 80-90 years (191,192). The only treatment that improves survival in symptomatic severe AS is AVR (50,193).

The natural history of AS involves a long, latent period and in general does not cause symptoms until the development of severe AS (valve area $\leq 1\text{cm}^2$, jet velocity $\geq 4\text{ m/sec}$ and/or mean aortic gradient $\geq 40\text{mmHg}$) (139,194). Progression to AVR occurs earliest in those with a unicuspid valve, later in those with a bicuspid valve and later still with a tricuspid valve(195). As the severity of AS gradually progresses and becomes hemodynamically significant, it increases the pressure afterload on the LV and raises ventricular systolic pressure (191,196). With time, compensatory concentric LV hypertrophy develops to maintain normal wall stress and cardiac output. As stenosis severity progresses, compensatory LV remodelling responses become maladaptive, which if left unchecked lead to irreversible LV fibrosis that can drive LV decompensation (9,10,14,175). These changes can lead to diastolic and/or systolic dysfunction, which in turn contribute to the development of dyspnoea and heart failure (197).

Without AVR, patients with symptomatic severe AS have a poor outlook (198,199). In an analysis of individuals with severe, symptomatic AS who were not eligible for transcatheter aortic valve replacement (TAVR), medical treatment which included aortic balloon valvuloplasty in the majority, was associated with a one-year mortality of 32% (199). In 179 patients with severe AS and heart failure symptoms assigned to medical therapy (also included aortic balloon valvuloplasty in the majority) in the PARTNER trial, the mortality rate at one year was 51% (198).

There is now increasing recognition that the onset of symptoms does not occur until valve obstruction is critical, making it a crude and late surrogate of disease progression (191). In fact, symptom onset can be preceded by subclinical changes in myocardial structure and function, which may be progressive and irreversible (14).

2.4.2.2 Contemporary challenges in the management of aortic stenosis

There are two major outstanding challenges in the management of severe AS. The first concerns the decision with regards to offering AVR in asymptomatic individuals. The second concerns the optimal timing of AVR in these individuals.

2.4.2.2.1 Aortic valve replacement in asymptomatic individuals

The decision to intervene with AVR in AS is usually governed by one of two reasons: achieving symptomatic relief or improving prognosis. For the most part, decision-making to intervene in *symptomatic* individuals with severe AS is not controversial (139,190). However, deciding to proceed with AVR in an individual with asymptomatic, severe AS solely to improve prognosis is more complicated. Arguments against performing AVR in asymptomatic, severe AS predominantly relate to peri-procedural complications and longer-term prosthesis-related issues. Periprocedural complications include surgical mortality and stroke whereas long-term complications include conduction system disturbance, prosthetic valve endocarditis, paravalvular leak, patient prosthesis mismatch and finite bioprosthetic valve durability particularly as they relate to younger patients undergoing AVR (200). As such, current guidelines recommend AVR only for

individuals with LV decompensation (139,190) based on the premise that asymptomatic patients with normal LV contractility have an excellent prognosis without AVR (201).

However, current management guidelines have become a matter of intense scrutiny for several reasons. Firstly, the onset of symptoms, which is considered by the guidelines as a clinical indicator of LV decompensation, is challenging to recognise in the elderly and less active patients frequently encountered in the clinic (191). Therefore, this can lead to under-recognition of LV decompensation and thus a delayed referral for AVR. Secondly, there is observational data to suggest that asymptomatic, severe AS is not as benign as previously thought with increased outcomes such as death and heart failure reported in individuals who are managed without AVR (201-205). Furthermore, there is now randomised controlled trial evidence from that report better outcomes in specific subsets of patients with asymptomatic, severe AS. The RECOVERY trial reported superior outcomes in asymptomatic individuals with very severe AS ($AVA \leq 0.75 \text{ cm}^2$, peak velocity $\geq 4.5 \text{ m/sec}$ or mean aortic gradient $\geq 50 \text{ mmHg}$) who have early surgery followed for over six years (HR for operative mortality and cardiovascular death: 0.09; 95% CI 0.01 – 0.67) (206). Lastly, with improved methods and perioperative care, surgical and transcatheter AVR can now increasingly be performed with low complication rates and high prosthetic valve durability.

2.4.2.2.2 Optimal timing of AVR

The optimal timing of AVR remains uncertain with current best practice recommendations based on observational data and expert consensus. Nonetheless, there is universal agreement that optimal timing of AVR is of paramount importance. The risk

of performing AVR too early is that these individuals are subjected to the risk of periprocedural and prosthetic related complications (200). These have to be balanced against the competing risks of performing AVR too late which is 1) associated with a small risk of sudden death (occurs in <1% per year) (201); and perhaps more crucially 2) associated with the development of irreversible myocardial damage.

The detrimental impact of AS on myocardial structure and function has long been demonstrated. Histological changes include myocyte hypertrophy in conjunction with elevated interstitial collagen deposition and eventually replacement fibrosis (169,207). The presence of fibrosis is significant because increasing fibrosis correlates with the degree of LVEF improvement and predicts adverse cardiovascular events even after AVR (9,14,175,208). Notably, some of these changes are only partially reversible. Krayenbuehl et al. demonstrated that at 18 months post-surgery, myocardial mass decreased primarily due to regression in myocyte hypertrophy, although with a relative increase in myocardial fibrosis (207). These findings have been consistently demonstrated in other histological reports and more recently on CMR studies (10,174,209). Recent developments in CMR have permitted the non-invasive characterisation and differentiation of AS-related myocardial fibrosis into two main patterns: 1) irreversible focal replacement fibrosis seen on LGE imaging and; 2) partially reversible diffuse interstitial fibrosis that can be identified using T1 mapping (see section 2.3.5).

Ideally, AVR should be performed just as LV decompensation occurs. Although the management guidelines define LV decompensation by an impaired LVEF (139,190), this marker appears to be a late surrogate of disease progression and is often irreversible (208).

Thus, there is need to identify novel biomarkers, beyond the use of LVEF and clinical symptoms alone to detect early LV decompensation.

2.4.3 Atrial fibrillation

2.4.3.1 Epidemiology, natural history and risk factors

AF is the most frequently encountered arrhythmia and is associated with adverse outcomes such as thromboembolic events, heart failure and death. The prevalence and incidence of AF are increasing with a substantial effect on health budgets (210-212). Worldwide, there are an estimated 34 million patients with AF (213). It is known that the prevalence of AF is higher in older age groups, men, and those of European ancestry.

AF diagnosis is suspected in individuals with an irregular pulse on physical examination and confirmed on ECG. As AF can be paroxysmal, a normal 12-lead ECG does not rule it out. AF can be classified as paroxysmal (terminates within 7 days of onset), persistent (lasting more than seven days), longstanding persistent (up to 12 months) and permanent when chronic presence is accepted, and no rhythm control is attempted (214).

There is a prevailing notion that “AF begets AF” based on early experimental data demonstrating that tachycardia led to electrophysiological remodelling of the atria resulting in longstanding AF. Thus, AF that started as paroxysmal episodes progressively become more frequent and prolonged, ultimately leading to chronic AF. In the Framingham Heart Study AF cohort, only 10% had AF without 2-year recurrence (215). However, findings from other studies challenge this notion (216,217). For example, in

the Canadian Registry of Atrial Fibrillation, progression of paroxysmal to chronic AF was only 8.6% per year (216).

Epidemiological studies indicate that AF is associated with several comorbid conditions (e.g. diabetes, obesity, hypertension and obstructive sleep apnoea), many contributing to unique structural changes that generate a vulnerable atrial substrate or cardiomyopathy (218-220). Remarkably, persistent AF may also switch to paroxysmal AF (221), highlighting the dynamic nature of the underlying mechanisms that dictate the natural history of AF (218). The observation that persistent subtypes of AF may regress to paroxysmal phenotypes may reflect a favourable shift in the exposure or severity of underlying AF risk factors; these need to be better understood.

2.4.3.2 Importance of risk factor modification

There is increasing recognition of the contributory role of cardiovascular risk factors to procedural success after catheter ablation for AF (222-224). For instance, Jacobs et al. demonstrated an association between AF recurrence post ablation and increasing risk scores (CHADS2 and CHA2DS2-VASc) in a cohort of 2,179 individuals followed for 5 years after catheter ablation (225). There is now major interest in addressing the modifiable cardiovascular risk factors in AF particularly as some interventions have been shown to confer a reduction in AF burden (222,223,226). Middeldorp and colleagues have aptly described the targeting of risk factors as an “essential fourth pillar of AF care alongside appropriate anticoagulation and rate and rhythm control” (227). More prospective randomised controlled trials are needed to allow better ascertainment of treatment targets for the individual risk factors. The degree and precise mechanisms by

which risk factor modification alters the atrial substrate and prevents arrhythmogenesis also remains to be understood.

2.4.3.3 Long term outcomes in atrial fibrillation

2.4.3.3.1 Stroke and systemic thromboembolism

AF is strongly associated with the development of systemic thromboembolism such as stroke and transient ischemic attack. In the Framingham Heart Study, the attributable risk of AF to cause stroke approached 25% among those greater than 80 years old (228). The absolute risk of stroke varies among patients and is modulated by risk factors that form the CHA₂DS₂VASC stroke risk prediction score (229). These include age ≥ 65 years, hypertension, previous stroke/ transient ischemic attack/ systemic thromboembolism, vascular disease, heart failure and female gender. In addition, as shown in the Copenhagen Stroke Study, stroke in patients with AF are associated with increased morbidity (longer hospital stay, 50 vs. 40 days) and mortality (in-hospital death, odds ratio: 1.7, 95% CI 1.2 – 2.5) compared to patients without AF (230). Similarly, in the Framingham Heart Study, there was increased one-month mortality post stroke in patients with AF versus patients without AF (odds ratio: 1.84; 95% CI 1.04 – 3.27) (231).

The prevailing theory that stasis of blood in the fibrillating atrium leads to thrombus formation which then leads to a stroke is certainly intuitive but incompletely accounts for stroke risk. If the arrhythmia was the sole contributor of stroke risk, then maintaining sinus rhythm should attenuate stroke risk, which has not been demonstrated in a meta-analysis of eight randomised controlled studies (odds ratio: 0.99, 95% CI 0.76 – 1.30)

(232). Independent of the presence of AF, there is overwhelming evidence linking incident stroke with markers of abnormal atrial substrate including premature atrial contractions, paroxysmal supraventricular tachycardia, left atrial abnormalities on ECG or echocardiography (233-237). Further understanding of the role of the atrial substrate is therefore expected to lead to the development of novel approaches to more optimally recognise and treat patients at risk of stroke.

2.4.3.3.2 Heart Failure

Heart failure and AF often co-exist and share common risk factors. The presence of one appears to increase the likelihood of developing the other (238). Common risk factors include hypertension, diabetes, CAD and valve disease.

Heart failure as a risk factor for AF has been demonstrated in several epidemiological studies (239-241). In the Framingham Heart Study, the risk of AF was 4.5- to 5.9-fold higher in patients with heart failure (240). Furthermore, there appears to be a graduated increase in AF risk with decreasing LVEF (242) and worsening diastolic dysfunction (243). Although the precise relationship is likely complex, epidemiological data suggest that AF contributes more to heart failure than heart failure contributing to AF. In a more recent Framingham Heart Study population, the incidence of heart failure was significantly higher in individuals with AF (57%) compared to the incidence of AF in those with pre-existing heart failure (37%) (244). Notwithstanding, the combination of AF and HF is associated with increased mortality (245).

2.4.3.3.3 Coronary artery disease and other vascular disorders

Similar to that observed with heart failure, there is a bidirectional relationship between AF and vascular disorders such as CAD. Indeed, AF and CAD share common risk factors such that CAD is associated with an increased risk of AF and vice versa (246,247). Beyond the presence of shared risk factors that can predispose patients to both AF and CAD, other mechanisms have been posited to explain this relationship. One theory is that repeated atrial ischaemia in those with CAD may contribute to atrial fibrosis and electric remodelling ultimately leading to AF formation (248,249). The relationship between AF and CAD was evaluated in the REGARDS cohort involving over 20,000 individuals without CAD (250). It was shown that baseline AF was independently associated with incident MI (HR 1.7, 95% CI 1.26-2.30), with the risk being greatest in women and those of African descent (250). In addition to increased risk of CAD, there is accumulating evidence demonstrating the high prevalence of several other vascular disorders in AF, such as peripheral artery disease and TAAD (251-253).

2.4.3.3.4 Mortality

There is accumulating evidence that AF is a risk factor for increased mortality independent of age and gender. In a secondary analysis of the AFFIRM randomised trial (rhythm vs. rate control), the presence of sinus rhythm was associated with a reduction in mortality (254). Similar findings were reported by the DIAMOND investigators (dofetilide vs. placebo) (255). A study from the United States of individuals ≥ 65 years demonstrated that death was the most common outcome in AF occurring at a rate of approximately 20% at one year and 50% at five years from the time of diagnosis (256).

In summary, AF is strongly associated with major CVD such as heart failure, CAD and other vascular diseases, which may contribute to increased mortality. Screening strategies may help to identify the patients with AF at highest risk of developing these conditions. The development of these comorbid conditions is likely driven by ‘shared’ risk factors that also contribute to AF progression. Therefore, the benefits of risk factor modification may extend beyond reducing arrhythmia burden to also reduce the occurrence the other major CVD in AF.

2.5 Conclusion

Previous studies have demonstrated that conventional risk stratification approaches have limitations and there is a need to supplement traditional risk factor assessment with circulating and imaging biomarkers. There are at present a plethora of available circulating CVD biomarkers. Aside from the traditional metabolic parameters deeply embedded in risk stratification algorithms (for example total cholesterol and glucose levels), the two cardinal circulating markers endorsed by guidelines include BNP and cardiac troponin, reflecting myocyte load and injury respectively. Although these biomarkers provide actionable information on key pathophysiological aspects of CVD such as myocardial stretch and cardiomyocyte damage, other important mechanisms of cardiovascular pathophysiology such as calcification or fibrosis remain mostly unsatisfactorily assessed by circulating markers alone. The ability to detect some of these mechanisms using imaging biomarkers is therefore a major clinical interest as they may facilitate early preventative strategies.

There are opportunities to enhance risk stratification in common CVD. For the long-term treatment of chronic CAD, management guidelines offer mostly generic recommendations with limited opportunities for personalised titration of pharmacological therapy beyond the first few years of diagnosis. In AS, there are ongoing efforts to search for earlier and more objective myocardial decompensation markers to gauge optimal AVR timing. In AF, there is a need to identify patients at risk of other CVD states such as heart failure and vascular diseases.

Chapter 3: Circulating angiotensin converting enzyme 2 activity and adverse cardiac events in patients with obstructive coronary artery disease

Ramchand J, Patel SK, Srivastava PM, Farouque O, Burrell LM. Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. PLoS One 13(6): e0198144

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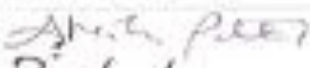
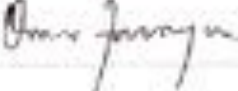
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RESEARCH ARTICLE

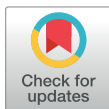
Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease

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Abstract

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Background

Angiotensin converting enzyme 2 (ACE2) is an endogenous regulator of the renin angiotensin system. Increased circulating ACE2 predicts adverse outcomes in patients with heart failure (HF), but it is unknown if elevated plasma ACE2 activity predicts major adverse cardiovascular events (MACE) in patients with obstructive coronary artery disease (CAD).

Methods

We prospectively recruited patients with obstructive CAD (defined as $\geq 50\%$ stenosis of the left main coronary artery and/or $\geq 70\%$ stenosis in ≥ 1 other major epicardial vessel on invasive coronary angiography) and measured plasma ACE2 activity. Patients were followed up to determine if circulating ACE2 activity levels predicted the primary endpoint of MACE (cardiovascular mortality, HF or myocardial infarction).

Results

We recruited 79 patients with obstructive coronary artery disease. The median (IQR) plasma ACE2 activity was 29.3 pmol/ml/min [21.2–41.2]. Over a median follow up of 10.5 years [9.6–10.8 years], MACE occurred in 46% of patients (36 events). On Kaplan-Meier analysis, above-median plasma ACE2 activity was associated with MACE (log-rank test, $p = 0.035$) and HF hospitalisation ($p = 0.01$). After Cox multivariable adjustment, log ACE2 activity remained an independent predictor of MACE (hazard ratio (HR) 2.4, 95% confidence interval (CI) 1.24–4.72, $p = 0.009$) and HF hospitalisation (HR: 4.03, 95% CI: 1.42–11.5, $p = 0.009$).

Conclusions

Plasma ACE2 activity independently increased the hazard of adverse long-term cardiovascular outcomes in patients with obstructive CAD.

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Introduction

Cardiovascular (CV) disease is a major cause of morbidity and mortality,[1] and is associated with activation of the renin-angiotensin system (RAS). Within the RAS, angiotensin converting enzyme (ACE) converts angiotensin (Ang) I to the vasoconstrictor and pro-atherosclerotic peptide Ang II,[2] whilst ACE2 is an endogenous inhibitor of the RAS through its major action to degrade Ang II.[3] ACE2 is highly expressed in the heart and blood vessels[4] and is cleaved from the cell surface to release the catalytically active ectodomain[5] into the circulation through the action of tumour necrosis factor alpha converting enzyme (TACE).[6] In human myocardium, ACE2 is localized to the endothelium of the microcirculation,[7] and is also present in the media of atherosclerotic radial arteries.[8] In healthy individuals, circulating ACE2 activity levels are low[9, 10] but increase in the presence of CV disease or risk factors including heart failure (HF),[11] atrial fibrillation (AF),[12] kidney disease[13, 14] and type 1 diabetes.[15] To date, there is limited information on the prognostic role of circulating ACE2 activity levels and the results are conflicting. For example, increased ACE2 activity predicted adverse CV outcomes in heart failure,[16] but not in patients after emergency orthopedic surgery[17] or with chronic kidney disease.[13, 14] These differences may reflect the patient population, the relative cardiovascular risk of the patient population or the length of follow up. The aim of this study was to investigate the utility of plasma ACE2 activity levels to predict CV events in a high-risk cohort of patients with angiographically proven obstructive CAD with more than 10 years of follow-up.

Materials and methods

Consecutive patients aged >18 years were prospectively recruited between November 2004 and January 2006 after referral to a tertiary cardiovascular centre for a coronary angiogram to investigate suspected CAD. Only those with significant obstructive CAD were eligible for this study. Patients in cardiogenic shock, with a past history of congestive heart failure or with a left ventricular (LV) ejection fraction < 30% on angiography were excluded. Ethical approval was obtained from the Human Research Ethics Committee at Austin Health, Melbourne and the study complied with the Declaration of Helsinki. All patients gave informed written consent.

A standardised medical questionnaire was completed and verified with the hospital medical record. Blood pressure was measured and anthropometric measurements were taken. Obstructive CAD was defined as $\geq 50\%$ stenosis of the left main coronary artery and/or $\geq 70\%$ stenosis in ≥ 1 other major epicardial coronary artery by visual assessment on invasive coronary angiography.[18] Diabetes was diagnosed based on a documented history, treatment with glucose lowering therapy or if fasting blood glucose was >7 mmol/L. Hypertension was defined if previously diagnosed by a physician and/or current use of anti-hypertensive medication. Dyslipidaemia was defined if previously diagnosed by a physician and/or current use of lipid lowering agents. Cigarette smoking was defined as smoking within the preceding 12 months.

Fasting blood samples were collected at the time of admission for measurement of kidney function, lipids, and troponin. The Access AccuTnI assay (Beckman-Coulter, Chaska, MN, USA) was used to measure plasma troponin with the 99th percentile of a healthy reference population of 0.04 $\mu\text{g/L}$. Levels of ≥ 0.04 $\mu\text{g/L}$ (99th percentile) were considered abnormal in this study.

For plasma ACE2 measurement, blood was collected within 48 hours of presentation into lithium heparin tubes, and plasma was obtained by centrifuging blood at 3000 rpm at 4°C for 10 minutes and stored at -80°C until tested. Plasma ACE2 activity was measured within 2 years after all samples were collected. Samples were batched and ACE2 assays were run over a

period of 2 days. The catalytic activity of ACE2 was measured using a validated, sensitive quenched fluorescent substrate-based assay as previously described.[9] Briefly, plasma (0.25 ml) was diluted into low-ionic-strength buffer (20 mmol/L Tris-HCl, pH 6.5) and added to 200 μ l ANXSepharose 4 Fast-Flow resin (Amersham Biosciences, GE Healthcare, Uppsala, Sweden) that removed a previously characterized endogenous inhibitor of ACE2 activity.[9] After binding and washing, the resulting eluate was assayed for ACE2 catalytic activity. Duplicate samples were incubated with the ACE2-specific quenched fluorescent substrate, with or without 100 mM ethylenediaminetetraacetic acid. The rate of substrate cleavage was determined by comparison to a standard curve of the free fluorophore, 4-amino-methoxycoumarin (MCA; Sigma, MO, USA) and expressed as μ mole of substrate cleaved/mL of plasma/min. The intra-assay and inter-assay coefficient of variation was 5.6 and 11.8% respectively.

The primary endpoint was a composite of major adverse cardiac events (MACE) defined as CV death, hospitalisation for HF or myocardial infarction (MI). The secondary endpoint was HF hospitalisation. Endpoints were described according to the 2014 American College of Cardiology/ American Heart Association definitions for CV endpoints in clinical trials.[19] CV death was defined as death due to sudden cardiac death, HF, acute MI, cerebrovascular accident, CV haemorrhage, CV procedures or other CV causes, that is death not included in the previous categories but with a specific, known CV cause such as pulmonary embolism.[19] Hospitalisation for HF was defined as an event where the patient is admitted to the hospital with a primary diagnosis of HF where the length of stay is at least 24 hours, where the patient exhibits new or worsening symptoms of heart failure on presentation, has objective evidence of new or worsening heart failure and receives intensification of treatment specifically for heart failure.[19] Myocardial infarction was defined as the clinical diagnosis of ST elevation or non-ST elevation myocardial infarction according to established criteria.[20, 21]

Clinical outcomes were collected by an experienced blinded investigator via medical records review and by contacting each patient and/or the nominated general practitioner for additional information.

Statistical analysis was performed using STATA, version 14.2 (Statacorp., College Station, TX, USA). Normally distributed continuous variables are expressed as mean $\pm$ standard deviation and non-normally distributed data (Plasma ACE2 activity, triglycerides, troponin and glomerular filtration rate) are expressed as the median and inter-quartile range (IQR). Student *t* test or the Mann Whitney *U* test (for non-normally distributed data) was used to assess differences in continuous variables between patients with above and below median ACE2 activity. Categorical variables are expressed as counts and percentages and compared using Fisher's exact or chi-square tests. Multiple regression analysis was used to identify variables that may independently influence plasma ACE2 activity. Plasma ACE2 activity, troponin levels and glomerular filtration rate were natural-logarithm transformed for analysis because of their skewed distribution. This rendered a more normal distribution by visual inspection of the distribution of the variables and Q-Q plots.

Cumulative incidence of MACE was estimated by the Kaplan Meier method and the log-rank test used to evaluate differences between patients with below and above median plasma ACE2 activity. When multiple end-points occurred during follow-up, the time to the first event was considered for analysis of MACE. Cox proportional hazard modelling was used to estimate the adjusted hazard ratio (HR) and 95% confidence interval (CI) for MACE. Significant variables ($p < 0.1$) from univariate analysis were entered into the final multivariate model to identify independent predictors of MACE. Conventional prognostic variables were used including age, history of diabetes, log troponin and treatment with statin, beta-blocker, ACE inhibitor or angiotensin receptor blocker in addition to log ACE2. Two-tailed *p*-values < 0.05 were considered significant.

Results

We recruited 79 patients with angiographically proven obstructive CAD. No patient was lost to follow up and the median follow-up was 10.6 years (IQR 9.6–10.9 years). The clinical and biochemical characteristics of the study population are presented in Table 1. The cohort comprised 65% males with a mean $\pm$ SD age of 66 ± 12 years and BMI of 27.4 ± 4.4 kg/m². Patients were at significant CV risk with 69% having a smoking history, and a history of CAD in 66%, dyslipidaemia in 60%, hypertension in 82%, diabetes in 24% and AF in 11%. With regard to pharmacological therapy at the time of presentation, 59% were on angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB), 58% on beta-blockers, 72% on statins and 100% on aspirin.

Table 1. Participant characteristics in total cohort and according to plasma ACE2 activity.

Variable	All patients	ACE2 < median (n = 39)	ACE2 > median (n = 40)	P-value
Age (years)	66 $\pm$ 12	67 $\pm$ 11	63 $\pm$ 13	0.27
Male gender [n (%)]	51 (65%)	18(46%)	33 (83%)	0.001
BMI (kg/m ²)	27.4 $\pm$ 4.4	27.4 $\pm$ 5.2	27.3 $\pm$ 3.6	0.939
SBP (mmHg)	130 $\pm$ 17	131 $\pm$ 15	130 $\pm$ 19	0.746
DBP (mmHg)	70 $\pm$ 13	71 $\pm$ 13	68 $\pm$ 14	0.321
Presentation with ACS	51 (65%)	23 (59%)	28 (70%)	0.306
Previous history [n (%)]				
CAD	51 (66%)	24 (63%)	27 (69%)	0.573
Dyslipidemia	48 (60%)	23 (59%)	25 (63%)	0.748
Hypertension	64 (82%)	30 (77%)	34 (87%)	0.238
Diabetes	19 (24%)	11 (29%)	8 (20%)	0.357
Atrial fibrillation	9 (11%)	1 (3%)	8 (20%)	0.015
Smoking history	54 (69%)	24 (63%)	30 (75%)	0.257
Medical treatment [n (%)]				
ACEi/ARB	45 (59%)	24 (63%)	21 (55%)	0.484
Beta-blocker	44 (58%)	23 (61%)	21 (55%)	0.642
Statin	56 (72%)	27 (69%)	29 (74%)	0.615
Aspirin	79 (100%)	39 (100%)	40 (100%)	1
Serum biochemistry				
LDL (mmol/L)	2.5 $\pm$ 0.9	2.6 $\pm$ 1.1	2.5 $\pm$ 0.8	0.736
Triglycerides (mmol/L)	1.2 [0.9–1.9]	1 [0.8–1.9]	1.4 [0.9–2]	0.119
eGFR (ml/min/1.73m ²)	70 [48–96]	76 [59–98]	69 [46–95]	0.675
Troponin I (μ g/L)	0.6 [0–14.5]	0.54 [0–8.2]	2.3 [0–21.2]	0.211
LVEF < 50% [n (%)]	42 (58%)	18(53%)	24 (62%)	0.459
Culprit vessel [n (%)]				
Right coronary	27 (34)	15 (39)	12 (30)	0.428
Left anterior descending	23 (23)	13 (33)	10 (25)	0.415
Left circumflex	20 (25)	8 (21)	12 (30)	0.332
Left main	1 (1)	0	1 (3)	0.320
Inpatient revascularisation [n (%)]	61 (77)	31 (79)	30 (75)	0.635
MACE [n (%)]	36 (46%)	12 (31%)	24 (60%)	0.009

Values are mean $\pm$ standard deviation, n (%) or median [interquartile range].

ACS = acute coronary syndrome; BMI = body mass index; ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CAD = coronary artery disease; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction; LDL = low-density lipoprotein; MACE = major adverse cardiovascular events; SBP = systolic blood pressure

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There was no difference in plasma ACE2 levels between patients with ST elevation acute coronary syndrome (ACS) [$n = 24$, median 31.7 pmol/ml/min (IQR 5.6–43.9)], non-ST elevation ACS [$n = 27$, median 29.1 pmol/ml/min (IQR 23.1–46.4)] or stable angina [$n = 28$, median 26.7 pmol/ml/min (IQR 18.7–39.8), $p = 0.386$] and so further analysis was conducted in the whole cohort.

The median ACE2 level in the whole cohort was 29.3 pmol/ml/min [IQR 21.2–41.2]. Patients were categorized according to plasma ACE2 activity above / below the median ACE2 level. Patients with above-median plasma ACE2 activity were more likely to be male and have AF (Table 1, both $p < 0.05$). Multiple regression analysis was performed to identify variables that influence plasma ACE2 activity. Male gender was the only independent predictor of higher ACE2 activity ($p = 0.022$). The prevalence of CAD and cardiac risk factors including dyslipidaemia, hypertension, diabetes and cigarette smoking were similar in the two groups, as was LVEF $< 50\%$, the use of pharmacological agents, low density lipoprotein cholesterol, triglycerides levels, kidney function and troponin level (all $p > 0.05$).

Over the follow-up period, there were 18 deaths, 19 myocardial infarcts and 16 hospitalisations with HF. The primary endpoint of MACE, a composite of CV mortality, HF hospitalisation or MI occurred in 36 patients (46%).

Above median levels of ACE2 (> 29.3 pmol/ml/min) were significantly associated with a higher incidence of MACE (log-rank test, $p = 0.035$; Fig 1A) and HF hospitalisation ($p = 0.01$; Fig 1B) compared with those with below-median ACE2. There was no significant difference in the incidence of CV death ($p = 0.195$) or MI ($p = 0.35$). In a subgroup analysis including male patients only, there was no significant difference in the incidence of MACE according to median levels of ACE2 ($p = 0.124$).

Survival analysis using the Cox regression model indicated that age, history of atrial fibrillation, history of diabetes and log ACE2 were univariate predictors of the primary endpoint of MACE. On multivariable Cox regression analysis, Log ACE2 activity remained the only significant predictor of MACE (HR: 2.4; 95% CI: 1.24 to 4.72; $p = 0.009$) (Table 2).

With regard to the secondary endpoint of HF, both log ACE2 (HR: 4.03; 95% CI: 1.42–11.5; $p = 0.009$) and age (HR: 1.06; 95% CI: 1.01–1.12, $p = 0.026$) were independent predictors of heart failure.

Discussion

The major finding of the current study was that plasma ACE2 activity independently increased the hazard for adverse cardiovascular events in patients with significant obstructive CAD. In this study in high-risk patients followed for a median of 10.6 years, elevated ACE2 activity remained an independent predictor of CV mortality and morbidity even after comprehensive multivariable adjustment in a model that included prognostically meaningful variables. The median ACE2 level in patients with CAD was 29 pmol/ml/min which is higher than levels we previously reported in young healthy volunteers (4.44 ± 0.56 pmol/ml/min)[9] and in elderly patients (median 19.4 pmol/ml/min).[17] We excluded patients with known HF or severe LV systolic dysfunction as both are associated with increased circulating ACE2 levels.[11, 16] Consistent with results of other studies,[13–15, 22] plasma ACE2 activity was higher in male patients but we found no other independent predictors of plasma ACE2 activity levels.

There are conflicting findings regarding the prognostic value of circulating ACE2 levels likely reflecting the differences in follow-up period and risk of CV events across the study populations. In a cohort of patients with HF ($n = 113$), 23% had an adverse CV event (death, cardiac transplant, HF hospitalisation) over a 34 month follow up and circulating ACE2 levels remained an independent predictor after adjustment for reduced ejection fraction and

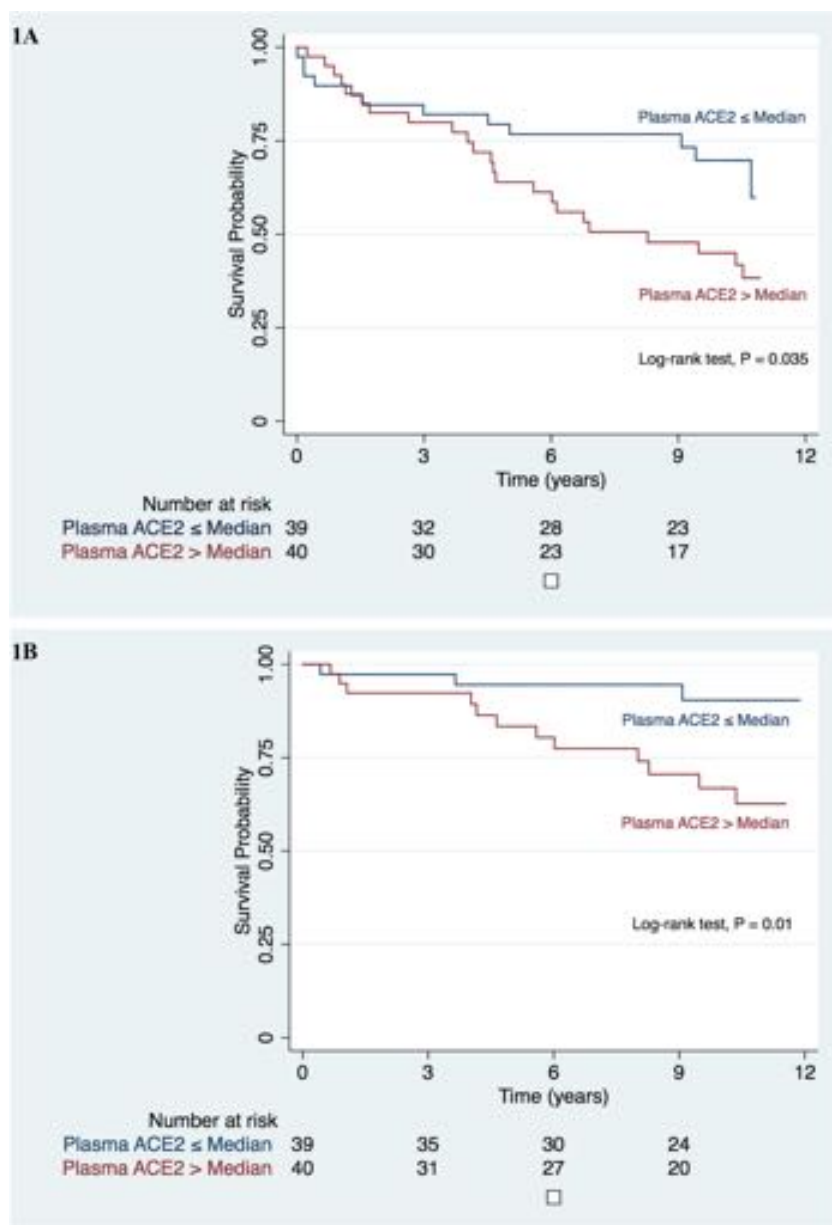


Fig 1. Clinical events at follow-up. Kaplan Meier Survival plot illustrating event free survival from major adverse cardiac events (A) and heart failure hospitalisation (B) in patients with coronary artery disease, stratified according to median plasma ACE2 level of 29.3 pmol/ml/min. ACE2 = angiotensin converting enzyme 2.

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increased N-terminal-pro brain natriuretic peptide.[16] In another cohort of patients with chronic kidney disease (CKD) without prior CV disease, circulating ACE2 activity was not an independent predictor of CV mortality or events over a follow-up period of 24 months.[13, 14] In concordance, our group reported no significant associations between elevated circulating ACE2 activity and adverse CV outcomes in patients with CKD stage III/IV, haemodialysis patients or kidney transplant recipients.[13] We also found that in elderly patients undergoing emergency orthopaedic surgery, elevated ACE2 levels did not predict CV events after 12 months of follow-up ($p = 0.051$).[17] We ascribe the significant association between increased plasma ACE2 activity and adverse CV outcomes seen in the present study to a higher rate of CV outcomes observed in the study cohort and longer follow-up duration.

Severe lines of evidence suggest that plasma ACE2 activity may serve as a marker of atherosclerosis. In non-dialysis patients with CKD, circulating ACE2 activity was associated with silent atherosclerosis in carotid and peripheral vessels.[14] In patients with type 1 diabetes and a history of CAD, circulating ACE2 activity was increased.[15] The same pattern was observed in kidney transplant recipients with a history of CAD,[23] further supporting the association between raised circulating ACE2 activity and coronary atherosclerosis. In another study of patients with angiographically confirmed CAD, Ortiz-Perez et al. demonstrated elevated levels of circulating ACE2 at baseline (24–48h) in patients presenting with ST-elevation myocardial infarction compared to a control group of patients without known CAD.[24] It is not therefore clear from the Ortiz-Perez et al. study whether the increase in circulating ACE2 reflects acute cardiac injury or underlying atherosclerosis. Our study extends knowledge in this regard as we included only patients with angiographically proven obstructive coronary artery disease, both with and without an acute presentation. As there was no difference in ACE2 according to presentation, our results suggest that the increase in plasma ACE2 reflects underlying atherosclerosis rather than acute myocardial injury.

The importance of the RAS in the pathogenesis of atherosclerosis is well established and indeed targeted pharmacological inhibition of the classic RAS improves outcomes in atherosclerotic disease including CAD.[25] In experimental models of atherosclerosis, we and others reported that ACE2 is expressed in vascular endothelial cells, macrophages and smooth muscle

Table 2. Cox regression analysis for MACE in patients with obstructive CAD.

Variable	Unadjusted HR (95% CI)	Unadjusted p Value	Adjusted HR (95% CI)	Adjusted p Value
Age	1.03 (1.0–1.06)	0.068	1.03(1–1.06)	0.129
Male	1.36 (0.65–2.83)	0.413		
Atrial fibrillation	2.26 (0.98–5.21)	0.053	1.49 (0.63–3.52)	0.364
Diabetes	1.92 (0.96–3.87)	0.067	1.88 (0.93–3.78)	0.078
ACEi/ARB	0.64 (0.32–1.26)	0.195		
β -blocker use	0.57 (0.29–1.13)	0.107		
Statin use	0.65 (0.32–1.32)	0.237		
Log troponin	1.07 (0.97–1.18)	0.194		
Log ACE2	2.56 (1.31–5)	0.006	2.42 (1.24–4.72)	0.009

ACE2 = angiotensin converting enzyme 2; ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; MACE = major adverse cardiovascular events

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cells within atherosclerotic plaques.[26, 27] We also reported that ACE2 was present in atherosclerotic blood vessels in patients with CAD undergoing coronary artery bypass surgery.[8] Experimental studies have shown that ACE2 overexpression promotes atherosclerotic plaque stability and attenuates atherosclerotic lesions[28, 29]. Activation of TACE results in increased ACE2 shedding from tissue into the circulation.[6] Shedding and hence loss of ACE2 from the tissue is mediated by angiotensin II and results in the pro-inflammatory effects of angiotensin II being unopposed.[6] Certainly in a rabbit model of atherosclerosis, gene silencing of TACE enhanced plaque stability and improved vascular remodelling,[30] possibly via reduced tissue ACE2 shedding. These findings reinforce the important counter-regulatory role of ACE2 in atherosclerosis and suggest that modulation of ACE2 could offer a future therapeutic option in patients with atherosclerotic disease.

The relationship between tissue and circulating levels of ACE2 is not yet understood. It has been postulated plasma ACE2 levels may parallel tissue ACE2 expression with a constant rate of shedding in normal physiology[16], although there are no studies that have concurrently measured both tissue and circulating ACE2 and TACE levels to address this hypothesis. Our findings raise the possibility that in human atherosclerosis, increased plasma ACE2 activity in those with adverse cardiovascular outcomes reflect a persistent albeit insufficient counter-regulatory process to shift the balance away from the deleterious effects of sustained Ang II activation. Genetic variation in and around the gene encoding ACE2 may account for differences in ACE2 expression or activity. Indeed, the location of the ACE2 gene within the X chromosome in an area where genes are known to escape X-inactivation may contribute to phenotypic differences between sexes and tissue-specific differences in X-inactivation.[31] Furthermore, the rs1978124 polymorphism in the ACE2 gene has been associated with poorer outcomes in two separate CAD cohorts of Chinese Han[32] and European[33] ancestry but there are not yet studies that combine genetic approaches with measurement of plasma ACE2 activity.[34]

The study has a number of limitations including the relatively small sample size and the use of a conventional troponin assay, as a high sensitivity assay was not available at the time of patient recruitment. Furthermore, the finding of elevated plasma ACE2 activity and its association with adverse outcomes only suggest a possible relationship and does not determine cause or effect. However major strengths include the detailed angiographic assessment and the long term follow up.

In conclusion, our study demonstrates that elevated plasma ACE2 activity is an independent predictor of MACE in patients with obstructive CAD.

Future

This study has identified ACE2 as a potential surrogate marker of CV outcomes, and possibly a target for therapeutic intervention. Whether targeting patients with increased plasma ACE2 levels for more intensive therapy would lead to improved outcomes has yet to be tested.

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Chapter 4: Circulating angiotensin converting enzyme 2 activity predicts mortality in aortic stenosis and is associated with severe myocardial fibrosis

Ramchand J, Patel SK, Kearney LG, Matalanis G, Farouque O, Srivastava PM and Burrell LM. Plasma ACE2 Activity Predicts Mortality in Aortic Stenosis and Is Associated With Severe Myocardial Fibrosis. *JACC Cardiovasc Imaging*. 2020; 13:655-664.

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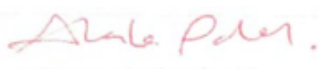
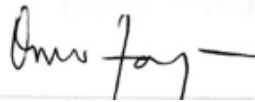
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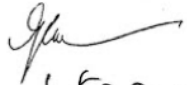
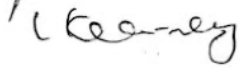
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ORIGINAL RESEARCH

Plasma ACE2 Activity Predicts Mortality in Aortic Stenosis and Is Associated With Severe Myocardial Fibrosis



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ABSTRACT

OBJECTIVES This study investigated the relationship between plasma angiotensin-converting enzyme 2 (ACE2) activity levels and the severity of stenosis and myocardial remodeling in patients with aortic stenosis (AS) and determined if plasma ACE2 levels offered incremental prognostic usefulness to predict all-cause mortality.

BACKGROUND ACE2 is an integral membrane protein that degrades angiotensin II and has an emerging role as a circulating biomarker of cardiovascular disease.

METHODS Plasma ACE2 activity was measured in 127 patients with AS; a subgroup had myocardial tissue collected at the time of aortic valve replacement.

RESULTS The median plasma ACE2 activity was 34.0 pmol/ml/min, and levels correlated with increased valvular calcification ($p = 0.023$) and the left ventricular (LV) mass index ($r = 0.34$; $p < 0.001$). Patients with above-median plasma ACE2 had higher LV end-diastolic volume (57 ml/m² vs. 48 ml/m²; $p = 0.021$). Over a median follow-up of 5 years, elevated plasma ACE2 activity was an independent predictor of all-cause mortality after adjustment for relevant clinical, imaging, and biochemical parameters (HR: 2.28; 95% CI: 1.03 to 5.06; $p = 0.042$), including brain natriuretic peptide activation (integrated discrimination improvement: 0.08; $p < 0.001$). In 22 patients with plasma and tissue, increased circulating ACE2 was associated with reduced myocardial ACE2 gene expression (0.7-fold; $p = 0.033$) and severe myocardial fibrosis ($p = 0.027$).

CONCLUSIONS In patients with AS, elevated plasma ACE2 was a marker of myocardial structural abnormalities and an independent predictor of mortality with incremental value over traditional prognostic markers. Loss of ACE2 from the myocardium was associated with increased fibrosis and higher circulating ACE2 levels. (J Am Coll Cardiol Img 2020;13:655–64) Crown Copyright © 2020 Published by Elsevier on behalf of the American College of Cardiology Foundation. All rights reserved.

Aortic stenosis (AS) is the leading cause of valvular heart disease and its prevalence is increasing as the population ages (1). Aortic valve replacement (AVR) improves survival in severe

AS and is recommended once AS-related symptoms and/or left ventricular (LV) decompensation occur (2) because the risk of death increases 25-fold with symptom onset (3). Because evaluation of symptoms

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ABBREVIATIONS AND ACRONYMS

ACE2 = angiotensin converting enzyme 2
ARB = angiotensin receptor blocker
AS = aortic stenosis
AVR = aortic valve replacement
BNP = brain natriuretic peptide
CI = confidence interval
CVD = cardiovascular disease
CVF = collagen volume fraction
GLS = global longitudinal strain
HR = hazard ratio
ID1 = integrated discrimination improvement
IHD = ischemic heart disease
IQR = interquartile range
LV = left ventricular
NRI = net reclassification improvement
RAS = renin-angiotensin system
ROC = receiver-operating characteristic
TACE = tumor necrosis factor- α converting enzyme

can be challenging (1), there is a role for the measurement of objective markers of risk to improve the prediction of myocardial decompensation (4-6) and facilitate timelier AVR. Current guidelines for risk stratification in AS suggest that measurement of natriuretic peptides may be useful in asymptomatic patients to determine the optimal timing of AVR (2).

Our group is interested in the role of angiotensin-converting enzyme 2 (ACE2) as a circulating biomarker of cardiovascular disease (CVD) (7-10). ACE2 is a member of the counter-regulatory axis of the renin-angiotensin system (RAS), and its main role is to degrade the pro-hypertrophic and pro-fibrotic peptide, angiotensin II, and act to limit the adverse effects of angiotensin II. ACE2 is an integral membrane protein expressed in the heart and vascular system (11,12) and exists in both a membrane bound and a soluble form; the latter results from proteolytic cleavage of the ectodomain by the proteinase ADAM17 (a disintegrin and metalloproteinase) (13).

The precise relationship between tissue and circulating levels of ACE2 is not yet understood. In experimental models, deletion or loss of ACE2 from the myocardium is associated with increased levels of tissue angiotensin II, which leads to cardiac fibrosis and dysfunction (14), as well as increased levels of circulating ACE2 activity (15). In healthy individuals without apparent CVD, circulating ACE2 activity is low (16). Circulating ACE2 levels are increased and predict adverse events in the presence of CVD, including coronary artery disease (8,9), atrial fibrillation (10), and heart failure (7). To our knowledge, there are no studies of both circulating and myocardial levels of ACE2 in humans, and there are no studies of ACE2 in common valvular heart diseases such as AS.

We hypothesized that plasma ACE2 activity levels would reflect AS severity and/or maladaptive myocardial structural changes and have incremental prognostic usefulness to predict long-term mortality. We also examined the relationship between plasma ACE2 activity, myocardial ACE2 gene expression, and myocardial fibrosis in a subgroup of patients who underwent AVR.

METHODS

The study protocol was approved by the Human Research Ethics Committee at Austin Health,

Melbourne and complied with the Declaration of Helsinki. Patients gave informed written consent. Consecutive patients ages older than 18 years who attended a follow-up in a cardiology valve clinic and who had mild, moderate, or severe AS were recruited (June 2008 to May 2010) as part of a prospective study that evaluated the development, progression, and outcomes of AS (5). Patients with another valvular lesion of greater than moderate severity were excluded. Valvular calcification and AS severity were classified using echocardiography according to current guidelines (17). Dobutamine stress echocardiography and/or valvular calcium score by multislice computed tomography were used to determine AS severity in those with discordant findings on baseline echocardiography (17). An abnormal LV ejection fraction was defined using sex-specific cutoff points (<52% for men and <54% for women) (18).

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Baseline demographic, clinical, and biochemical data were obtained, and blood collected for the measurement of ACE2 in 127 patients. Of the total cohort, 58 patients underwent AVR, with 22 patients having myocardial tissue collected at the time of AVR.

BLOOD SAMPLING AND ANALYSIS. At recruitment, blood was collected into cold lithium heparin tubes and spun at 3,000 rpm at 4°C for 10 min. Plasma was stored at -80 °C until measurement of plasma ACE2 activity, which was performed using a validated, sensitive quenched fluorescent substrate-based assay as previously described (16). Briefly, plasma (0.25 ml) was diluted into low-ionic strength buffer (20 mmol/l Tris-HCl, pH 6.5) and added to 200 μ l ANX-Sepharose 4 Fast-Flow resin (Amersham Biosciences, GE Healthcare, Uppsala, Sweden) that removed a previously characterized endogenous inhibitor of ACE2 activity (16). After binding and washing, the resulting eluate was assayed for ACE2 catalytic activity. Duplicate samples were incubated with the ACE2-specific quenched fluorescent substrate, with or without 100-mM ethylenediaminetetraacetic acid. The rate of substrate cleavage was determined by comparison to a standard curve of the free fluorophore, 4-amino-methoxycoumarin (Sigma, St. Louis, Missouri) and expressed as picomole of substrate cleaved per milliliter of plasma per minute. The intraassay and interassay coefficients of variation were 5.6% and 11.8%, respectively.

Plasma creatinine, hemoglobin, and brain natriuretic protein (BNP) were measured by Austin Health Pathology. Plasma BNP levels were measured using the AxSYM BNP assay (Abbott Laboratories, Abbott

Park, Illinois). Normal values of BNP specific to age and sex were derived based on available data from Abbott Laboratories from a cohort of 890 individuals without a diagnosis of heart failure (19). Patients with values above the maximal limit (95th percentile) for age and sex were considered to have elevated BNP; these patients were classified as having BNP clinical activation as previously described (20).

MYOCARDIAL TISSUE COLLECTION. In patients who underwent AVR (n = 22), right atrial appendage (RAA) tissue was obtained at the site of cannulation for cardiopulmonary bypass. The sample was snap frozen in isopentane and stored at -80°C for mRNA extraction, with remaining tissue fixed in 4% paraformaldehyde and embedded in paraffin for determination of interstitial collagen.

MYOCARDIAL ACE2 GENE EXPRESSION BY QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION. Total RNA was isolated from frozen RAA using the RNeasy kit method (Qiagen, Hilden, Germany). cDNA was synthesized with a reverse transcriptase reaction using Superscript III (ThermoFischer Scientific, Victoria, Australia), as described previously (11). Cardiac ACE2 and tumor necrosis factor- α converting enzyme (TACE) (also known as ADAM17) gene expression was determined using pre-designed human TaqMan assays (ThermoFischer Scientific, Victoria, Australia). Quantitative real-time polymerase chain reaction was performed in triplicate using the ViiA 7 Real Time PCR System (Applied Biosystems, Foster City, California). Gene expression was normalized to 18S VIC. The gene expression experiments (both RNA extraction and cDNA synthesis) were performed twice using 2 separate atrial tissue samples from each subject and average values used for statistical analysis. Patients with plasma ACE2 activity less than or equal to the median were treated as the calibrator group and given an arbitrary value of 1.

MYOCARDIAL INTERSTITIAL COLLAGEN VOLUME FRACTION. Myocardial fibrosis was determined as previously described (21). Briefly, RAA paraffin sections 4- μm thick were deparaffinized, rehydrated, and stained with 0.1% Sirius Red (Polysciences, Warrington, Pennsylvania) in saturated picric acid (picrosirius red) for 1 h, differentiated in 0.01% hydrogen chloride for 30 s, and rapidly dehydrated. Staining was quantified using computerized image analysis (Imaging Research, Ontario, Canada). Interstitial collagen volume fraction (CVF) was determined by measuring the area of stained tissue within a given field, excluding epicardium, vessels, artefacts, minor scars, or incomplete tissue fields; 10 to 20 fields were analyzed per sample by a blinded reviewer. As per

previous classification of fibrosis (22), CVF $\leq 6\%$ was classified as nonsevere fibrosis and CVF $>6\%$ as severe fibrosis.

STATISTICAL ANALYSIS. Statistical analysis was performed using STATA version 15.1 (Statacorp., College Station, Texas). Normality of distribution was tested using the Shapiro-Wilk test. Descriptive analysis was presented as either number (proportion), mean $\pm$ SD or median (interquartile range [IQR]) where appropriate to compare the baseline characteristics between patients with above and below median ACE2 activity. Student's *t*-test or the Mann-Whitney *U* test (for non-normally distributed data) was used to assess differences in continuous variables. Cumulative incidence of the main outcome (all-cause mortality) was estimated by the Kaplan-Meier method and the log-rank test used to evaluate the effect of ACE2 and BNP on all-cause mortality. Cox proportional hazard modeling was used to estimate the adjusted hazard ratio (HR) and 95% confidence interval (CI) for all-cause mortality. Multivariable forward stepwise regression was performed using significant variables from univariate analysis and those with clinical relevance to identify independent predictors of all-cause mortality. Plasma ACE2 was entered in the model categorically, dichotomized according to an optimal discriminatory value determined from receiver-operating characteristic (ROC) curve analysis, which was selected to detect the primary outcome of all-cause mortality with a combined sensitivity and specificity closest to 100% and 100%, respectively (Supplemental Figure 1) (7,23). A $p < 0.05$ was required for a variable to enter the multivariate Cox model, and a $p > 0.10$ was required for exclusion. The proportional hazards assumption was tested on the basis of Schoenfeld residuals using the "estat phtest" command in STATA. No significant violations of the proportionality assumption were detected. Multicollinearity between variables was assessed using the variance inflation factor; this showed absence of multicollinearity among variables in the final model. The discriminative value of increased plasma ACE2 in the prediction of mortality was assessed using Harrell's C-statistic, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) methods (24). The IDI summarizes a new model's ability to improve integrated (average) sensitivity without sacrificing integrated (average) specificity. For ACE2 gene expression analysis, patients were divided according to median plasma ACE2 activity. ACE2 mRNA expression for the group with plasma ACE2 activity less than or equal to the median was

TABLE 1 Baseline Characteristics of the Study Population				
	Whole Cohort (N = 127)	ACE2 ≤ Median (n = 64)	ACE2 > Median (n = 63)	p Value
Demographics				
Age, yrs	75 ± 11	73 ± 11	76 ± 10	0.080
Male	81 (64)	32 (50)	49 (78)	0.002
Caucasian	119 (94)	58 (91)	61 (97)	0.270
Body mass index (kg/m ²)	29 ± 6	30 ± 6	28 ± 5	0.010
Systolic blood pressure (mm Hg)	131 ± 19	133 ± 21	130 ± 16	0.290
Diastolic blood pressure (mm Hg)	64 ± 13	66 ± 13	63 ± 14	0.190
Comorbidities				
Smoking history	61 (48.0)	30 (47)	31 (49)	0.860
Hyperlipidemia	95 (74.8)	48 (75)	47 (75)	1.00
Diabetes	33 (26.0)	14 (22)	19 (30)	0.320
Hypertension	103 (81.1)	51 (80)	52 (83)	0.820
Ischemic heart disease	47 (37.0)	18 (28)	29 (46)	0.044
Percutaneous coronary intervention	13 (10.2)	4 (6)	9 (14)	0.150
Coronary bypass surgery	20 (15.7)	8 (12)	12 (19)	0.340
Atrial fibrillation	35 (27.6)	16 (25)	19 (30)	0.560
Heart failure	40 (31.5)	15 (23)	25 (40)	0.0580
Medications				
ACEI/ARB	95 (75)	46 (72)	49 (78)	0.540
Aldosterone antagonist	8 (6)	4 (6)	4 (6)	1.00
Statins	89 (71)	44 (69)	45 (73)	0.700
Beta-blockers	49 (39)	27 (42)	22 (35)	0.470
Echocardiography				
Aortic valve area index (cm ² /m ²)	0.5 (0.4–0.7)	0.5 (0.4–0.7)	0.5 (0.4–0.7)	0.760
Mean aortic valve gradient (mm Hg)	37 (20–51.5)	37.6 (17.5–52)	36 (24–50)	0.710
Dimensionless performance index	0.26 (0.21–0.34)	0.26 (0.22–0.32)	0.25 (0.19–0.34)	0.220
LV end-diastolic volume (mL/m ²)	50 (41–62)	48 (41–56)	57 (43–69)	0.021
LV stroke volume index (mL/m ²)	48 ± 13	48 ± 12	49 ± 14	0.540
LV mass index (g/m ²)	111 (89–138)	106 (83–123)	119 (103–151)	<0.001
Left atrial volume index (mL/m ²)	46 (38–57)	44 (37–52)	47 (40–64)	0.072
E/A ratio	0.75 (0.67–0.99)	0.75 (0.67–0.99)	0.75 (0.67–0.99)	0.780
E/E' (average) ≥15	66 (57)	29 (50)	37 (64)	0.190
PASP (mm Hg)	39 (34–47)	38 (32–46)	40 (35–47)	0.420
LV ejection fraction (%)	60.5 (53–66)	61.3 (55–66.6)	59.4 (49–66)	0.160
Global longitudinal strain* (%)	-15 ± 4	-16 ± 3	-15 ± 4	0.120
Biochemical markers				
Hemoglobin (g/L)	132 ± 17	130 ± 16	135 ± 18	0.510
Creatinine (μmol/L)	84 (67–111)	83 (63–106)	92 (70–117)	0.180
Brain natriuretic peptide (ng/L)	130 (74–348)	93 (53–251)	141 (94–401)	0.021

Values are mean ± SD, n (%), or median (interquartile range). *Data only available for 119 individuals (n = 64 angiotensin-converting enzyme 2 [ACE2] below median group and n = 55 ACE2 more than median group). Median plasma ACE2 activity = 34 pmol/mL/min. **Bold** values denote statistical significance at the p < 0.05 level.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; AS = aortic stenosis; LV = left ventricular; PASP = pulmonary artery systolic pressure.

arbitrarily standardized to 1 by taking the average of the results in this group. Gene expression in the group with plasma ACE2 activity more than the median was expressed relative to this group. Two-tailed p values <0.05 were considered significant.

RESULTS

The cohort consisted of 127 patients with mild (25%), moderate (24%), or severe AS (51%). The baseline clinical, biochemical, and echocardiographic characteristics are presented in **Table 1** (63% men; mean age

75 ± 10 years). There was a high prevalence of cardiovascular risk factors and/or disease, including hyperlipidemia (75%), diabetes (26%), hypertension (81%), ischemic heart disease (IHD) (37%), atrial fibrillation (28%), and heart failure (32%). With regard to baseline medical therapy, 75% of subjects were on ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), 71% were on statins, and 39% were on beta-blockers.

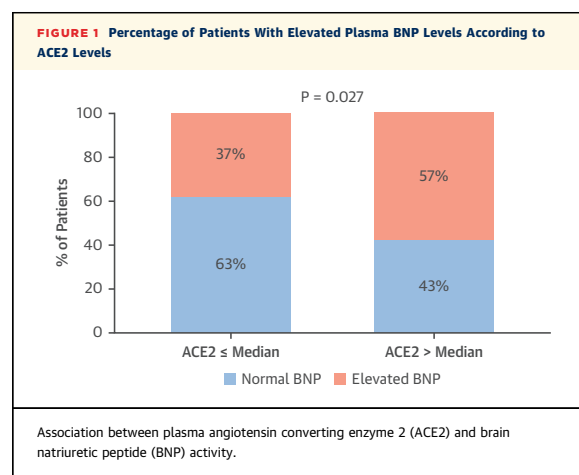
Table 1 also shows characteristics when categorized according to the median level of plasma ACE2 activity in the whole cohort of 34.0 pmol/mL/min. Patients

with above-median plasma ACE2 activity were more likely to be men ($p = 0.02$) with a history of IHD and higher body mass index ($p < 0.05$). There were no significant differences in baseline pharmacological therapy. On multivariable regression analysis, male sex was the only independent clinical predictor of higher plasma ACE2 activity ($p = 0.001$).

PLASMA ACE2 ACTIVITY AND IMAGING MARKERS OF AORTIC VALVE CALCIFICATION, AS SEVERITY, AND MYOCARDIAL DISEASE. Patients with above-median plasma ACE2 activity were more likely to have moderate or more aortic valve calcification assessed qualitatively compared with those with below-median plasma ACE2 activity (86% vs. 69%; $p = 0.023$). There were no differences in measures of AS severity, including aortic valve area, mean aortic valve gradient (Supplemental Figure 2), and dimensionless performance index between groups (all $p > 0.05$). A bicuspid aortic valve was present in 12 subjects (9.4%) and a dilated aortic root was present in 34 subjects (26.5%). There was no significant difference in ACE2 levels according to the presence of bicuspid aortic valve ($p = 0.563$) or a dilated root ($p = 0.392$). With regard to myocardial structure, there was a moderate positive correlation between plasma ACE2 activity and LV mass index ($r = 0.337$; $p < 0.001$) (Supplemental Figure 3). Patients with above-median plasma ACE2 had higher LV end-diastolic volume (57 vs. 48 ml/m²; $p = 0.021$). There were no significant differences in left atrial volume, LV diastolic indexes, pulmonary artery systolic pressure, or LV systolic function assessed using LV ejection fraction and LV global longitudinal strain (GLS) (reliably measurable only in 93% of the cohort) (Table 1).

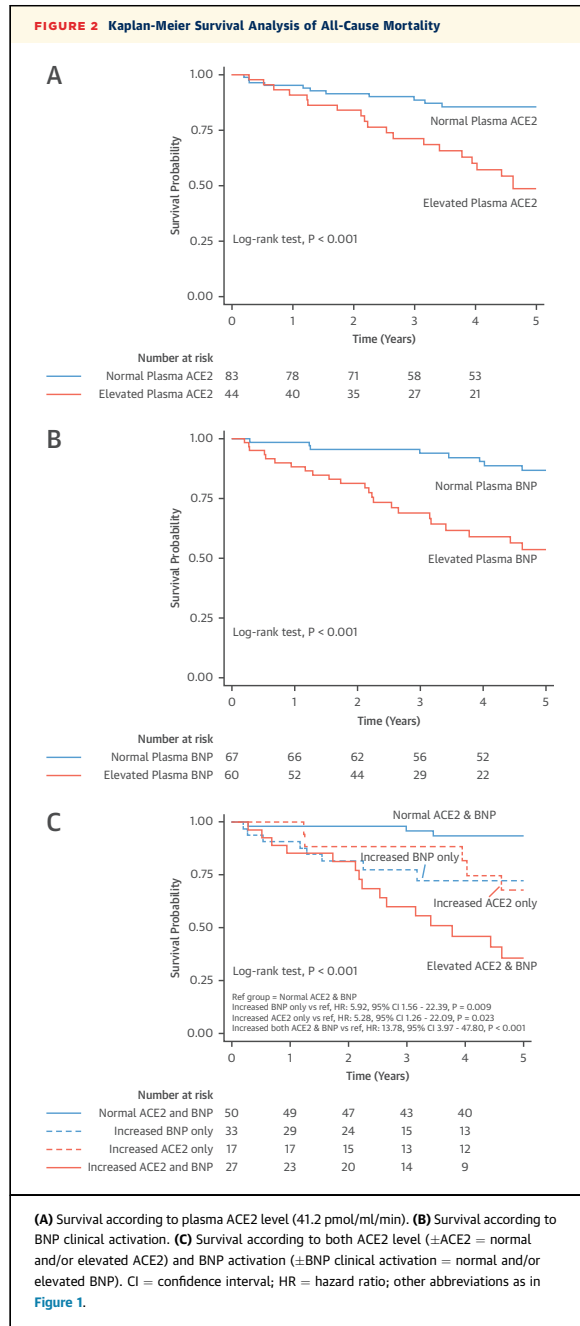
PLASMA ACE2 ACTIVITY AND BIOCHEMICAL MARKERS. The median (IQR) of BNP was higher in those with above-median plasma ACE2 activity (141 [IQR: 94 to 401] pg/ml vs. 93 [IQR: 53 to 251] pg/ml; $p = 0.021$). The proportion of patients with BNP clinical activation was also significantly higher in those with above-median plasma ACE2 activity (57% vs. 37%; $p = 0.027$) (Figure 1). There was no difference in hemoglobin concentration and creatinine levels between the 2 groups ($p > 0.05$).

PLASMA ACE2 ACTIVITY AND ALL-CAUSE MORTALITY. The median duration of follow-up was 5 years (IQR: 0.19 to 5 years). Over this period, 58 patients underwent AVR, and 31 patients (24%) died. There was a higher incidence of all-cause mortality in those with above-median plasma ACE2 activity compared with below-median plasma ACE2 (38% vs. 11%; log-rank $p < 0.001$). ROC analysis was used to determine the plasma ACE2 value with the best combination of



sensitivity and specificity for all-cause mortality. The ROC area under the curve was 0.73 (95% CI: 62% to 82%) and a plasma ACE2 cutoff value of 41.2 pmol/ml/min had a sensitivity of 65% and a specificity of 75% for detecting all-cause mortality (Supplemental Figure 1). Patients with plasma ACE2 activity above this threshold had a significantly increased likelihood of all-cause mortality compared with those without activity (HR: 3.86; 95% CI: 1.85 to 8.06; $p < 0.001$) (Figure 2A); therefore, this ROC-derived value was used for subsequent analysis. When analyzed according to sex, elevated plasma ACE2 activity >41.2 pmol/ml/min was associated with a higher incidence of all-cause mortality in both male (log rank test; $p = 0.01$) and female patients (log-rank test; $p < 0.001$) respectively. Patients with BNP activation had a significantly increased likelihood of death (HR: 4.64; 95% CI: 2.06 to 10.42; $p < 0.001$) (Figure 2B). All-cause mortality was highest in those with the combination of elevated plasma ACE2 activity and BNP clinical activation (HR: 13.78; 95% CI: 3.97 to 47.8; $p < 0.001$) (Figure 2C).

Other univariate predictors of mortality included age, heart failure, IHD, beta-blocker use, LV mass, abnormal LV ejection fraction (sex-specific), BNP clinical activation, and log serum creatinine level (Table 2). Cox multivariable regression analysis demonstrated that elevated plasma ACE2 activity (HR: 2.28; 95% CI: 1.03 to 5.06; $p = 0.042$) was an independent predictor of mortality in addition to age (HR: 1.07; 95% CI: 1.01 to 1.13; $p = 0.015$), a history of heart failure (HR: 3.07; 95% CI: 1.39 to 6.77; $p = 0.006$), and a history of IHD (HR: 2.96; 95% CI: 1.29 to 6.82; $p = 0.011$). In the subgroup of



patients with normal LV ejection fraction and normal stroke volume, elevated plasma ACE2 activity remained associated with a higher incidence of all-cause mortality (HR: 3.25; 95% CI: 1.21 to 8.74; $p = 0.019$).

In a nested model that included the independent predictors of mortality (age, history of IHD, history of heart failure), the addition of elevated plasma ACE2 activity (above the ROC-derived threshold of 41.2 pmol/ml/min) had incremental prognostic value to predict all-cause mortality, with the chi-square of the model increasing from 40.2 to 45.2 ($p = 0.025$). Harrell's C-statistic for all-cause mortality increased slightly from 0.80 to 0.82. The IDI improved significantly (IDI: 0.084; SE: 0.030; $p < 0.001$). The category free (continuous) NRIs were 0.290 (95% CI: 0.04 to 0.625) for deaths and 0.479 (95% CI: 0.296 to 0.653) for survivors; this means that there was a 29.0% improvement in the prediction of deaths and a 47.9% net improvement in the prediction of survivors. The total NRI was 0.769 (95% CI: 0.390 to 1.165). The categorical reclassification calibration was 12.60% (95% CI: 3.15% to 29.53%) (Supplemental Figure 4).

CIRCULATING AND TISSUE ACE2 GENE EXPRESSION AND MYOCARDIAL FIBROSIS. Myocardial tissue was available for analysis in 22 patients. There were no significant differences in clinical characteristics between those who did and did not have myocardial tissue collected (data not shown). Patients with above-median circulating ACE2 levels had significantly reduced myocardial ACE2 gene expression (0.7-fold; $p = 0.033$) (Figure 3). In 18 patients with assessment of myocardial fibrosis, nonsevere fibrosis (CVF $\leq 6\%$) was present in 11 (61%) patients and severe fibrosis (CVF $> 6\%$) in 7 (39%) patients. The only clinical predictor of myocardial fibrosis was a history of atrial fibrillation. Among those with atrial fibrillation, 80% had severe fibrosis compared with 20% with no atrial fibrillation ($p = 0.026$). Patients with severe fibrosis had significantly elevated plasma ACE2 activity ($p = 0.027$) (Figure 4). In subjects without IHD ($n = 9$), plasma ACE2 levels were numerically, but not significantly, higher in those with significant myocardial fibrosis compared with those without significant fibrosis (42.0 pmol/ml/min [IQR: 28.4 to 63 pmol/ml/min] vs. 24.1 pmol/ml/min [IQR: 6.6 to 41.4 pmol/ml/min]). There was no significant association between LV GLS and atrial fibrosis quantification ($\rho = 0.351$; $p = 0.218$).

DISCUSSION

Activation of the classic RAS plays a role in the pathophysiology of AS and the accompanying adverse

LV remodeling and myocardial fibrosis (25). RAS inhibition has a positive impact on regressing hypertrophy, improving myocardial physiology, and slowing the rate of progression of valvular stenosis (26). In 2000, our understanding of the RAS was significantly broadened with the discovery of the ACE2 gene (27), which changed the simplistic vertical view of the RAS and led to the concept of a counter-regulatory arm with opposing effects in cardiovascular biology (28).

The key findings of this study in patients with AS were that increased plasma ACE2 levels were associated with myocardial structural abnormalities and provided added incremental prognostic information to predict long-term mortality over and above conventional risk markers, including BNP activation (Central Illustration). Plasma ACE2 levels correlated with the extent of valvular calcification but not AS severity. The median ACE2 level in the AS cohort was 34 pmol/ml/min, which was similar to levels we reported in a high-risk cohort with coronary artery disease (9) (median: 29 pmol/ml/min) and higher than levels in patients with atrial fibrillation (10) or in young healthy volunteers (mean: 4 pmol/ml/min) (16). Consistent with previous reports, there was no correlation between ACE2 activity and treatment with ACE inhibitors, and male sex was an independent predictor of increased plasma ACE2 activity (9,10).

MARKER OF MYOCARDIAL STRUCTURAL ABNORMALITIES.

These results add to the growing body of evidence that plasma ACE2 activity levels may serve as a marker of myocardial abnormalities and/or adverse outcomes in cardiovascular disease, including coronary artery disease, atrial fibrillation, and heart failure (7-10). We recently reported that elevated plasma ACE2 activity was an independent predictor of major adverse cardiovascular events (cardiovascular mortality, heart failure, myocardial infarction) over a median follow-up of 10.5 years in patients with angiographically proven obstructive coronary artery disease (9). Plasma ACE2 levels also reflect cardiac structural alterations. For example, in human atrial fibrillation, increased plasma ACE2 activity was associated with more advanced left atrial structural remodeling assessed by electroanatomic mapping (10). In patients with heart failure, elevated circulating ACE2 levels independently predicted cardiovascular events (death, cardiac transplantation, or heart failure hospitalization) after 34 months of follow-up and were positively correlated with imaging indexes of ventricular remodeling and

TABLE 2 Cox Regression Analysis for All-Cause Mortality in Patients With AS

	Unadjusted HR (95% CI)	Unadjusted p Value	Adjusted HR (95% CI)	Adjusted p Value
Age	1.10 (1.04-1.16)	<0.001	1.07 (1.01-1.13)	0.015
Male	1.05 (0.50-2.18)	0.906		>0.10
Diabetes	1.23 (0.57-2.69)	0.592		>0.10
Atrial fibrillation	1.46 (0.69-3.10)	0.327		>0.10
IHD	5.48 (2.52-11.93)	<0.001	2.96 (1.29-6.82)	0.011
Heart failure	4.14 (2.02-8.46)	<0.001	3.07 (1.39-6.77)	0.006
LVMI	1.01 (1.01-1.02)	0.001		>0.10
Abnormal LVEF (sex-adjusted)	5.06 (2.46-10.43)	<0.001	2.11 (0.90-4.93)	0.086
Log creatinine	1.71 (1.01-2.91)	0.047		>0.10
Elevated BNP	4.64 (2.06-10.42)	<0.001		>0.10
Plasma ACE2 > 41.2 pmol/ml/min	3.86 (1.85-8.06)	<0.001	2.28 (1.03-5.06)	0.042

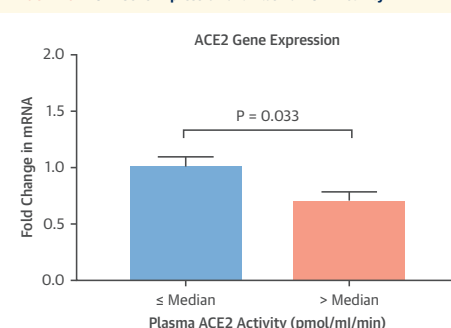
A p value <0.05 was required for a variable to enter the multivariate Cox model and a value of p > 0.10 was required for exclusion. **Bold** values denote statistical significance at the p < 0.05 level.

BNP = brain natriuretic peptide; CI = confidence interval; HR = hazard ratio; IHD = ischemic heart disease; LVEF = left ventricular ejection fraction; LVMI = left ventricular mass index; other abbreviation as in Table 1.

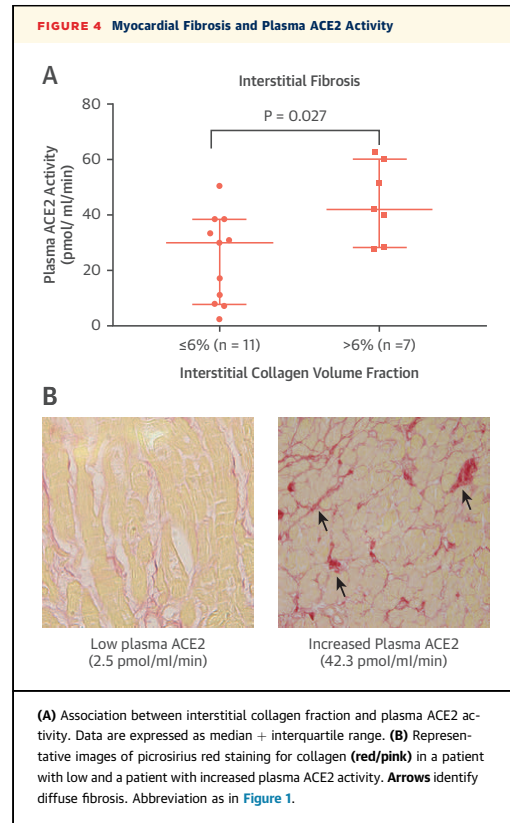
dysfunction (7). Similarly, following ST-segment elevation myocardial infarction, circulating ACE2 activity increased in relation to the infarct size and correlated with more severe LV remodeling on cardiac magnetic resonance (8).

The present study extended knowledge in this regard. We found a positive correlation between plasma ACE2 activity and LV mass, although the relationship

FIGURE 3 ACE2 Gene Expression and Plasma ACE2 Activity



Relative myocardial ACE2 mRNA in patients according to median plasma ACE2 activity (19.5 pmol/ml/min in cohort of 24 patients). Gene expression for the group with plasma ACE2 activity less than or equal to the median was arbitrarily standardized to 1. Gene expression in the group with plasma ACE2 activity above the median was expressed relative to this group. Data are expressed as mean ± SEM. Abbreviation as in Figure 1.



in this patient population with AS was likely influenced by the presence of hypertension and/or anti-hypertensive therapies. Furthermore, we found that increased plasma ACE2 activity was associated with increased LV diastolic volume but not LV ejection fraction or GLS. These data suggested a potential role for measurement of plasma ACE2 activity levels as a marker of early myocardial decompensation in AS.

BIOMARKERS FOR RISK STRATIFICATION IN AS. Guidelines for risk stratification in AS emphasize the importance of recognizing early myocardial decompensation and suggest that natriuretic peptide levels may be useful in asymptomatic patients to determine the optimal timing of AVR (Class IIa, Level of Evidence: C) (2). Plasma BNP activation was shown to independently predict long-term mortality in a prospective study of 1,953 patients with at least

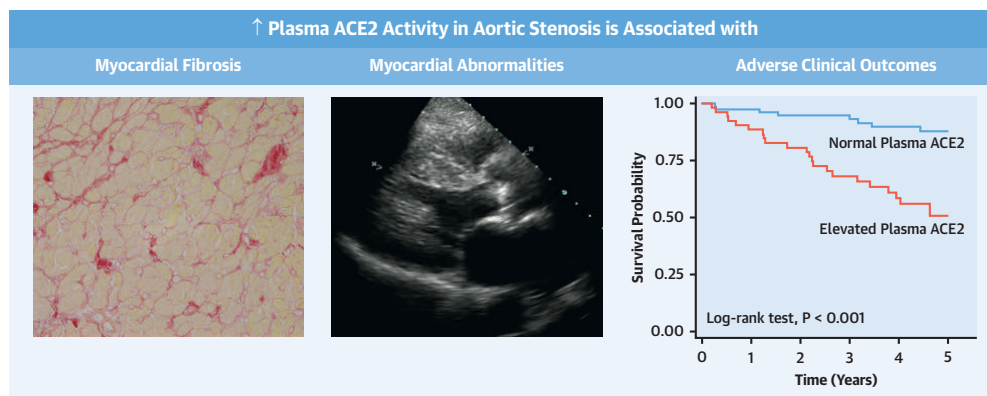
moderate AS who were followed up over 4.3 years (20). More recently, plasma BNP measurement enhanced the prediction of all-cause mortality in patients with low-flow, low-gradient AS (29).

In the present study, patients with BNP activation and elevated plasma ACE2 activity had increased mortality compared with those without elevation of either ACE2 or BNP or with elevation of only 1 biomarker. Because plasma ACE2 measurement had prognostic value not captured by BNP alone, the combination of the 2 biomarkers might enhance clinical decision-making in AS. Importantly, >40% of patients with above-median ACE2 activity had normal BNP levels suggesting different pathophysiological mechanisms and/or stimuli for the release of the 2 markers. BNP is a 32-amino acid peptide secreted from the heart in response to myocardial stretch from cardiac volume or pressure overload (29) and has a well-established role in risk stratification in valvular heart disease (2). ACE2 is highly expressed in the heart and undergoes cleavage or “shedding” to release the catalytically active ectodomain into the extracellular milieu (13), a process that involves the proteinase ADAM17, also known as TACE (13,30).

Release of ACE2 from the heart is regulated by various stimuli, including angiotensin II and the loss of ACE2 from the tissue leads to unopposed pro-inflammatory and pro-fibrotic effects of angiotensin II in the myocardium (31). In an experimental mouse model of targeted ACE2 disruption (14), loss of myocardial ACE2 led to increased cardiac angiotensin II levels, cardiac hypertrophy and fibrosis, and impaired cardiac function. In human tissue, ACE was upregulated (32) and ACE2 downregulated in stenotic aortic valves (33). This is the first report in patients with AS to describe that myocardial ACE2 gene expression was reduced, that reduced tissue levels were associated with elevated plasma ACE2 activity, and that those with the highest plasma ACE2 activity had more severe myocardial fibrosis, an important predictor of death in AS (34).

STUDY LIMITATIONS. The strengths of this study includes the detailed cardiac phenotyping and long-term follow-up, as well as the assessment of circulating and myocardial ACE2, and cardiac fibrosis. Some important limitations should be acknowledged. First, the finding of elevated plasma ACE2 activity and the association with abnormal myocardial function and increased mortality suggests a possible relationship but does not determine a causal relationship. Second, the LV is the first extra-valvular cardiac structure to be affected in the earliest stages

CENTRAL ILLUSTRATION Prognostic Value of Plasma ACE2 in AS



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In patients with aortic stenosis (AS), elevated plasma angiotensin-converting enzyme 2 (ACE2) activity was associated with reduced myocardial ACE2 gene expression, myocardial structural abnormalities, and more severe myocardial fibrosis, and independently predicted mortality.

of AS (35), but to avoid the low but not insignificant risks of septal endomyocardial biopsy, we studied right atrial tissue accessible at time of AVR. Finally, because of the relatively small number of patients, findings from the subgroup analyses findings should be interpreted with caution.

CONCLUSIONS

We present the original observations that increased plasma ACE2 activity in patients with AS was: 1) associated with myocardial structural abnormalities; 2) independently predicted mortality with incremental value over traditional prognostic markers; and 3) was associated with reduced myocardial ACE2 expression and more marked myocardial fibrosis. Our results need confirmation in large independent cohorts before measurement of plasma ACE2 activity plays a role in risk stratification of patients with AS to guide optimal timing of AVR, but our findings will stimulate further research into the pathophysiologic mechanisms of AS, particularly as it relates to the alternative arm of the RAS.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: ACE2 is a critical regulator of the RAS and functions to degrade the pro-fibrotic peptide angiotensin II. Plasma ACE2 levels have an emerging role as a biomarker of cardiovascular disease. In patients with AS, elevated plasma ACE2 activity was associated with increased myocardial structural abnormalities, more severe myocardial fibrosis, and independently predicted mortality with incremental value over traditional prognostic markers.

TRANSLATIONAL OUTLOOK: The role of plasma ACE2 measurement in combination with known plasma and imaging biomarkers in AS merits further investigation and has the potential to foster timely AVR.

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KEY WORDS angiotensin II, cardiac fibrosis, myocardial dysfunction, renin-angiotensin system, valve disease

APPENDIX For supplemental figures, please see the online version of this paper.

Chapter 5: Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with severe aortic stenosis

Ramchand J, Iskandar JP, Puri R, Chetrit M, Burrell LM, Krishnaswamy A, Griffin B, Yun J, Flamm S, Kapadia S, Kwon D, Harb S. **Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with chronic kidney disease and severe aortic stenosis** (manuscript under revision)

Journal of Cardiovascular Magnetic Resonance

Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with chronic kidney disease and severe aortic stenosis --Manuscript Draft--

Manuscript Number:	JCMR-D-21-00054
Full Title:	Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with chronic kidney disease and severe aortic stenosis
Article Type:	Research
Funding Information:	
Abstract:	Background Amongst patients with chronic kidney disease (CKD), aortic stenosis (AS) is associated with a significantly higher rate of mortality. We aimed to evaluate if diffuse myocardial fibrosis determined using native T1 mapping harbors prognostic utility to predict major adverse cardiovascular events (MACE: all-cause mortality or heart failure hospitalization) in patients with CKD and severe AS being evaluated for transcatheter aortic valve replacement (TAVR). Methods Cardiac magnetic resonance with T1 mapping using the modified Look-Locker inversion recovery technique was performed in 117 consecutive individuals with severe AS and CKD stage > 3. Patients were followed up to determine the occurrence of MACE. Results The cohort of 147 individuals had a mean age of 82±8 years. Native T1 time was 1055ms (25th -75th percentiles 1031-1078), higher than previously reported values in healthy controls. Patients with higher T1 times were more likely to have higher NT-proBNP levels [4122(1578-7980) vs. 1678(493-2851) pg/mL, P=0.005] and a history of heart failure [33% vs 9%, P= 0.034]. After median follow-up of 3.4 years, MACE occurred in 71 individuals (61%). The STS-PROM score (HR:1.07,95%CI:1.02-1.1,P=0.006), native T1 time>1024ms (HR:2.1;95%CI:1.09-4.06, P=0.028) and NYHA class (HR:1.56;1.09-2.34, P=0.016) were independent predictors of MACE. Conclusions Longer native T1 time associated with MACE occurrence in individuals with CKD and severe AS. Further studies are needed to establish if interstitial fibrosis identified using T1 mapping can be used to guide timing of AVR.
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Is this study a clinical trial? A clinical trial is defined by the World Health Organisation as 'any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes'.	No

Myocardial tissue characterization using native T1 time predicts the occurrence of adverse events in patients with chronic kidney disease and severe aortic stenosis

Running title: T1 mapping in CKD and AS

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Abstract

Background

Amongst patients with chronic kidney disease (CKD), aortic stenosis (AS) is associated with a significantly higher rate of mortality. We aimed to evaluate if diffuse myocardial fibrosis determined using native T1 mapping harbours prognostic utility to predict major adverse cardiovascular events (MACE: all-cause mortality or heart failure hospitalization) in patients with CKD and severe AS being evaluated for transcatheter aortic valve replacement (TAVR).

Methods

Cardiac magnetic resonance with T1 mapping using the modified Look-Locker inversion recovery technique was performed in 117 consecutive individuals with severe AS and CKD stage ≥ 3 . Patients were followed up to determine the occurrence of MACE.

Results

The cohort of 147 individuals had a mean age of 82 ± 8 years. Native T1 time was 1055ms (25th-75th percentiles 1031-1078), higher than previously reported values in healthy controls. Patients with higher T1 times were more likely to have higher NT-proBNP levels [4122(1578–7980) vs. 1678(493-2851) pg/mL, $P=0.005$] and a history of heart failure [33% vs 9%, $P=0.034$]. After median follow-up of 3.4 years, MACE occurred in 71 individuals (61%). The STS-PROM score (HR:1.07, 95%CI:1.02-1.1, $P=0.006$), native T1 time >1024 ms (HR:2.1; 95%CI:1.09-4.06, $P=0.028$) and NYHA class (HR:1.56; 1.09–2.34, $P=0.016$) were independent predictors of MACE.

Conclusions

Longer native T1 time associated with MACE occurrence in individuals with CKD and severe AS. Further studies are needed to establish if interstitial fibrosis identified using T1 mapping can be used to guide timing of AVR.

Keywords:

Aortic stenosis, biomarkers, cardiac magnetic resonance, echocardiography, fibrosis, kidney disease

INTRODUCTION

Aortic stenosis (AS) is the leading cause of valvular heart disease in the Western world and is associated with increased morbidity and mortality (1). Aortic valve replacement (AVR) improves survival in severe AS and is indicated if there is evidence of left ventricular (LV) decompensation (2, 3). With progressive valve narrowing, compensatory left LV remodelling mechanisms become maladaptive, which if left unchecked lead to irreversible LV fibrosis.

Myocardial fibrosis is a pathological process that drives LV decompensation in AS (4, 5). It is desirable to identify such pathological myocardial changes before it becomes irreversible, as targeted early AVR may improve outcomes (6). Two patterns of fibrosis can be observed using cardiac magnetic resonance (CMR): 1) focal replacement fibrosis evident on T1-weighted late gadolinium enhancement imaging that appears irreversible, and 2) diffuse interstitial fibrosis that is potentially reversible following AVR (5). Myocardial interstitial fibrosis changes the longitudinal relaxation properties (T1 values) of tissue and, using CMR T1 mapping techniques, the abnormal relaxation properties can be exploited to measure the extent of diffuse fibrosis (7). Myocardial T1 mapping allows quantitative tissue characterization, yielding a value unlike relying on visually recognizable contrast differences (8). The T1 relaxation time corresponds to the specific time decay constant when the proton recovers approximately 63% of its longitudinal magnetization equilibrium value. Native T1 mapping produces a voxel-based map of the myocardium that estimates absolute myocardial T1 values. The two most important factors associated with longer T1 values include increase in tissue oedema and/or greater deposition of interstitial collagen or amyloid protein (7, 9, 10). On the contrary, lipid (Anderson–Fabry) or iron overload diseases are responsible for shorter T1 values. In human

AS, prior histological studies have reported a consistent relationship between a variety of T1-based indices and the extent of myocardial fibrosis (7, 8, 11).

Amongst patients with chronic kidney disease (CKD), AS is associated with a high rate of adverse outcomes compared to those without CKD (12, 13). It is posited that in CKD, there is further amplification of LV remodelling and fibrosis in addition to the expected changes seen in AS (14). We therefore aimed to investigate if a greater extent of diffuse interstitial fibrosis reflected by longer non-contrast T1 values predicted poorer outcomes in individuals with CKD and severe AS.

METHODS

Study Population

This was an observational, retrospective cohort study of consecutive adults with severe native valvular AS and CKD stages 3-5 who underwent TAVR and who underwent pre-TAVR non-contrast CMR evaluation. This study was approved by the Cleveland Clinic institutional review board, with a waiver of individual consent.

Stages of CKD were defined according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative Classification, Prevalence, and Action Plan for Stages of Chronic Kidney Disease: Stage 3 = GFR 30 to 59 mL/min per 1.73 m²; Stage 4 – GFR 15 to 29 mL/min per 1.73 m²; G5 – GFR <15 mL/min per 1.73 m² or treatment by dialysis (15).

Since cardiac amyloidosis is associated with high native T1 values (16) and is prevalent in elderly patients with severe aortic stenosis (17, 18), clinical records were searched to assess for the presence of cardiac amyloidosis. This was specifically achieved by entering the search term “amyloid” into EPIC, the electronic medical records of our institution, which captures all cardiac imaging investigations as well as any outpatient, inpatient and emergency room encounters.

CMR assessment

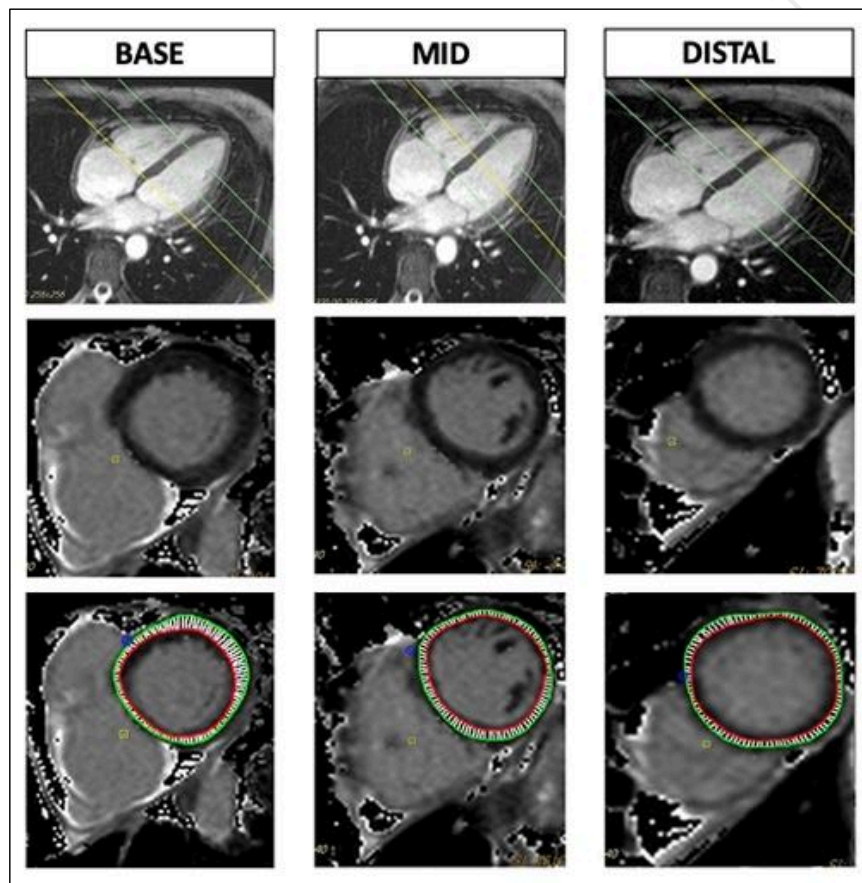
CMR examinations were performed on a 1.5-T MR scanner (Achieva, Philips Health Care, Mass, USA), using 45 mT/m maximum gradient strength, 200 T/m per second maximum slew rate. Electrocardiographic gating was used as previously described (19, 20). For assessment of cardiac function, steady-state free-precession (SSFP) images were acquired (echo time 1.6 ms, repetition time 3.3 ms, flip angle 70°, and slice thickness 8 mm in contiguous short-axis images. For short- axis images, the spatial resolution was 1.5–2.1 mm × 1.1–1.4 mm. LV volumes, LV ejection fraction (EF) and LV mass were calculated on the basis of short-axis SSFP images. CMR images were analyzed using commercially available software (cvi42; Circle Cardiovascular Imaging, Calgary, Alberta, Canada). Normal values for ventricular size and function were determined using published data from Kawel-Boehm et al (21). LV concentricity was calculated and 95th percentile cut-offs were determined according to values published by Chuang et al (22). Gadolinium contrast was not administered as the main purpose of the CMR examination was to assess aortic annular dimensions for TAVR valve sizing in this cohort of patients with CKD where contrast CT angiography was not performed.

T1 mapping

T1 imaging was performed using a Modified Look-Locker Inversion (MOLLI 5(3)3) (FA 35°) sequence in three short axis images (basal, mid and distal LV) during breath-hold in end-expiration. The MOLLI sequence was not adjusted for high heart rate though this strategy has been reported to have significantly improved heart rate sensitivity compared to original sampling strategies (23, 24). Native T1 maps were generated and analysed using a commercial software package for cardiovascular image analysis (cvi42, V.5.11.2, Circle Cardiovascular Imaging, Calgary, Canada) as previously described (Supplementary Figure 1) (25). Endocardial and epicardial borders were defined manually to only include the myocardium, using an offset of 15% to avoid partial-volume effects in the subendocardial and subepicardial layers. Global myocardial T1 values were calculated as a mean of all segments with respect to the segment's area. T1 times were categorized into above or below 1024ms, representing the mean + 2 SD of control patients on a 1.5-T MR scanner in our institution.

Supplementary Figure 1. T1 mapping

Native T1 maps were generated and analysed using a commercial software package for cardiovascular image analysis (cvi42, V.5.11.2, Circle Cardiovascular Imaging, Calgary, Canada). Endocardial (red tracing) and epicardial borders (green tracing) were defined manually to avoid partial-volume effects in the subendocardial and subepicardial layers.



Echocardiographic assessment

Transthoracic echocardiography (TTE) was performed using commercially available ultrasound systems in the left lateral decubitus position by experienced sonographers. Measurements and recordings were obtained according to the American Society of Echocardiography (ASE) guidelines. (26, 27) Dobutamine stress echocardiography, transoesophageal echocardiography, invasive hemodynamic assessment and/or aortic valvular calcium score by multi slice computed tomography were used to determine AS severity in those with discordant findings on baseline echocardiography. (27) Final arbitration of AS severity and all management decisions were made following a consensus decision from a multidisciplinary Heart Team. Following current guidelines, four subtypes of severe AS were defined: (1) Preserved EF high-gradient AS; (2) paradoxical normal EF, low-gradient AS; (3) Low EF, high-gradient AS; (4) low EF, low-gradient AS ('classic' low-flow, low-gradient AS) (3).

Echocardiographic left atrial volume index was collected routinely using standard methods recommended by the ASE guidelines (26). Although CMR is the reference gold standard for measurement of chamber volumes due to its enhanced accuracy, the process of accurate LA volume assessment requires tomographic slices through the atrial chambers. This is not routinely used in clinical practice as it can increase scanning times and decrease scan tolerance, particularly in symptomatic individuals with dyspnoea (28). Furthermore, there is accumulating data regarding the prognostic role of left atrial volume as measured on echocardiography in aortic stenosis to support its use (29, 30).

Plasma NT-proBNP analysis

N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured using a validated, commercially available assay using established methodologies (Elecsys® ProBNP assay, Roche Diagnostics, Indianapolis, IN, USA)

Clinical outcomes

The duration of follow-up was calculated from initial CMR visit to event/last office follow-up. The primary endpoint was a composite of major adverse cardiac events (MACE) defined as all-cause mortality or heart failure hospitalization. We ascertained events by reviewing medical records. Heart failure hospitalization was defined “as an event in which the patient is admitted to the hospital with a primary diagnosis of HF, the length of stay is at least 24 h (or extends over a calendar date), the patient exhibits new or worsening symptoms of HF on presentation, has objective evidence of new or worsening HF, and receives initiation or intensification of treatment specifically for HF” (31).

Statistical analysis

Statistical analysis was performed using STATA version 15.1 (Statacorp., College Station, Texas). Normality of distribution was tested using the Shapiro-Wilk test. Descriptive analysis was presented as either number (proportion), mean $\pm$ SD or median (25th, 75th percentile) where appropriate to compare the baseline characteristics between patients with above and below median native T1 values. Student’s t-test, Mann-Whitney U (for non-normally distributed data) or Kruskal Wallis tests were used to assess differences in continuous variables.

Cumulative incidence of the main outcome, MACE (all-cause mortality or heart failure hospitalization) was estimated by the Kaplan-Meier method and the log-rank test.

Cox proportional hazard modelling was used to estimate the adjusted hazard ratio (HR) and 95% confidence interval (CI) for MACE. Multiple variable forward stepwise regression was performed using significant variables from univariate analysis and those with clinical relevance to identify independent predictors of MACE. Native T1 value was entered in the model categorically, dichotomized according to a cut-off value of 1124 which represented the mean + 2SD of control subjects in our institution. A $p < 0.05$ was required for a variable to enter the multiple variable Cox model, and a $p > 0.10$ was required for exclusion. Proportional hazards assumptions were tested on the basis of Schoenfeld residuals using the “estat phtest” command after fitting the models with stcox. No significant violations of the proportionality assumption were detected. Multicollinearity was assessed using “Collin” to determine variance inflation factors (VIFs) and tolerance; this showed absence of multi- collinearity among variables in the final model. The discriminative value of native T1 time in the prediction of MACE was assessed using Harrell’s C-statistic and integrated discrimination improvement (IDI) methods. The IDI summarizes a new model’s ability to improve integrated (average) sensitivity without sacrificing integrated (average) specificity (32). Intra and interobserver reproducibility of T1 mapping measurements was assessed by using the Bland–Altman method as well as intra-class coefficient analysis. Two-tailed p values <0.05 were considered significant.

RESULTS

A total of 117 individuals were included in the analysis (Figure 1). Baseline clinical variables, medications, biochemical, and imaging parameters are listed in Table 1. There was a high prevalence of cardiovascular comorbidities, including hypertension (92%), diabetes (44%), chronic heart failure (28%), and previous cardiac surgery (coronary bypass surgery in 33%). The median Society of Thoracic Surgeons predicted risk of mortality (STS-PROM) score was 5.8%, reflective of an intermediate to high-risk cohort (33). With regard to baseline medical therapy, there was increased use of heart failure or hypertension related therapy, including ACE inhibitors or angiotensin receptor blockers (22%), beta-blockers (74%) and diuretics (68%). The majority of the patients had stage 3 CKD (63%), and the remaining had stages 4 (33%) and 5 (4%) CKD.

Figure 1. Flow diagram of study participants

Flow diagram demonstrating derivation of study cohort.

CMR = cardiovascular magnetic resonance; AVR surgical aortic valve replacement

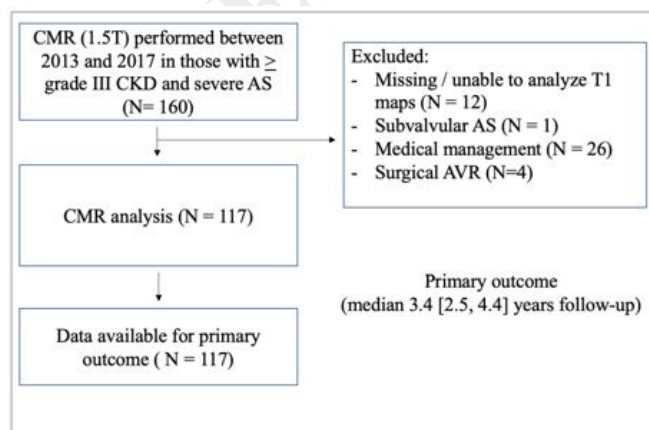


Table 1. Baseline Characteristics

	Entire cohort	T1 time $\leq$ 1024ms	T1 time > 1024ms	P value
	N=117	N=22	N=95	
Age	81.6 (8.3)	79.6 (9.1)	82.0 (8.1)	0.23
Male	70 (59.8%)	16 (72.7%)	54 (56.8%)	0.23
Body mass index (kg/m ²)	27.6 (5.0)	28.9 (3.8)	27.3 (5.2)	0.19
Clinical history / risk factors				
Hypertension	108 (92.3%)	21 (95.5%)	87 (91.6%)	1.00
Diabetes	52 (44.4%)	9 (40.9%)	43 (45.3%)	0.81
Atrial fibrillation	38 (32.5%)	4 (18.2%)	34 (35.8%)	0.13
History of heart failure	33 (28.2%)	2 (9.1%)	31 (32.6%)	0.034
Coronary artery disease	95 (81.2%)	19 (86.4%)	76 (80.0%)	0.76
Coronary bypass surgery	39 (33.3%)	11 (50.0%)	28 (29.5%)	0.081
Valvular surgery	5 (4.3%)	1 (4.5%)	4 (4.2%)	1.00
Dialysis	6 (5.1%)	0 (0.0%)	6 (6.3%)	0.59
NYHA functional class $\geq$ III	85 (72.6%)	14 (63.6%)	71 (74.7%)	0.30
STS-PROM score, %	5.8 (3.3-8.1)	5.5 (4.0-7.8)	5.8 (3.1-8.3)	0.91
Medications				
ACEi or ARB use	26 (22.2%)	4 (18.2%)	22 (23.2%)	0.78
Beta blocker	87 (74.4%)	19 (86.4%)	68 (71.6%)	0.18
Calcium channel blocker	43 (36.8%)	6 (27.3%)	37 (38.9%)	0.34
Diuretics	79 (67.5%)	14 (63.6%)	65 (68.4%)	0.80
Laboratory markers				
Hemoglobin, g/dL	11.0 (1.8)	11.5 (1.6)	10.9 (1.8)	0.17
NT-ProBNP, pg/mL	3186 (1337-6684)	1678 (493-2851)	4122 (1578-7980)	0.005

Serum creatinine, mg/dL	1.8 (1.5-2.2)	1.9 (1.7-2.3)	1.8 (1.5-2.2)	0.17
GFR, ml/min/1.73 m ²	32.0 (26.0-37.0)	31.5 (28.0-41.0)	33.0 (26.0-37.0)	0.72
Echocardiography parameters				
LV ejection fraction, %	58.0 (50.0-63.0)	60.5 (57.0-64.0)	57.0 (50.0-63.0)	0.10
LA volume index, ml/m ²	44.0 (34.0-54.0)	43.7 (29.0-53.0)	44.0 (35.0-54.0)	0.34
AV mean gradient, mmHg	41 (32-47)	41.5 (35-51)	41 (31-47)	0.22
AV area, cm ²	.73 (.63-.86)	.675 (.62-.84)	.74 (.65-.88)	0.34
Cardiac Magnetic Resonance parameters				
LV ejection fraction (%)	56.0 (48.0-63.0)	57.5 (53.0-65.0)	56.0 (45.0-62.0)	0.12
LV concentricity (g/mL)	0.98 (0.28)	1.04 (0.30)	0.97 (0.27)	0.34
LVEDVi, ml/m ²	82.0 (67.0-98.0)	76.5 (63.0-97.0)	82.5 (67.0-100.0)	0.29
LVESVi, ml/m ²	37.5 (26.0-47.5)	33.0 (20.0-40.0)	38.5 (26.0-49.0)	0.098
LV mass index, g/m ²	76.5 (64.5-93.0)	76.5 (65.0-93.0)	76.5 (64.0-93.0)	0.99

Data are presented as mean (SD) or median (25th- 75th percentile) for continuous measures, and n (%) for categorical measures.

ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; AV = Aortic valve; LVEDVi = Left ventricular end diastolic volume indexed; LVESVi = Left ventricular end systolic volume indexed; NYHA = New York Heart Association; STS-PROM = Society of Thoracic Surgeons Predicted Risk of Mortality.

Laboratory markers

There was high NT-proBNP levels in the cohort with a median of 3186 (1337- 6684) pg/mL.

The median glomerular filtration rate was 32 (26 -37) ml/min/1.73 m².

Imaging findings

Although the median LV ejection fraction (LVEF) was normal (56%), there was a high prevalence of individuals with myocardial structural abnormalities. A total of 44 individuals (38%) had increased LV end diastolic volume index and 44 (38%) had increased LV mass on CMR. The majority (N=90, 77%) had a dilated left atrium $> 34 \text{ ml/m}^2$. Abnormal concentricity index ($>1.15 \text{g/mL}$ for men, $>0.95 \text{g/mL}$ for women) was found in 38 individuals (32%). LV concentricity increased with age ($r = 0.193$, $P = 0.038$). There was no correlation between T1 values and LV concentricity ($r = -0.123$, $P = 0.186$).

With regards to echocardiographic parameters of AS severity, median aortic valve area was $0.73 (0.63 - 0.86) \text{ cm}^2$ and mean gradient was $41 (32-47) \text{ mmHg}$. A total of 60 individuals (51%) had preserved EF high-gradient AS; 31 (27%) paradoxical normal EF, low-gradient AS; 9 (8%) low EF, high-gradient AS; 17 (14%) reduced EF, low-gradient AS.

T1 mapping

T1 values were measurable in 117 of 129 studies (feasibility 91%) of suitable patients. Table 1 also shows characteristics when categorized according to those with T1 times above or below 1024ms. Patients with increased T1 times were more likely to have higher NT-proBNP levels [$4122(1578-7980)$ vs. $1678(493-2851) \text{ pg/mL}$, $P=0.005$] and a history of heart failure [33% vs 9%, $P= 0.034$]. There were otherwise no significant differences in baseline demographics, kidney function, comorbidities, or pharmacological therapy. There were also no significant associations with echo, CMR, and CT parameters of increased AS severity. There was no overall significant difference in T1 values amongst the different subtypes of AS ($P = 0.432$). There was an overall negative correlation between T1 values and mean gradient ($r = -0.231$, $P = 0.013$, Figure 2A). The correlation between T1 values and the different subtypes of AS were as following: (1) Preserved EF high-gradient AS ($r = -0.145$, $P = 0.270$); (2) paradoxical normal

EF, low-gradient AS ($r = -0.506$, $P = 0.004$, Figure 2B); (3) Low EF, high-gradient AS ($r = 0.452$, $P = 0.260$); (4) low EF, low-gradient AS ($r = -0.07$, $P = 0.768$).

Figure 2A. Correlation between T1 values and mean gradient

Scatter plot depicting a negative relationship between T1 time and mean aortic gradient in the entire cohort.

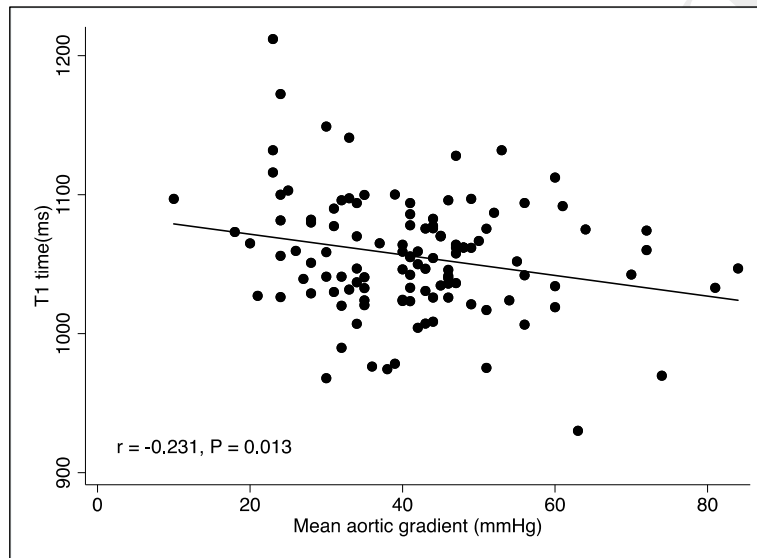
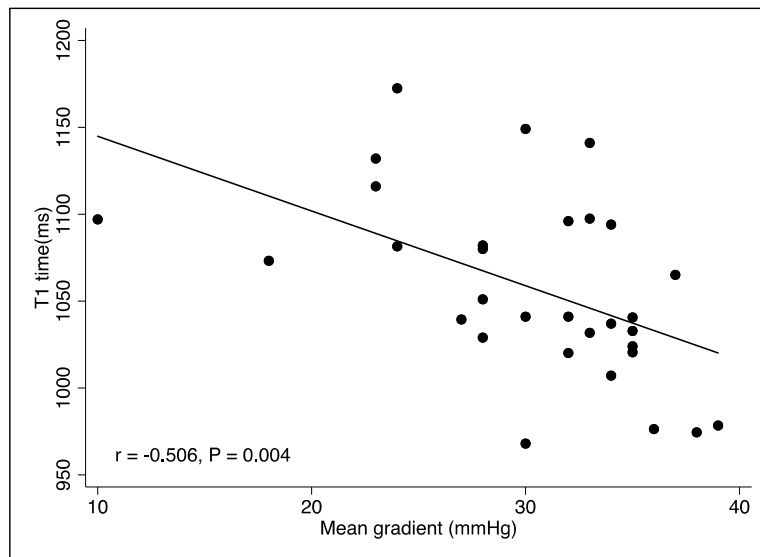


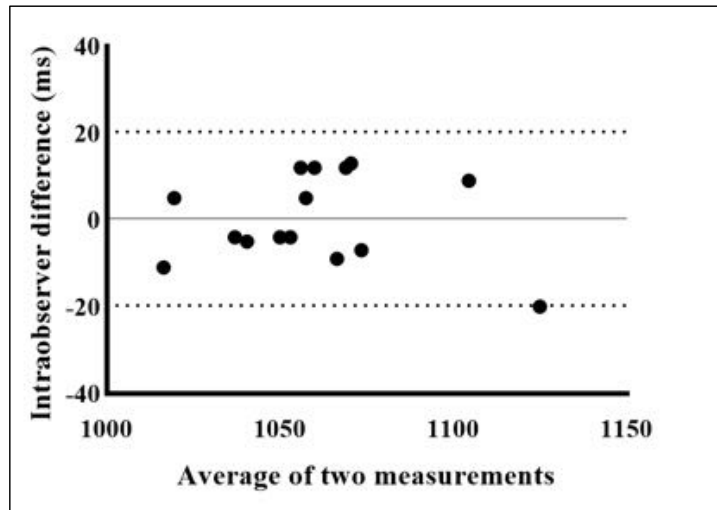
Figure 2B. Correlation between T1 values and mean gradient

Scatter plot depicting a negative relationship between T1 time and mean aortic gradient in the patients with paradoxical, normal ejection fraction, low grade aortic stenosis

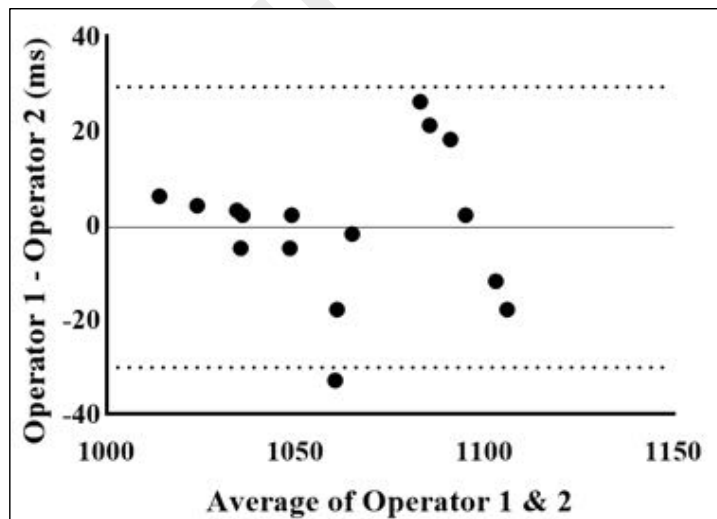


There was acceptable intraobserver and interobserver variability of T1 time measurements. The intraclass correlation coefficient was 0.94 (95% CI: 0.83 – 0.98) for intraobserver variability and 0.88 (95% CI: 0.69 – 0.96) for interobserver variability. Bland-Altman plots for intra- and interobserver variability are shown in Supplementary Figure 2.

Supplementary Figure 2A. Bland-Altman plot of intraobserver differences



Supplementary Figure 2B. Bland-Altman plot of interobserver differences



AORTIC VALVE REPLACEMENT

Post TAVR, the majority of individuals (N= 84, 72%) had trace or less aortic insufficiency whilst 29 (25%) had mild, and 4 (3%) had moderate aortic insufficiency.

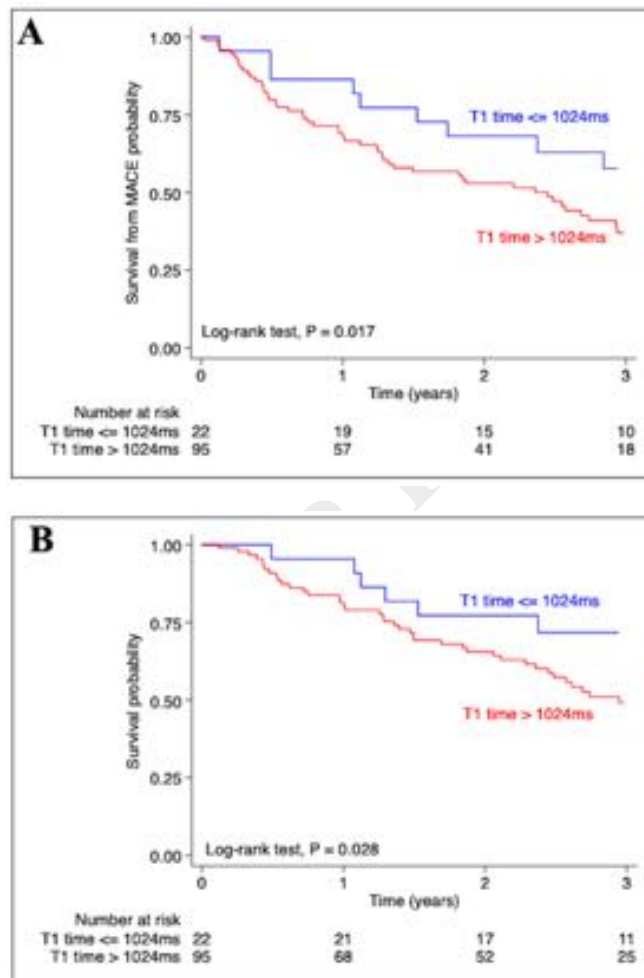
Outcomes

MACE occurred in 71 of the 117 subjects (61%). The median follow-up time was 3.4 years [2.5, 4.4]. There were 62 deaths (53%) and 31 heart failure admissions (27%) during the follow-up period.

On Kaplan-Meier analysis, patients with elevated T1 were more likely to experience MACE ($P = 0.017$, Figure 3A). On Kaplan-Meier analysis of the components of MACE, patients with elevated T1 were also more likely to experience all-cause mortality ($P = 0.028$, Figure 3B) but not heart failure hospitalization ($P = 0.104$, Figure 3C). Univariate predictors of MACE ($P < 0.10$) included STS-PROM score (HR: 1.06 per 1% increase; 95% CI: 1.02 to 1.11), mean aortic valve gradient (HR: 0.98 per 1mmHg increase; 95% CI: 0.96 to 1.0), native T1 time > 1024ms (HR: 2.18; 95% CI: 1.13 to 4.22) and NYHA class (HR: 1.48 per class increase; 95% CI: 1.05 to 2.10) (Table 2). On multiple variable Cox regression analysis, the STS-PROM score (HR: 1.07 per 1% increase, 95%CI: 1.02 - 1.1, $P = 0.006$), native T1 time > 1024ms (HR: 2.1; 95%CI: 1.09-4.06, $P = 0.028$) and NYHA class (HR: 1.56 per class increase; 1.09 – 2.34, $P = 0.016$) were independent predictors of MACE. There were no independent predictors of the individual components of MACE when considered separately (all-cause mortality or heart failure hospitalization).

Figure 3. Clinical outcomes

Kaplan Meier Survival plots illustrating event-free survival from A) major adverse cardiac events (MACE) B) all-cause mortality and C) heart failure hospitalization, according to native T1 values



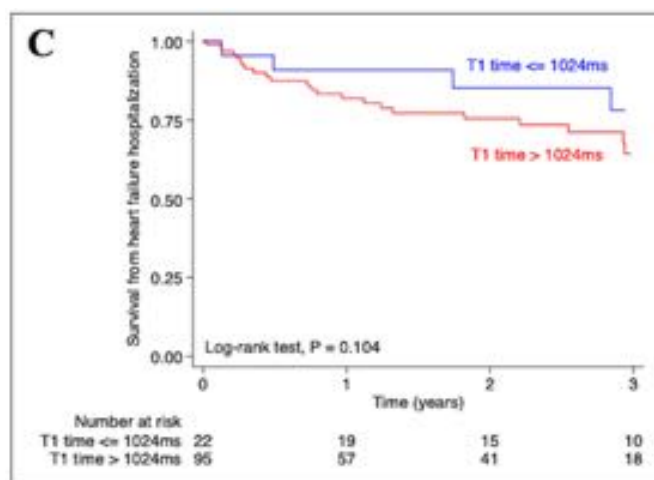


Table 2. Cox Regression Analysis for MACE

Variables	Unadjusted HR (95% CI)	Unadjusted P Value	Adjusted HR (95% CI)	Adjusted P value
Age, per year	1.01 (0.99 – 1.04)	0.364		>0.10
Male gender	1.09 (0.67 – 1.75)	0.738		>0.10
STS-PROM Score, per 1%	1.06 (1.02 – 1.11)	0.008	1.07 (1.02 – 1.12)	0.006
Diabetes	1.37 (0.85 – 2.20)	0.197		>0.10
History of heart failure	1.39 (0.81 – 2.38)	0.226		>0.10
Known coronary artery disease	0.65 (0.36 – 1.16)	0.143		>0.10
NYHA class, per class increase	1.48 (1.05 – 2.10)	0.025	1.56 (1.09 – 2.34)	0.016
Atrial fibrillation	0.96 (0.59 0 1.57)	0.873		>0.10
Mean AV gradient, per 1mmHg	0.98 (0.96 0 1.00)	0.076		>0.10
AV area, per 1 cm ²	2.11 (0.55 – 8.13)	0.279		>0.10
LAVI (echo), per 1 mL/m ²	1.00 (0.98 – 1.01)	0.522		>0.10
LVEF (CMR), per 1%	0.99 (0.97-1.00)	0.122		>0.10
LVEDVi (CMR), per 1 mL/m ²	1.01 (1.00 – 1.02)	0.299		>0.10
LVMI (CMR), per 1 g/m ²	1.00 (0.99 – 1.01)	0.532		>0.10
NT-ProBNP, per 1pg/mL	1.00 (1.00 – 1.00)	0.995		>0.10
Creatinine, per 1mg/dL	1.06 (0.89 – 1.27)	0.528		>0.10
GFR, per 1ml/min/1.73 m ²	0.99 (0.97 – 1.01)	0.374		>0.10
Hemoglobin, per 1 g/dL	0.96 (0.84 – 1.10)	0.575		>0.10
Native T1 time > 1024ms	2.18 (1.13 – 4.22)	0.020	2.10 (1.09 – 4.06)	0.028

AV = aortic valve; GFR = glomerular filtration rate; LA: left atrial; LVEDVi = Left ventricular end diastolic volume indexed; LVEF= Left ventricular ejection fraction; NYHA = New York Heart Association; STS-PROM = Society of Thoracic Surgeons Predicted Risk of Mortality.

In a nested model that included the independent predictors of MACE (STS-PROM score, NYHA class and native T1 >1024ms), the addition of increased native T1 time had incremental prognostic value to predict MACE, with the chi-square of the model increasing from 12.4 to 17.85 (P = 0.020, Figure 4). Harrell's C-statistic for MACE increased slightly from 0.61 to 0.63. There was no significant improvement in IDI with the addition of native T1 time to predict MACE.

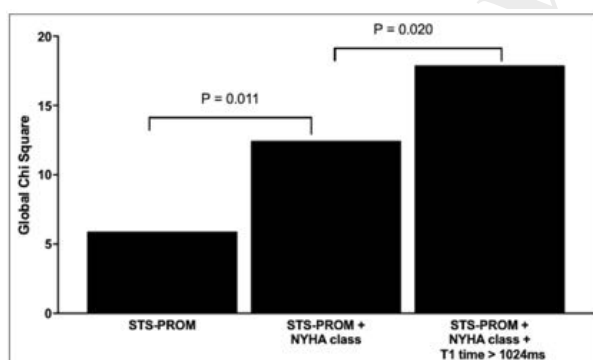


Figure 4. Incremental prognostic value of higher native T1 times to predict occurrence of major adverse cardiovascular events

Incremental prognostic value of higher native T1 times to predict occurrence of major adverse cardiovascular events.

NYHA class = New York Heart Association; STS-PROM = Society of Thoracic Surgeons Predicted Risk of Mortality.

DISCUSSION

It is increasingly evident that AS is also a disease of the myocardium rather than solely a disease of the aortic valve (4-7, 34). There is a transition from compensatory LV hypertrophy to fibrosis that results in eventual myocardial decompensation. AS is associated with significantly reduced survival in individuals with CKD (12, 13), possibly through accelerated myocardial remodelling. It is therefore desirable to identify LV remodeling at a stage when pathological myocardial changes are potentially reversible and AVR may improve patient outcomes in the concomitant setting of CKD.

The key findings of the present study in patients with CKD and severe AS were that following adjustment for a range of baseline clinical, biochemical, and imaging variables, higher T1 times were significantly associated with myocardial functional abnormalities and provided incremental prognostic information to predict long-term outcomes over conventional risk markers. The risk of adverse outcomes was high compared to previously published cohorts of patients with AS without CKD (12, 13). This risk was attenuated following aortic valve replacement and increased in the setting of higher STS-PROM score and advancing age. Patients with a history of congestive heart failure, higher BNP levels, and low EF-low gradient AS were more likely to have increased T1 times. Amongst the subjects with paradoxical normal EF, low-flow, low-gradient AS, there was a negative correlation between T1 values and mean gradient.

These results add to the growing body of evidence that increased native T1 times may serve as a marker of myocardial abnormalities and/or adverse outcomes in various cardiovascular

diseases (34). In AS, there has been increasing interest in assessment of myocardial fibrosis as an earlier and more objective marker of LV decompensation (4-7, 34). There is evidence that the extent of myocardial fibrosis on CMR predicts recovery of LV function and mortality after AVR (11, 35). Taken together, these studies emphasize the detrimental impact of delayed intervention and hence the need to use additional markers of myocardial decompensation, particularly in asymptomatic stages of AS, where current AVR guidelines rely on nonrandomized data and expert consensus (2, 5).

To the best of our knowledge, this is the first study to measure T1 values in individuals with severe AS and CKD. T1 times did not appear to be influenced by the degree of renal dysfunction. Furthermore, the relative simplicity of the method means that native T1 mapping has the potential to lend itself to routine use for assessment. Consistent with previous reports, we found that non-contrast T1 values were feasibly performed in the vast majority of patients (92%), and are known to be reproducible (7, 34). Additionally, there are advantages over contrast imaging; contrast administration is associated with increased costs, longer scan times and carry a small risk of nephrogenic systemic fibrosis in patients with advanced CKD. (7)

Inconsistent AS severity grading with discordance of aortic valve area and mean gradient can be particularly problematic in CKD where common comorbidities such as obesity and hypertension can increase the complexity of AS assessment and lead to under-identification of severe AS (36). In this study, the identification of severe AS was supplemented by invasive assessment and/or advanced echocardiography, in addition to the near universal use of MDCT (92%). Our findings importantly revealed that over 40% (N = 48) had discordant findings on echocardiography with low (<40mmHg) mean gradients; 31 such individuals (64%) had preserved LVEF. Hence, several aspects require emphasis. First, such patients should receive

particular attention because severe AS can be conceivably under-recognized with even comprehensive transthoracic echocardiography alone. Secondly, there was an overall negative correlation between T1 values and mean gradient in the entire cohort though this was most pronounced amongst the subset with paradoxical normal EF, low-gradient AS. This raises the hypothesis that left ventricles with higher T1 values cannot generate higher gradients despite the presence of seemingly normal contractile function as assessed using LVEF. Indeed, echocardiographic studies confirm that patients with paradoxical normal EF, low-gradient AS in fact do have myocardial decompensation as reflected by substantially reduced global longitudinal strain (37). Thus, patients with lower gradients who may have symptoms suggestive of severe AS merit further investigations such as aortic valve calcium scoring by MDCT to confirm the stenosis severity and the need for AVR.

In patients with AS without CKD, concentrations of BNP rise with disease severity and have been shown to predict survival (32). We did not find that NT-ProBNP independently predicted adverse outcomes in the present study suggesting that NT-ProBNP levels may be less useful in discerning prognosis in the subset of patients with AS and CKD.

Several caveats of the present analysis warrant further consideration and are limitations. Whilst the detailed cardiac phenotyping (using echocardiography, CT, CMR, blood biomarkers) and the long-term follow-up represent strengths of the present study, this retrospective observational study from a quaternary cardiac centre could have selection bias. Given the high-volume and increased complexity of patients seen in our centre, our findings may not be generalizable to lower volume centres. Before the findings can be incorporated into routine clinical practice, these data need to be replicated in independent cohorts. Secondly, patients on dialysis are under-represented in this patient cohort likely due to referral patterns for CMR.

Third, our study focused on the value of non-contrast T1 mapping in AS which can be sensitive to concurrent processes such as edema, volume overload or amyloidosis. Low oncotic pressure though anemia or decompensated heart failure in this cohort of patients with CKD and aortic stenosis may have contributed to increased generalized edema. Precise assessment of hemodynamic status using invasive methods were not performed and thus the impact of volume overload on T1 values cannot be determined in this study. Although a comprehensive review of the chart and all imaging results were performed as part of the TAVR evaluation, cases of cardiac amyloidosis could conceivably be missed without use of contrast CMR. Additional measurement of postcontrast T1 provides data on extracellular volume fraction. This was not undertaken in this study as contrast use was not required for our pre-TAVR CMR protocol performed mainly to measure aortic annular dimensions for TAVR valve sizing in these patients with CKD who could not have contrast CT angiography. We note recent findings in AS, however, that extracellular volume quantification did not correlate with histological myocardial fibrosis (4). Precontrast T1 mapping may thus better reflect the native pathophysiologic state of tissue and is a consistent, reproducible method not confounded by choice and dose of the contrast agent, measurement timing during the dynamic redistribution of the contrast, and renal function (34). It must be noted that setups of available T1 mapping methods are presently site and vendor specific and thus our values cannot be extrapolated to other sites and vendors. Finally, the association between higher T1 times and adverse cardiovascular outcomes in this patient cohort does not indicate a causal relationship. Our findings will stimulate further investigations into understanding myocardial disease biology and LV mechanics in AS and CKD.

CONCLUSIONS

In individuals with CKD and severe AS, native T1 time was increased and associated significantly with the occurrence of MACE. Further studies are needed to establish if the presence of interstitial fibrosis identified using native T1 mapping can be used to guide the optimal timing of aortic valve replacement.

ABBREVIATIONS:

AS: Aortic stenosis; BNP: B-type natriuretic peptide; CKD: chronic kidney disease; CMR: cardiac magnetic resonance; LVEF: left ventricular ejection fraction; MACE: major adverse cardiovascular events; STS-PROM: Society of Thoracic Surgeons predicted risk of mortality; TAVR: transcatheter aortic valve replacement

DECLARATIONS

Ethics approval and consent to participate

This study was approved by the institutional review board, with a waiver of individual consent.

Consent for publication

Not applicable

Availability of data and materials

In order to comply with our institutional review board agreements, we cannot make our data, which consists of potentially identifying and sensitive patient information, publicly available.

Competing interests

The authors declare that they have no competing interests

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Chapter 6: Incidental thoracic aortic dilation and coronary calcification on chest computed tomography in patients with atrial fibrillation

Ramchand J, Bansal A, Saeedan MB, Wang TKM, Agarwal R, Kanj M, Wazni O, Svensson LG, Desai MY, Harb SC, Schoenhagen P, Burrell LM, Griffin BP, Popovic ZB and Kalahasti V. **Incidental Thoracic Aortic Dilation on Chest Computed Tomography in Patients With Atrial Fibrillation.** *Am J Cardiol.* 2021;140:78-8

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Incidental Thoracic Aortic Dilation on Chest Computed Tomography in Patients With Atrial Fibrillation



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Patients with atrial fibrillation (AF) have risk factors that predispose to thoracic aneurysmal disease (TAD) and atherosclerosis. In this study in patients with AF, we assessed the occurrence of incidental TAD and assessed if a validated predictive score used to predict AF, the CHARGE-AF score, was associated with greater aortic dimensions. We also assessed the prevalence of coronary calcification. We conducted a cross-sectional study of 1,000 consecutive patients with AF undergoing chest multidetector CT during evaluation for pulmonary vein isolation. A dilated aortic root or ascending aorta (AA, dimension/body surface area $>2.05 \text{ cm/m}^2$) were found in 195 (20%). A total of 12 (1%) had significant aortic aneurysmal enlargement of $>5.0 \text{ cm}$. Advancing age, a bicuspid aortic valve, hypertension, and male gender were associated with increased aortic dimensions. Aortic root dimensions increased linearly ($p < 0.001$) and ascending aortic dimensions increased nonlinearly across CHARGE-AF deciles ($p < 0.001$). Nearly two-thirds (63%) had coronary calcification, 38% of whom were not on lipid-lowering therapy. In conclusion, in patients with AF undergoing gated chest CT, 1 in 5 had previously undetected TAD, with a small proportion having significantly aneurysmal dimensions approaching surgical thresholds. Risk factors previously established to increase the propensity to develop AF are also associated with increased TAD. These findings raise the need to consider a surveillance strategy for TAD in patients with AF, particularly in those with other risk factors for aortic disease. A high prevalence of coronary calcium was also detected, representing an opportunity to optimize statin therapy in patients with AF. © 2020 Elsevier Inc. All rights reserved. (Am J Cardiol 2021;140:78–82)

Thoracic aneurysmal disease (TAD) leads to devastating complications and is a leading cause of death in the United States.¹ Reduction in mortality in recent years is related to improved surveillance strategies that permit earlier detection and treatment.² TAD is associated with several risk factors such as advancing age and hypertension.³ Although patients with atrial fibrillation (AF) also have risk factors that would predispose to TAD and atherosclerosis,^{4,5} the prevalence of occult TAD in patients with AF has not been defined. The aim of this study in AF was to assess the presence of TAD detected incidentally on chest multidetector computed tomography (CT) as part of evaluation for pulmonary vein isolation. We assessed if a validated predictive score used to predict AF, the CHARGE-AF score,^{6,7} was associated with greater aortic dimensions. As a secondary

aim, we also sought to assess the prevalence of coronary artery calcification.

Methods

We conducted a cross-sectional, single-center study of patients with AF after institutional review board approval. As this was a pilot, observational study, the sample size was predefined arbitrarily as 1,000 consecutive patients. This sample size has a power of 89% to detect a correlation coefficient r of 0.1 or more. We included consecutive patients undergoing a standardized pulmonary venous CT as part of work-up for pulmonary venous isolation between December 2018 and October 2019. Baseline demographic and clinical data were obtained. No patient had a documented history of connective tissue disorder or TAD.

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

The CT protocol comprised sequential axial imaging with prospective triggering in the systolic phase with 1.5 mm slice reconstruction following intravenous contrast administration (Siemens scanner, Siemens, Erlangen, Germany). Aortic dimensions were measured by the investigators retrospectively for the purpose of the present study. Using multiplanar reconstruction, aortic root (AR) and

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See page 82 for disclosure information.

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proximal ascending aorta (AA) dimensions were measured perpendicular to the centre line.⁸ Diameters were measured from the outside to outside wall using electronic calipers with a zoom function.^{8,9} The AR was measured using the sinus-to-sinus technique. With standard centerline techniques, the “diameter” of the aorta at the sinuses of Valsalva is determined by running a line from the deepest point of one sinus perpendicularly through the “center” to the adjacent sinus.^{8,9} This technique is recommended by contemporary guidelines owing to similarity to monoplane CT measurements, close correlation with echocardiographic measurements, and easier application to bicuspid aortic valves.^{8,9} Furthermore, from a biomechanical perspective, this diameter is believed to be most representative of the highest wall tension in the aorta and thus more naturally predictive of aortic events.¹⁰

For both AR and AA, aortic size index (dimension/body surface area, dilated >2.05 cm/m²), aortic height index (dimension/height, dilated >2.43 cm/m), aorta area/height ratio and AR Z-score were calculated as previously described.^{1,11} Presence of coronary calcification was recorded (present / absent).

The CHARGE-AF risk score was developed in 2013 to predict risk of AF in the general population.⁶ It was derived from the Framingham Heart Study, the Cardiovascular Health Study, and the Atherosclerosis Risk In Communities study and validated in the Age, Gene/Environment Susceptibility Study, and the Rotterdam Study, with more than 26,000 patients of European, European-American, and African-American ancestry in total.⁶ The CHARGE-AF risk score was calculated as follows: $0.508 \times \text{age (5 years)} + 0.248 \times \text{height (10 cm)} + 0.115 \times \text{weight (15 kg)} + 0.197 \times \text{systolic blood pressure (20 mm Hg)} - 0.101 \times \text{diastolic blood pressure (10 mm Hg)} + 0.359 \times \text{current smoker} + 0.349 \times \text{antihypertensive medication} + 0.237 \times \text{diabetes} + 0.701 \times \text{congestive heart failure} + 0.496 \times \text{myocardial infarction}$. The coefficients for each risk factor are from the derivation study for the CHARGE-AF risk score.^{6,7} We did not include race because it was not disclosed by many of the study participants though where collected, the majority of the participants were of European-American ancestry where the CHARGE-AF score has previously been independently validated.⁷

Baseline demographic data, risk factors, and clinical variables were descriptively summarized with continuous variables expressed as mean $\pm$ SD and categorical data presented as percentage frequency. The primary outcome of interest was the incidence of a dilated AR or AA. Continuous data are reported as mean $\pm$ SD or median with interquartile range and categorical data as percentage. We assessed potential predictors of aortic size using General Linear Model analysis of variance (ANOVA). To circumvent the correlation between gender and body size, ($p < 0.0001$ by Spearman's rho for body height in our sample), we normalized AR and AA diameters by height. Given that cross correlation matrix revealed a very significant correlation between hypertension and age ($p < 0.0001$ by Spearman's rho), separate multivariable models were built for these 2 variables. To assess the relation between the CHARGE-AF score and diameters of AR and AA, patients were first divided into 10 equal groups based on the deciles of the CHARGE-AF score.¹² We then applied ANOVA

with polynomial contrast analysis. A 2-tailed $p < 0.05$ was interpreted as significant. Statistical analysis was performed using STATA version 15.1 (Statacorp., College Station, Texas) and SPSS version 23.0 (IBM, Armonk, New York)

Results

Baseline characteristics are shown in Table 1. Mean age was 66 years and the cohort was mostly male. Cardiovascular risk factors were prevalent such as diabetes, hypertension, and hyperlipidemia. A total of 40 patients had a bicuspid aortic valve (4%).

A dilated AR or AA (dilated aortic size index >2.05 cm/m²) was found in 195 (20%). A total of 96 (10%) had AR or AA > 4.5 cm and 12 (1%) had significant AR or AA aneurysmal enlargement of > 5.0 cm. A total of 26 patients (2.6%) had an AR or AA area/ height ratio of > 10 cm²/m, an index shown to be predictive of death¹³ and is included

Table 1
Baseline characteristics

Variable	N = 1,000
Age (years)	66 $\pm$ 11
Men	681 (68%)
Height (cm)	176 [168–183]
Weight (kg)	92 [78–106]
Body surface area (m ²)	2.1 [1.9–2.3]
Body mass index (kg/ m ²)	29 [26–34]
Hypertension	645 (65%)
Smoking history	415 (42%)
Diabetes mellitus	194 (19%)
Hyperlipidemia	510 (51%)
Bicuspid aortic valve	40 (4%)
Beta-blockers	706 (71%)
Lipid lowering therapy	516 (52%)
ACE-I / ARB	414 (41%)
Aortic root diameter (cm)	3.7 [3.4–4.1]
Aortic root size index (cm/ m ²)	1.8 [1.6–1.9]
Aortic root height index (cm/ m)	2.1 [2.0–2.3]
Aortic root diameter ≥ 4.5 cm	78 (8%)
Aortic root diameter ≥ 5.0 cm	10 (1%)
Aortic root Z score > 2	146 (15%)
Aortic root height index ≥ 2.43 cm/m	118 (12%)
Aortic root cross-sectional area/height ratio ≥ 10 cm ² / m ²	23 (2%)
Ascending aorta diameter (cm)	3.5 [3.2–3.8]
Ascending aorta size index (cm/ m ²)	1.7 [1.5–1.8]
Ascending aorta height index (cm/ m)	2.0 [1.9–2.2]
Ascending aorta diameter > 4.5 cm	32 (3%)
Ascending aorta diameter > 5.0 cm	3 (0.3%)
Ascending aorta Height index ≥ 2.43 cm/m	52 (5%)
Ascending aortic cross-sectional area/height ratio ≥ 10 cm ² / m ²	6 (0.6%)

ACE-I=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker.

Data are presented as median [25th–75th percentile] or mean (standard deviation) as noted for continuous variables and frequencies for categorical variables.

Hyperlipidemia was defined according to the fulfillment of one of the following criteria: clinical history of hyperlipidemia, total cholesterol levels >200 mg/dL, LDL cholesterol ≥ 130 mg/dL, HDL cholesterol <40 mg/dL or lipid lowering therapy

Table 2
Predictors of aortic size using General Linear Model Analysis of variance

N = 1,000	Variables	B (95% Confidence Interval)	p Value
Model 1: Ascending aorta indexed to height	Age	0.005 (0.004–0.006)	<0.001
	Bicuspid valve	0.178 (0.102–0.254)	<0.001
	Male	–0.19 (–0.51–0.013)	0.248
Model 2: Ascending aorta indexed to height	Hypertension	0.083 (0.052–0.115)	<0.001
	Bicuspid valve	0.183 (0.106–0.259)	<0.001
	Male	–0.033 (–0.066– –0.001)	0.042
Model 3: Aortic root indexed to height	Age	0.002 (0.001–0.004)	0.004
	Bicuspid valve	0.097 (0.021–0.173)	0.013
	Male	0.110 (0.078–0.142)	<0.001
Model 4: Aortic root indexed to height	Hypertension	0.013 (–0.019–0.044)	0.427
	Bicuspid valve	0.097 (0.021–0.174)	0.013
	Male	0.104 (0.072–0.136)	<0.001

in the most recent guidelines as an indication for elective aortic repair in high-risk patients.¹ In the patients with thoracic aorta dilation (n = 195), a subsequent chest x-ray or echocardiogram was available in 144 patients and identified thoracic aortic dilation in only 44 (31%)

General Linear Model (ANOVA) was used to assess potential predictors of aortic size adjusted to height (Table 2). Patients with bicuspid aortic valve had a 1.78 mm/m larger AA compared with those with trileaflet aortic valves (Table 2, model 1, $p < 0.001$). Ascending aortic size adjusted to height increased by 0.5mm/m for increase in age by a decade (Table 2, model 1, $p < 0.001$). Gender did not consistently influence height adjusted-ascending aortic dimension. The presence of hypertension increased the size of the height-adjusted aorta by 0.83 mm/m (Table 2, model 2, $p < 0.001$). With regard to measurements at the level of the AR, height adjusted AR was larger in men by 1.1 mm/m (Table 2, model 3, $p < 0.001$). Similarly, AR normalized for height increased by 0.2 mm/m for increase in age by a decade (Table 2, model 3, $p = 0.001$). There was no effect of hypertension on AR measures (Table 2, model 3, $p = \text{NS}$).

The relation between AR and ascending aortic dimensions across deciles of CHARGE-AF score are shown in Figures 1 and 2 respectively. AR dimensions increased linearly across CHARGE-AF deciles ($p < 0.001$). Mid ascending aortic dimensions increased nonlinearly with CHARGE-AF deciles ($p < 0.001$), with significant first and second order components ($p < 0.001$ and $p = 0.003$).

A total of 631 (63%) participants had coronary calcification, and 242 (38%) of these patients were not on lipid-lowering therapy.

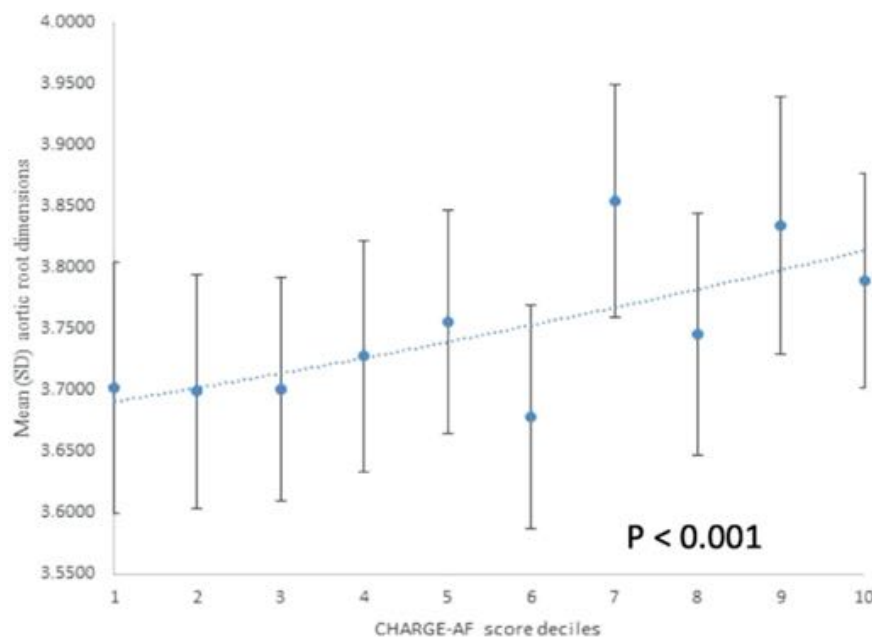


Figure 1. Aortic root dimensions according to CHARGE-AF score. The relation between aortic root dimensions across deciles of CHARGE-AF score. Aortic root dimensions increased linearly across CHARGE-AF deciles ($p < 0.001$). The y-axis represents mean and standard deviation of aortic dimensions across CHARGE = AF deciles (x-axis). SD = standard deviation.

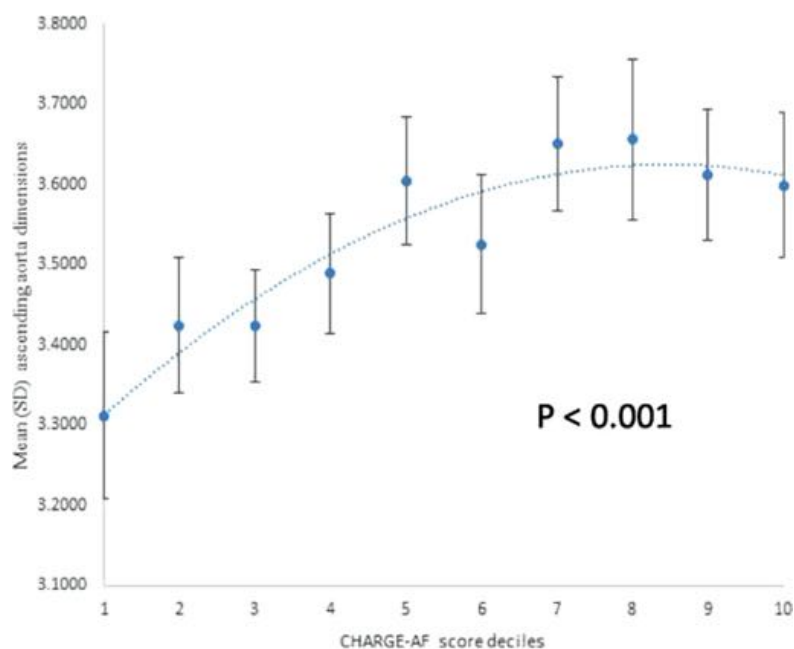


Figure 2. Ascending aortic dimensions according to CHARGE-AF score. Mid ascending aortic dimensions increased nonlinearly with CHARGE-AF deciles ($p < 0.001$), with significant first and second order components ($p < 0.001$ and $p = 0.003$). The y-axis represents mean and standard deviation of aortic dimensions across CHARGE-AF deciles (x-axis). SD = standard deviation.

Discussion

The key finding in this study is that in patients with AF who underwent gated chest CT as part of evaluation for pulmonary vein isolation, 1 in 5 had a dilated proximal thoracic aorta, and 1% of the cohort had aneurysmal dimensions approaching surgical repair thresholds. The prevalence of significantly aneurysmal dimensions (>5.0 cm) in this cohort with AF is numerically higher compared with a separate primary prevention 'control' population without AF that we previously reported (1% vs 0.1%).¹¹

The underlying mechanisms underpinning high aortic dimensions in AF remains to be understood. AR dimensions and mid ascending aortic dimensions increased across CHARGE-AF deciles suggesting that factors previously established to increase the propensity to develop AF are also associated with increases in aortic dimensions.⁶

These findings raise the need to consider a surveillance strategy for thoracic aortic enlargement in patients with AF particularly in those with concurrent risk factors for aortic disease. Our results are expected to stimulate further research in this patient population and the findings need confirmation in large, independent cohorts if routine screening for TAD is to be recommended in this patient group. To balance the risk of ionizing radiation and costs, the optimal surveillance strategy should be targeted at patients with the highest risk for TAD for example in those who also have other risk factors identified on the present study such as advancing age, a bicuspid aortic valve, hypertension, and male gender.

Remarkably, nearly two-thirds of the population had coronary calcification, a known powerful predictor of atherosclerotic cardiovascular disease events.¹⁴ A large subset (38%) of these patients were not on lipid lowering therapy. Our findings thus emphasize the added utility of pulmonary venous CT to optimize atherosclerotic cardiovascular disease primary prevention strategies. This is of particular relevance considering the most recent cholesterol clinical practice guidelines recommend initiation of statin therapy in those with intermediate risk of atherosclerotic cardiovascular disease in the presence of coronary artery calcification.¹⁵ Randomized controlled trials are needed to determine if more intensive lifestyle and/or pharmacological interventions improve outcomes in this cohort of patients with AF. Until then, it would seem reasonable to use this information to complement guideline driven clinical strategies in cardiovascular risk screening and help improve the timely initiation of cardiovascular risk management in eligible patients.

The strengths of this study include the large patient population, precise measurement of the aorta using gated CT with contrast and multiplanar reconstruction to measure aortic size perpendicular to the centre line. The study has a number of limitations. Our results are applicable only to the subset of patients underwent CT as part of evaluation for pulmonary vein isolation for AF. Results may therefore not be applicable to the patients who are not candidates for pulmonary vein isolation AF. Secondly, the entire aorta was not completely assessed due to the range included as part of the pulmonary vein CT protocol and as such dilation in

regions not covered such as the aortic arch or distal descending thoracic aorta may have been missed. Lastly, coronary artery calcium was assessed qualitatively using a contrast-enhanced CT scan. This method avoids the additional costs/ radiation exposure with a dedicated coronary artery calcium score scan.

To conclude, to our knowledge this study of patients with AF underwent gated chest CT demonstrates that 1 in 5 patients has a dilated proximal thoracic aorta, with 1% of the cohort having incidental but significant aneurysmal dimensions approaching surgical thresholds. None of those with dilated aortas had dissection or rupture negating the need for acute operative intervention on these patients.

Authors contribution

Jay Ramchand: Conceptualization, Methodology, data curation, formal analysis, writing – original draft **Agam Bansal:** investigation – data collection **Mnahi Bin Saeed:** investigation – data collection **Tom Kai Ming Wang:** investigation – data collection **Ritu Agarwal:** investigation – data collection **Mohamed Kanj:** data interpretation and the critical revision of the manuscript. **Ousama Wazni:** data interpretation and the critical revision of the manuscript. **Lars G. Svensson:** data interpretation and the critical revision of the manuscript. **Milind Y. Desai:** data interpretation and the critical revision of the manuscript. **Serge C. Harb:** resources, data interpretation and the critical revision of the manuscript. **Paul Schoenhagen:** data interpretation and the critical revision of the manuscript. **Louise M. Burrell:** data interpretation and the critical revision of the manuscript. **Brian P Griffin:** resources, data interpretation and the critical revision of the manuscript. **Zoran B. Popović:** supervision, formal analysis and the critical revision of the manuscript. **Vidyaagar Kalahasti:** supervision, project administration, data interpretation and the critical revision of the manuscript

Disclosures

The authors have reported that they have no relations relevant to the contents of this study to disclose.

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Chapter 7: Summary, clinical perspectives and future directions

CVD is a leading cause of morbidity and mortality in Australia, and globally. With the aging population, the number of individuals at risk of CVD complications continues to rise, making CVD preventative strategies an ongoing public health priority. In addition to traditional risk factor assessment, physicians rely on biomarkers to identify individuals at high risk of CVD to effectively risk stratify and treat patients. Current risk-stratification approaches incorporating traditional risk factors and biomarkers have limited predictive usefulness which poses a significant challenge to deliver preventative therapies. Therefore, finding new biomarkers for CVD is of interest and can improve the identification of individuals who would benefit most from preventative therapies. The main aim of this thesis was to identify novel approaches to risk stratification in common CVDs using circulating and imaging biomarkers.

This thesis is divided into four studies evaluating:

- 1) The prognostic role of circulating ACE2 in coronary artery disease (**Chapter 3**);
- 2) The prognostic role of circulating and myocardial ACE2 in AS (**Chapter 4**);
- 3) The prognostic role of abnormal myocardial tissue characterisation using native T1 time, a CMR technique in AS (**Chapter 5**); and
- 4) The role of opportunistic screening using cardiac CT to identify markers of vascular disease such as coronary artery calcium and thoracic aortic dilation in atrial fibrillation (**Chapter 6**).

The first study (**Chapter 3**) was a prospective evaluation of the utility of circulating ACE2 levels in a high-risk cohort of patients with angiographically proven obstructive

CAD. The study was the first in patients with CAD where patients with known HF or severe LV systolic dysfunction were excluded as both are associated with increased circulating ACE2 levels. Over a median follow up of 10.5 years, circulating ACE2 activity predicted the occurrence of MACE even after comprehensive multivariable adjustment in a model that included prognostically meaningful variables. Consistent with results of other studies (83,85,87,95,257), circulating ACE2 activity was higher in male patients. The median ACE2 level in patients with CAD was 29 pmol/ml/min which is higher than levels we previously reported in young, healthy volunteers (4.44 ± 0.56 pmol/ml/min) (7) and in elderly patients (median 19.4 pmol/ml/min) (258).

The second study (**Chapter 4**) investigated the relationship between circulating ACE2 activity levels and all-cause mortality in patients with aortic stenosis. Additionally, the relationship between circulating ACE2 activity, myocardial ACE2 gene expression, and myocardial fibrosis were examined. Higher circulating ACE2 levels were associated with increased myocardial structural and functional abnormalities such as increased LV mass index and increased LV volumes. Circulating ACE2 levels correlated with the extent of valvular calcification but not AS severity. Over a median follow-up of 5 years, elevated circulating ACE2 activity was an independent predictor of all-cause mortality. Circulating ACE2 improved reclassification of mortality risk, incremental to conventional clinical, imaging, and biochemical parameters that included BNP. In a subset of patients with myocardial tissue collection, increased circulating ACE2 was associated with reduced myocardial ACE2 gene expression and severe myocardial fibrosis. There was no correlation between ACE2 activity and treatment with RAS inhibitors, and male sex was an independent predictor of increased circulating ACE2 activity.

The third study (**Chapter 5**) investigated whether diffuse myocardial fibrosis characterised by greater native T1 times harbours prognostic utility to predict MACE (all-cause mortality or heart failure hospitalisation) in patients with CKD and severe AS being evaluated for TAVR. Native T1 times were higher than previously reported values in healthy controls. Patients with higher T1 times were more likely to have a history of congestive heart failure, higher NT-proBNP levels and lower echocardiographic LVEF. Following adjustment for baseline clinical, biochemical, and imaging variables, higher T1 times provided incremental prognostic information to predict long-term outcomes over 3.4 years of follow-up. In an analysis of the CT findings, the aortic valve calcification score was significantly higher in men and those with high-gradient phenotypes.

The fourth study (**Chapter 6**) investigated the prevalence of incidental TAAD and coronary atherosclerosis, both leading causes of cardiovascular mortality, in patients with AF. In a cross-sectional study of 1,000 consecutive patients with AF, one in five were found to have newly identified TAAD on multidetector chest CT. A small proportion (1%) had large aneurysmal dimensions which approached surgical thresholds. The prevalence of aneurysmal dimensions >5.0cm in this cohort with AF is numerically higher compared with a separate primary prevention ‘control’ population without AF (259). A validated predictive score used to predict AF, the CHARGE-AF score (260,261) was associated with greater aortic dimensions. It was also found that although nearly two-thirds had coronary calcification, 38% of whom were not on lipid-lowering therapy.

7.1 Clinical perspectives

In the literature review chapter, the limitations of current CVD risk stratification approaches were highlighted, justifying research into additional biomarkers. Overall, the findings of the thesis suggest that the circulating and imaging biomarkers performed as part of the studies provide incremental diagnostic and/or prognostic information in the selected CVD states.

7.1.1 Circulating angiotensin converting enzyme 2 studies

The results of this thesis add to the growing body of evidence that circulating ACE2 activity levels may serve as a marker of myocardial abnormalities and/or adverse outcomes in CVD, including AF, heart failure and CAD (4-6). The present thesis extends the knowledge in this regard and affirms the role of circulating ACE2 as a readily measurable marker of RAS dysregulation. The report was the first in humans to demonstrate that higher circulating ACE2 activity was associated with reduced myocardial ACE2 and, that those with the highest circulating ACE2 had more severe myocardial fibrosis, an important predictor of death in AS. Therefore, these data suggest a potential role for the measurement of circulating ACE2 activity as a marker of early myocardial decompensation in AS.

The European guidelines for the management of valvular heart disease provide a weak recommendation to use BNP as a decision tool to guide AVR timing in severe AS (50). Further insight has been gained from the concurrent measurement of circulating ACE2 activity alongside BNP. In the present study, patients with increases in both BNP and

ACE2 activity had increased mortality compared with those without elevation of either ACE2 or BNP or with the elevation of only one biomarker. Because circulating ACE2 measurement had prognostic value not captured by BNP alone, the combination of the two biomarkers might enhance clinical decision-making in AS. Importantly, >40% of patients with increased ACE2 activity had normal BNP levels suggesting different pathophysiological mechanisms and/or stimuli to release the two markers.

Another essential piece of information from the thesis in the setting of the ongoing COVID-19 pandemic is the absence of any association between RAS inhibitors and circulating ACE2. These results have subsequently been supported by recently published evidence that suggest that at present, RAS inhibitors should not be withheld in patients with COVID-19 for the sole purpose of modifying ACE2 (61,84).

7.1.2 Cardiac magnetic resonance imaging

It has been increasingly evident that AS is also a disease that involves the myocardium rather than solely a disease of the aortic valve (15,262-265). There is a transition from compensatory LV hypertrophy to fibrosis that results in eventual myocardial decompensation (15,262-265).

Several insights have been gained from the present thesis with regards to myocardial fibrosis in individuals with severe AS, where outcome data is presently limited. Firstly, diffuse myocardial fibrosis identified using T1 time was significantly associated with increased MACE in patients with CKD and severe AS though it remains unclear if this contributes to poorer outcomes previously reported in these individuals (18,19).

Secondly, nearly half of these patients had discordant findings on echocardiography (severely reduced AVA $\leq 1\text{cm}^2$ and mean gradient $\leq 40\text{mmHg}$). These patients should therefore receive close follow-up because severe AS can be conceivably under-recognized in the presence of low gradients with transthoracic echocardiography alone. Thirdly, in the subset of patients with paradoxical normal EF, low-flow, low-gradient, there was an inverse correlation between T1 values and mean aortic gradient suggesting that the fibrotic ventricle cannot generate higher gradients despite the presence of seemingly normal contractile function assessed using LVEF. Notably, others have shown that this subset of patients demonstrate evidence of myocardial decompensation as reflected by substantially reduced LV GLS (266). Taken together, the findings suggest that patients with lower gradients who may have symptoms suggestive of severe AS merit further investigations such as aortic valve calcium scoring by CT to more comprehensively evaluate AS severity and the need for AVR.

7.1.3 Cardiac computed tomography

AF is strongly associated with other major CVD such as stroke, heart failure, CAD and other vascular diseases. In this thesis, opportunistic screening using cardiac CT in a large cohort of patients with AF undergoing catheter ablation remarkably demonstrated that one in five patients had a dilated aorta and that 1% had significant aneurysmal dimensions nearly meeting or meeting criteria for surgical intervention. These findings raise the need to consider a surveillance strategy for vascular disease in these patients. Furthermore, it was identified that nearly two-thirds of the patient population had CAC, yet a large subset (38%) of these individuals were not on lipid-lowering therapy. The findings therefore represent an opportunity to substantially optimise preventative measures in these

individuals considering that CAC is strongly associated with the development of adverse cardiovascular events. It is therefore envisaged that cardiac CT may have a role as a screening tool to detect the individuals with AF at the highest risk of cardiovascular events.

7.2 Future directions

Several avenues for future research have arisen from this work. If circulating ACE2 activity, either as a cause or an effect, is associated with CVD, it is important to understand intrinsic factors that might influence ACE2 activity. Variations in *ACE2* may account for differences in ACE2 activity. This area is worth exploring particularly as *ACE2* genetic polymorphisms are associated with myocardial structural abnormalities and worsened outcomes (88,267). Studies investigating the potential role of genetic variation in *ACE2* are therefore underway and preliminary analysis has commenced.

The role of interstitial fibrosis on CMR in patients with AS requires further study. It should be noted that although the presence of fibrosis may label an individual as being at high risk, this does not necessarily suggest that the risk can be attenuated through a specific intervention such as AVR. Only randomised controlled trials can provide this data. Indeed, studies are underway to determine if early AVR is beneficial in asymptomatic patients with AS, in all-comers (EARLY TAVR, NCT03042104) or in those who have biomarker evidence of early LV decompensation (EVoLVeD, NCT03094143).

Finally, coronary artery calcium scoring in AF may significantly impact risk stratification in AF and needs further exploration. Beyond its utility to predict general atherosclerotic cardiovascular risk, evidence of vascular disease on CT would lead to higher calculated CHA₂DS₂-VASc stroke prediction scores and consequently, influence the use of anti-thrombotic therapy for stroke prevention. A prospective study is being planned to screen all-comers with AF with a coronary calcium score CT. To more precisely understand the effect of AF on the risk of vascular disease, a matched control group of individuals without AF will be recruited. It is anticipated that the study findings will be reported by mid 2023.

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APPENDICES

Other publications related to this PhD thesis are included in the following appendices

Appendix 1

Ramchand J and Burrell LM. Circulating ACE2: a novel biomarker of cardiovascular risk. *Lancet*. 2020;396:937-939.

Appendix 2

Klein AL, Ramchand J and Nagueh SF. Aortic Stenosis and Diastolic Dysfunction: Partners in Crime. *J Am Coll Cardiol*. 2020;76:2952-2955.

Appendix 3

Ramchand J, Jaber W and Hachamovitch R. Identifying Likelihood of Obstructive Coronary Disease in Patients With a Calcium Score of Zero: Separating the Wheat From the Chaff. *Circ Cardiovasc Imaging*. 2019;12:e009649.

Appendix 4

Ramchand J, Sampaio Rodrigues T, Kearney LG, Patel SK, Srivastava PM and Burrell LM. The Peguero-Lo Presti Electrocardiographic Criteria Predict All-Cause Mortality in Patients With Aortic Stenosis. *J Am Coll Cardiol*. 2017;70:1831-1832.

Appendix 5

Nagaraja V, Ramchand J (co-first author), Bolen MA, Das R, Krishnaswamy A, Kapadia SR, Puri R, Harb S. What is the Role of Cardiac Magnetic Resonance Imaging in Transcatheter Management of Aortic Valve Stenosis? *Structural Heart (in press)*, DOI: 10.1080/24748706.2021.1881194

is unlikely that hydroxychloroquine has any effect on disease progression, but its use might bias estimates towards the null compared with treatment with azithromycin alone.

The results of COALITION II corroborate those of COALITION I,¹⁰ which was done by the same study group and evaluated hydroxychloroquine with or without azithromycin in patients admitted to hospital with mild or moderate COVID-19. In COALITION I, there was no significant difference in outcomes in patients receiving hydroxychloroquine with or without azithromycin, and no evidence of an increase in adverse events. The results of these trials suggest that azithromycin might not provide benefit to patients once the disease has progressed and patients require hospitalisation. Because azithromycin is currently the most commonly prescribed outpatient therapy for COVID-19, establishing whether azithromycin is helpful earlier in the disease course is an important research priority. If azithromycin does not have a role in the treatment of COVID-19, avoiding its use would reduce unnecessary antibiotic consumption.

The results of COALITION II are an important contribution to the randomised trials evaluating therapeutics for COVID-19. For patients with COVID-19, the addition of azithromycin to existing standard of care regimens does not appear to improve outcomes. Additional placebo-controlled trials in hospitalised patients, and earlier in the disease course, would strengthen the

evidence and provide a comprehensive understanding of the role of azithromycin in COVID-19.

We declare no competing interests.

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Circulating ACE2: a novel biomarker of cardiovascular risk

Dysregulation of the renin-angiotensin system plays a major role in the progression of cardiovascular disease in humans. The enzymatic reactions within the renin-angiotensin system generate angiotensin II, which promotes vasoconstriction and inflammation and deleterious cardiovascular effects.¹ Angiotensin-converting enzyme 2 (ACE2) acts to counterbalance the renin-angiotensin system by degrading angiotensin II.^{2,3} In 2005, ACE2 was identified as the cellular receptor for severe acute respiratory syndrome coronavirus (SARS-CoV),⁴ and we now know that ACE2 also facilitates viral entry of SARS-CoV-2, leading to widespread systemic illness in COVID-19.⁵ Notably, ACE2 is present on endothelial cells and can undergo so-called shedding into the circulation. In patients with

cardiovascular disease, increased circulating ACE2 activity predicts adverse cardiovascular outcomes in patients with heart failure, coronary artery disease, and aortic stenosis.^{6–8} However, in the general population, the role of circulating ACE2 as a biomarker of risk is not well established.

In *The Lancet*, Sukrit Narula and colleagues⁹ present one of the largest epidemiological datasets on plasma ACE2 concentration in the general population. They did a case-cohort study involving 10753 participants from the multinational Prospective Urban Rural Epidemiology study, including 5084 patients randomly selected as the subcohort and 5669 with an incident event of interest. In the subcohort, 2935 (57.7%) were men and 2149 (42.3%) were women; the mean age



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was 50.79 years (SD 9.58). They report that ACE2 concentration was the highest-ranked independent predictor of deaths compared with standard cardiovascular risk markers (smoking, diabetes, systolic blood pressure, non-HDL cholesterol, and body-mass index).⁹ Increased concentration of plasma ACE2 was associated with increased risk of all-cause mortality (hazard ratio [HR] 1.35 per 1 SD increase [95% CI 1.29–1.43]), incident heart failure (HR 1.27 per 1 SD increase [1.10–1.46]), stroke (HR 1.21 per 1 SD increase [1.10–1.32]), myocardial infarction (HR 1.23 per 1 SD increase [1.13–1.33]), and incident diabetes (HR 1.44 per 1 SD increase [1.36–1.52]).

We commend the authors for the enormity of the data that was compiled. Study strengths include the large sample size, the well phenotyped patient population, and long duration of follow-up (median 9.42 years [IQR 8.74–10.48]). A notable feature of the study is the use of mendelian randomisation analyses to understand how antecedents of cardiovascular disease and drugs might causally affect circulating ACE2 levels. These analyses in combination with phenotypic data suggest a possible causal relationship between increased body-mass index and history of diabetes with increased circulating ACE2. The main study limitation is the absence of external replication and ultimately, validation of the findings by Narula and colleagues in independent cohorts is needed before the use of circulating ACE2 as a biomarker of risk can be recommended. Validation in independent cohorts is essential because the performance of biomarkers is rarely as good in the validation cohort as in the cohort in which they were initially assessed.¹⁰ Furthermore, only a solitary ACE2 measurement was obtained in each individual, and it is unknown whether circulating ACE2 levels change with disease progression, or if they can be used to guide response to therapy. If circulating ACE2 is to be used as a marker of risk in the clinical setting, then standardised methods with gender-specific reference ranges that take into account the effect of different disease states on ACE2 levels will need clarification.

Other questions remain. There is a long history of using blood biomarkers to predict disease and adverse outcomes in cardiovascular medicine. These markers, the original personalised medicine, permit patient-specific prediction of risk. However, is there a need for yet another biomarker? Screening for biomarkers

such as circulating ACE2 will probably compete for scarce health-care funding, and only those with excellent performance characteristics will be used in primary-care settings.¹⁰ Only cost-effective biomarkers with therapeutic implications to prevent the adverse sequelae of cardiovascular disease will survive such competition. A dedicated cost-utility analysis is needed to compare the cost-effectiveness of plasma ACE2 measurements over standard clinical care—eg, based on the outcome of incremental cost per additional quality-adjusted life-year (QALY).¹¹ This type of analysis is particularly important because cost-effectiveness of biomarkers in terms of QALYs is likely to be smaller than those associated with direct interventions.¹¹

Perhaps one of the most important pieces of information from the study by Narula and colleagues in the setting of the ongoing COVID-19 pandemic is the absence of any association between ACE2 levels and the use of ACE inhibitors, angiotensin-receptor blockers (ARBs), β blockers, calcium channel blockers, and diuretics.⁹ These results, validated by simultaneously performed mendelian randomisation studies, add support to the evidence that renin-angiotensin system inhibitors should not be withheld in patients with COVID-19 for the sole purpose of modifying ACE2.¹² They are also in line with our previous findings that neither ACE inhibitors nor ARBs alter plasma ACE2 activity in patients with cardiovascular disease.^{8,13} Although observational data show that ACE inhibitors and ARBs have no adverse effects in patients with COVID-19, we must await the results of ongoing randomised controlled clinical trials in this area that are assessing the effects of moving patients off or onto renin-angiotensin system blockers. The CLARITY trial, a controlled evaluation of ARBs for COVID-19 respiratory disease, will randomly assign renin-angiotensin system inhibitor naive patients with COVID-19 to an ARB or placebo, with sites in India and Australia (NCT04394117).

Narula and colleagues state that plasma ACE2 might be a marker of renin-angiotensin system dysregulation.⁹ Indeed, this characteristic could guide both preventive and therapeutic approaches in the future, with specific targeting of individuals with increased circulating ACE2 levels for more intensive lifestyle or pharmacological interventions to improve outcomes—a hypothesis that needs testing in the clinical setting.

We declare no competing interests.

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Reframing the NCD agenda: a matter of justice and equity



The international development community has never taken non-communicable diseases (NCDs) seriously. Seen largely as a challenge for high-income nations, NCDs and their community of advocates have met with, at best, warm words and, more commonly, indifference. There have been moments for optimism. The landmark 2005 WHO report, *Preventing Chronic Diseases: a Vital Investment*;¹ the 2011 Political Declaration on NCDs;² and the inclusion of NCDs in the Sustainable Development Goals (SDG) in 2016 (SDG 3.4: by 2030, reduce premature mortality from NCDs by a third and promote mental health and wellbeing). But, despite an increasingly well organised civil society response, NCDs have not broken through into the mainstream of development and global health.

One reason could lie in the framing of NCDs. A biomedical model, for example, emphasises genetic and other biological risk factors and processes. When viewed through the lens of epidemiological transitions, changes in NCD prevalence are associated with industrialisation, growing economic prosperity, and rises in life expectancy. And when NCDs are viewed as lifestyle conditions, attention is paid to individual behaviours rather than to wider social and commercial determinants of health.³

The current 5 × 5 approach to NCDs, favoured by WHO, focuses on five diseases (cardiovascular disease, cancer,

diabetes, chronic respiratory diseases, and mental ill-health) and five risk factors (tobacco use, unhealthy diets, physical inactivity, harmful use of alcohol, and air pollution).⁴ But, as the NCD Countdown 2030 showed, “Although premature mortality from NCDs is declining in most countries, for most the pace of change is too slow to achieve SDG target 3.4”.⁵ The global NCD community needs to consider a different approach to the framing of chronic diseases.

The central argument of *The Lancet NCDs and Injuries (NCDI)*⁶ Poverty Commission is that although the

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EDITORIAL COMMENT

Aortic Stenosis and Diastolic Dysfunction



Partners in Crime*

Allan L. Klein, MD,^{a,b} Jay Ramchand, MBBS,^{a,b} Sherif F. Nagueh, MD^c*"The future depends on what you do today."*

—Mohandas K. Gandhi (1)

Aortic stenosis (AS) is the leading cause of valvular heart disease in the Western world and is associated with increased morbidity and mortality (2). Progressive valvular narrowing leads to left ventricular (LV) pressure overload, LV hypertrophy, and fibrosis, which can ultimately culminate to LV systolic and/or diastolic dysfunction (DD) (3,4).

Aortic valve replacement (AVR), historically a high-risk endeavor, is a solution to relieve AS and AS-related myocardial dysfunction and can now be carried out with lower operative mortality with either modern surgical or transcatheter techniques (2). Despite the lower risk of adverse outcomes with modern AVR approaches, contemporary management guidelines only recommend AVR after development of overt myocardial systolic dysfunction and/or AS-related symptoms, which if missed, can lead to detrimental consequences (2). There is now increasing appreciation for the need for a more refined approach to AS management with the incorporation of more objective and sensitive markers of myocardial decompensation using biochemical (e.g.,

N-terminal pro-B-type natriuretic peptide) or imaging-based parameters.

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There is accumulating evidence (Table 1) that pre-operative DD is a marker of myocardial decompensation and represents an important determinant of outcomes in patients undergoing transcatheter aortic valve replacement (TAVR), although data have been predominantly derived from relatively small studies (5-12). In this issue of the *Journal*, Ong et al. (12) provide important insights to clarify the relationship between baseline and follow-up DD, with adverse outcomes in patients undergoing TAVR from the PARTNER-2 SAPIEN3 registry (13). The registry comprised patients with severe, symptomatic AS who were considered inoperable, high risk, or intermediate risk. Baseline, 30-day, and 1- and 2-year transthoracic echocardiograms (final included n = 1,253) from the PARTNER 2 SAPIEN 3 registry were analyzed by a consortium of core laboratories, and diastolic function was graded using American Society of Echocardiography/European Association of Cardiovascular Imaging criteria (14).

There are 3 key findings (13). First, the presence of grade ≥ 2 DD was common, with 42% of the cohort affected. As DD increased from grades 1 to 3, there was a significant increase in abnormalities involving indices of myocardial structure and function. Second, incremental grades of DD at baseline were associated with an increase in combined cardiovascular (CV) death/rehospitalization at 1 year. Third, improvement by ≥ 1 grade in DD or grade 1 DD at 30 days was common (71%), and on multivariable analysis was independently associated with reduced CV death/rehospitalizations at 1 year (hazard ratio [HR]: 0.63; 95% confidence interval [CI]: 0.42 to 0.92) but not at 2 years. Baseline DD3 group was an independent predictor of 1-year CV death/rehospitalization (HR: 2.24;

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TABLE 1 Recent Key Studies Assessing Diastolic Function Post-TAVR and Clinical Outcomes

First Author (Ref. #), Year	Number of Cases	Study Type	Results Summary
Blair et al. (4), 2017*	90	Retrospective, single-center	Baseline diastolic dysfunction grade, but not post-TAVR or changes in diastolic dysfunction grade, was associated with 1-yr death (HR: 1.163; 95% CI: 1.049 to 1.277) and combined death/cardiovascular hospitalization (HR: 1.174; 95% CI: 1.032 to 1.318).
Muratori et al. (5), 2015	358	Prospective, single-center	Survival was better in those with DD grade 3 with improvement in diastolic function grade vs. in those without improvement post TAVR (unadjusted).
Sato et al. (6), 2018*	237	Retrospective, single-center	Over a median follow-up of 1,320 days, neither pre- nor post-TAVR DD grade were associated with prognosis. In patients with grade III DD detected before TAVR and AR ≥ 2 after TAVR had poorer survival ($p < 0.008$). Patients with grade III DD detected after TAVR and AR ≥ 2 after TAVR had poorer prognosis ($p = 0.002$).
Thaden et al. (7), 2020*	1,383	Retrospective, single-center	Over a mean follow-up period of 7.3 yrs, increased left ventricular filling pressure (using ASE guidelines) remained an independent predictor of mortality after successful AVR (HR: 1.45; 95% CI 1.16 to 1.81).
Kampaktsis, et al. (8), 2020	359	Retrospective, single-center	Over a mean follow-up of 13 months, DD identified using an E/A ratio cut-off of 1.8 was associated with an increased risk for the outcome measure (HR: 2.02; 95% CI: 1.23 to 3.30). This association was lost in a propensity-matched cohort.
Anantha-Narayan et al. (9), 2020*	222	Prospective, single-center	Over a median follow-up of 385 days, advanced (Grades II-III) and indeterminate DD were associated with increased long-term mortality (25%-28% vs. 5%; $p = 0.02$).
Kampaktsis, et al. (10), 2017	195	Retrospective, single-center	At mean follow-up of 14 months, patients with severe baseline DD (E/A >1.5) who developed $\geq$ mild post-TAVR AI was independently associated with increased mortality compared to all other patients (HR: 3.89; 95% CI: 1.76 to 8.60; $p = 0.001$).
Asami et al. (11), 2018*	777	Prospective, single-center	1-yr all-cause mortality was higher in patients with LVDD grades I (16.3%; HR: 2.32; 95% CI: 1.15 to 4.66), II (17.9%; HR: 2.58; 95% CI: 1.43 to 4.67), and III (27.6%; HR: 4.21; 95% CI: 2.25 to 7.86) than in those with normal diastolic function (6.9%).

*Study assessed diastolic function using 2016 American Society of Echocardiography/European Association of Cardiovascular Imaging criteria guidelines (13).
AR = aortic regurgitation; CI = confidence interval; DD = diastolic dysfunction; HR = hazard ratio; TAVR = transcatheter aortic valve replacement.

95% CI: 1.34 to 3.76) but not at 2 years (HR: 1.48; 95% CI: 0.85 to 2.58).

The authors should be commended for the enormity of data that was compiled. Study strengths include the large sample size, the well-phenotyped patient population, and the availability of follow-up echocardiography at 30 days. The main study limitations include the retrospective nature of the analysis and the lack of external replication. This is essential, as in general, the performance of risk markers is rarely as good in the validation cohort compared with the cohort in which they were initially assessed. Second, it was assumed that all the patients with severe AS had evidence of myocardial pathology and DD; thus, algorithm B from the 2016 American Society of Echocardiography diastology guidelines was used. This assumption may not hold true in all patients with AS, especially post-TAVR, where diastolic function may normalize in a proportion of patients. Further in the study, it appears that peak E-wave velocity was not considered in DD assessment. Thus, study participants with E/A ≤ 0.8 but with E > 50 cm/s would have been classified as having grade I DD (defined in the study as E/A ≤ 0.8), whereas in the guidelines, the classification may have been either grade II or indeterminate depending on the presence of ancillary criteria. Third, as acknowledged by the authors, the presence of mitral annular calcification

was not collected. Calcification is known to influence mitral annular movement used to assess diastology, and as such may have introduced bias in grading a higher E/A ratio and lower tissue Doppler imaging annular velocities. This limitation is in part mitigated by the exclusion of patients with mitral peak velocity > 1.5 m/s, though it may have been useful to have incorporated known parameters of DD in this setting such as isovolumic relaxation time (15). Fourth, the presence of antecedent pathologies known to influence diastolic function, such as cardiac amyloidosis, is not reported and may have influenced the occurrence of adverse outcomes (16). Indeed, on multivariable analysis, neither the baseline grade 3 diastolic function nor improvement by ≥ 1 grade in DD/grade 1 DD at 30 days independently predicted clinical outcomes at 2 years. This suggests that long-term, the prognostic value of DD post-TAVR may not be incremental to pertinent structural parameters and/or comorbidities associated with advanced DD. Finally, nearly 30% of the cohort was excluded because of reasons such as the presence of significant mitral valve disease and/or atrial fibrillation; thus, the findings may not be reflective of a real-world cohort of AS and reflect important challenges of assessing diastolic function in AS.

Other questions remain. It is intriguing why, post-TAVR, DD improves in some but not others. In

those who do not improve, this may potentially reflect irreversible alterations in myocardial architecture that may be better understood through histology studies or cardiac magnetic resonance imaging (3,4). Notwithstanding, the present study supports the concept that once advanced DD develops, there is potential for progression and even irreversibility despite reduction of afterload with AVR. This provides an opportunity to identify patients who would benefit from AVR earlier than using traditional metric of symptoms before the development of irreversible ventricular remodeling and/or advanced DD. Indeed, in patients post-surgical AVR, Krayenbuehl et al. (4) demonstrated that at 18 months post-surgery, myocardial mass decreased primarily due to regression in myocyte hypertrophy, although with relative increase in myocardial fibrosis known to be associated with worsening myocardial stiffness/DD.

The field has evolved and has identified various promising blood and imaging biomarkers previously, but none have ever been fully embraced in current practice guidelines. The findings of Ong et al. (13) describe increasing baseline grades of DD associated with increasing gradations of risk. Timely detection of more advanced stages of DD is therefore important, and could be a marker for poor prognosis that should trigger consideration for closer evaluation in patients who do not meet traditional indications for AVR. For example, stress testing to assess exercise capacity should be considered in those without symptoms but with evidence of significant DD. Furthermore, DD should not be solely attributed to severe AS, as in

many circumstances, causes are typically multifactorial. The importance of identifying and managing concurrent pathologies known to coexist with AS that can influence diastolic function, such as hypertension and amyloidosis (16), particularly in elderly patients, cannot be underestimated.

To conclude, the study by Ong et al. (13) supports that the presence of advanced DD has prognostic utility in those with severe AS undergoing TAVR, although it remains to be seen if diastolic function assessment can translate into better clinical outcomes. In the meantime, there is little doubt in our minds that the words by Mr. Mohandas Gandhi are true: “The future depends on what you do today” (1). The same principle applies to the assessment of DD in aortic stenosis. To advance the assessment of diastolic function in the future, we will need to continuously adapt and refine our approaches, for example, through the incorporation of emerging parameters such as LV diastolic strain and left atrial strain (17).

AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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EDITORIAL

Identifying Likelihood of Obstructive Coronary Disease in Patients With a Calcium Score of Zero

Separating the Wheat From the Chaff

See Article by Alshahrani et al

The lesson I've learned the most often in life is that you're always going to know more in the future than you know now.

—Taylor Swift

Don't think, look!

—Ludwig Wittgenstein

The presence of coronary artery calcium (CAC)—a pathognomonic marker of atherosclerosis—has emerged as a factor strongly associated with the development of adverse cardiovascular events. It is established that a zero CAC score is a unique negative risk marker, aptly referred to as the power of zero given its association with an exceedingly low event rate.¹ This result permits a reclassification of risk incremental to conventional risk markers alone. However, as noncalcified plaque can occur, the presence of a zero CAC score is not identical to the absence of atherosclerosis. Noncalcific plaque formation can be dynamic, with a preponderance to develop and bring about symptoms in younger patients.² The optimal evaluative approach to the symptomatic individual with a CAC score of zero is undefined.

In the current issue, Alshahrani et al³ report a new clinical score predicting the presence of coronary computed tomography angiography (CCTA)-defined obstructive coronary artery disease (CAD) in patients with zero CAC. The score was based on a derivation cohort from a computed tomography registry⁴ of predominantly symptomatic patients (82%) with a CAC score of zero (n=4903) and validated in an independent cohort of 4290 mostly symptomatic patients (73%) from the Coronary CT Angiography Evaluation for Clinical Outcomes: an International Multicenter Registry multinational registry.⁵ Logistic regression modeling was applied to patient characteristics—patient sex, age, typical chest pain, dyslipidemia, hypertension, family history of CAD, and diabetes mellitus—to identify CCTA-defined obstructive CAD (stenosis $\geq 50\%$; 3.5% of patients). Based on weights of the β -coefficients, a score of ≤ 6 was considered low risk (negative predictive value, 99%; negative likelihood ratio, 0.42), scores of 7 to 13 were intermediate risk, and scores >14 were high risk for obstructive CAD (specificity, 98%; positive likelihood ratio, >5). The prognostic characteristics were examined in an underpowered small subanalysis with all-cause mortality over a median 2-year follow-up was 0.51% and 0.58% in the low- and intermediate-risk groups, respectively, versus 2% in the high-risk group. The results of the current study potentially aid the decision-making process in symptomatic patients with zero CAC. Study findings suggest that in low-risk individuals (risk score, ≤ 6), CCTA-defined obstructive CAD can be excluded with a high degree of certainty, potentially avoiding further investigation.

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Study strengths include the large sample size, the well-phenotyped patient population, and validation of findings in an independent cohort. The study limitations include a 22% frequency of asymptomatic patients, possibly obfuscating the accuracy of the score. The prevalence of CAD was very low, 3.5% overall, and only 2.8% of patients were reclassified by the score as high likelihood of CAD. The use of a 50% CCTA stenosis threshold has been shown to be associated with a significant false positive rate⁶; thus, the low CAD prevalence may well be an overestimation. Finally, as many as 47% of patients from the validation cohort were excluded from analysis, which may have influenced the results of the study. Although there are currently no guideline recommendations to support the use of CAC in symptomatic individuals,⁷ it is unclear whether the current results sway the reader toward or away from its use given the extremely low risk of obstructive CAD even in the high-risk zero CAC patients.

A basic axiom of noninvasive testing is that the utility of the test depends on the pretest probability of the population tested.^{8,9} The importance of conditional probability still holds true in a seemingly low-risk population with a zero CAC. A low pretest CAD probability combined with a zero CAC is associated with low CAD prevalence and risk.¹⁰ However, in the presence of symptoms, with increasing age and risk factors,¹¹ a zero CAC is increasingly associated with myocardial ischemia on functional testing and a significantly higher risk of coronary-related adverse events. A zero CAC in this context does not rule out obstructive CAD and begs the question of the appropriateness and value of the CAC assessment in greater than low-risk, symptomatic patient groups. The PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain)^{12,13} and SCOT-HEART¹⁴ trials (CT Coronary Angiography in Patients with Suspected Angina due to Coronary Heart Disease) demonstrated the potential utility of CCTA to overcome shortcomings of CAC. In SCOT-HEART, the use of CCTA in addition to standard care in patients with stable chest pain resulted in a lower rate of death from coronary heart disease or nonfatal myocardial infarction at 5 years compared with standard care alone. Indeed, the 2016 update to the National Institute for Health and Care Excellence CG95 no longer recommends CAC scoring.¹⁵ Moreover, this document does not recommend CAC scoring in symptomatic individuals with low pretest probability, particularly given the risks of noncalcific plaques.

Given the avalanche of available data, clinicians often rely on heuristics—an educated guess or rule of thumb. These cognitive shortcuts provide quick and efficient decision-making when faced with a complex cognitive problem, albeit imperfect and likely flawed. A solution for this frustrating challenge is the use of predictive instruments. There is a long history of scores for pre-

dicting CAD probability or risk of adverse outcomes.^{8,16} These scores, the original personalized medicine, permit patient-specific prediction via weighted combination of all available data. The prerequisites for a predictive instrument include calibration to an observed outcome, generalizability, portability to an outside population, and acceptability by clinicians with an impact on practice.¹⁷ Independently of the generalizability of CAC to symptomatic low-risk patients, is there a need for another clinical score? Indeed, the variables included in the model proposed by Alshahrani et al³ are similar to those used in more ubiquitous tools used to determine pretest probability.¹⁸ Although the CAD risk model proposed by Alshahrani et al³ was able to categorize risk for obstructive CAD and mortality, the patient population was dominated by younger individuals with atypical symptoms and otherwise low pretest CAD probability. Therefore, the applicability of this model in other patient populations such as the elderly or those without symptoms, or extension to other cohorts, requires further evaluation. Although the analysis of net reclassification or integrated discriminative value is not provided, most individuals (≈58%) were stratified into the intermediate-risk category, which does not suggest an improved predictive utility of the present model compared with already available risk scores. Nevertheless, the study represents an important step forward to develop an optimal approach in the assessment of patients with CAC score of zero.

At present, routine use of CAC assessment cannot be recommended for the assessment of symptomatic individuals. That said, there is little doubt in our minds that words by Swift are true—we will know more in the future than the past. Future studies will refine our approaches to best select candidates for CAC versus CCTA testing. It will be particularly interesting to see what the role will be in the future for CAC in the era of modern day CCTA technology.

ARTICLE INFORMATION

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Disclosures

None.

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Appendix 4

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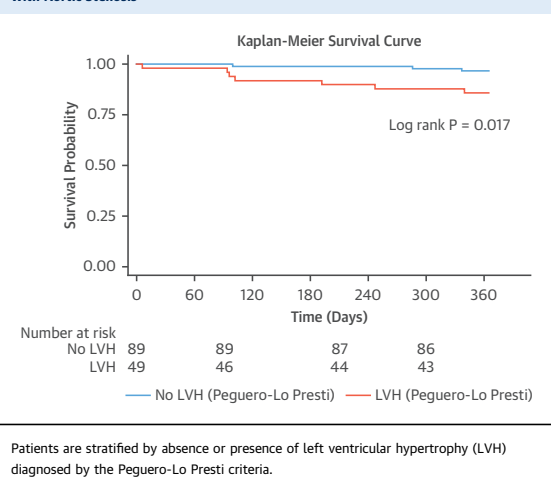
The Peguero-Lo Presti Electrocardiographic Criteria Predict All-Cause Mortality in Patients With Aortic Stenosis



Peguero et al. (1) have described new electrocardiographic (ECG) criteria (the $S_D + SV_4$ -Peguero-Lo Presti) for the diagnosis of left ventricular hypertrophy (LVH), which improved the sensitivity and accuracy of the test. We addressed 2 outstanding issues with regard to the new criteria in patients at risk of LVH due to aortic stenosis (AS). First, we assessed its ability to predict all-cause mortality. Second, we tested its performance against LVH measured using cardiac magnetic resonance (CMR). Patients were prospectively recruited for an ECG and echocardiogram ($n = 138$), and a subset also had CMR ($n = 41$). All analyses were reviewed by 2 investigators blinded to other imaging results or clinical outcomes.

The mean age was 74 ± 11 years, 61% were men, and comorbidities were common (hypertension, 80%; diabetes, 25%; heart failure, 31%; coronary artery disease, 38%). On echocardiography, AS was severe in 52%, moderate in 24%, and mild in 24%. Echocardiographic-LVH (defined according to American Society for Echocardiography guidelines) was present in 81 (59%) patients. The Peguero-Lo Presti LVH criteria had nominally the best sensitivity (49%; 95% confidence interval [CI]: 39% to 60%) and specificity (84%; 95% CI: 73% to 91%), followed by the Cornell criteria (sensitivity: 26% [95% CI: 18% to 36%]; specificity: 82% [95% CI: 71% to 90%]) and Sokolow-Lyon criteria (sensitivity: 14% [95% CI: 8% to 23%]; specificity: 93% [95% CI: 83% to 97%]) (all $p < 0.001$, McNemar test). All-cause mortality was increased in those with Peguero-Lo Presti LVH (Figure 1). On Cox regression, after adjustment for age, coronary artery disease, and heart failure, Peguero-Lo Presti LVH was an independent predictor

FIGURE 1 Kaplan-Meier Survival Curve Illustrating the 1-Year Survival of Patients With Aortic Stenosis



of all-cause mortality (hazard ratio: 4.2; 95% CI: 1.1 to 16.4; $p = 0.036$).

We then tested the diagnostic utility of the Peguero-Lo Presti criteria against CMR-LVH. Again, the Peguero-Lo Presti LVH criteria had nominally the best sensitivity (48%; 95% CI: 31% to 66%) and specificity (92%; 95% CI: 65% to 99%); followed by the Cornell criteria (sensitivity: 24% [95% CI: 12% to 42%]; specificity: 92% [95% CI: 65% to 99%]) and Sokolow-Lyon criteria (sensitivity: 17% [95% CI: 8% to 35%]; specificity: 100% [95% CI: 76% to 100%]) (all $p < 0.001$, McNemar test).

In summary, in patients with AS, the Peguero-Lo Presti criteria is a more sensitive method to diagnose both echocardiographic-LVH and CMR-LVH compared with existing ECG criteria. We report for the first time that the Peguero-Lo Presti LVH criteria predicts all-cause mortality. We agree with Peguero et al. (1) that the $S_D + SV_4$ -Peguero-Lo Presti criteria is a very useful addition to our diagnostic strategies in patients at risk of LVH.

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REPLY: Electrocardiographic Criteria for Diagnosing Left Ventricular Hypertrophy and Predicting Mortality in Patients With Aortic Stenosis



We congratulate Ramchand and colleagues for putting together a prospective study that further validates the Peguero-Lo Presti criteria (1) as a more accurate predictor of left ventricular hypertrophy (LVH) as assessed by cardiac magnetic resonance. Interestingly, their calculated sensitivities and specificities had almost no variation when comparing the transthoracic echocardiogram versus cardiac magnetic resonance as a gold standard for the diagnosis of LVH (49% to 84% vs. 48% to 92%, respectively). Their reported results correlate well with the validation cohort of our initial study (57% to 90%). Moreover, they have shown the outstanding relationship between electrocardiographic (ECG) criteria and all-cause mortality in patients with aortic stenosis, which further emphasizes the importance of having an accurate ECG-LVH criteria when evaluating the cardiac patient.

By analyzing the electrocardiogram of a male patient with possible rate-related left anterior fascicular block (LAFB), Betjeman and colleagues have pointed out a potential pitfall in the ECG diagnosis of LVH by the Peguero-Lo Presti criteria, assuming this patient had no proven diagnosis of LVH.

It is well documented that LAFB and other conduction abnormalities have been shown to distort the ECG diagnosis of LVH (2). The 2009 American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society Recommendations for the Standardization and Interpretation of the Electrocardiogram propose that LVH criteria that include the R-wave in limb leads I or aVL (e.g., Cornell voltage criteria) are unreliable in the presence of LAFB (2).

Furthermore, they suggest that criteria utilizing an S-wave in the precordial leads (e.g., Sokolow-Lyon voltage criteria) may improve detection of LVH in the presence of this conduction abnormality. However, a more current investigation by Ravi and colleagues concluded that there was no difference in overall accuracy between aVL- and non-aVL-based criteria for diagnosing LVH in the presence of LAFB (3). Moreover, LAFB is usually considered a diagnosis of exclusion after LVH, left bundle branch block, and inferior wall Q-wave myocardial infarction have been ruled out.

We agree that the electrocardiograms shown by Betjeman and colleagues depict an example that may be encountered in clinical practice where the Peguero-Lo Presti criteria may not be applicable. However, the Cornell voltage criteria have the same limitations in their example: negative in the first electrocardiogram (R-wave in aVL 12 mm + S-wave in V₃ 13 mm = 25 mm) and positive in the second (R-wave in aVL 16 mm + S-wave in V₃ 12 mm = 28 mm). Given the dynamic nature of the Peguero-Lo Presti criteria, it may be difficult to predict the precise impact that LAFB could have on its overall diagnostic accuracy. We commend the work of Betjeman and colleagues and encourage further study with perhaps a larger population to tease out the relationship between LAFB and LVH.

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REVIEW ARTICLE



What Is the Role of Cardiac Magnetic Resonance Imaging in Transcatheter Management of Aortic Valve Stenosis?

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ABSTRACT

Transcatheter aortic valve replacement (TAVR) has changed the management paradigm of severe aortic stenosis. Cardiac magnetic resonance (CMR) has an emerging role in the evaluation of aortic stenosis. MRI provides excellent tissue characterization, endocardial definition, and is considered the gold standard for the assessment of left and right ventricular volumes, remodeling, mass, and chamber function. In addition, CMR is free from ionizing radiation, noninvasive, and an iodine-contrast sparing modality. CMR has a role in the assessment of annular dimensions, especially in individuals in whom computed tomography is contraindicated. This review discusses the contemporary role of CMR in patients with aortic stenosis, particularly in those being considered for TAVR.

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KEYWORDS Transcatheter aortic valve replacement; cardiac magnetic resonance imaging; aortic stenosis

Introduction

Transcatheter aortic valve replacement (TAVR) has fundamentally altered the management paradigm of aortic stenosis. TAVR has been systematically adopted worldwide, initially in elderly, high-risk individuals, then in those at intermediate risk, and now in low surgical risk patients.^{1–4} Imaging is essential in the diagnosis, planning, and guidance of TAVR.^{5,6} Cardiac computed tomography angiography (CCTA) evolved as the imaging modality of choice prior to TAVR, though it is relatively contraindicated in patients with severe renal dysfunction.^{5–7} Cardiac magnetic resonance (CMR) is increasingly utilized in the assessment of patients under consideration for TAVR, allowing assessment of the remodeling response of the heart, as well as aiding in assessment of aortic stenosis severity and aortic annular dimensions. CMR is considered the noninvasive gold standard for ventricular volumes, mass, chamber function as well as tissue characterization.^{8–10} It is a modality free of ionizing radiation, an attractive feature for use in surveillance, as well as for use with increasingly younger patients under consideration for TAVR. The review focuses on the practical application of CMR in periprocedural planning and risk stratification of patients with aortic valve disease undergoing TAVR.

Discussion

Pre-TAVR assessment

Annular sizing

CCTA is the current gold standard for annular measurement in individuals undergoing TAVR.¹¹ However, the administration of iodinated contrast is relatively contraindicated in patients with advanced chronic kidney disease and contrast allergy. In such individuals, transesophageal echocardiography is a common alternative method to size the annulus; however, this is a relatively invasive imaging modality with sedation. There are multiple publications reporting the use of three-dimensional transesophageal echocardiography for annular sizing.^{12–14} CMR has shown promising results in annular depiction, without the requirement for intravenous contrast material. In a prospective observational study of patients with severe aortic stenosis, there were no significant propensity-adjusted differences in perimeter, area, and average, maximum, and minimum diameters found between non-contrast-CMR derived and cardiac computed tomography angiography diastolic measurements.^{15–18} Furthermore, with the exemption of the minimum annulus diameter being significantly smaller with CMR (difference 0.4139 mm, 95% confidence interval [CI]: 0.0721 to 0.7556, $p = 0.02$), similar results were observed when CMR was compared to CCTA end-systolic measurements. Annular sizing using CMR can be obtained with several different

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approaches. A free-breathing sequence using respiratory navigator-gating and ECG triggering (so-called “whole heart” sequence) can provide a three-dimensional data set similar to CCTA, though typically requiring 4–6 minutes acquisition time. Additionally, direct planimetry using steady state free precession (SSFP) cine imaging (Figures 1 and Figures 2) obtained as a stack, using double oblique orientation in plane with the aortic annulus. A relative limitation of CMR is the inability to precisely assess the amount of leaflet and annular calcification, typically appearing as signal void. Furthermore, the longer scan time compared to CCTA may be less well tolerated in patients with severe, decompensated AS.

Aortic stenosis severity and valve morphology

Doppler echocardiography is usually the modality of choice¹⁹ in the imaging assessment of aortic stenosis, though it has inherent limitations.²⁰ The flow continuity equation is commonly used to calculate the aortic valve area (AVA) on transthoracic echocardiography. This approach requires Doppler measurement of the velocity time integral at LVOT and aortic valve levels as well as

measurement of LVOT diameter. Assuming equal flow at both levels, the AVA can be calculated. The continuity equation approach has some disadvantages, given that accurate assessment requires good acoustic windows and is often user (sonographer skill level) dependent. Additionally, the presence of subvalvular flow acceleration can render this technique suboptimal.²⁰ The left ventricular outflow tract (LVOT) in this equation is assumed to be circular, while in reality it is elliptical in shape. Accurate estimation of LVOT area can be further confounded by poor windows, or severe calcification of the aortic valve.²¹ The left ventricular outflow tract dimension should be measured at the base of the aortic valve leaflets and ideally pulse wave Doppler measurement should be performed at the same point.²² Hybrid calculation of aortic valve area has been proposed using peak velocities from echocardiography and left ventricular outflow tract dimensions from CCTA/CMR though prospective evaluations confirming the utility of such an approach are lacking.^{23,24} Alternatively, LV stroke volume or LVOT forward flow can be obtained using CMR techniques, and applied directly to the continuity equation to obtain the aortic valve area. Lastly, visualization of the aortic

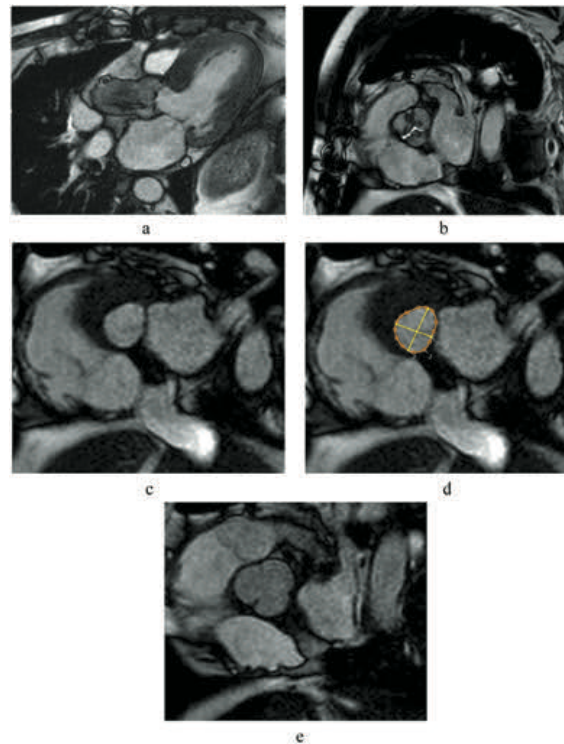


Figure 1. **A and B:** Three chamber and short axis steady state free precession images showing a severely thickened and restricted aortic valve. **C and D:** demonstrate through plane views of the aortic annulus with corresponding measurements, in addition mild aortic regurgitation and possible fusion of the right and left coronary cusps. **E:** Short-axis view through the aortic root, perpendicular to the aortic valve plane.

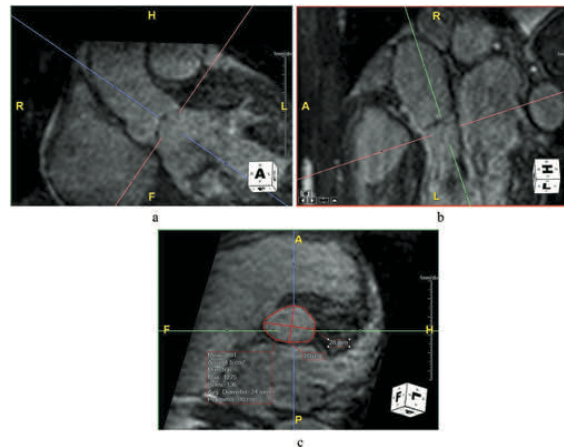


Figure 2. (A) and (B) Orthogonal long-axis views of the aortic valve. (C) Short-axis views of the aortic annulus. Small and large diameters and area are measured for transcatheter aortic valve replacement valve sizing.

valve using CMR can complement the assessment of valve morphology, particularly in those with challenging echocardiographic windows (Video 1 and 2).

Planimetry AVA

AVA planimetry involves direct visualization of the anatomical valve area, defined as the area encompassed by planimetry of the maximal systolic opening of the valve leaflets at the level of the leaflet tips. Good correlation has been demonstrated between CMR planimetry, transthoracic and transesophageal echocardiography in the quantification of aortic stenosis severity.^{20,25–30} It has been found to be consistent in bicuspid, and trileaflet valves.^{20,31} In addition, there is good intra- and inter-observer reproducibility in measurement of effective orifice area and planimetry.²⁰ Planimetry using two-dimensional echocardiography has certain disadvantages, as it can be difficult

to delineate leaflet edges, particularly in setting of heavily calcified valves. Planimetry should be measured at the valve tips using planes orthogonal to the aortic annulus ideally in both oblique transversal long axis and oblique coronal views. To avoid assessing AVA beyond or above the leaflet tips, both of which will result in overestimation of the AVA, the imaging plane should be moved up and down off-center shiftwise in 2 mm steps for at least 4 mm in each direction. Lastly, manual planimetry of the maximum visible AVA in systole should be obtained from each series and values should then be averaged.²⁹ Figure 3 depicts planimetry using the short axis views in mid systole.

Potential limitations of CMR planimetry are difficulties in the precise visualization of the aortic leaflet tips due to partial volume effects, the presence of calcifications, or flow-related artifacts. Patients with irregular cardiac rhythms, or those

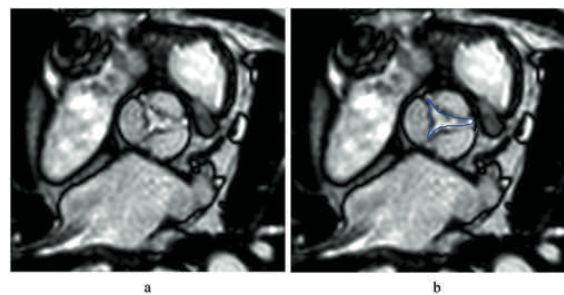


Figure 3. A. Short axis through plane steady state free precessions images of the aortic valve perpendicular to the valve plane showing a thickened and restricted aortic valve in mid systole B. Corresponding measurement of anatomical aortic valve area.



having difficulty performing breath holds may also be challenging to image precisely. In practice, because calcium typically appears as a signal void, this generally leads to underestimation of annular and leaflet calcification. Cine SSFP sequences are generally preferred because of their superior signal-to-noise ratio, optimal blood-tissue contrast, and high spatial and temporal resolutions, allowing the accurate identification of the valvular cusps. Older generation spoiled gradient echo cine images may be of use in some circumstances, as flow-related enhancement can delineate leaflet opening, and the decreased artifact in stronger magnetic field settings.

Phase contrast MRI

Velocity encoded phase-contrast imaging is a MR technique which allows peak flow velocity through the aortic valve to be estimated. This assessment can be obtained by in plane or through plane, with orientation established through reference to previously obtained cine images. Care must be taken to ensure that the imaging plane is closely aligned to orthogonal or in plane flow direction, as an offset >20 degrees can lead to inaccurate measurements.³² The maximum velocity typically occurs just above the valve, so the slice should be acquired accordingly. The expected peak velocity encoding (VENC) value and direction (through or in plane) are defined by the user. The amplitude of the flow-sensitizing gradients is calculated so that the peak velocity corresponds to a phase shift of 180 degrees. Imaging should start with a VENC according to the estimated maximum velocity, and then adjusted accordingly and should ideally not be >20% higher than the actual velocity. If selected VENC is lower than the actual velocity encountered, aliasing occurs, and if VENC is significantly higher than the actual velocity, the sensitivity to detect the maximum velocity may be suboptimal.

CMR flow measurements show good correlation with Doppler echocardiographic findings, although peak velocity measurements are commonly underestimated.³³ There are several potential sources of error with PC-MRI measurements. The impact of improper VENC settings has already been delineated. Other potential sources of error in PC-MRI measurement include deviation of the imaging plane during data acquisition (e.g., cardiac or respiratory motion), inadequate temporal or spatial resolution, and field inhomogeneity (e.g., susceptibility artifact from metallic implants).³⁴ PC-MRI is also optimal in measurement of laminar flow, which is often not present in setting of stenosed valves.

Assessment of aortic regurgitation

Aortic regurgitation can be quantified with PC-MRI as well. To quantify aortic regurgitation, forward and reverse flow measured during a cardiac cycle can be used to calculate the regurgitant fraction, defined as follows: (reverse flow volume/forward flow volume) $\times$ 100%. Quantitative through plane flow measurements in the ascending aorta has been performed at valve level, sinotubular junction (STJ), and at the mid-ascending aorta. Our own experience suggests that the sinotubular junction may be the preferred location (Figure 4). There is no broad consensus on the correlation between aortic regurgitation as measured by MRI and regurgitation graded in multiparametric fashion by echocardiography. However, in a prospective evaluation of 107 patients, the regurgitant values that best defined AR severity using phase-contrast CMR compared with surface echocardiography were as follows: grade 0 to 1+, <8%; grade 2+, 8% to 19%; grade 3+, 20% to 29%; and grade 4+, >30%.³⁵ A regurgitant fraction greater than 33% had a sensitivity of 85% and a specificity of 92% and detected patients who became symptomatic needing surgery.³⁶ Additionally, a combination of two indices namely LV end-

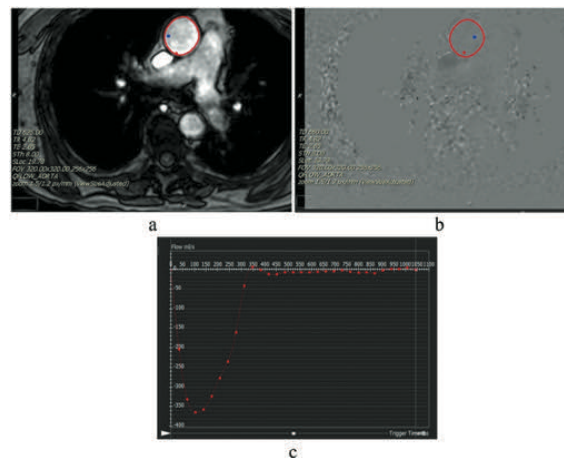


Figure 4. 68-year-old man with moderately severe aortic stenosis. Through-plane phase contrast MR images an ideal encoding velocity (400 cm/s) at mid ascending aorta. A) Magnitude image, B) corresponding phase-contrast image shows flow pattern in ascending aorta without aliasing (region of aorta shown in red circle), C) corresponding ascending aorta flow profile showing a peak velocity of 365 cm/s.

diastolic volume of more than 246 ml and a regurgitant fraction >33% was strongly correlated with clinical outcomes.³⁶ Finally, aortic regurgitation assessment by comparison of the forward aortic flow and pulmonary forward flow by CMR can also be useful for patients with combined aortic stenosis, in whom the higher velocity encoding may lead to underestimation of regurgitant volume.

Myocardial assessment

Ventricular function

In addition to accurate direct and objective quantitation of aortic regurgitant volume and fraction, LV volumes can be precisely assessed, a key feature in the management of aortic regurgitation. LV dysfunction is an independent predictor of poor outcomes post aortic valve replacement.³⁷ The most common method to assess LV systolic function on CMR is using the Simpson's technique.³⁸ In brief, diastolic and systolic LV volumes are determined by summing the endocardial area within multiple short axial slices spanning the base to the apex of the heart and multiplying each area by slice thickness. LV ejection fraction is then computed (end-diastolic volume – end systolic volume)/end-diastolic volume. This technique allows reproducible calculations of volumes and function without assumptions of LV shape or geometry. Similarly, RV function is an important predictor of outcomes in those undergoing TAVR and can be measured using the aforementioned Simpson technique.³⁹ Normative values of ventricular size and function have been published elsewhere.⁴⁰

Myocardial remodeling and perfusion

CMR has a crucial role in identifying various patterns of ventricular remodeling response to severe aortic stenosis. Different patterns include normal ventricular geometry, concentric remodeling, asymmetric remodeling, concentric hypertrophy, asymmetric hypertrophy, and decompensation.⁴¹ These patterns are not necessarily related to the severity of aortic stenosis.⁴¹ A recent study⁴² demonstrated heterogeneous adaptation to severe aortic stenosis among males and females. Women had lower LV mass (indexed), smaller LV end-diastolic volume (indexed), and lower concentric remodeling and LGE compared to gentlemen. However, the LV ejection fraction was similar across the two cohorts, and no significant differences were observed with regards to reverse remodeling. Another multicenter study⁴³ of 674 patients demonstrated men were more likely to have advanced remodeling on CMR.

Patients with aortic stenosis can present with a multitude of symptoms, making it challenging to determine the etiology of the presentation. Myocardial perfusion using CMR may highlight if symptoms are also contributed by myocardial ischemia related to flow limiting coronary artery disease. This can be performed using vasodilators (adenosine or regadenoson), dobutamine, or exercise. Future research is required to help delineate the role of CMR perfusion imaging to aid in planning coronary revascularization before TAVR. Myocardial subendocardial or global ischemia can be seen as a result of microvascular dysfunction and/or hypertrophied left ventricle in the context of non-obstructive coronary artery disease.^{44,45} Myocardial perfusion reserve is a predictor of

aerobic exercise capacity (VO_2 max) and negatively correlates to New York Heart Association functional class in individuals with severe aortic stenosis with non-obstructive coronary artery disease.⁴⁶ Lower myocardial perfusion reserve predicts symptom onset in asymptomatic individuals.⁴⁷

Replacement fibrosis

Pressure overload and increased ventricular wall stress due to severe aortic stenosis results in ventricular hypertrophy. This is frequently associated with adverse ventricular remodeling in the form of intramyocardial fibrosis resulting in systolic and diastolic dysfunction.⁴⁸ CMR is an excellent tool to detect myocardial fibrosis and necrosis noninvasively.⁴⁹ Replacement fibrosis is permanent and recognized using late gadolinium enhancement (LGE) on cardiac magnetic resonance, while diffuse fibrosis happens earlier, and is possibly reversible.^{50–52} Late gadolinium enhancement^{53,54} is an independent predictor of all-cause mortality and cardiovascular mortality in patients with severe aortic stenosis. A midwall configuration of LGE is nonspecific, but can help differentiate this from enhancement due to other etiologies like ischemic damage or cardiac amyloidosis.⁵⁵ Once this process is established in the midwall, rapid increase in scar burden can be seen, and becomes permanent despite valve replacement.⁵⁶ A meta-analysis⁵⁴ of over 600 patients suggested that LGE in patients with aortic valve disease had a higher all-cause mortality (OR: 3.28, 95% CI: 2.02–5.32) compared to patients with no LGE. LGE is a good risk stratification tool especially in individuals undergoing TAVR. One particular study suggested the dose-dependent effect of LGE on the rates of major adverse cardiac events and midwall pattern of LGE was superior to left ventricular function in predicting mortality.⁵⁷

Myocardial fibrosis can be quantified by determining the extracellular myocardial volume fraction by CMR using the modified Look-Locker inversion recovery based T1 mapping.⁵⁸ Everett et al.⁵⁸ reported outcomes from 440 patients undergoing aortic valve intervention (isolated surgical aortic valve replacement 71%, combined coronary artery bypass grafting with surgical aortic valve replacement 14%, and transcatheter aortic valve replacement 15%). They found that extracellular myocardial volume fraction was an independent predictor of all-cause mortality (HR: 1.09, 95% CI: 1.01–1.17).⁵⁸

Additional research is required to establish optimal timing of aortic valve intervention, which may be informed by the presence of LGE. Currently, the EVOLVD study (NCT03094143) is recruiting individuals with asymptomatic severe aortic stenosis with midwall LGE that will be randomized to early intervention (surgical or transcatheter) or surveillance. The primary composite endpoint of the trial is all-cause mortality and unforeseen hospital readmissions due to aortic stenosis. An early report suggests that diffuse fibrosis post aortic valve replacement on T1 mapping CMR is associated with poor outcomes and potentially be used as a biomarker.⁵⁹

Diffuse fibrosis

Diffuse fibrosis, in contradistinction to replacement fibrosis, is characterized by a disproportionate accumulation of collagen diffusely in the heart, and is an important contributing factor to sudden cardiac death.⁶⁰ T1 mapping is an emerging CMR



imaging technique used to identify diffuse fibrosis. Magnetic resonance imaging exploits the magnetic properties of hydrogen nuclei protons within a determined magnetic field. T1 value is specifically defined as the time when longitudinal proton magnetization recovers approximately 63% of its equilibrium value, and may represent a valuable biomarker in aortic stenosis.⁶⁰ T1 is influenced by the molecular constitution of tissue, largely determined by the proportion of water content. As discussed above, LGE has been widely used to detect and assess qualitatively for the presence of replacement myocardial fibrosis. A limiting factor with the use of LGE is the diffuse infiltrative nature of fibrosis in aortic stenosis, thereby lacking the presence of normal non-fibrotic myocardium as a frame of visual reference. This may result in underestimation of the true burden of interstitial fibrosis. To address this, native and contrast enhanced T1 mapping have been developed to quantify diffuse myocardial fibrosis in a manner that does not rely on contrasting signal intensity.

By combining native and contrast enhanced T1 maps of blood and myocardium, extracellular volume (ECV) can be measured. ECV reflects diffuse interstitial remodeling and expansion. Shortened post-contrast T1 times as well as increased ECV have been consistently demonstrated in patients with aortic stenosis, correlating with myocardial disarray and interstitial fibrosis on histology.⁶¹ There is emerging evidence that increased native T1 times are associated with adverse outcomes in aortic stenosis⁶² and potentially predict post-TAVR conduction defects.⁶³ Furthermore, post AVR, replacement fibrosis does not resolve, but diffuse fibrosis and myocardial hypertrophy can regress.⁶⁴ These changes are accompanied by structural and functional improvements suggesting that diffuse fibrosis, measurable by CMR may represent a potential therapeutic target. Although developments in T1 mapping represent an important step forward in the noninvasive assessment of diffuse fibrosis, at the present time, there is insufficient evidence to recommend its routine use in AS. Furthermore, although acquisition of pre- and post-contrast T1 mapping images can be performed in a timely fashion using the current rapid protocols, offline post-processing remains laborious.

Infiltrative pathology

Wild-type transthyretin cardiac amyloid and severe aortic stenosis often coexist.⁶⁵⁻⁶⁸ The prevalence of wild-type transthyretin cardiac amyloid is nearly 6%-16% of patients with severe aortic stenosis.^{65,66,69,70} The existence of transthyretin cardiac amyloid is an independent predictor mortality (Hazard ratio: 9.5, 95% confidence interval: 2.5 to 35.8) post-surgical aortic valve replacement.⁶⁶ The effect of cardiac amyloid on mortality with or without aortic valve replacement in this cohort is deleterious.^{67,71} Cardiac amyloid is associated with low-flow, low-gradient severe aortic stenosis with mildly reduced ejection fraction.⁶⁵ Cardiac magnetic resonance⁷²⁻⁷⁴ is an excellent imaging modality in detecting amyloidosis with robust sensitivity (85%-87%) and specificity (92%-96%) (Figure 5).⁶⁷ TAVR is the preferred management strategy in severe AS patients with cardiac amyloid.^{70,75}

Assessment of concurrent aortopathies

Bicuspid aortic valves are commonly associated with aortopathy⁷⁶ and pose a higher risk of aortic dissection (Figure 6).⁷⁷ CMR angiography provides accurate aortic dimensions, and can be obtained without contrast.¹⁰ Other congenital anomalies such as coarctation of the aorta can be detected and monitored. The flow dynamics and pathophysiology of aortopathies can be further assessed on 4D flow imaging, predominantly utilized in the research realm to this point. The flow patterns/shear stress can be assessed across the valve morphologies (trileaflet and bicuspid), and subtypes of bicuspid aortic valves.⁷⁸ Bicuspid aortic valves are commonly associated with aortic dilatation and concomitant valve regurgitation.^{79,80}

Hypertrophic cardiomyopathy

CMR has superior temporal resolution when compared with CCTA. This is optimal for functional assessment, like LV- and RV-function, volume, and mass calculation. In addition, CMR is better at tissue characterization and helps differentiate etiologies of LV hypertrophy in the context of severe AS (hypertrophic cardiomyopathy, cardiac amyloidosis). CMR has a well-established role in the assessment of other causes

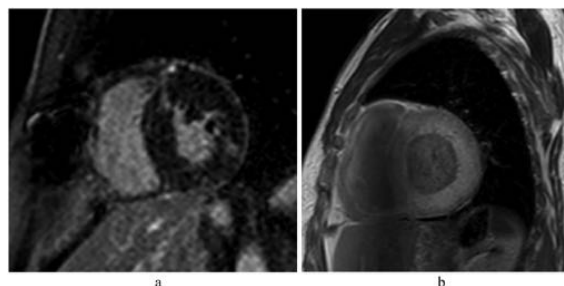


Figure 5. Phase-sensitive inversion-recovery late gadolinium short axis imaging showing A) focal non-infarct late gadolinium enhancement in the mid inferolateral wall typical of the replacement fibrosis seen in aortic stenosis. B) In another patient with significant aortic stenosis, there are abnormalities in myocardial nulling highly suggestive of cardiac amyloidosis (late gadolinium enhancement (LGE) imaging kinetics demonstrate suboptimal myocardial nulling).

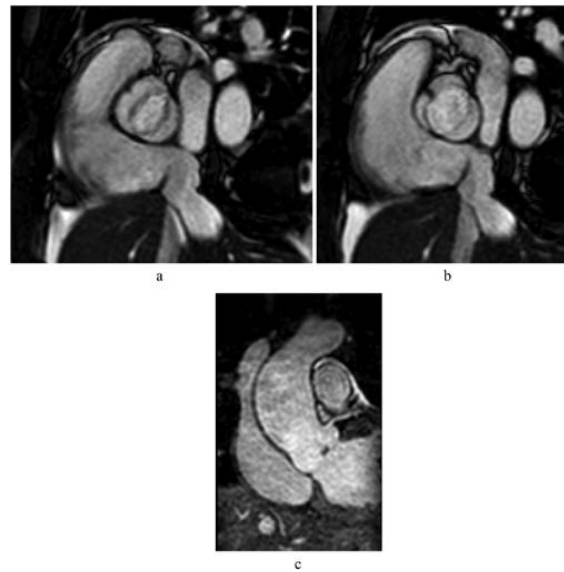


Figure 6. (A-C) Bicuspid aortic valve type 1 and concomitant aortopathy measuring 4.7 cm.

of aortic outflow obstruction such as obstructive hypertrophic cardiomyopathy.⁸¹ Masked LVOT physiology in addition to severe AS can lead to challenging hemodynamics post TAVR. The majority of cases should be identified readily using echocardiography, though this may be enhanced on pre-TAVR CMR. One important implication is in the setting of decreased afterload post TAVR, as preexisting LVOT obstructive physiology may worsen, or new dynamic intraventricular gradients may develop. Typical late gadolinium enhancement patterns (small punctuate, noncoronary in distribution, patchy mid-wall hyper-enhancement typically involving anterior and posterior RV insertion points/mid to basal anteroseptal segments of the interventricular septum) on CMR clinch the diagnosis of hypertrophic cardiomyopathy.^{82,83}

Vascular access and coronary anatomy

The evaluation of vascular access sites is crucial before TAVR. The role of CMR is limited in this arena as most patients undergoing TAVR are elderly. This cohort has substantial vascular calcification resulting in suboptimal visualization with CMR. Gadolinium contrast CMR can provide lumen dimensions; however, Gadolinium can result in nephrogenic systemic fibrosis in individuals with advanced kidney disease.⁸⁴ Recent consensus statements from the American College of Radiology and the National Kidney Foundation suggest that the risk of this occurring with Type II Gadolinium based contrast agents is low.⁸⁵ Novel imaging techniques like non-contrast 3-dimensional MR can be

utilized to assess aortoiliac system in individuals at high risk for contrast-induced nephropathy.^{86–88} Non contrast CT is an essential additional imaging modality along with MRI for identifying calcium-free windows for transaortic access. CT continues to be the imaging modality of choice to detect a porcelain aorta.⁸⁹ CMR coronary angiography has a low yield as severe coronary calcification is observed in patients with severe AS. CMR coronary angiography may be feasible and accurate, mainly in the assessment of proximal epicardial coronary arteries like the left main coronary artery.⁹⁰ CMR can help assess the height of coronary ostia from the aortic annulus.

Post TAVR assessment

Myocardial injury

Periprocedural myocardial damage post TAVR is a poor prognostic sign as well as an independent predictor of mortality.^{91,92} Multiple hypotheses have been proposed, including global myocardial ischemia secondary to hypotension, embolization of aortic valve debris, concomitant coronary atherosclerosis, obstruction of the coronary ostia and direct trauma during the procedure.^{91,93} Myocardial injury has also been linked to higher rate of permanent pacemaker implantation.⁹² Investigators at the Kerckhoff Heart Center⁹⁴ quantified the amount of myocardial injury using cardiac magnetic resonance imaging and biomarkers post TAVR. Sixty-one patients underwent cardiac magnetic resonance imaging before and after TAVR along with measurement of troponin T and creatine



kinase-myocardial band before and after TAVR (at 1, 2- and 3-days post TAVR). Nearly 20% of the cohort developed new onset ischemic pattern late gadolinium enhancement with a statistically significant reduction in left ventricular function compared to the cohort that did not develop a new onset ischemic pattern on CMR. The myocardial injury was presumed to be embolic. Further research in this area is essential to clarify the implications of this study.

Paravalvular aortic regurgitation

Paravalvular regurgitation post TAVR is linked to adverse LV remodeling,⁹⁵ and moderate to severe paravalvular aortic regurgitation post TAVR is a poor prognostic factor.⁹⁶ Echocardiography is most commonly used to assess paravalvular regurgitation though accurate assessment may be difficult. Acoustic shadow/drop out caused by the TAVR prosthesis, native aortic valve calcification, and multiple eccentric jets can make echocardiographic assessment of paravalvular regurgitation challenging.^{97,98} (Video 3) A study performed at the Emory University⁹⁸ reported a reclassification of paravalvular regurgitation severity based on CMR in more than half of the symptomatic paravalvular regurgitation cohort and nearly a third were graded by phase-contrast cardiac magnetic resonance imaging as being higher than conventional echocardiography. This study⁹⁸ concluded that CMR had greater prognostic value compared to semi-quantitative echocardiography. More than mild paravalvular regurgitation based on CMR (regurgitant fraction >20%) had a greater incidence of adverse events compared to grading by echocardiography. These results were replicated by a German center⁹⁹ with only a fair agreement regarding paravalvular regurgitation between echocardiography and cardiac magnetic resonance imaging (weighted $\kappa = 0.33$). Similarly, a Canadian study¹⁰⁰ reported that patients with aortic regurgitant fraction >30% post-TAVR (N = 135) based on cardiac magnetic resonance imaging had worse clinical outcomes and higher 2 year mortality (area under the curve: 0.678, sensitivity = 39%, specificity = 70%; $p = 0.001$) compared to those without. A novel angiographic based videodensitometric aortic regurgitation index has been used to assess paravalvular regurgitation.¹⁰¹ As with native valvular regurgitation, there is also a need to standardize grading of paravalvular regurgitation severity using CMR.

Reverse remodeling

Reverse remodeling is defined as reduction in left ventricular mass, end systolic and end diastolic volumes, along with improvement in cardiac function.¹⁰² The eradication of pressure overload post TAVR leads to cardiac function enhancement, followed by delayed reverse remodeling.¹⁰³ Reverse remodeling is an independent predictor of outcomes post TAVR, and patients without LV reverse remodeling were observed to have a higher one year mortality post TAVR¹⁰³ compared to patients who had LV reverse remodeling. Early regression has also been linked to lower hospitalization rate.¹⁰⁴ It has been observed post TAVR that women compared to men experience better and earlier reverse remodeling, as well as more LV mass regression on echocardiography.¹⁰⁵ CMR has been utilized to evaluate reverse remodeling post TAVR.^{106,107} TAVR and surgical aortic valve replacement

(SAVR) have shown similar impact in LV reverse remodeling.¹⁰⁶ Interestingly, TAVR was associated with more robust right ventricular reverse remodeling compared to SAVR. LV reverse remodeling typically occurs 6 months post TAVR.¹⁰⁷ Larger pre TAVR ventricular volumes¹⁰⁶ and post TAVR paravalvular regurgitation⁹⁵ were associated with poor LV reverse remodeling. A recent study was published from the Leeds Cardiovascular Institute of 85 post TAVR patients that underwent CMR to assess the influence of mitral regurgitation (MR) severity on cardiac reverse remodeling and patient outcomes.¹⁰⁸ They found that concomitant significant MR at baseline, even though frequent did not prevent LV reverse remodeling and that the degree of MR commonly improved post-TAVR.¹⁰⁸ Myocardial T2 relaxation/T2 mapping and left-ventricular strain using CMR before and after TAVR has been investigated on a small scale so far to assess reverse remodeling¹⁰⁹ and predicts positive remodeling.^{110,111} A meta-analysis of ten studies confirmed reduction in LV end-diastolic volume index, LV end-systolic volume index, LV mass index, and improvement in left ventricular ejection fraction post TAVR.¹¹² A study of 51 patients showed that the lotus valve and CoreValve had similar valve hemodynamics and LV reverse remodeling 6 months post TAVR.¹¹³ CMR could potentially provide insights in comparison of different TAVR devices and their impact on reverse remodeling, and valve hemodynamics. Future research is needed to evaluate the role of CMR in assessing long-term prognostic implications and its role in improving patient selection for valvular intervention.

Relative limitations to CMR

CCTA has a higher spatial resolution and isotropic voxel size in all axes compared with CMR. This makes CCTA better for multiplanar reconstruction of the aortic annulus, providing superior image quality with better clinical reliability. CMR requires skilled technologists and physicians for scan acquisition and interpretation, which are less widely available in comparison to CCTA. Additionally, imaging with CMR takes longer compared to CCTA, though is comparable to echocardiography. CMR may be challenging in setting of patients with irregular heart rhythms, and in patients unable to comply with repeated breath hold instructions. Where CMR is not contraindicated, patients with implanted cardiac devices require special considerations, due to possible imaging artifacts. Significant pleural effusions may also compromise image quality in CMR.

Recommendations

CMR is a useful modality in patient assessment prior to TAVR, particularly in those patients with contraindications to CCTA especially in patients with advanced renal dysfunction. CMR provides robust imaging of the aortic annulus, and can provide supplemental information on leaflet opening. Further, the entirety of the aorta can be accurately measured in the setting of suspected aortopathy. Post TAVR CMR can be considered in the diagnosis and

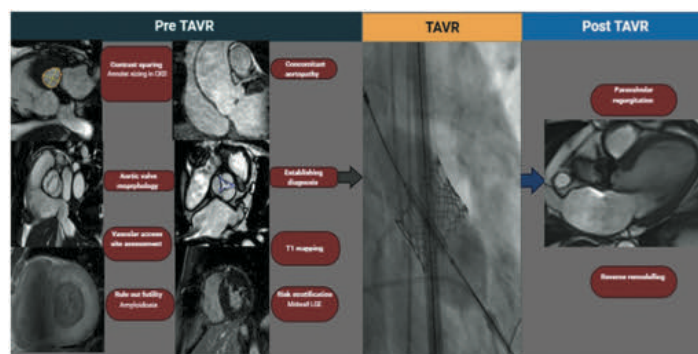


Figure 7. The role of CMR in severe aortic stenosis.

surveillance of paravalvular regurgitation. Additionally, CMR provides gold standard assessment of LV volumes and mass for pre and post procedural assessment. Aortic annulus, sinus of Valsalva, sinotubular junction, ascending aorta, LV size, and flow measurements can be performed without intravenous contrast. The patient does not incur the burden of ionizing radiation. Use of intravenous contrast allows for scar, as well as diffuse fibrosis assessment, an emerging area of study for risk stratification, and identification of patients with diffuse infiltrative processes such as amyloidosis. There are relative limitations to the use of CMR in some instances, and familiarity with these will help direct referral of appropriate patients to this modality. In addition to CMR, a noncontrast CT is an important adjunct to assess calcification patterns which are very important for device selection. (Video 4) **Figure 7** is the central illustration demonstrating the role of CMR in severe aortic stenosis.

Conclusions

CMR is an emerging imaging modality with proven utility in the setting of TAVR. Reliable measurements can be obtained for the aortic annulus without the use of contrast, a point of interest in patients with relative contraindications to CCTA. The versatility of CMR allows for concomitant evaluation of dimensions of the entire aorta, and direct quantitation aortic insufficiency, as well as LV size, mass and function, both prior to and following valve intervention, with implications for patient outcomes. Further research will help inform the utility of LV scar assessment, commonly obtained with use of intravenous contrast, both in pre procedural risk assessment, and identification of entities such as amyloidosis.

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