REVIEW

Mineralocorticoid receptor antagonists, heart failure and predictive biomarkers

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Abstract

The mineralocorticoid receptor is a steroid hormone receptor that is well known for its involvement in fluid and electrolyte homeostasis in epithelial cells present in the distal nephron. The inappropriate activation of this receptor is now known to be implicated in various pathophysiological mechanisms in heart failure. Mineralocorticoid receptor antagonists offer substantial clinical benefit in patients with heart failure with reduced ejection fraction; however, for patients with heart failure with preserved ejection fraction, the treatment benefit is less clear. Biomarkers that can predict response to mineralocorticoid receptor antagonist treatment do not currently exist. Potential biomarkers may be modulated either directly by the mineralocorticoid receptor or indirectly via downstream effects and be able to reflect treatment outcomes, particularly changes in key parameters of cardiac health and function. A biomarker or set of biomarkers that can reliably predict responsiveness to mineralocorticoid receptor antagonist treatment at an early stage may allow for the selection of patients who are most likely to benefit from treatment thereby avoiding any unnecessary side effects associated with the use of these medications.

Introduction

The purpose of this review is to explore whether the use of existing and novel biomarkers may have the potential to assist in the identification of patients with heart failure (HF) expected to respond to mineralocorticoid receptor antagonist (MRA) treatment.

HF is a complex, clinical syndrome caused by structural or functional cardiac disease, resulting in impaired ventricular filling and/or contraction. HF affects more than 60 million individuals worldwide (GBD 2017, 2018), with increasing prevalence as a result of global population ageing (Strait & Lakatta 2012), increasing rates of diabetes and obesity (Carbone et al. 2017) and improved survival following acute coronary events (Mensah et al. 2017). HF is often classified into two phenotypes, determined by left ventricular ejection fraction (LVEF); HF with reduced ejection fraction (HFrEF, LVEF < 40%) and HF with preserved ejection fraction (HFpEF, LVEF ≥ 50%) (Yancy et al. 2013). For patients with HFrEF, several proven effective pharmacological treatment options are available, such as beta-blockers (BBs), angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), angiotensin receptor neprilysin inhibitors (ARNIs) and MRAs (Yancy et al. 2013, 2017, Group et al. 2018). However for patients with HFpEF, targeted therapies have not yet been established (Berbenetz & Mrkobrada 2016), apart from recent evidence demonstrating the benefit of the
sodium–glucose cotransporter 2 inhibitor empagliflozin on cardiovascular morbidity and mortality in this group (Anker et al. 2021).

In some clinical situations, the diagnosis of HF can be challenging, particularly HFrEF where the presenting symptoms (breathlessness, fatigue and lower limb swelling) may be mistaken for alternative diagnoses or attributed to co-existent comorbidities. To tackle the diagnostic challenge of HF, algorithms have been developed to aid the diagnosis, thereby leading to improved patient outcomes (Yancy et al. 2017). These algorithms consist of conventional diagnostic modalities such as chest x-ray and echocardiography as well as biomarkers including B-type natriuretic peptide (BNP) and N-terminal fragment of proBNP (nt-proBNP) (Group et al. 2018). Biomarkers such as these and future biomarkers may enable a cost-effective and personalised approach to HF diagnosis. Similarly, biomarkers may help to guide the management of patients with HF.

**Mineralocorticoid receptor antagonists**

The mineralocorticoid receptor (MR) is activated by its steroid hormone ligands, primarily aldosterone but also cortisol in non-epithelial tissues. Activation of the receptor in the renal tubules leads to increased expression of the epithelial sodium channel and the Na+/K+ pump, with the net effect of increasing Na+ resorption and increasing K+ secretion into the urine. Many studies have investigated the importance of structure–function relationships of the MR for receptor transactivation. Like other members of the nuclear receptor superfamily of transcription factors, the MR is modulated by interactions with coregulators proteins that show considerable diversity between cell types. Recruitment of coregulators proteins to the MR is dependent upon the ligand and target gene promoter, the net effect of which impacts endogenous agonist and antagonist ligand actions at the MR. Several activating and inactivating mutations have also been described for the MR that modulate receptor activity and are reviewed in detail elsewhere (Zennaro & Fernandes-Rosa 2017). The S810L mutation, for example, is a rare cause of early onset hypertension that is markedly exacerbated in pregnancy due to progesterone serving as a potent agonist at the mutated receptor (Geller et al. 2000). Other mutations, in the ligand-binding domain for example, often reduce or abolish ligand activation and thus could impact the response of an MRA. However, to date, there is little evidence that MRA in broader clinical use for HF displays tissue-selective effects, and consideration of these mechanisms in future studies may provide useful insights into structure–function relationships in vivo.

MRAs such as spironolactone and eplerenone block the MR and have been demonstrated in randomised clinical trials to provide substantial clinical benefit in the treatment of patients with HFrEF (Pitt et al. 1999, 2003, Zannad et al. 2011). There may be some benefit in patients with HfPfEF; however, the evidence for this is far less robust (Pitt et al. 2014, Pfeffer et al. 2015). Initially used as potassium-sparing diuretics to treat conditions such as primary aldosteronism and hypertension (Gomez-Sanchez 2016), MRAs now have an established benefit in the treatment of HF and as a result, are currently a guideline-indicated treatment for the management of HFrEF; American College of Cardiology/American Heart Association/Heart Failure Society of America (ACC/AHA/HFSA), European Society of Cardiology (ESC), and Cardiac Society of Australia and New Zealand (CSANZ) HF management guidelines (Yancy et al. 2017, McDonagh et al. 2021, NHFA CSANZ Heart Failure Guidelines Working Group et al. 2018). The Randomized Aldactone Evaluation Study (RALES) demonstrated the diuretic-independent benefit of spironolactone in a group of 1663 patients with severe HFrEF (Pitt et al. 1999). In comparison to standard therapy, the addition of spironolactone significantly reduced the risk of cardiac-related mortality by 31% as well as the risk of all-cause mortality and hospitalisation for cardiac causes (Pitt et al. 1999). Eplerenone, an MRA with greater selectivity for the MR, has also been shown to benefit patients with HF in the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) trial. In comparison to placebo, eplerenone resulted in a significant reduction in morbidity and mortality in patients with acute myocardial infarction (MI) complicated by left ventricular dysfunction and HFrEF (Pitt et al. 2003). The benefit of eplerenone treatment in patients with HFrEF was corroborated by the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) randomised trial, which also demonstrated a morbidity and mortality reduction in patients with HFrEF and mild symptoms (Zannad et al. 2011).

Despite the clear benefits of MRAs in HFrEF, clinical usage is sometimes limited due to side effects of this drug class. Both spironolactone and eplerenone block the MR located in the distal nephron which may result in clinically significant hyperkalaemia and/or deterioration of renal function (Pitt et al. 2008). These side effects quite frequently impact the ability to use an MRA drug, particularly in patients with existing renal impairment. Hyperkalaemia in randomised, placebo-controlled trials...
was observed in 9.3% of patients taking MRA treatment compared to 4.3% of patients taking placebo (Vukadinovic et al. 2017). Following the publication of the RALES study, the prescription rate of spironolactone increased and an increase in the rate of hospitalisation for hyperkalaemia was observed; from 2.4 per 1000 patients pre-RALES to 11.0 per 1000 patients post-RALES (Juurlink et al. 2004) thus showing that this side effect is more than just a theoretical concern. In addition, spironolactone exhibits agonistic and antagonistic effects at the progesterone and androgen receptors, respectively, thereby resulting in gynecomastia, mastodynia, menstrual cycle irregularities and impotence in some patients (Gomez-Sanchez 2016). These adverse effects are not observed with eplerenone due to its specificity for the MR (Pitt et al. 2003).

The adverse side effects of the first- and second-generation MRAs prompted the development of novel non-steroidal MRAs including esaxerenone (CS-3150) and finerenone (BAY 94-8862). Esaxerenone monotherapy at a dose of 5 mg/day was shown to offer superior anti-hypertensive effects compared to eplerenone 50 mg/day in Japanese patients with essential hypertension (Ito et al. 2020). Importantly, esaxerenone was well tolerated despite a slightly higher incidence of elevated serum potassium levels (Ito et al. 2020). In patients with HFrEF and mild chronic kidney disease (CKD), finerenone (5–10 mg/day) was equally effective as spironolactone (25 or 50 mg/day) in decreasing BNP and NT-proBNP but more importantly, correlated with significantly lower incidence of hyperkalaemia and preservation of renal function (Pitt et al. 2013). A subsequent study revealed that in patients with HFrEF and CKD and/or type 2 diabetes, both finerenone and eplerenone shared similar efficacy in achieving a >30% reduction in plasma NT-proBNP levels after 90 days with a similar overall risk profile (Filippatos et al. 2016). However, the group treated with finerenone 10 mg/day uptitrated to 20 mg/day after 30 days showed the greatest reduction in mortality and morbidity compared to the group receiving eplerenone (Filippatos et al. 2016). Despite demonstrating significant potential, further research is required to thoroughly assess the safety and efficacy of these new generation MRAs in different patient populations.

In addition to the aforementioned side effects of MRAs, a proportion of treated patients fail to respond to treatment. Although the exact proportion of patients that demonstrate lack of response has not been fully elucidated, in the RALES, 152 of 822 (18.5%) patients in the spironolactone treatment arm discontinued treatment due to either lack of response or for administrative reasons (Pitt et al. 1999). The proportion of patients who failed to respond to MRA treatment in other large clinical trials including the EPHESUS and EMPHASIS-HF trials have not been documented.

We propose that a biomarker which demonstrates and/or predicts patients’ response to MRA at an early stage of HF treatment may allow clinicians to make better-informed decisions regarding the use of MRA therapy, especially in patients with renal impairment or those who experience adverse effects (Fig. 1). MRA should only be continued if beneficial outcomes are expected. A biomarker of MRA responsiveness may also be useful for patients with HFrEF if it enabled the identification of a sub-category of HFrEF that may benefit from MRA treatment. This personalised approach to treatment has the potential to maximise benefit while reducing the potential for adverse side effects.

### Biomarkers

Biological markers or ‘biomarkers’ can be broadly defined as biological parameters that offer deeper clinical insight in a more convenient and potentially cost-effective manner than measuring traditional evaluation. Biomarkers may include simple biological measurements such as blood

![Figure 1](https://joe.bioscientifica.com)

**Figure 1**

Proposed schematic of biomarkers for predicting MRA treatment response. Inappropriate MR activation is implicated in many of the pathophysiological mechanisms that contribute to HF. Modulation of the proposed biomarkers, via these mechanisms or directly via the MR, may be able to inform clinicians about treatment outcomes at an early stage and thereby predict response to treatment. MR - mineralocorticoid receptor, MRA - mineralocorticoid receptor antagonist, sST2 - soluble suppression of tumorigenicity, HFrEF - heart failure with reduced ejection fraction, HfPEF - heart failure with preserved ejection fraction, LVEF - left ventricular ejection fraction, Echo - echocardiography, CMR - cardiac magnetic resonance imaging. A full colour version of this figure is available at https://doi.org/10.1530/JOE-21-0323.
pressure, measurement of proteins or other markers in the blood, genetic variations, or measurements derived from imaging. An ideal biomarker should be easy to measure, reproducible and add substantial predictive information above standard evaluation. The interest in biomarker discovery has skyrocketed over the last decade, with the current focus being on seeking novel and specific biomarkers which can enhance medical care.

**Diagnostic and prognostic biomarkers in heart failure**

**Natriuretic peptides**

Natriuretic peptides are released in response to atrial or ventricular stretch resulting in the secretion of atrial natriuretic peptide (ANP) or BNP, respectively. These peptide hormones counteract the effects of pathophysiological neurohormonal mechanisms of HF via vasodilation, natriuresis/diuresis as well as inhibition of cardiac fibrosis, hypertrophy, and of renin/aldosterone secretion (Fu et al. 2018). Elevated plasma BNP concentration is considered to be one of the early signs of HF and the measurement of this peptide hormone is currently regarded as the ‘gold standard’ of diagnostic and prognostic biomarkers in HF (Yancy et al. 2017).

Numerous studies have demonstrated the utility of both BNP and NT-proBNP, the biologically inactive counterpart of BNP, in improving HF diagnosis (Maisel et al. 2002, McCullough et al. 2002, Januzzi et al. 2005). The utility of BNP in improving HF diagnostic accuracy was demonstrated by the Breathing Not Properly (BNP) Multinational Study which revealed that out of the patients with acute dyspnoea presenting to the emergency department, BNP levels were elevated in those with clinically diagnosed HF compared to those without HF (Maisel et al. 2002, McCullough et al. 2002). Furthermore, increasing BNP levels correlated significantly with increasing HF severity (Maisel et al. 2002). BNP has also been shown to be a useful predictor of prognosis in patients with asymptomatic or mildly symptomatic left ventricular dysfunction and its utility in establishing prognosis/disease severity is supported by current heart failure guidelines (Yancy et al. 2017).

However, circulating natriuretic peptide levels can be influenced by various factors including other cardiac and non-cardiac conditions (Yancy et al. 2013) and by the use of routine HF medications (Troughton et al. 2007), which can diminish their accuracy and reliability as biomarkers. The use of natriuretic peptides alone as biomarkers is insufficient without other additional diagnostic methods in both routine clinical care and in clinical trials. While natriuretic peptides are routinely used in clinical practice, other potential biomarkers such as soluble suppression of tumorigenicity 2 (ST2) and galectin-3 (Gal-3) have shown promising prognostic utility in the experimental setting.

**Suppression of tumorigenicity 2**

Soluble ST2 (sST2), the soluble, circulating form of the ST2 protein, has recently been recognised as a potential biomarker in HF, even resulting in this biomarker earning a class Ib recommendation for consideration as part of the 2017 ACC/AHA/HFSA HF guidelines (Yancy et al. 2017) for improving prognostic stratification of patients with HF. Weinberg et al. first demonstrated potential ST2 expression in cardiac cells in response to myocardial stress (Weinberg et al. 2002), which led to further investigation into its potential role as a biomarker of cardiac damage. Activation of the transmembrane isoform of this receptor (ST2L) by interleukin (IL)-33 induces cardioprotective actions including downregulation of apoptosis and hypertrophic and pro-fibrotic pathways. However, these cardioprotective actions are lost when ST2 binds to IL-33 thereby preventing IL-33/ST2L ligand-receptor binding (Schmitz et al. 2005).

Several studies have explored the prognostic utility of sST2 in HF; with a recent meta-analysis of seven studies, and more than 6000 patients demonstrating that sST2 is an independent predictor of all-cause mortality (adjusted hazards ratio (HR): 1.75, P < 0.001) and cardiovascular mortality (adjusted HR: 1.79, P < 0.001) (Aimo et al. 2017). Elevated sST2 concentration >35 ng/mL is correlated with an increased risk of mortality and morbidity (Emdin et al. 2018). Importantly, unlike natriuretic peptides, the accuracy of sST2 levels is not affected by factors such as age and renal function (Ibrahim & Januzzi 2018). However, the accuracy of sST2 levels can be affected by guideline HF medications including BBs, ARBs and MRAs. This characteristic may actually prove beneficial in guiding HF treatment; sST2 has been suggested to identify patients with HF that may potentially benefit from a higher dosage of BBs (Gaggin et al. 2013); however, further investigation is required for other treatments.

**Galectin-3**

Galectin-3 (Gal-3) is a galactosidase-binding protein found in many different cell types and is implicated in various physiological functions including inflammation and fibrosis, which are hallmark characteristics of HF. Like ST2,
this biomarker also received a class IIb recommendation for consideration as part of the 2017 ACC/AHA/HFSA HF guidelines (Yancy et al. 2017) for its additive prognostic utility in patients with HF. Elevated Gal-3 has been associated with aspects of HF pathophysiology, such as cardiac extracellular matrix turnover (Lin et al. 2009). The prognostic capabilities of this biomarker have been substantiated by a meta-analysis of 18 studies comprising of more than 32,000 patients, which revealed that increased Gal-3 is associated with a higher risk of all-cause mortality, CVD mortality and HF (Imran et al. 2017). However, various factors such as cardiac rehabilitation, comorbidities and renal function can influence plasma Gal-3 levels and therefore its predictive power (Gehlken et al. 2018).

**Biomarkers to guide treatment of heart failure**

Following the commencement of HF treatment, its efficacy needs to be monitored to allow clinicians to make well-informed decisions regarding adjustments to ongoing treatment strategy. Traditional clinical parameters such as LVEF obtained from echocardiography can provide information about cardiac function in a non-invasive, real-time manner, which may reflect the response to treatment (Mikkelsen et al. 2006). Although baseline echocardiography is used to select appropriate treatment options and monitor the patients response to treatment, it is limited in its ability to predict/demonstrate a beneficial response to MRA therapy, or any other treatment, at an early time-point (Miyazaki et al. 2010).

Natriuretic peptide-guided HF treatment, targeting a pre-defined plasma BNP concentration, has been thoroughly considered. BNP has been utilised as a surrogate endpoint of treatment-mediated improvement in acute HF with decreased BNP levels correlating with better prognosis (Gackowski et al. 2004). Troughton et al. were the first to show a reduction in CV events, including death and hospitalisation, as a result of BNP-guided treatment (Troughton et al. 2000). This study however only consisted of a relatively small number of patients with the treatment consisting of ACEIs, diuretics but not BBs (Troughton et al. 2000), which is now a recommended treatment combination. Subsequent studies building upon this BNP-guided treatment strategy have demonstrated a reduction in HF-related deaths and hospitalisation via uptitration of ACEIs and BBs (Jourdain et al. 2007). The Strategies for Tailoring Advanced Heart Failure Regimens in the Outpatient Setting: BRAin Natriuretic Peptide vs the Clinical CongesTion ScorE (STARBRITE) trial, a randomised, multi-centre pilot study which evaluated whether BNP-guided diuretic treatment improved outcomes in patients with HFrEF. Interestingly, this study found that the BNP-guided treatment strategy, in comparison to the standard treatment strategy, yielded no additional days alive or days not hospitalised (Shah et al. 2011). Although no improvements were detected, an increase in the usage of ACEIs and BBs was reported, which is associated with increased survival. Meta-analyses further substantiate the benefit of BNP-guided therapy in HF patients with the observed benefit being attributed to the increased usage of beneficial pharmacological treatments, with an approximately 10% increase (BNP group vs control group) in the number of patients that achieved target doses of ACEIs as well as BBs (Felker et al. 2009, Porapakkham et al. 2010).

**Potential role of biomarkers in demonstrating response to MRA treatment**

Blockade of the MR can be reflected by an increase in plasma renin concentration; however, this does not provide insight into clinical response to MRA treatment. Biomarkers that can demonstrate and/or predict patients’ response to MRA treatment do not currently exist. If available, they may allow clinicians to be more selective regarding MRA prescription by targeting those patients most likely to respond to the therapy, thereby minimising the risk of unnecessary adverse effects in patients unlikely to benefit. This theoretical benefit was supported by an *in silico* analysis of The BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) data (Voors et al. 2016), which found that in patients with HFrEF, MRA uptitration hypothetically guided by a panel of 161 biomarkers would lead to slightly lower mortality or hospitalisation event rate, in comparison to MRA uptitration to >50% of recommended doses in all patients (Ouwerkerk et al. 2018). The suggested benefit of this biomarker-guided approach is promising especially given that MRA uptitration was not shown to be beneficial in 13% of patients (Ouwerkerk et al. 2018). However, none of the 161 biomarkers tested in *silico* have been evaluated in a prospective clinical study.

Despite the lack of specific trials examining biomarker-based titration of MRA treatment, various biomarkers have been evaluated in trials of MRA in HF. A sub-study of the RALES demonstrated that compared to placebo, a significant decrease in procollagen type III aminoterminal
peptide (PIIINP), a serum marker of collagen turnover, was observed after 6 months of spironolactone treatment in patients with severe HFrEF and baseline levels of PIIINP above the median but not in patients with baseline levels of PIIINP below median (Zannad et al. 2000). Furthermore, spironolactone-associated morbidity and mortality benefit was significant only in those with baseline levels of PIIINP above the median (Zannad et al. 2000), suggesting the potential to differentiate response to MRA treatment. Similarly, a significant decrease in plasma PIIINP was observed after 4 months in a small cohort of patients with mild to moderate non-ischaemic HF receiving spironolactone treatment but not placebo (Tsutamoto et al. 2001). Moreover, significant improvements in LV volume and mass were observed; both of which had a moderate, positive correlation with the decrease in PIIINP (Tsutamoto et al. 2001). These observations are important given that the inhibition of fibrosis is a central mechanism of MRA treatment and a marker that measures collagen turnover is a plausible correlate of improvements in ventricular remodelling (Pitt et al. 1999). Furthermore, using data from the EPHEUS and Impact Of Eplerenone On Cardiovascular Outcomes In Patients Post Myocardial Infarction (REMINDER) trials, Stienen et al. were able to identify patients with acute MI (with or without HF) who had a greater decrease in PIIINP in response to eplerenone treatment using certain clinical factors and baseline PIIINP levels (Stienen et al. 2020), which aligns with previous findings and further suggests the potential of baseline PIIINP to differentiate response to the ‘anti-fibrotic’ effects of MRA treatment. However, in patients with mild stabilised HF, plasma PIIINP levels between placebo and canrenone treatment groups were unchanged (Boccanelli et al. 2009). This was consistent with the small but statistically insignificant decrease in plasma PIIINP levels in patients with mild to moderate stabilised HF treated with eplerenone (9 months), compared to placebo (Udelson et al. 2010), suggesting that the potential utility of PIIINP in demonstrating response to MRA may only applicable in moderate to severe HF. Of note, serum PIIINP is not a cardiac-specific marker of fibrosis as serum levels may be affected by non-cardiac fibrosis. As a result, a more robust cardiac-specific marker would have value in assessing the cardiac response to MRA treatment.

In mild to severe HF, decreases in BNP levels following MRA treatment (compared to placebo) have been previously demonstrated by several studies. For example, there was a significant decrease in plasma BNP following 4 months of spironolactone (Tsutamoto et al. 2001), a 23% decrease in plasma BNP levels after 3 and 6 months of spironolactone treatment (Rousseau et al. 2002), a significant reduction in plasma BNP following 6 months of canrenone treatment (Boccanelli et al. 2009) and a significant decrease in plasma BNP following 9 months of eplerenone (Udelson et al. 2010). Despite these findings, the utility of BNP in guiding MRA treatment is limited. The PRIMA II study investigating NT-proBNP-guided therapy in the setting of acute decompensated HF found no significant improvement in the composite endpoint of all-cause mortality and readmission for HF (Stienen et al. 2018). More importantly, changes to MRA treatment were not significantly different between the NT-proBNP-guided group and the standard group. While BNP levels have previously been shown to correlate with cardiac function and improved patient outcomes, the lack of improved outcomes observed in the PRIMA II study may be attributed to the use of NT-proBNP to guide therapy instead of BNP. Furthermore, compared to the standard group, in the NT-proBNP-guided group, ACEIs/ARBs and diuretics were either significantly more often initiated or uptitrated (Stienen et al. 2018). This suggests the utility of BNP-guided treatment for ACEIs, ARBs and diuretics but simultaneously highlights the insufficiencies of this biomarker for guiding MRA treatment.

Although the prognostic utility of Gal-3 is well explored, there is limited and inconclusive evidence supporting its utility in guiding HF therapy, particularly MRA therapy in patients. A rodent model of HF demonstrated that treatment with either spironolactone or eplerenone reduced Gal-3 gene expression in rats with infarcted myocardium, and also downregulated levels of sST2 without affecting the protective actions of IL-33 (Lax et al. 2015). Additionally, decreased levels of these biomarkers correlated with a reduction in fibrosis and inflammation (Lax et al. 2015). In adult male Wistar rats, aldosterone-salt treatment resulted in an increase in: blood pressure, cardiac fibrosis and renal Gal-3 expression which was prevented with spironolactone co-treatment (Calvier et al. 2015). Similarly, spironolactone co-treatment was able to prevent aldosterone-mediated increases in intracellular Gal-3 protein expression *in vitro* in human cardiac fibroblasts (Martinez-Martinez et al. 2015). In contrast, human studies appear to show a weak relationship between Gal-3 and MRA therapy. A sub-analysis of the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION) study revealed no interaction between Gal-3 and MRA treatment effect (Fiuzat et al. 2014), and usage or uptitration of MRA treatment in a small cohort of patients with HFrEF was found to have no significant effect on plasma Gal-3 levels (Gandhi et al. 2000).
Interestingly, in patients with mild, stabilised HF, a marginally greater benefit of canrenone treatment was observed in those with above the median concentrations of Gal-3 (Clemenza et al. 2017). Moreover, in patients with acute HF treated with spironolactone, treatment was significantly beneficial in those with elevated levels of NT-proBNP, sST2, Gal-3 or creatinine; with the treatment interaction effects for sST2 and creatinine approaching significance, thereby suggesting a treatment benefit in patients with elevated levels of these biomarkers (Maiel et al. 2014). The use of these biomarkers to guide MRA treatment in HF patients has potential as there is evidence of a correlation between changes in biomarker levels and the aforementioned characteristics of cardiac health, that is inflammation and fibrosis (Nadar & Shaikh 2019). However, further research and appropriate clinical studies are needed to elucidate the utility of these biomarkers to guide heart failure therapy.

The effects of MRA treatment on circulating protein biomarkers have not only been identified in patients with mild to severe HF but also in patients at risk of developing HF with the most notable study being the Heart ‘OMics’ in AGEing (HOMAGE) study. Spironolactone treatment was not able to significantly reduce serum PIINP levels in patients at risk of HF (Cleland et al. 2021), similar to the results observed in patients with mild HF treated with MRAs. However, spironolactone treatment led to a decrease in serum procollagen type I carboxy-terminal propeptide (PICP) and an increase in serum collagen type-1 C-terminal telopeptide (CITP) (Cleland et al. 2021), which are markers of type 1 collagen synthesis and degradation, respectively. These changes were seen at the early 1 month time-point and were largely sustained even at the 9-month time-point along with increases in LVEF (Cleland et al. 2021), highlighting the potential of these biomarkers’ potential to demonstrate response to MRA at early stage. Although the tissue origin of circulating PICP is unclear, an increase in coronary and peripheral PICP was demonstrated in patients with hypertensive heart disease with/without HF (Querejeta et al. 2004). Moreover, a significant but weak correlation has been shown between plasma and myocardial PICP levels in patients with hypertrophic cardiomyopathy (Yang et al. 2019). A more comprehensive analysis of proteomic biomarkers in patients of the HOMAGE trial was performed by a secondary analysis (Ferreira et al. 2021). Plasma levels of other markers of collagen metabolism; collagen type I alpha 1 chain (COL1A1) and matrix metalloproteinase 2 (MMP2) as well as BNP; were significantly decreased at both 1 month and 9 months in those treated with spironolactone compared to control (Ferreira et al. 2021). The reduction in MMP2 observed between the two time-points was similar suggesting that the earlier effects are stabilised and sustained over a longer period of time. This finding could potentially be leveraged to provide insight into the therapeutic reversal of cardiac remodelling and consequentially may allow MMP2 to guide MRA treatment. It would be particularly interesting to examine the correlation between MMP2 and BNP and whether both biomarkers can be used together to improve treatment guidance.

**Use of biomarkers for heart failure with preserved ejection fraction**

HFpEF accounts for approximately half of HF cases, but the complex and heterogeneous nature of this condition has made the diagnosis and management of disease progression even more difficult. Currently, evidence-based HFpEF treatments that reduce morbidity or mortality are limited and there are no clear guidelines regarding the use of MRAs in this population (Yancy et al. 2017). Initial findings from the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial found that compared to placebo, spironolactone did not significantly reduce the composite endpoint of cardiovascular mortality, abort cardiac arrest or HF hospitalisation (Pitt et al. 2014). However, posthoc analysis revealed controversial regional variations between the two study locales, Eastern Europe and the Americas. Results from only the latter locale showed a significant reduction in the composite endpoint with spironolactone treatment in comparison to placebo (Pfeffer et al. 2015). Meta-analyses have demonstrated the use of MRAs in HFpEF to be of no benefit in reducing adverse cardiovascular outcomes (Berbenetz & Mrkobrada 2016) but useful in terms of improving cardiac structure and function (Kapelios et al. 2019). Amidst the uncertainty and ambiguity of MRA use in HFpEF, a biomarker of MRA treatment responsiveness may prove beneficial for selecting patients with potential for treatment benefit. Posthoc analysis of the TOPCAT study has shown that patients with lower baseline natriuretic peptides levels were more likely to benefit from MRA treatment compared to those with higher baseline natriuretic peptides levels (Anand et al. 2017). Further research into how natriuretic peptides and additional novel biomarkers can be used to predict MRA treatment response may be useful in the context of HFpEF.
Approach to identifying novel blood-based biomarkers of the patient response to MRA treatment

A promising approach that may help further elucidate the potential to predict treatment response of existing and novel biomarkers, is the use of blood cell-based transcriptomic analysis. While this is still an emerging area of research in the context of HF, the feasibility and utility of this approach have been demonstrated in other disease contexts such as oncology. As early as the 2000s, microarray technology was used to identify novel prognostic markers of acute myeloid leukaemia (Valk et al. 2004). A few studies have explored the use of peripheral blood mononuclear cell (PBMC) based transcriptomic analysis in the context of various HF phenotypes (Meier et al. 2021) and have yielded differentially expressed genes and microRNA (miRNA) between different phenotypes. The translation potential of this approach has been previously demonstrated in the field of coronary artery disease, where whole-genome microarray analysis of PBMCs was used to identify genes in the Corus® CAD Gene Expression biomarker panel (Vargas et al. 2013). Gene expression profiles derived from monocyte/macrophage analyses offer new opportunities for analysing an MR-sensitive cell type in a targeted manner that may be incorporated into a routine blood test. Several aldosterone-regulated genes have previously been identified in rat cardiomyocytes (H9C2 cells) stably expressing the MR, including genes associated with extracellular matrix regulation such as tenasin-XB (TNXB), ADAM metallopeptidase with thrombospondin type 1 motif 1 (ADAMTS1) and plasminogen activator inhibitor (PAI-1) (Fejes-Tóth & Náray-Fejes-Tóth 2007). Some of these genes have previously been shown to be regulated by MRAs, albeit differentially by different MRAs (Gruné et al. 2018). The increased cardiac gene expression of TNX observed in a mouse model of cardiac fibrosis was significantly reduced by treatment with finerenone but not with eplerenone (at equinatriuretic doses) (Gruné et al. 2018). Similarly, in H9C2 cells stably expressing the MR, finerenone treatment was able to inhibit aldosterone-induced induction of TXN and ADAMTS1 gene expression to a greater degree than eplerenone or spironolactone treatment (Gruné et al. 2018). Moreover, in a mouse model of pressure-induced cardiac hypertrophy, finerenone and eplerenone were shown to differentially modulate cardiac gene expression of genes regulated by pressure overload, including BNP and troponin T2, cardiac type (TNNT2) (Gruné et al. 2016). These aforementioned targets could be potentially explored as biomarkers of responsiveness to MRAs but will be dependent upon whether similar MR-dependent changes are observed in PBMCs. As gene expression in PBMCs can be regulated by many stimuli including tissue dysfunction and medication use, it remains to be seen whether transcriptional responses in PBMCs can be differentiated from MR-dependent gene expression profiles, in a robust and reliable manner.

miRNAs, which are small, non-coding RNAs, are involved in regulating gene expression. miRNAs have been proposed as potential biomarkers for various diseases including CVD. However, the potential of these biomarkers to demonstrate and/or predict response to treatment has not been thoroughly explored. Circulating blood-based miRNAs have previously been demonstrated to be differentially expressed in various subtypes of CVD (Min & Chan 2015, Zhou et al. 2018). Moreover, the high levels of cardiac-specific miRNAs miR-208a, miR-208b, miR-499 and miR-1 observed in patients with advanced HF (Akat et al. 2014) were reduced as early as 3 months after left ventricular assist device implantation, and mostly continued to approach normal levels after 6 months (Akat et al. 2014). This finding suggests the potential utility of circulating miRNAs for demonstrating an early therapeutic benefit of HF treatment. Furthermore, given the recent evidence of miR-181a being a novel regulator of aldosterone-MR mediated cardiac remodelling (Garg et al. 2020), the use of circulating miRNAs may offer a new avenue for demonstrating a response to MRA treatment in future studies. Circulating miRNAs associated with cardiac fibrosis have also been investigated to determine whether these biomarkers can identify patients that will have a clear anti-fibrotic response to eplerenone treatment (Stienen et al. 2021). In a sub-study of the EPHEUS, it was found that miR-133a was associated with decreased PICP levels; however, none of the miRNAs that were measured were able to predict the anti-fibrotic response to eplerenone treatment (Stienen et al. 2021). Moreover, in patients with HFPeF, miR-181c was able to differentiate high and low responders of exercise training, prior to the commencement of training (Gevaert et al. 2021).

High-throughput proteomic biomarker discovery is another methodology that has been employed to identify biomarkers in the context of HF. As detailed earlier, the proteomic studies from the HOMAGE trial were able to show changes in proteomic biomarkers in response to MRA treatment in patients at risk of HF. Verdonschot et al. used a similar approach to show differential expression of plasma proteins in patients at risk of HF with/without diabetes (Verdonschot et al. 2021). In diabetic patients, the plasma concentration of COL1A1 and PICP decreased, and
plasma concentration of MMP7 increased after 9 months of spironolactone treatment (Verdonschot et al. 2021). Another study to employ the use of high-throughput proteomic profiling is the ongoing MyoVasc study; a large, prospective, observational cohort study aiming to use multi-omics profiling to gain a better understanding of HF pathophysiology (Gobel et al. 2021).

Plasma amino acid (AA) profiling is another approach that can be potentially utilised to identify markers of treatment response. Despite the number of studies exploring this relatively novel approach being limited, the existing evidence is encouraging. Fasting AA profiling in patients with stable HF revealed that of the 41 AAs measured, 17 were different compared to control patients (Hakuno et al. 2015). Moreover, plasma levels of AAs monoethanolamine and glutamate demonstrated a significant, negative correlation with LVEF, and histidine demonstrated a significant, negative correlation with BNP (Hakuno et al. 2015). The inclusion of branched chain AAs to the Global Registry of Acute Coronary Events (GRACE) risk score was shown to significantly enhance the prediction of in-hospital adverse cardiovascular events in patients with ST-segment elevation MI following primary percutaneous coronary intervention (Du et al. 2018). Similarly, a recently published study also examining plasma AA biomarkers reported that the inclusion of 3-methylhistidine and either β-alanine or valine was able to improve prognostic accuracy (Kouzu et al. 2021). Further research exploring the changes in plasma AA levels in response to treatment, as well as together with correlations with cardiac functional response may enable the identification of biomarkers that demonstrate response to treatment including MRAs.

**Conclusion**

MRAs have proven beneficial in the treatment of patients with HFrEF, but their widespread use is sometimes hindered by drug-related side effects. BNP and NT-proBNP are considered the ‘gold standard’ of biomarkers in HF and are crucial in the diagnosis and management of HF but possess limitations, particularly in their treatment guiding utility. Preclinical models of HF have demonstrated promising interactions of other biomarkers such as sST2 and Gal-3 with MRAs; however, this finding is yet to be translated into human studies. A novel and specific MRA-sensitive biomarker would allow clinicians to identify patients within HF who will respond to MRA treatment, thereby enabling optimised treatment in a personalised manner to ultimately improve patient outcomes.

**Declaration of interest**

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