

# Serum Biomarkers in Valvular Heart Disease

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## LEARNING OBJECTIVES

1. Understand the pathophysiological pathways involved in deteriorating valve disease, which may provide useful targets for biomarker development
2. Understand the currently available serum biomarkers in valve disease, and their utility in clinical practice.
3. The potential for future biomarkers, based on the known metabolic and biological pathways

## INTRODUCTION

Valvular heart disease (VHD) is an important cause of morbidity and mortality whose prevalence is set to increase dramatically as a consequence of the increase in life expectancy in middle- and high-income nations. Community echocardiographic screening has identified a major burden of undiagnosed VHD in older people, with some degree of valve degeneration in almost 50% and undiagnosed clinically significant (moderate-severe) disease affecting 1 in 10 [1]. Population statistics project an increase in the elderly population, such that there will be an estimated 4 million people aged between 75 and 84 in the UK by 2018, while the population >85 years is set to double by 2028.[2] Meanwhile, rheumatic heart disease accounts for a much higher proportion of valve pathology in developing nations and contributes to the growing worldwide prevalence.[3, 4]

Despite wider appreciation of the emerging importance of VHD, the mechanisms underlying its development (particularly in degenerative pathology) are poorly understood, and the role of serum biomarkers in guiding clinical management in individual patients is relatively unexplored. European guidelines [5] for the diagnosis and management of VHD make reference to the use of serum B-type natriuretic peptide (BNP) as a marker of prognosis in aortic stenosis (AS) and mitral regurgitation (MR), but thresholds are poorly defined and there are no definitive recommendation on its use.

As the prevalence of VHD increases, it will be increasingly important to develop our understanding of the factors that determine the rate of progression (allowing the planning of careful and efficient follow up and timely intervention). A large proportion of patients with VHD remain asymptomatic and most have mild disease. It is therefore fundamentally important to identify these patients at an earlier stage prior to the development of complications and risk stratify them according to the likelihood of developing significant disease that requires intervention. In this review, we outline current knowledge concerning existing and emerging serum biomarkers that have an established or potential role in the assessment and management of patients with VHD. Imaging (echocardiography, CT and cardiac magnetic resonance [CMR]) and physiological (e.g. cardiopulmonary exercise testing) are also vital in this clinical endeavour but are beyond the scope of the article.

## THE ROLE OF SERUM BIOMARKERS IN THE MANAGEMENT OF VALVE DISEASE

The potential roles of circulating biomarkers are:

1. Identification of baseline disease activity and prediction of progression - allows the identification of higher risk patients early, and potentially a subset of patients who might benefit from early surgery (even in the absence of symptoms).
2. Identification of those patients who are less likely to benefit from valve repair/replacement – some have severely impaired left ventricular function that is unlikely to recover after surgery.
3. Understanding the aetiology of VHD - to facilitate the development of future treatment strategies (e.g. tailored medical therapy).

Most existing biomarkers are of myocardial origin (reflecting their established clinical and research pedigree in heart failure) and are likely to provide prognostic utility since morbidity and mortality are largely related to myocardial dysfunction. Conversely, biomarkers of valve leaflet pathology are lacking as a consequence of the relatively small mass of tissue, the rudimentary blood supply, and current poor understanding of the biological stimuli for the development of degenerative VHD.

Myocardial biomarkers may provide significant advantages over existing imaging techniques. For example, whilst CMR is able to identify discrete and diffuse myocardial fibrosis, current techniques are relatively insensitive and only able to identify diffuse fibrosis once it is significantly advanced. Moreover, identification of myocardial wall stress prior to the development of fibrosis would arguably be of greater clinical value.

Biomarkers capable of predicting VHD progression would also be of major clinical value, even if only to determine the frequency of follow-up. For example, the faster rate of progression of calcific aortic stenosis (AS) in subjects with renal disease might be monitored via measurement of factors reflecting the systemic process rather than disease activity affecting the valve itself.

We have focussed on the circulating biomarkers for which reasonable data exists. Several other biomarkers have been examined in an effort to understand the underlying biology of VHD (e.g. serum fetuin-A) [6] but these are generally small cohorts with limited or no clinical follow-up and are not discussed further here.

### PATHOPHYSIOLOGY

Since existing serum biomarkers associated with VHD are mostly related to secondary effects on the ventricular myocardium, a brief overview of the myocardial effects of VHD is helpful. Figure 1 (adapted from reference [7]) also highlights the myocardial physiological processes that may be suitable targets for biomarkers.

#### A) PRESSURE OVERLOAD IN AORTIC STENOSIS

Left ventricular systolic pressure is elevated in AS to overcome outflow obstruction with resulting myocyte hypertrophy (to reduce wall stress) and eventual interstitial fibrosis.[8] However, the overall pattern of this adaptive response is highly heterogeneous and may include concentric remodelling and concentric or eccentric hypertrophy.[8] Patterns of left ventricular adaptation and

degree of hypertrophy correlate poorly with the severity of valve narrowing and asymmetric patterns of wall thickening are common.[9] Left ventricular hypertrophy also leads to reduced density of coronary arteriolar vessels and increased transmural pressures, resulting in increased coronary vascular resistance and reduced coronary flow reserve.[10, 11] The reduction of coronary flow reserve limits the ability of the coronary circulation to increase flow to match myocardial oxygen demand, especially during exercise, and is a key factor in the development of myocardial ischaemia and the occurrence of symptoms.[12] Repetitive myocardial ischaemia related to the exhaustion of coronary flow reserve can lead to apoptosis of myocytes and to the development of 'replacement' myocardial fibrosis. This type of fibrosis occurs predominantly in the subendocardial and mid-wall layers of the left ventricle wall and is generally not reversible following relief of left ventricular pressure overload by AVR.[12] Diastolic dysfunction occurs early in the disease course and worsens with progression of stenosis severity and myocardial fibrosis. In more advanced stages, raised left ventricular filling pressures lead to secondary pulmonary hypertension.[13, 14] LV systolic function, measured by ejection fraction (LVEF) and cardiac output, are generally well preserved even in the presence of severe AS, likely due to the increase in LV wall thickness which normalises wall stress whereas the reduced LVEF or cardiac output occurs only in late-stage disease and is usually preceded by clinical symptoms [8]. Subtle LV systolic dysfunction may occur prior to a reduction in ejection fraction however - the occurrence of myocardial fibrosis initially in the subendocardium, in which the myocardial fibres are oriented longitudinally, may result in a reduction in longitudinal function which is compensated for by relatively well preserved radial and circumferential function.[15, 16, 17] Measures of long axis function (e.g. systolic mitral annular velocities on tissue Doppler echocardiography) may be an indicator of subclinical left ventricular systolic dysfunction despite preserved LVEF and the absence of symptoms, and can be related to adverse outcomes. [18]

The factors that determine the 'tipping' point at which myocardial compensation becomes dysfunction are not entirely clear, and are likely to be variable between individuals. Left ventricular mass does not seem to correlate with the severity of valve disease [9], but has shown some association with future events [19, 20], albeit with significant overlap between LV mass groups. The processes of myocyte stress and ultimately degeneration to necrosis/cell death and replacement fibrosis, seem to be better indicators of decompensation, and are associated with reduced LV function on histological assessment of cell degeneration and biochemical/DNA markers [21]. Patchy fibrosis is also a predictor of mortality.[22] These would therefore seem to be suitable targets for biomarker development.

## **B) VOLUME OVERLOAD IN AORTIC AND MITRAL REGURGITATION**

The left ventricle is volume overloaded in aortic regurgitation (AR) as a result of combined elevation of preload and afterload. The excess preload reflects the volume overload that is in turn directly related to the severity of AR. Afterload is increased since the increased stroke volume is ejected into the high-impedance aorta and systolic hypertension is a frequent consequence. In addition, elevated end-diastolic volume increases LV wall stress. Combined elevation of preload and afterload excess ultimately leads to progressive left ventricular dilatation with resulting systolic dysfunction.[23, 24, 25, 26, 27]

The left ventricle is also volume overloaded in mitral regurgitation (MR) though afterload is normal. The adaptive changes of the ventricle include dilatation with normal wall thickness and proportional ('eccentric') hypertrophy [23, 24, 25, 26, 27] while the left atrium also enlarges to accommodate the

regurgitant volume.[28] Left ventricular ejection fraction may be supra-normal in chronic MR as a result of increased preload and the afterload-reducing effects of ventricular ejection into the low-impedance left atrium. A low normal ejection fraction can therefore be misleading as a measure of contractile function.[29] Severe MR also leads to pulmonary hypertension (associated with worse outcomes) [30] right ventricular pressure overload and ultimate right ventricular failure.[30] The precise mechanism underlying myocardial decompensation in aortic and mitral regurgitation remains unclear, however it is likely to be related to chronic volume overload leading to eccentric hypertrophy and progressive LV dilatation leading to LV impairment [31].

### **C) RIGHT VENTRICULAR EFFECTS OF MITRAL STENOSIS AND TRICUSPID REGURGITATION**

Left atrial pressure is increased and compliance is reduced in mitral stenosis (MS) leading to transmission of the trans-mitral pressure gradient to the pulmonary circulation and eventual pulmonary hypertension.[32] Severe MS is associated with pulmonary arteriolar vasoconstriction, intimal hyperplasia and medial hypertrophy and median survival less than 3 years once severe pulmonary hypertension is established.[23, 33]

Tricuspid regurgitation (TR) is usually a secondary phenomenon as a consequence of left sided heart disease or intrinsic pulmonary pathology, rather than resulting from a primary tricuspid valve lesion. Secondary TR is mainly caused by dilation of the tricuspid annulus and/or tethering of the valve leaflets secondary to right ventricular dysfunction. The right ventricle is very tolerant of increasing volume overload well and TR can remain clinically silent for a prolonged period before eventual progressive right ventricular dilatation and dysfunction.[34]

## **BIOMARKERS ASSOCIATED WITH MYOCARDIAL STRESS**

### **NATRIURETIC PEPTIDES**

Natriuretic peptides are the most widely used markers of myocardial strain and are primarily synthesized in the heart and regulated by myocardial stress in response to volume or pressure overload [35]. They include B-type natriuretic peptide (BNP) and the N-terminal fragment of its prohormone (NT-proBNP), atrial natriuretic peptide (ANP), adrenomedullin and the mid-regional fragment of the prohormone (MR-proANP). These prohormones are released under conditions of hemodynamic stress and processed into biologically active natriuretic peptides which induce vasodilation, natriuresis and diuresis.[36]

Various studies have demonstrated that BNP is a marker of AS severity and a predictor of poor prognosis in both symptomatic and asymptomatic patients [37, 38, 39, 40, 41, 42] (Table-1). The underlying mechanism triggering BNP activation and release seem to be chronic pressure overload leading to left ventricular hypertrophy, increased wall stress, fibrosis, diastolic dysfunction and raised filling pressures [43]. However, increasing wall thickness/hypertrophy normalises wall stress and may limit the rise in BNP until late in the disease process. In addition, BNP is related to age and there is considerable overlap between different groups of patients with AS, which further limit its utility in clinical practice.

Aortic regurgitation usually progresses slowly with increasing volume overload and left ventricular adaptation by means of dilatation and hypertrophy. Elevation of BNP levels has been associated with severe AR and left ventricular dysfunction on exercise echocardiography, as reflected by a higher

end-systolic volume index and lower longitudinal strain rate [44], but again this is a late stage of the disease. Various well designed studies have shown that NT-proBNP levels correlate closely with the severity of AR and the patient's functional status [44, 45, 46, 47] (Table-1), but with significant overlap between groups limiting individual utility.

In mitral regurgitation, plasma natriuretic peptide levels increase with increasing severity in patients with both asymptomatic and symptomatic mitral regurgitation, independent of left ventricular systolic function [48, 49] and are also associated with higher mortality and the combined end point of death or heart failure in both chronic organic (primary) as well as degenerative MR [50], [51], [52] BNP levels relate to the both the severity as well as being a marker of poor prognosis in patients with MS. [53, 54]

Data on BNP assays in TR remain limited. In a small study of patients undergoing surgery for severe isolated TR, BNP levels correlated directly with right ventricular volume (and inversely with left ventricular ejection fraction) and were helpful in predicting one year mortality.[55]

In summary, BNP is a potential marker of disease severity and overall prognosis within groups, in patients with AS, AR and MR. However, thresholds of abnormality (which might trigger clinical concern or the need for intervention) are poorly defined and there is significant overlap between groups in the studies to date. Moreover, BNP levels may be elevated in various other conditions, including renal failure, chronic obstructive airways disease, obesity, atrial fibrillation, liver cirrhosis, myocardial infarction, and vary according to age, exercise and fluid status.[56] These factors likely limit its potential for the assessment of individual patients and serial measurement following the definition of baseline levels remains inappropriate until further data emerge.

## **GROWTH DIFFERENTIATION FACTOR (GDF)-15**

The cytokine GDF-15 (also known as MIC-1) is a member of the transforming growth factor (TGF)-*b* superfamily [57] and produced at low levels via autocrine or paracrine pathways under baseline conditions by most tissues.[57, 58] It provides endogenous protection against ischemia and reperfusion-induced cardiomyocyte apoptosis and is strongly induced via PI3K–Akt-dependent signalling pathways following ischaemic myocardial injury.[59] GDF-15 has been associated with heart failure with preserved ejection fraction and with the degree of myocardial fibrosis in patients with end-stage non-ischaemic dilated cardiomyopathy.[60, 61, 62] Various studies have confirmed its role as a useful predictor of both cardiovascular and all-cause mortality.[63, 64, 65, 66]

## **BIOMARKERS ASSOCIATED WITH MYOCARDIAL HYPERTROPHY AND FIBROSIS**

### **ST2**

ST2 is an inflammatory cytokine member of the interleukin (IL-1) receptor family that predicts mortality and heart failure in patients with acute myocardial infarction and may play a vital role in cardiac pathophysiology. ST2 is thought to be involved in modifying immunologic processes, specifically mediated by T-helper lymphocytes. Interleukin-33, a hormone which may protect against left ventricular hypertrophy and myocardial fibrosis has recently been identified as the ligand for ST2.[36]. In the Framingham Study, ST2 was associated with death, heart failure and major cardiovascular events, but not with echocardiographic indices of myocardial dysfunction. Furthermore, patients with elevated levels of both ST2 and BNP were found to be at considerably

higher risk of death than those with elevation of one or neither marker.[57] In the emergency setting, the PRIDE study has also found ST2 levels to be higher in patients presenting with acute heart failure [67].

As indicated above, ST2 is one of several markers (alongside GDF-15 & NTproBNP) which are elevated in patients with severe calcific aortic stenosis and which predict higher mortality after valve replacement.[68] Furthermore, in a recent study of 86 patients with moderate to severe AS (aortic valve area <1.5cm<sup>2</sup>) and preserved left ventricular ejection fraction (>50%), ST2 was related to the severity of valve stenosis the extent of diastolic dysfunction and the ability to distinguish between symptomatic and asymptomatic patients.[69] (Table-1)

ST2 is a promising serum biomarker which correlates well with the severity of AS and poor clinical outcomes. Large prospective studies are now required to validate its utility in patients with significant mitral valve disease.

### GALECTIN 3

Galectin-3 (Gal-3) is a 26-kDa, 3-galactoside-binding lectin [57] found in a wide variety of cells and tissue surfaces which is thought to represent a link between inflammation and fibrosis. Galectin-3 is secreted by activated macrophages, especially at sites of fibrosis and fibroblast deposition, [58] and may therefore play a role in cardiac pathophysiology and act as a surrogate indicator of cardiac remodelling and fibrosis. Expression appears to occur before evidence of heart failure is established and it may therefore be a particularly useful marker in strategies to predict and prevent advanced disease [36].

Serum galectin-3 levels are increased in myocardial biopsies from AS patients with reduced ejection fraction as well as being associated with adverse outcomes after TAVI [70, 71]. Raised galectin-3 levels are also detected in the serum of patients with degenerative mitral valve disease.[72] In contrast, galectin-3 was not associated with AS severity or functional status in a large, single centre, population cohort study (COFRASA-GENERAC) of 583 patients with AS over a wide range of severity.[73] However, one of the limitations of this study was that a large number of participants had only mild or moderate AS.

Interestingly, *in vitro* and preclinical *in vivo* studies suggest that Gal-3 may have a role in the disease process at the valve level in AS,[74] but further studies are required to examine this further.

### MICRORNA

MicroRNAs (miRNA) are short (approximately 21 nucleotide) non-protein coding RNAs which are responsible for altering the expression of approximately 30% of the human genome.[75, 76] miRNAs are highly stable in the circulation, either packaged in extracellular vesicles or micro-particles, or bound to molecules such as Argonaute-2. molecules are released into plasma following the liberation of cell contents secondary to necrosis where they remain highly stable [76] and therefore provide an excellent serum marker. Some have tissue specific expression and play a significant role in cellular growth, proliferation and apoptosis. Significant reduction in myocardial hypertrophy was observed following alteration in the expression of miR-21 by gene knockdown, or antisense-mediated depletion [77] suggesting that miR-21 is involved in the process of myocardial hypertrophy and may be a possible therapeutic target[76] (Figure-1) . miR-122 levels were found to be down regulated whereas miR-29c, miR-125b & miR-21 levels were found to be upregulated in studies



involving patients with AS [78, 79, 80, 81]. In a prospective study of 74 patients with severe AS undergoing AVR surgery preoperative plasma levels of miR-133a were found to predict the regression potential of LV hypertrophy after AVR[82]. However, these small studies rarely take major confounding factors into account, and another study found limited reproducibility once stratified by history of coronary artery disease [83].

## BIOMARKERS ASSOCIATED WITH MYOCARDIAL CELL DAMAGE/NECROSIS

### TROPONIN

Cardiac troponins would seem a good choice of biomarker to explore, given their release into the circulation only after myocardial cell death – one of the potential mechanisms of decompensation identified earlier. However, minor elevation of cardiac troponin I is relatively common in patients with AS (even those with normal coronary arteries) and is conceivably related to higher left ventricular wall thickness and pulmonary artery systolic pressure.[84] Elevated levels of high sensitivity cardiac troponin I & T (hs Tnl/TnT) have been associated with advanced left ventricular hypertrophy, ECG strain, replacement myocardial fibrosis, and the need for aortic valve replacement as well as predicting survival after transcatheter aortic valve implantation, poor prognosis in patients with AS, and the occurrence of post-operative fatal arrhythmia and cardiac death [85, 86, 87, 88],[89], although the latter three groups involve patients who have already developed symptoms and therefore would not support the use of troponin as an earlier marker of decompensation. Troponins therefore appear to be promising biomarkers to evaluate prognosis in VHD although the overlap between groups is significant, and combining these markers with other factors may help [90]. Larger prospective studies are needed for further validation before incorporation into clinical practice.

### METABOLOMICS

Metabolomics is the systematic study of small molecules in biological fluids [91, 92, 93]. In general, these are assessed in a broad-based fashion, incorporating numerous potential metabolic molecules, and employing advanced statistical methods to identify the signature metabolic profiles in particular cohorts [94]. In this manner, metabolomics has the potential to elucidate poorly understood pathophysiological pathways, identify potential therapeutic targets, and facilitate risk-stratification in individual patients [94]. The metabolic requirements of the myocardium make it feasible that circulating metabolomic profiling could allow quantification of degree of pathological volume or pressure overload in valvular disease, but current knowledge is limited by small sample numbers [95].

### GENOMIC BIOMARKERS

Data from a genome-wide association study including patients from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium identified a single-nucleotide polymorphism in the lipoprotein(a) locus which significantly correlated with aortic valve calcification [96]. These findings were subsequently confirmed for clinical aortic stenosis [97]. Another multi-centre study involving 220 patients with mild-to-moderate AS showed that elevated oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) and Lp(a) levels were independently associated with an increased risk of echocardiographically determined AS progression rate, translating to a higher need for AVR accentuated among younger patients.[98] Previous randomised controlled



trials and a recently published meta-analysis (including 5 randomised control trials and 9 observational studies) have shown no benefit of statins in aortic stenosis [99], but these genomic and serum studies suggest that trials of therapies likely to increase Lp(a) levels might be more successful.

## COMBINING MULTIPLE BIOMARKERS

It is likely, given the multiple processes involved in myocardial degeneration, that a combination of biomarkers are likely to be more useful than any single target. There are few existing studies of multiple biomarkers in VHD, and this remains an area for research. One study in AS examined three biomarkers (GDF-15, ST2 and NTproBNP) in a prospective cohort of 345 intermediate or high risk surgical patients with severe AS undergoing surgical or percutaneous valve replacement. Individual elevated biomarker levels were associated with higher mortality, elevation of all three biomarkers demonstrated a stronger predictive ability, with a 10-fold higher 1- and 2-year mortality than those with normal levels. [68] A multimarker approach representing diverse biological pathways thus seems likely to be more beneficial.

## CONCLUSION

Established biomarkers such as BNP and high sensitivity troponin can play a partial role in predicting the progression of VHD in patient groups. However, overlap between groups with differing severity and prognosis is significant and further research will be necessary to determine if meaningful clinical thresholds exist for individual patients. Emerging biomarkers, in particular mi-RNA, GDF-15, ST2, Galectin-3 and DNA profiling, hold promise for risk stratification and tailored disease management. Ultimately, it is likely that a combination of biomarkers will be more useful than a single factor, and the development of risk scores combining the serum biomarkers and other parameters, such as cardiac imaging may prove most beneficial in improving risk stratification in VHD.

## KEY POINTS

1. The likely processes involved in the transition from compensated to decompensated valve disease include cardiomyocyte stress, apoptosis/cell death and replacement fibrosis, which form suitable targets for biomarker development.
2. Natriuretic peptides are the most widely studied biomarkers in valve disease, but they are not specific to valve disease and there is considerable overlap in serum levels between different clinical groups.
3. Combinations of biomarkers are likely to prove more beneficial than any single marker, and have been successfully utilised in other disease areas, and to a limited extent in valve disease
4. Aortic stenosis is the most widely researched individual valve lesion, and useful biomarkers for predicting prognosis include a combination of ST2, GDF-15 & NTproBNP, in addition to high-sensitivity troponins.
5. Future biomarkers will likely include micro-RNAs and metabolomic markers, as more specific markers of valve decompensation

## COMPETING INTERESTS

The authors have no competing interests

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**Table-1**

A summary of the key studies showing the role of serum biomarkers in valvular heart disease

<b>Biomarker</b>	<b>VHD</b>	<b>First author (Ref. #)</b>	<b>N</b>	<b>Year</b>	<b>Restricted to severe disease?</b>	<b>Study details</b>
Natriuretic peptides	<b>Pros:</b>	a) Readily available b) Large no. of studies c) Marker of severity and prognosis in AS,AR,MR			<b>Cons:</b>	a) Poorly defined thresholds b) Significant overlap between groups c) Lacks specificity for VHD - can be elevated in other unrelated medical conditions
	AS	Gerber et al.[37]	74	2003	No	NT-proBNP and BNP both associated with symptoms (AUC: 0.84 and 0.83, respectively)
	AS	Bergler-Klein et al.[42]	130	2004	Yes	NT-proBNP and BNP both predicted the presence of symptoms as well as the risk of symptom onset or death among subjects with severe AS
	AS	Nessmith et al.[41]	124	2005	No	BNP predicted presence of symptoms (AUC: 0.87) Optimal cut off was 190 pg/ml
	AS	Monin et al.[40]	107	2009	No	BNP independently predicted outcomes (cardiac death, hospitalization for CHF, or AVR)
	AS	Clavel et al.[39]	1953	2014	Yes	BNP independently predicted long term mortality even among asymptomatic subjects with at least moderate aortic stenosis
	AS	Henri et al.[38]	69	2016	No	Annual change in BNP levels predicted outcomes (symptoms, AVR, or death)
	AR	Gerber et al.[47]	40	2003	Yes	Natriuretic peptide (ANP, BNP & N-BNP) levels correspond with symptomatic status among patients with moderate to severe AR and preserved LV systolic function
	AR	Gabriel et al.[44]	39	2007	Yes	Increased BNP level associated with increased severity of regurgitation & early LV dysfunction on exercise echo among asymptomatic or mildly symptomatic patients with moderate to severe AR with preserved LV systolic function
	AR	Weber et al.[46]	60	2008	No	NT-proBNP linked to disease severity and functional status. It also predicted prognosis & clinical outcomes in patients with chronic aortic regurgitation & preserved LV systolic function
	AR	Pizarro et al.[45]	294	2011	Yes	BNP independently predicted prognosis among asymptomatic patients with severe AR and preserved LV systolic function
	MR	Sutton et al.[48]	49	2003	No	Natriuretic peptide (BNP, ANP, N-BNP) levels correspond with increasing severity of MR and symptomatic status (AUC 0.9, 0.89, 0.89 respectively) even among patients with preserved LV function

	MR	Detaint et al.[50]	124	2005	No	BNP was found to be an independent predictor of adverse events including death and heart failure
	MR	Pizarro et al.[49]	269	2009	Yes	BNP was an independent predictor of adverse events including heart failure, LV dysfunction and death among patients with severe asymptomatic MR
	MR	Clavel et al.[52]	1331	2016	No	BNP-ratio(ratio to upper limit of normal for age, sex, and assay) was found to be an independent, and incremental predictor of long-term mortality in patients with degenerative MR on medical therapy
	MS	Sharma et al.[54]	44	2011	Yes	Raise serum BNP levels were associated with left atrial dilatation, raised pulmonary artery pressure, reduced mitral valve area and treadmill exercise capacity as well as being a marker of adverse events among patients with moderate to severe MS
	TR	Yoon et al.[55]	39	2010	Yes	An elevated BNP level was determined by the LVEF and RVESVI, a baseline BNP level of >200pg/ml predicted poor outcomes and increased mortality and morbidity among patients with isolated, severe TR undergoing corrective surgery
<b>GDF-15, ST2</b>	<b>pros</b>	a) markers of prognosis in AS undergoing AVR b) ST2 reflects symptom status, severity, diastolic function as well as being an independent predictor of cardiovascular events in AS			<b>cons</b>	a) Limited available data for GDF-15 b) Available data only in AS
	AS	Lancellotti et al.[69]	86	2015	Yes	ST2 levels were related to symptomatic status, AS severity & diastolic dysfunction; furthermore ST2 was identified as an independent predictor of cardiovascular events among patients with moderate to severe AS and preserved LV function
Combination GDF15, ST2, NT-proBNP	AS	Lindman et al.[68]	345	2015	Yes	Elevated baseline GDF-15, ST2 and NTproBNP levels were associated with a higher post-operative mortality among patients with severe AS undergoing surgical or percutaneous valve replacement. Patients with elevation of all three biomarkers had a 10-fold higher 1- and 2-year mortality than those with normal levels
<b>Galectin-3</b>	<b>pros</b>	a) Marker of prognosis in AS b) Potential biotarget to delay AV calcification			<b>cons</b>	a) Limited data b) Only AS assessed
	AS	Sadaba et al.[74]	77	2016	Yes	Galectin-3 overexpressed in aortic valve tissue & in vitro blockade of Galectin-3 among vascular interstitial cells extracted from AV obtained from patients with severe AS undergoing AVR surgery lead to a delay in valve calcification.

<b>Troponins</b>	<b>pros</b>				<b>cons</b>	
	a) readily available b) various large studies c) marker of prognosis in AS, MR				a) Not specific for VHD - can be elevated in other conditions b) Limited evidence of utility outside AS	
AS	Rosjo et al.[85]	57	2011	Yes	hs-cTnT levels were universally detectable, LV mass and systolic function were independent determinants of hs-cTnT levels & high hs-cTnT levels were associated with a poor prognosis among patients with moderate to severe AS	
AS	Frank et al.[86]	107	2013	Yes	hsTnT was found to be superior to NTproBNP in predicting survival & hsTnT alongside pre-procedural AR were identified as the independent predictors of survival among patients with severe AS undergoing TAVI	
AS	Chin et al.[87]	253	2014	No	Plasma cTnI concentrations detectable in 98% of subjects and were increased when compared with age- and sex-matched healthy volunteers. cTnI concentrations were found to be associated with advanced LV hypertrophy and replacement fibrosis as well as AVR or cardiovascular death among patients with AS	
AS	Shah et al.[88]	242	2014	No	ECG LVH with strain was identified as a marker of midwall myocardial fibrosis and it was also associated with increased myocardial injury, impaired left ventricular function and was found to be an independent predictor of cardiovascular death or AVR among patients with AS	
(Troponins & MR-proANP)	MR	Wohrle et al.[51]	34	2015	Yes	MR-proANP & hsTnT were strong predictors of cardiovascular death and rehospitalization for HF among patients with severe MR undergoing percutaneous repair for MR (MitraClip procedure)
<b>miRNA</b>	<b>pros</b>				<b>cons</b>	
	a) Several small studies b) can help in understanding pathophysiology of VHD c) potential biotarget in myocardial reverse remodelling				a) expensive, lengthy processing b) lack of standardised protocols c) reproducibility is affected by confounding factors d) higher technical expertise needed for sample processing	

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AS	Garcia et al.[82]	74	2013	Yes	Preoperative plasma levels of miR-133a were found to predict the regression potential of LV hypertrophy post AVR among patients with severe AS undergoing AVR AUC 0.89, p-value<0.001
AS	Villar et al.[81]	107	2013	Yes	Myocardial and circulating levels of miR-21 were higher and correlated directly with the transvalvular gradients among patients with severe AS when compared to controls
AS	Beaumont et al.[78]	38	2014	Yes	miR-122 was found to be down regulated in endomyocardial biopsies taken from 28 patients using necropsies of 10 subjects as controls ,possibly leading to myocardial fibrosis through TGF-β1 up-regulation among subjects with severe AS
AS	Derda et al.[79]	120	2015	Yes	miR-29a levels were found to be increased in patients with hypertrophic obstructive cardiomyopathy while miR-29c were found to be increased in patients with aortic stenosis (awaiting TAVI) in a study involving patients with aortic stenosis and hypertrophic cardiomyopathy

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Abbreviations: miR = microRNA, TAVI= transcatheter aortic valve intervention, GDF=Growth differentiation factor, TGF= transforming growth factor, TnI= Troponin-I, TnT= Troponin-T, hs=high sensitivity, MR-proANP=mid-regional pro atrial natriuretic peptide, NTproBNP=N-terminal Pro-BNP, N-BNP=N-terminal BNP