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Direktor: Professor Dr. med. Stefan Frantz

Mineralocorticoid-receptor antagonism and its metabolic consequences in haemodialysis patients

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Tobias Gregor Hauser

aus München

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Referent:	Professor Dr. med. Christoph Wanner
Koreferent:	Professor Dr. med. Martin Nentwich
Dekan:	Professor Dr. med. Matthias Frosch
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Der Promovend ist Arzt.

to my parents and my brother

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1 Introduction

1.1 Chronic kidney disease, haemodialysis and heart failure

1.1.1 Chronic kidney disease and haemodialysis treatment

The beginnings of dialysis treatment date back almost a century. In 1923, Georg Ganter – physician at Würzburg university hospital – pioneered peritoneal dialysis.[1] Two decades later, the first successful haemodialysis therapy was carried out treating a young woman in uremic coma.[2] Today, different stages of chronic kidney disease affect 10% of the global population with an estimate of 82 per 100,000 Europeans receiving dialysis treatment according to the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) Registry 2016 Annual Report.[3-6] Chronic kidney disease was defined by the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease as impaired kidney function or abnormal kidney morphology present for a minimum of three months and affecting patient health.[7] It is differentiated into five categories distinguished by glomerular filtration rate (GFR). GFR is high or normal in category one (>90ml/min/1.73m²) and decreases along categories two (60-89 ml/min/1.73m²), three (30-59 ml/min/1.73m²), four (15-29 ml/min/1.73m²) and five (<15 ml/min/1.73m²). Stage five is also referred to as kidney failure or end-stage kidney disease (ESKD). This group comprises patients with minimal residual kidney function on or about to be started on haemodialysis treatment.

1.1.2 End-stage kidney disease and heart failure

Chronic kidney disease and subsequent end-stage kidney disease often occur secondary to diseases of civilisation including arterial hypertension and diabetes mellitus with the latter causing renal function to decline relevantly in every third patient.[3,8,9] In the course of their illness, haemodialysis patients are likely to additionally develop symptoms of heart failure, partly due to overlaps in disease aetiology, partly as a result of their renal impairment. This interplay of chronic kidney disease and subsequent decline in cardiac health was described as ‘Cardiorenal syndrome type 4’ or ‘chronic renocardiac

syndrome'.[10] It is commonly characterized by cardiac remodelling, left ventricular hypertrophy and diastolic dysfunction. Correspondingly, the prevalence of heart failure with preserved ejection fraction (HFpEF) is particularly high in haemodialysis patients and overall, cardiovascular events are considered responsible for more than 40% of mortality in this group of patients.[11,12] The strong interaction between diseases of the cardiovascular system and chronic kidney disease leads to a substantial deterioration in prognosis making heart failure a major health burden in patients on haemodialysis treatment.[13-18]

1.1.3 Radiological evaluation of heart failure in haemodialysis patients

Radiological evaluation of cardiac morphology and function is a key pillar of heart failure diagnostics and of great importance in haemodialysis patients. When haemodialysis is initialised, almost 90% of patients already present with at least one structural or functional cardiac abnormality as seen in echocardiography (e.g., left ventricular hypertrophy, diastolic dysfunction grade ≥2 etc.).[19] In past trials, left ventricular hypertrophy assessed by cardiac magnetic resonance imaging was found to be an independent predictor of cardiovascular events including sudden cardiac death. It was further associated with several cardiovascular risk factors present in haemodialysis patients (e.g., hypertension, volume overload).[20-22] Echocardiographic assessment of cardiac function is an integral part of current guidelines on the evaluation of diastolic function and HFpEF.[23,24] In particular, the ratio between early peak trans-mitral flow rate (E) and early peak mitral annular tissue velocity (e') (E/e') was associated independently with hospitalization and mortality in ESKD patients.[25,26] Moreover, septal E/e' was proven to be especially robust with regard to haemodialysis-dependent volume overload.[27]

1.2 Mineralocorticoid-receptor antagonists

Mineralocorticoid-receptor antagonists (MRA) are a group of drugs comprised of spironolactone, eplerenone and similar substances. They function by inhibiting the final step of the renin-angiotensin-aldosterone system and counteracting the biological

functions of aldosterone. As aldosterone controls a variety of body functions, MRAs are a viable treatment option for a broad spectrum of conditions in different medical fields including ophthalmology, hepatology, nephrology and cardiology.

1.2.1 Mineralocorticoid-receptor antagonists in heart failure

Aldosterone is a key mediator in different processes associated with the development of heart failure including myocardial fibrosis and endothelial dysfunction. In past trials, MRA intake reduced fibrosis as well as the degree of myocardial stiffness and improved systolic and diastolic cardiac function.[28-33] Moreover, MRA were proven to reduce morbidity and mortality in mildly and severely symptomatic systolic heart failure patients as well as after acute myocardial infarction complicated by heart failure.[34-36] According to the 2016 European Society of Cardiology (ESC) Guidelines for the diagnosis and treatment of acute and chronic heart failure, MRA are highly recommended in patients suffering from heart failure with reduced ejection fraction (HFrEF) presenting with symptoms compliant with New York Heart Association (NYHA) functional class of two or higher despite treatment with angiotensin-converting enzyme inhibitors (ACEi) and a beta-blocker.[37] Even though high levels of aldosterone are associated with left ventricular hypertrophy in HFpEF patients, there is no clear recommendation regarding the use of MRA in this patient collective as several trials investigating the beneficial effect of MRA intake yielded ambiguous results.[38-41]

1.2.2 Mineralocorticoid-receptor antagonists in haemodialysis

The inhibition of aldosterone leads to an increased sodium excretion and potassium retention.[42] To meet the risk of severe hyperkalaemia, the use of spironolactone is to date contraindicated in patients with impaired renal function with a glomerular filtration rate of 30 mg/dl or lower.[43] Therefore, despite proven benefits in the treatment of heart failure patients, MRA were not to be used in haemodialysis patients in the past. In recent years however, several trials demonstrated a moderate increase in serum potassium levels and no significant increase in events of severe hyperkalaemia upon application of MRAs within a setting of adequate care in maintenance haemodialysis.[40,44,45] At the

same time, positive effects of spironolactone on cardiovascular morphology in patients with mild and moderately impaired kidney function were confirmed.[46] Spironolactone was also able to reduce left ventricular mass, improve blood pressure and overall cardiac function in some trials focusing on ESKD patients.[47-49] However, due to limited data from large randomized-controlled trials and some conflicting results, the value of MRA intake in patients on haemodialysis treatment remains subject to investigation.[50]

1.3 Biomedical markers

The risk of cardiovascular diseases in patients on maintenance haemodialysis is high. Tools to detect cardiac complications as early as possible are of paramount interest in realizing optimal care. Traditional clinical scores like the NYHA functional classes are of limited use in ESKD patients since observed dyspnoea can only partly be attributed to deteriorating cardiac functionality and is often caused by recurring volume overload.[51] Finding biomarkers that help estimate the grade of illness in patients on haemodialysis suffering from cardiac diseases offers a potential solution and is therefore of high value.

1.3.1 Definition of biomarkers

Modern medicine has access to a multitude of non-invasive approaches enabling in-vivo diagnostics that go beyond clinical examination and imaging. In oncology, for instance, magnetic resonance spectroscopy and metabolomics present a tool to help understand cellular processes that characterize malignancies.[52] Most commonly, a variety of serum substance concentrations are used to diagnose a condition or predict the progression of an illness. In 2001, a biomarker was defined by the 'Biomarkers Definitions Working Group' 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".*[53]

Ideally, a biomarker test should demonstrate a high sensitivity and specificity, should not be easily compromised, widely accessible, reasonably priced, standardized and well reproduceable to be of maximum usability.[54]

1.3.2 Biomarkers in heart failure

The enormous impact of biomarker tests in cardiovascular medicine is proven by a number of established biomarkers that help evaluate critically ill patients. Troponin T/I tests reflect cell death in myocardial tissue and revolutionized diagnostics in acute coronary syndrome, N-terminal pro-B-type natriuretic peptide (NT-proBNP) tests help evaluate and diagnose different stages of heart failure and C-reactive protein (CRP) tests mirror inflammatory processes associated with long-term survival.[37,54-56] Novel molecules are currently evaluated regarding their use to predict heart-failure related mortality, hospitalisation and other outcomes. Galectin-3 and soluble source of tumorigenicity 2 (sST2) emerged as promising candidates, both being involved in cellular processes of cardiac fibrosis and structural changes of the heart. As MRAs are known to prompt reverse remodelling in heart failure and reduce fibrogenesis, the impact of MRA on these biomarkers is of special interest.

1.3.3 N-terminal pro-B-type natriuretic peptide (NT-proBNP)

NT-proBNP and B-type natriuretic peptide (BNP) are considered the gold standard diagnostic and prognostic biomarkers in heart failure and play an important role in the latest guidelines for the diagnosis of HFrEF and HFpEF.[24,37,54] Both are released as a result of end-diastolic wall stress reflecting volume overload and high intraventricular pressure and are seen equivalent in literature with regard to their general significance.[57-59] In kidney-healthy individuals, NT-proBNP demonstrated a very high negative predictive value of 99% for heart failure and high serum levels resistant to treatment were associated with increased cardiovascular event rates.[60,61] Recently, spironolactone was found to reduce NT-proBNP serum levels in HFpEF patients, strengthening the case for the value of MRA treatment in this patient collective.[62] In haemodialysis, NT-proBNP as well predicted mortality and cardiovascular events in several trials.[63,64] Notably, despite

their proposed prognostic value, the interpretation of NT-proBNP serum levels proves difficult under haemodialysis conditions as they are markedly elevated regardless of cardiac status and optimal ESKD-specific cut-off values are yet to be incorporated in current guidelines.[65] Coincidently, although serum levels of NT-proBNP correlated with renal clearance, they appeared to be only mildly affected by dialysis treatment.[66,67]

1.3.4 Galectin-3

The beta-galactoside-binding protein Galectin-3 is part of a lectin family and amongst others secreted from macrophages partaking in inflammatory processes involved with the development of cardiac fibrosis.[68,69] Increased serum levels of Galectin-3 were also observed in pneumonia, sepsis and chronic kidney disease stages three and four.[70] In a mouse model, Galectin-3 was seen as a prerequisite for aldosterone-induced fibrotic processes in the vascular system and associated with cardiac remodelling and myocardial fibrogenesis.[71,72] High serum levels of Galectin-3 were repeatedly associated with cardiac impairment and predicted all-cause as well as cardiovascular mortality in patients suffering from heart failure and were therefore incorporated as potentially value-adding in current guidelines.[43,73-75] Noteworthy, the predictive value for all-cause mortality and association with cardiovascular risk factors was also reported in the general population.[76] Furthermore, several trials pointed into the direction of Galectin-3 being useful in distinguishing and evaluating HFpEF patients in individuals presenting with dyspnoea. It further predicted near-term hospitalization and long-term outcome in connection as well as independent from assessment with natriuretic peptides.[73,77-80] Despite confirming its prognostic ability, some studies disputed the added value of Galectin-3 when compared to established biomarkers like natriuretic peptides reporting very limited usefulness in initial diagnostics and inability to differentiate types of heart failure.[81-84] This controversy is also reflected in recent studies aiming to confirm Galectin-3 related findings in haemodialysis patients. Whereas high levels of Galectin-3 were reportedly associated with deteriorating renal function and predictive of adverse events and cardiovascular mortality in chronic kidney disease, investigations into their validity in haemodialysis patients yielded inconclusive results.[85-88] Additionally, Galectin-

3 serum levels were proven to be heavily influenced by haemodialysis treatment further complicating their evaluation in ESKD patients.[89] Despite the suggested connection of Galectin-3 with fibrotic processes induced by aldosterone, no treatment effect of MRA on Galectin-3 serum levels could be observed.[90,91]

1.3.5 Soluble source of tumorigenicity 2 (sST2)

sST2 is involved in fibrogenesis across inflammatory and immunological processes, cancer and cardiovascular disease.[92] By binding to circulating Interleukin (IL) 33, sST2 prevents its interaction with the membranous source of tumorigenicity 2 (ST2) receptor and nihilates the protective properties of the IL33/ST2 system.[92] Elevated sST2 serum levels were connected with myocardial remodeling processes in the development of heart failure and discussed as potential therapeutic target to prevent the same.[93-96] Subsequently, the prognostic value of sST2 was demonstrated in several studies, associating high levels of sST2 with cardiovascular and all-cause mortality, proposing an added prognostic value beyond NT-proBNP and leading to the inclusion of sST2 into the latest clinical guidelines.[43,97-100] High levels of sST2 were independently associated with poor NYHA functional classes in HFrEF, presence of HFpEF, diastolic abnormalities and poor outcomes after myocardial infarction.[98,101-104] It nevertheless remains subject to discussion, whether adding sST2 assessment into prediction models of adverse events and mortality already including natriuretic peptides adds value, especially with regard to its usefulness in a healthy general population.[105,106] The strong association between elevated sST2 serum levels and mortality was confirmed in ESKD, without however being as strongly connected to cardiovascular disease in patients on maintenance haemodialysis.[87] In contrast to most established biomarkers, the prognostic power of sST2 does not appear to be negatively influenced by deteriorating renal function and sST2 serum levels are not affected by haemodialysis.[67,88,107] Although sST2 serum levels were reported to be correlated with aldosterone levels in acute myocardial infarction, serum levels were independent from MRA intake in one study.[103,108] In consequence, due to its good prognostic characteristics and described independence from renal

impairment and dialysis treatment, sST2 can be considered one of the most promising novel biomarkers in the context of heart failure in haemodialysis patients.

1.4 Aim of this work

This dissertation addresses two research questions that are answered based on data taken from the MiREnDa study.

Firstly, the ability of spironolactone treatment to impact serum levels of established and novel biomarkers associated with heart failure, fibrosis and inflammation along with serum electrolytes and hormones is investigated in haemodialysis patients.

Secondly, the discriminative properties of baseline values of NT-proBNP, Galectin-3 and sST2 regarding the degree of left ventricular hypertrophy and diastolic function are assessed and compared in the MiREnDa population. Further, the correlation of biomarker changes of NT-proBNP, Galectin-3 and sST2 with changes in cardiac morphology and function is explored.

2 Materials and Methods

All data included in this work were collected as part of the 'Mineralocorticoid Receptor Antagonists in End-Stage Renal Disease' (MiREnDa) trial. This section will focus on the trial design of MiREnDa, image acquisition, blood sample analysis and the used guidelines and statistical methods. The detailed study design and flow including predefined endpoints and imaging methods have been published previously.[109-111]

2.1 Trial registration and ethics approval

The MiREnDa trial was registered at clinicaltrials.gov (NCT01691053; first posted Sep. 24, 2012) and approved by the Ethics-committee at the Medical Faculty of the University of Würzburg (application number "AZ-181/11_ff"). It was carried out according to the principals determined in the Declaration of Helsinki and the International Conference on Harmonization – Good Clinical Practice. Safeguards were put in place to guarantee unblinding in case of emergency and all trial activities including privacy, data safety, drug handling and administration were continuously monitored by a dedicated board. All participants provided written informed consent before being enrolled in the study.

2.2 Trial design

2.2.1 Aim of the Mineralocorticoid Receptor Antagonists in End-Stage Renal Disease (MiREnDa) trial and study course

The MiREnDa trial was set up as a prospective double-blinded, randomized and placebo controlled multi-centre trial. Its primary aim was to investigate the ability of spironolactone to reduce left ventricular mass index (LVMi) as measured by cardiac magnetic resonance imaging in haemodialysis patients along with drug safety over a 40-week period. Predefined secondary endpoints included echocardiographic assessment of cardiac function parameters and analysis of changes in biomarkers of fibrosis, inflammation and heart failure indicating metabolic alterations. All participants were randomized to 50 mg of spironolactone once daily or matching placebo in a ratio of 1:1. Recruitment started in December 2012 and was carried out at 20 sites in central

Germany (Figure 1). A complete list of all involved facilities can be viewed in the appendix section (Supplemental table 1). Patient screening, examination, imaging and blood sample collection were carried out at the university hospital centres following predefined protocols and standardized operating procedures.

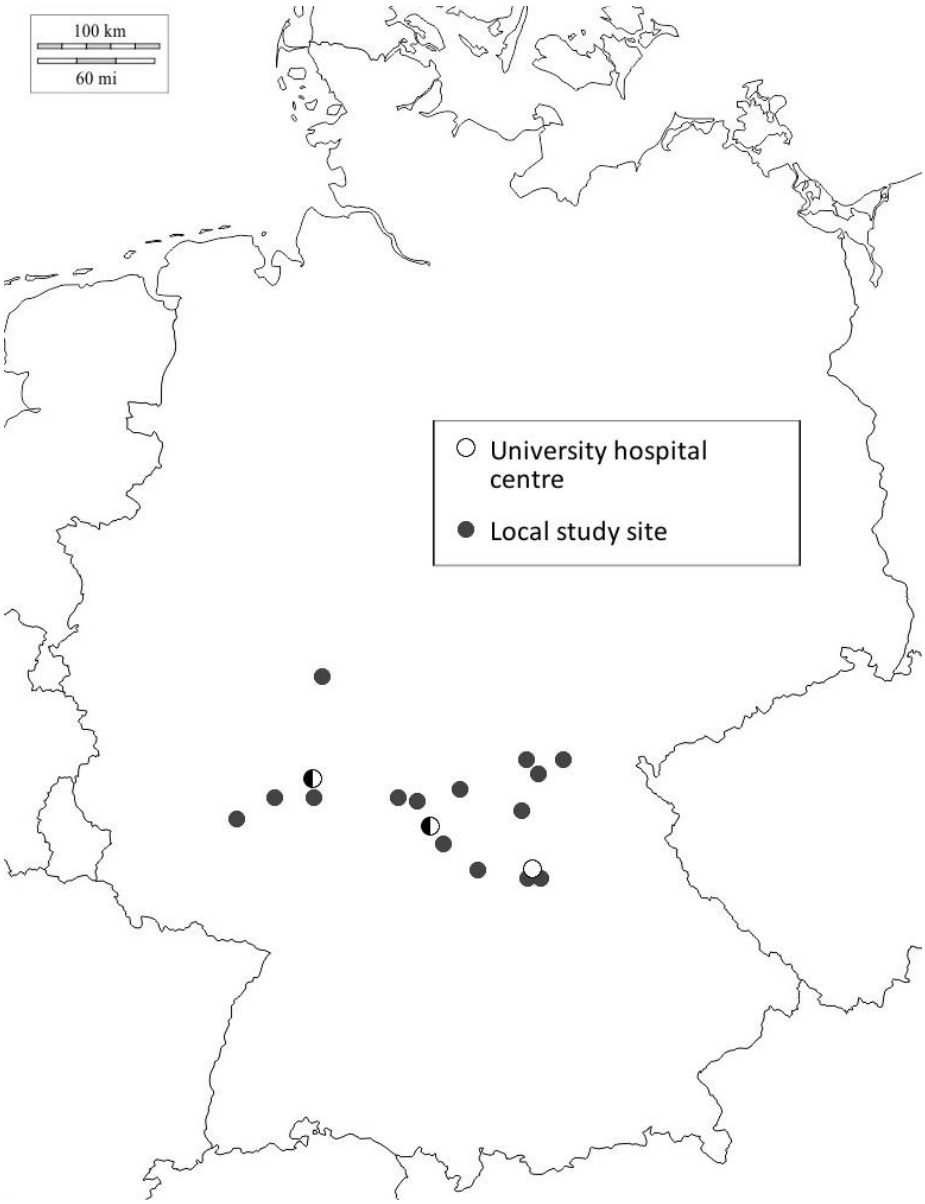

Figure 1: *Location of study sites in Germany*
map adapted from 'd-maps.com'[112]

During the trial, standardized questionnaires were used to collect additional information on medical history, sociodemographic factors and medication of all participants. Charts were provided by general practitioners and nephrologists to obtain information on

comorbidities. The trial was divided in three phases, a placebo run-in phase (2-4 weeks), a double-blinded treatment phase (40 weeks) and a safety follow-up phase (4 weeks). Blood samples and imaging data were collected at the beginning and end of the treatment phase on dialysis-free days (approximately 24 hours subsequent to the previous haemodialysis session). The active phase of the trial was completed in November 2015.

2.2.2 Inclusion and exclusion criteria

To be eligible to participate in the MiREnDa trial, patients had to be at least 18 years of age and on maintenance haemodialysis for three month or longer with a minimum of three dialysis sessions per week. Some comorbidities and patient-specific medical criteria as per the treating nephrologist led to the exclusion of screened patients. Participation was ruled out, if patients were unfit to undergo cardiac magnetic resonance (CMR) imaging, were expected to receive a kidney transplant from a living donor during the estimated trial duration, had an estimated life expectancy of less than a year or were likely to be non-compliant with the study protocol. In addition, individuals at risk of hyperkalaemia (defined as pre-dialysis potassium >6.0 mmol/L), a history of hyperkalaemia (defined as pre-dialysis potassium >6.5 mmol/L on at least four occasions during a three-month period prior to enrolment) or with a known intolerance to spironolactone were not eligible. MRA intake less than six months prior to trial commencement, manifest hypotension (defined as systolic blood pressure below 100mmHg) as well as an acute illness precluding participation within four weeks of trial commencement also led to the exclusion from the trial. Finally, premenopausal women without reliable contraception as well as pregnant or breastfeeding women were excluded.

2.3 Image acquisition and interpretation

2.3.1 Cardiac magnetic resonance imaging

Left ventricular mass (LVM) and left atrial volume (LAV) data were collected using cardiac magnetic resonance imaging according to a standardized protocol. LVM was

computed by determining the precise left ventricular myocardial volume without papillary muscles and trabeculae using a planimetric approach. After defining the ventricular myocardial area in each cross section, the left ventricular myocardial volume per specific slice was calculated (*'measured area' x 'slice thickness'*). Subsequently, the results of all measurements were summated. LVMi was calculated by normalising LVM to body surface area and left ventricular hypertrophy was defined as a LVMi of >79 g/m² in men and >61 g/m² in women.[113] LAV was measured following the 2005 American Society of Echocardiography (ASE) recommendation for chamber quantification utilizing the biplane area-length method (combining information from the four chamber view and two chamber view) and normalised to body surface area according to the Mosteller formula.[114,115] The obtained left atrial volume index (LAVi) data were then categorized in four groups using CMR-specific cut-off values proposed by Khan *et al*.; normal (21-52 ml/m²), mildly (52-62 ml/m²), moderately (63-73 ml/m²) and severely enlarged (>73 ml/m²).[116] By adapting the ASE recommendation for chamber quantification and the ESC 2019 consensus recommendation to CMR measurements, all LAVi values above the mark of 62 ml/m² were considered relevantly enlarged.[24,114] All scans were assessed by a radiology specialist blinded to treatment. A random subset of scans was sent to an external radiologist in order to confirm reading results and ensure data quality.

2.3.2 Echocardiography

Different morphological and functional parameters were gathered using transthoracic echocardiography (Figure 2). To ensure data comparability, the MiREnDa core lab at the University Hospital Würzburg, Germany, evaluated test recordings supplied by all university hospital centres prior to trial commencement. Close attention was directed to performing echocardiography on dialysis free days according to study protocol to minimize fluid status variations. The assessed parameters included left ventricular ejection fraction (LVEF, in %), septal early peak mitral annular tissue velocity (septal e', in cm/s), tricuspid regurgitation peak velocity (TR-velocity, in m/s), the ratio of Early peak trans-mitral flow rate (E, in ml/s) to e' (E/e') and the ratio of E to late peak trans-

mitral flow rate (A, in ml/s) (E/A) using pulsed wave (pw) doppler. Septal e' was used for E/e'. Measurements were acquired following a standardized protocol.

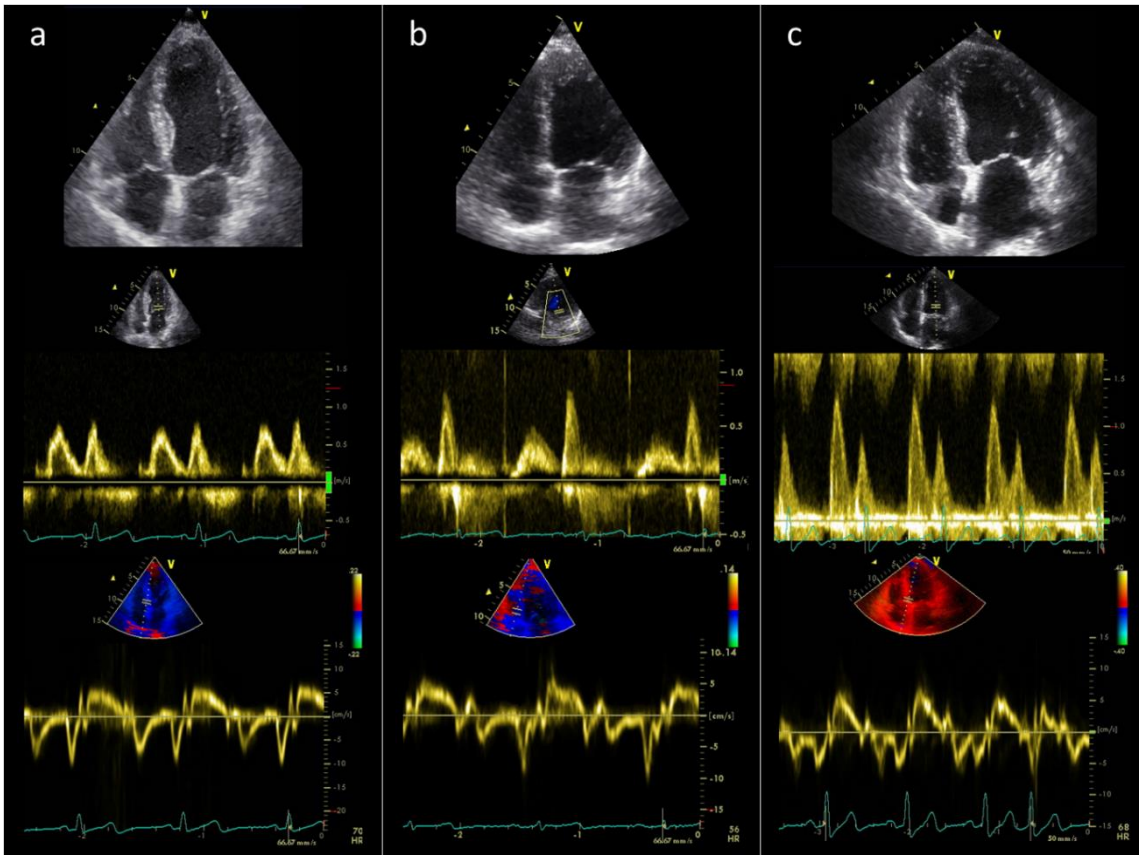

Figure 2: *Echocardiographic assessment of cardiac remodeling and diastolic dysfunction in three exemplary female ESKD patients with preserved ejection fraction*

Upper, middle, and lower panels depict apical 4-chamber view (upper panel), pulsed-wave Doppler evaluated diastolic filling pattern (middle panel), and tissue Doppler early diastolic mitral annular velocity (e´, lower panel); (a) Apparently healthy heart, LVMi=59 g/m², LVEF=72%, LAVi=33 ml/m², E/e'=8.9; (b) Beginning left ventricular hypertrophy and mild diastolic dysfunction, LVMi=103 g/m², LVEF=61%, LAVi=53 ml/m², E/e'=12.6; (c) Severely enlarged left ventricle and severely impaired diastolic function, LVMi=147 g/m², LVEF=67%, LAVi=56 ml/m², E/e'=33.0

ESKD: End stage kidney disease, LAVi: Left atrial volume index, LVEF: Left ventricular ejection fraction, LVMi: Left ventricular mass index

Hauser et al., 2021, Int J Cardiovasc Imaging[111] (copyright remaining with T. Hauser)

All examinations were performed by experienced university hospital staff and data was acquired either manually or semi-automatically with a dedicated software (GE Healthcare EchoPAC software, version 202, GE Healthcare, Chalfont St Giles, UK or

similar) using the GE Vivid E9 ultrasound system (GE Healthcare, Chalfont St Giles, UK) or a platform with comparable image quality. Data was analysed at the MiREnDa core lab and met standards proposed by Nagueh *et al.*[23]

2.3.3 Definition of diastolic dysfunction

The provided imaging data were utilized to evaluate diastolic cardiac function. Diastolic dysfunction was defined according to the algorithm proposed by the 2016 guidelines of the ASE and the European Association of Cardiovascular Imaging.[23] Patients were classified to one of the following five categories: normal diastolic function, diastolic dysfunction (DD) grade I, DD grade II, DD grade III or indeterminate (due to ambiguous data). The severity of cardiac impairment increased from DD grade I to III. As suggested, the diagnostic algorithms differentiated between patients with and without clinical or functional sings of HFpEF and HFrEF.

In patients presenting with a LVEF >50% and no clinical apparent dyspnoea, four variables were evaluated against the following thresholds: (1) E/e` septal >15, (2) septal e' velocity <7cm/s or lateral e' velocity <10cm/s, (3) TR- velocity >2.8m/s and (4) LAVi >62ml/m². If only one parameter reached the defined limit, a normal diastolic function was assumed. Participants were diagnosed with diastolic dysfunction with three or four positively tested criteria. With two prerequisites proven and two absent, the state of diastolic function could not be determined. Subjects who were diagnosed with diastolic dysfunction using the above criteria were subsequently regarded as suffering from grade I diastolic dysfunction, since they did not show any clinical symptoms of cardiac impairment. Patients showing a reduced LVEF (<50%) or a preserved LVEF but dyspnoea compliant with NYHA functional class II or higher were diagnosed using slightly adapted criteria. Grade I diastolic dysfunction was defined as a combined presence of E/A ≤ 0.8 and E ≤ 50cm/s. Diastolic dysfunction grade II was diagnosed, if E/A and E were elevated (> 0.8 and > 50cm/s, respectively) and at least two of the following three criteria were fulfilled: E/e` septal >15, TR- velocity >2.8m/s and LAVi >62ml/m². If thresholds were not reached in at least two of the additional criteria, diastolic dysfunction grade I was diagnosed. With only two criteria available, both had to point in the same direction to

determine diastolic dysfunction grades I or II, respectively. In all remaining cases, diastolic function was rated indeterminable. Diastolic dysfunction grade III was defined as E/A ≥ 2. Diastolic dysfunction grades II and III were defined as severe diastolic impairment.

2.4 Blood sample analysis

2.4.1 Specimen acquisition, processing and storage

Blood samples for biomarker analysis were collected at baseline and follow-up according to a predefined standard operation procedure at the university hospital trial centres following safety and hygiene regulations of the institutions. Ethylenediamine-tetraacetic acid (EDTA) and serum samples were collected by intravenous cannulation. Serum samples were let to clot for 10 to 60 minutes. In a next step, all samples were centrifuged using a refrigerated centrifuge (settings: 4 to 20°C, 1000 to 1500 g, 4000 cycles/min). Subsequently, the supernatant was aliquoted in up to five barcode-marked EDTA or serum trial tubes (holding 1.2 ml each). Specimens were frozen at -80°C and stored in a dedicated facility at the University Hospital Würzburg. All shipments (from local study sites to central storage unit and between central storage unit and analysing laboratory) were carried out by a dedicated medical express shipping service. Specimens were stored on dry ice during shipping. All stored tubes were catalogized using Microsoft Office 365 Access (Microsoft Corporation, Redmond, USA) to facilitate easy access and availability of specimens.

2.4.2 Laboratory analyses

The concentrations of 15 biomarkers, hormones and serum electrolytes were tested in patient blood serum at a certified central laboratory (Institute for Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany) (Table 1). Depending on the component, different materials, analysis systems and techniques were used to determine serum levels. These included the use of radioimmunoassays, spectrophotometry and enzyme-linked immunosorbent assays (ELISA). A complete list

of the utilized analysis systems can be viewed in the appendix section (Supplemental table 2).

Table 1: *List of all tested substances, standard units and range of standard values*

CRP: C-reactive protein, FGF: Fibroblast growth factor, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2, iPTH: Intact parathyroid hormone

Substance	Unit	Range of standard values[a]
Biomarkers		
CRP	mg/l	< 5.0
FGF-23	pmol/l	0.8[b]
Galectin-3	ng/ml	6.73 (2.40 – 15.7)[c]
NT-proBNP	pg/ml	< 125 (< 450, when age ≥ 75)
sST2	ng/ml	13.0 (6.74 – 20.4)[c]
Troponin T	ng/l	< 14
Hormones		
Aldosterone	pg/ml	5 – 188[d]
iPTH	pg/ml	9 – 65
25-OH-Vitamin-D	µg/l	> 30
Electrolytes and other components		
Sodium	mmol/l	135 – 145
Potassium	mmol/l	3.7 – 5.1
Calcium	mmol/l	2.12 – 2.52
Phosphate	mmol/l	0.60 – 1.60
Albumin	g/l	34 – 50
Urea	mmol/l	2.5 – 6.4

[a] *as supplied by the analysing laboratory*

[b] *non-standard ELISA analysis with no established standard range, only median value for apparently healthy individuals available according to test system handbook*

[c] *non-standard ELISA analysis with no established standard range, values are mean (range) for apparently healthy individuals according to test system handbook*

[d] *depending on age and sex of test subject smaller intervals may apply*

All measurements were conducted using serum samples of at least 800µl. With an amount of 600µl required to perform all analyses, all aliquots held a minimum of 200µl

additional serum to allow for remeasurement of individual values as judged by the specialist conducting the test.

2.5 Artwork and illustrations

Statistical illustrations were created with IBM Statistics package for the social sciences (SPSS) (version 26, IBM, Armonk, USA). Figures depicting echocardiographic data were designed with images obtained from GE Healthcare EchoPAC software, version 202, (GE Healthcare, Chalfont St Giles, UK) or similar and processed using Adobe Creative Suite 6 Photoshop (Adobe Systems, San Jose, USA). Some figures were adapted using GIMP 2 (GNU Image Manipulation Program 2) and Microsoft Office 365 PowerPoint (Microsoft Corporation, Redmond, USA).

2.6 Statistics

This thesis is based upon two research hypotheses. The first hypothesis was that nine months of daily treatment with 50 mg of spironolactone affected serum levels of biomarkers of heart failure, fibrosis and inflammation as well as additional serum parameters when compared to placebo. The second hypothesis was that patients presenting with left ventricular hypertrophy or a severe form of diastolic dysfunction (defined as grade II or grade III) had higher serum levels of NT-proBNP, Galectin-3 and sST2 at baseline than individuals without hints for the respective conditions. Furthermore, there was the assumption that changes in key imaging parameters of the named diseases (LVMi, LAVi, septal E/e') correlated with changes in biomarker serum levels over the study course. Baseline characteristics were examined per treatment group using suitable means of descriptive statistics. To compare mean changes in serum levels of biomarkers, electrolytes, albumin, urea and selected hormones from baseline to follow-up between treatment groups, one-way analyses of covariance (ANCOVA) with adjustment for the respective baselines (covariate) were performed. Even though the ANCOVA F test is robust in terms of moderate deviations from normal distribution assumption in balanced designs (such as in the MiREnDa study), in some cases basic assumptions underlying the ANCOVA method were violated. These violations concerned

firstly the occurrence of outliers or extreme values resulting in pronounced variance heterogeneity between the groups and secondly, the assumption of homogeneity of regression slopes. The latter was detected by significant interaction terms between treatment and covariate. Whenever an ANCOVA analysis could not be carried out for the above reasons, comparisons were alternatively conducted using the nonparametric Mann-Whitney-U-test without baseline adjustment. Nonparametric alternatives were also applied for simple univariate analysis to compare independent groups or to conduct paired difference tests of pre-treatment and post-treatment (baseline and week 40) measurements. The Mann-Whitney-U-test was applied to compare biomarker serum levels between patients with and without left ventricular hypertrophy or severe diastolic dysfunction. The Kruskal-Wallis-Test was used for the comparison of biomarker serum levels between patients with normal, mildly and severely impaired diastolic function and corrected for multiple comparisons using Bonferroni-correction. The distributions of LVMi, LAVi and septal E/e' at baseline and follow-up was compared using the Wilcoxon signed rank test. The assessment of the existence of possible linear correlations between selected biomarkers and measurement variables from imaging was also carried out graphically. Since a linear relationship could not always be assumed, the correlation between imaging data and biomarker data was statistically assessed with Spearman's rank correlation coefficients. The statistical null hypothesis for all applications of significance tests regarding underlying correlation coefficients ρ was "H0: ρ=0". Unless explicitly otherwise noted, statistical "significance" is generally defined as p-value < 0.05 (two-sided) without adjustment for multiple testing. The term "statistically significant" is used in this way throughout this work. Results were reported either as 'mean ± standard deviation (SD)', 'median [interquartile range (IQR)]' or 'mean difference of change (95% confidence interval)'. All statistical calculations and illustrations were carried out using IBM SPSS (versions 25 and 26, IBM, Armonk, USA).

3 Results

3.1 Descriptive analysis of the MiREnDa cohort

The MiREnDa trial enrolled a total of 118 haemodialysis patients. After a four-week run-in phase, 97 individuals were randomized to spironolactone (n=50) or placebo (n=47). With six dropouts in the placebo group and five dropouts in the spironolactone group during the study course, 86 subjects completed all steps of the trial including follow-up imaging and blood sample collection (Figure 3). Dropouts occurred for different reasons including withdrawal of consent, newly found deviation from inclusion criteria, death or non-medical patient-specific events that precluded study participation (e.g., relocation). Moreover, patients without complete biomarker workup were defined as dropouts.

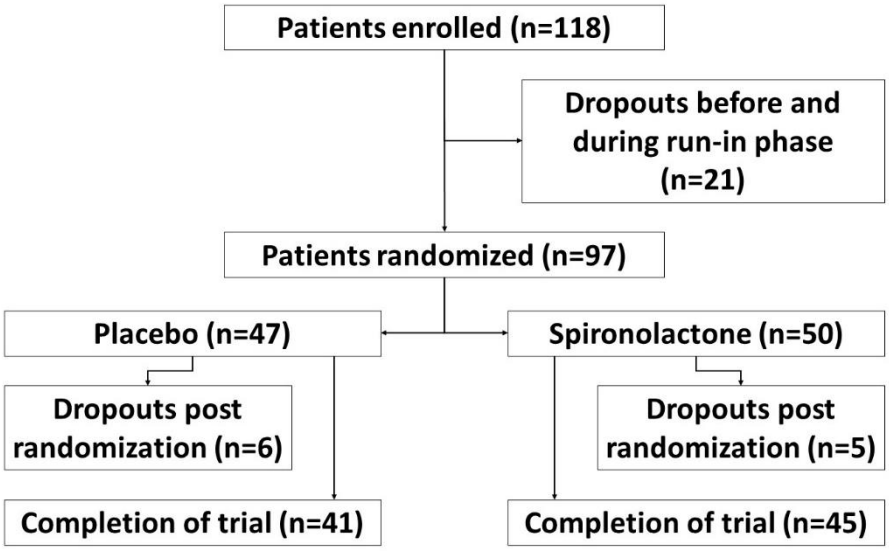

Figure 3: *Consort diagram depicting the MiREnDa trial flow*
Adapted from Hammer et al., 2019, Kidney Int.[110]

Mean age (± SD) at entry was 59.9 (± 12.9) years, 18 (20.9%) patients were female and 39 (45.3%) presented with symptoms compliant with NYHA functional class of two or higher (Table 2). Arterial hypertension was the most common secondary diagnosis (75 participants, 87.2%), followed by coronary artery disease (29 participants, 33.7%) and diabetes mellitus (25 participants, 29.1%).

Table 2: *Baseline characteristics of all patients with complete biomarker workup*

ACE: Angiotensin-converting enzyme, ARB: Angiotensin receptor blocker, BMI: Body mass index, BP: Blood pressure, IQR: inter quartile range, LVEF: Left ventricular ejection fraction, NYHA: New York Heart Association, SD: standard deviation

Characteristic	**Placebo (n=41)**	**Spironolactone (n=45)**
Age (years), mean ± SD	60.5 ± 13.0	59.3 ± 13.0
Female, n (%)	9 (22.0)	9 (20.0)
NYHA functional class, n (%)		
I	21 (51.2)	25 (55.6)
II	15 (36.6)	9 (20.0
III	5 (12.2)	10 (22.2)
LVEF (%), median (IQR)	67 (60; 72)	63 (56; 69)
Systolic BP (mmHg), mean ± SD	138 ± 22	139 ± 20
Diastolic BP (mmHg), mean ± SD	83 ± 15	82 ± 11
BMI (kg/m²), mean ± SD	27.2 ± 4.9	27.7 ± 5.1
Months on dialysis, median (IQR)	45 (11; 83)	37 (18; 61)
Comorbidities, n (%)		
Arterial hypertension	40 (97.6)	35 (77.8)
Atrial fibrillation	6 (14.6)	4 (8.9)
Coronary artery disease	13 (31.7)	16 (35.6)
Diabetes mellitus	12 (29.3)	13 (28.9)
Peripheral vascular disease	9 (22.0)	10 (22.2)
Congestive Heart Failure	2 (4.9)	3 (6.7)
Medication, n (%)		
ACE inhibitor / ARB	21 (51.2)	21 (46.7)
Beta blocker	27 (65.9)	27 (60.0)
Calcium antagonists	21 (51.2)	17 (37.8)
Loop diuretics	23 (56.1)	28 (62.2)
Phosphate binder	34 (82.9)	38 (84.4)
Potassium binder	3 (7.3)	4 (8.9)

At baseline, more than 80% of patients received antihypertensive medication with every third patient taking three or more different antihypertensive medicines. Comparison of baseline characteristics showed good randomization results. No major disparities between the placebo and spironolactone group were observed.

3.2 Effect of spironolactone on serum parameters

3.2.1 Effect of spironolactone on biomarkers of heart failure, fibrosis and inflammation

At baseline, elevated serum levels were observed across biomarkers of heart failure (NT-proBNP (median [IQR]): 3,250 [1,270; 11,640] pg/ml; Troponin T: 40 [26; 70] ng/l) and fibrosis (Galectin-3: 27.8 [20.3; 36.4] ng/ml; sST2: 20,300 [14,800; 29,700] pg/ml; FGF-23: 32 [11; 93] pmol/l).

Table 3: *Change in biomarker serum levels over 40 weeks compared between the spironolactone and placebo group*

CRP: C-reactive protein, FGF-23: Fibroblast growth factor 23, Gal-3: Galectin-3, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2, Trop T: Troponin T

Data are presented as 'median (interquartile range)' and 'mean difference (95% confidence interval)'

Missing data: FGF-23 (placebo group): n=8, FGF-23 (spironolactone group): n=4

	Placebo		**Spironolactone**		**mean difference in change[a]**	**p-value[b]**
	Baseline	Follow-up	Baseline	Follow-up		
CRP, mg/l	3.8 (1.7; 6.1)	5.4 (2.4; 8.5)	3.8 (2.1; 12.9)	2.7 (1.0; 7.4)	0.8 (-3.8; 5.3)	0.74
FGF-23, pmol/l	18 (8; 61)	23 (7; 65)	41 (14; 133)	35 (11; 140)	-34 (-87; 19)	0.68ǂ
Gal-3, ng/ml	25.2 (18.3; 35.0)	27.9 (19.8; 39.3)	30.5 (22.2; 37.8)	28.7 (23.1; 40.0)	0.2 (-3.0; 3.4)	0.60ǂ
NT-proBNP, pg/ml	2,800 (1,060; 6,660)	4,460 (1,750; 9,660)	4,220 (1,370; 14,520)	4,790 (1,940; 22,130)	-610 (-6,900; 5,670)	0.85
sST2, pg/ml	18,700 (15,400; 28,600)	20,900 (12,500; 33,700)	20,400 (14,100; 30,400)	22,600 (16,200; 30,000)	-1,200 (-8,100; 5,800)	0.52ǂ
Trop T, ng/l	41 (25; 78)	41 (28; 83)	39 (27; 70)	42 (25; 83)	-8 (-19; 4)	0.99ǂ

[a] Negative values indicate a relative enlargement in the spironolactone group compared to placebo

[b] One-way ANCOVA analyses with adjustment for baseline values when not indicated otherwise

ǂ Assumption of parallel slopes and/or homogeneity of variance violated; therefore, analysis was conducted by non-parametric Mann-Whitney-U test without adjustment for baseline values

Measured CRP levels gave no indication of the widespread presence of inflammatory processes. Compared to placebo, 40 weeks of spironolactone treatment did not affect mean change of any investigated biomarker (Table 3) including NT-proBNP (placebo vs. spironolactone: +760 [-660; +2,300] vs. +540 [-1,050; +5,580] pg/ml, p=0.85, Figure 4a), Galectin-3 (+1.6 [-1.7; +4.8] vs. +0.9 [-1.6; +4.4] ng/ml, p=0.60, Figure 4b) and sST2 (+400 [-2,800; +5,700] vs. +1,000 [-2,600; +7,100] pg/ml, p=0.52, Figure 4c). FGF-23 data was missing for a total of twelve patients due to serum levels outside the measuring range.

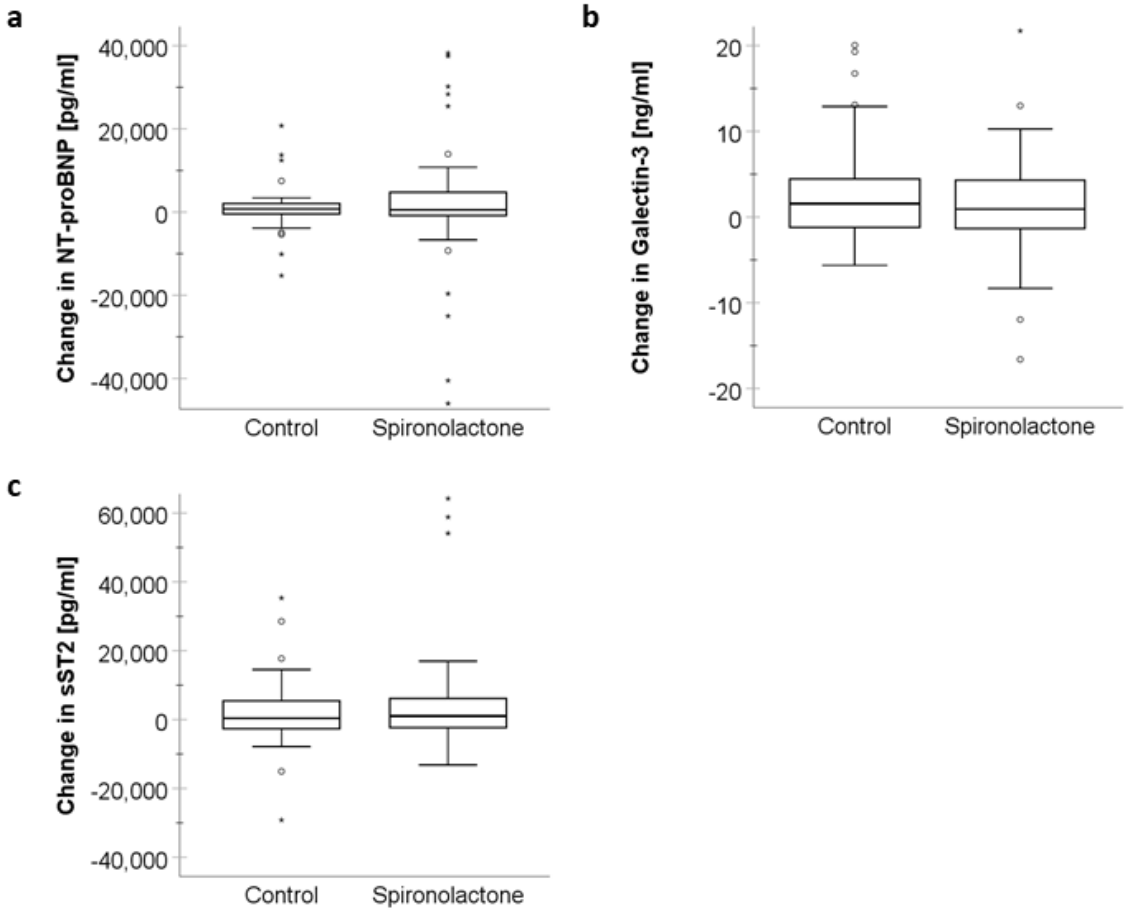

Figure 4: *Effect of 40 weeks of treatment with placebo or spironolactone on mean change of (a) NT-proBNP (p=0.85), (b) Galectin-3 (p=0.60) and (c) sST2 (p=0.52)*

NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

Three outliers are not depicted in the respective box plot charts for improved readability: NT-proBNP (Control): +84,600 pg/ml; Galectin-3 (Control): -44.9 ng/ml; sST2 (Spironolactone): -92,600 pg/ml

Whiskers represent 95% confidence interval

3.2.2 Effect of spironolactone on electrolyte and selected hormone serum levels

In addition to the analysis of biomarker serum levels, the effect of spironolactone treatment on serum electrolytes, albumin, urea, aldosterone, 25-OH-Vitamin D and parathyroid hormone (iPTH) was investigated (Table 4). At baseline, median serum levels of aldosterone, 25-OH-Vitamin D and urea were above the expected range. Serum electrolytes as well as albumin were inside their physiological range.

Table 4: *Change in electrolyte and aldosterone serum levels as well as additional serum parameters over 40 weeks compared between the spironolactone and placebo group*
iPTH: Intact parathyroid hormone, 25H-VitD: 25-Hydroxy-Vitamin D
Data are presented as 'mean ± standard deviation', 'median (interquartile range)' and 'mean difference (95% confidence interval)'

	Placebo		**Spironolactone**		**mean difference in change[a]**	**p-value[b]**
	Baseline	Follow-up	Baseline	Follow-up		
Sodium, mmol/l	136.7 ± 3.3	136.8 ± 2.5	135.9 ± 3.3	135.4 ± 2.8	1.2 (0.1; 2.2)	0.03*
Potassium, mmol/l	5.0 ± 0.5	5.1 ± 0.5	5.0 ± 0.5	5.1 ± 0.5	-0.1 (-0.3; 0.1)	0.43
Calcium, mmol/l	2.2 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	0.0 (-0.1; 0.1)	0.83
Phosphate, mmol/l	1.3 ± 0.4	1.4 ± 0.5	1.3 ± 0.3	1.3 ± 0.4	0.1 (-0.1; 0.2)	0.38
Albumin, g/l	37.2 ± 3.2	35.5 ± 3.1	36.2 ± 4.2	35.4 ± 3.2	-0.5 (-1.6; 0.6)	0.40
Urea, mmol/l	14.8 ± 5.2	14.1 ± 4.5	13.3 ± 5.0	13.3 ± 4.8	0.1 (-1.6; 1.7)	0.62ᵗ
Aldosterone, pg/ml	146 (67; 428)	121 (32; 485)	168 (74; 518)	124 (50; 634)	34 (-269; 337)	0.83
iPTH, pg/ml	274 (125; 457)	272 (119; 418)	263 (161; 429)	267 (182; 373)	-1 (-89; 87)	0.98
25H-VitD, µg/l	20.2 (12.8; 37.6)	19.4 (13.2; 40.8)	23.4 (14.8; 37.4)	22.5 (12.9; 38.4)	1.6 (-2.0; 5.2)	0.17ᵗ

[a] Negative values indicate a relative enlargement in the spironolactone group compared to placebo

[b] One-way ANCOVA analyses with adjustment for baseline values when not indicated otherwise

** significant (p<0.05)*

ᵗ Assumption of parallel slopes and/or homogeneity of variance violated; therefore, analysis was conducted by non-parametric Mann-Whitney-U test without adjustment for baseline values

After 40 weeks of treatment, only serum levels of sodium changed significantly between the placebo and spironolactone group (mean ± SD: +0.1 ± 3.3 vs. -0.5 ± 3.4, p=0.03). All

other serum parameters including potassium remained unaffected by treatment when compared to placebo (potassium: +0.1 ± 0.6 vs. +0.1 ± 0.7, p=0.43).

3.3 Biomarker distribution in left ventricular hypertrophy and diastolic dysfunction

3.3.1 Influence of left ventricular hypertrophy on biomarker serum levels

At baseline, 41 (47.7%) patients presented with left ventricular hypertrophy (LVH). Mean LVMi (± SD) was 78.3 (± 23.4) g/m². Comparison of biomarker serum levels between patients with and without enlarged left ventricle revealed a significant difference of NT-proBNP (normal vs. LVH (median [IQR]): 2,120 [810; 5,040] vs. 6,340 [2,410; 15,360] pg/ml, p<0.01, Figure 5a). No difference was observed for Galectin-3 (27.3 [20.1; 35.6] vs. 30.5 [19.7; 37.2] ng/ml, p=0.44, Figure 5b) and sST2 (20,000 [14,300; 29,300] vs. 20,500 [15,800; 32,000] pg/ml, p=0.37, Figure 5c).

There was a significant correlation of LVMi and NT-proBNP at baseline (Spearman's ρ=0.41, p<0.001, Figure 6a). No correlation between baseline Galectin-3 serum levels and baseline LVMi was observed (Spearman's ρ=0.06, p=0.58, Figure 6b). A mild correlation between baseline sST2 and LVMi reached borderline significance (Spearman's ρ=0.21, p=0.05, Figure 6c).

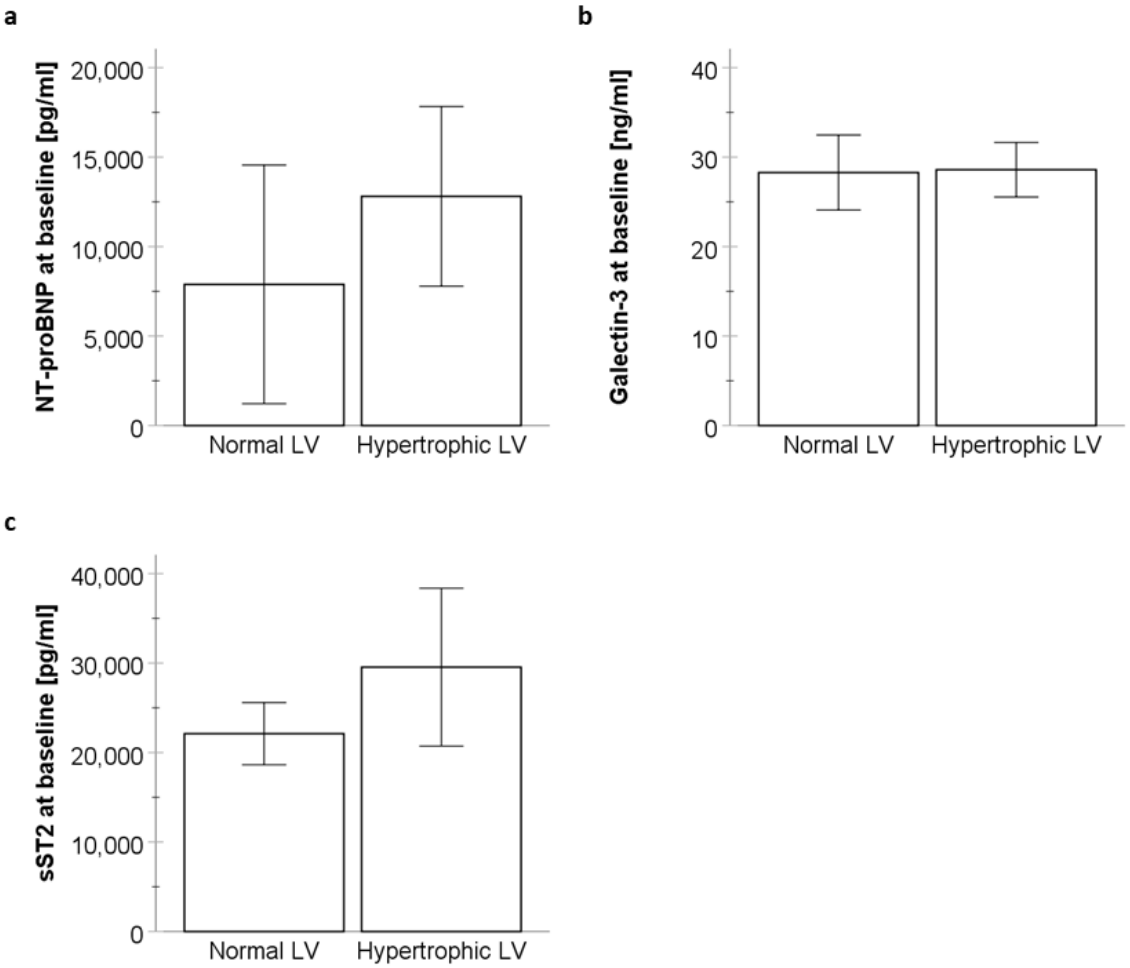

Figure 5: *Comparison of serum levels of NT-proBNP (a), Galectin-3 (b) and sST2 (c) between patient with and without hypertrophic left ventricle*

(a) NT-proBNP serum levels were significantly higher in patients presenting with left ventricular hypertrophy (LVH) compared to patients with normal left ventricular configuration (Mann-Whitney-U-test: p<0.01); (b) Galectin-3 serum levels did not differ between patients with and without LVH (Mann-Whitney-U-test: p=0.44); (c) sST2 serum levels did not differ between patients with and without LVH (Mann-Whitney-U-test: p=0.37)

Group size: Normal LV: n=45, hypertrophic LV: n=41

LV: Left ventricle, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

Whiskers represent 95% confidence interval

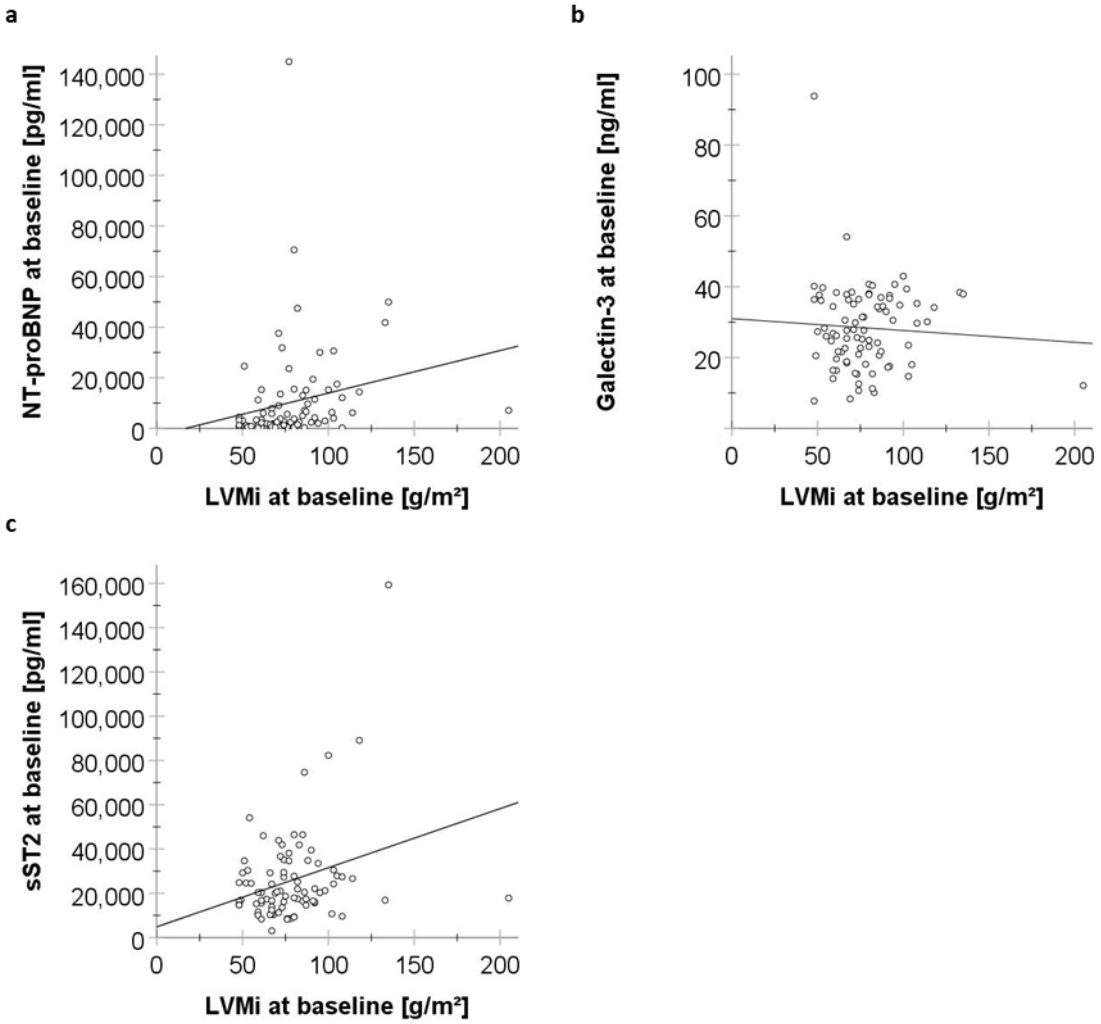

Figure 6: *Correlation between baseline serum levels of NT-proBNP (a), Galectin-3 (b) and sST2 (c) with left ventricular mass index (LVMi)*

(a) Baseline NT-proBNP serum levels correlated significantly with LVMi at baseline (Spearman's ρ=0.41, p<0.001); (b) Baseline Galectin-3 serum levels did not correlate with LVMi at baseline (Spearman's ρ=0.06, p=0.58); (c) Baseline sST2 serum levels did mildly correlate with baseline LVMi (Spearman's ρ=0.21, p=0.05)

LVMi: Left ventricular mass index, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

3.3.2 Influence of diastolic dysfunction on biomarker serum levels

At baseline, 32 (37.2%) patients presented with different grades of diastolic dysfunction (DD) including 10 (11.6%) individuals with severe diastolic impairment (DD grade II or DD grade III). Mean LAVi (± SD) was 47.2 (± 21.2) ml/m² and mean septal E/e' was 15.7 (± 7.6).

Serum levels of NT-proBNP were markedly elevated in patients presenting with severe diastolic dysfunction (normal diastolic function and DD grade I vs. DD grade II and DD grade III: 2,300 [850; 6,050] vs. 12,260 [3,340; 34,830] pg/ml, p=0.02, Figure 7a). Serum levels of Galectin-3 and sST2 did not differ across different levels of diastolic impairment (Galectin-3: 27.7 [20.0; 36.3] vs. 36.1 [22.8; 37.8] ng/ml, p=0.19, Figure 7b; sST2: 18,700 [13,400; 29,200] vs. 24,200 [17,000; 29,700] pg/ml, p=0.37, Figure 7c). Further investigation of the biomarker serum level distribution showed a significant difference of NT-proBNP measurements between patients with normal and severely impaired diastolic function (normal diastolic function vs. DD grade II and DD grade III, 2,030 [800; 4,180] vs. 12,260 [3,340; 34,830] pg/ml, p=0.04, Supplemental figure 1). NT-proBNP serum levels of patients with DD grade I were intermediate between those of patients with normal diastolic function and DD grade II & III, but not significantly different from either group. No further significant differences were observed for Galectin-3 and sST2.

There was a significant correlation of baseline NT-proBNP with LAVi at baseline (Spearman's ρ=0.55, p<0.001, Figure 8a) and septal E/e' at baseline (Spearman's ρ=0.45, p<0.001, Figure 8b). No significant correlations were observed for Galectin-3 and sST2 (Figure 8c-f). Correlation analysis of biomarkers to each other indicated a significant correlation between baseline NT-proBNP and baseline sST2 serum levels (Spearman's ρ=0.37, p<0.001, supplemental figure 2b). Baseline Galectin-3 correlated mildly with baseline NT-proBNP without reaching a level of significance (Spearman's ρ=0.19, p=0.08, supplemental figure 2a) and was independent from sST2 at baseline (Spearman's ρ=0.05, p=0.68, supplemental figure 2c).

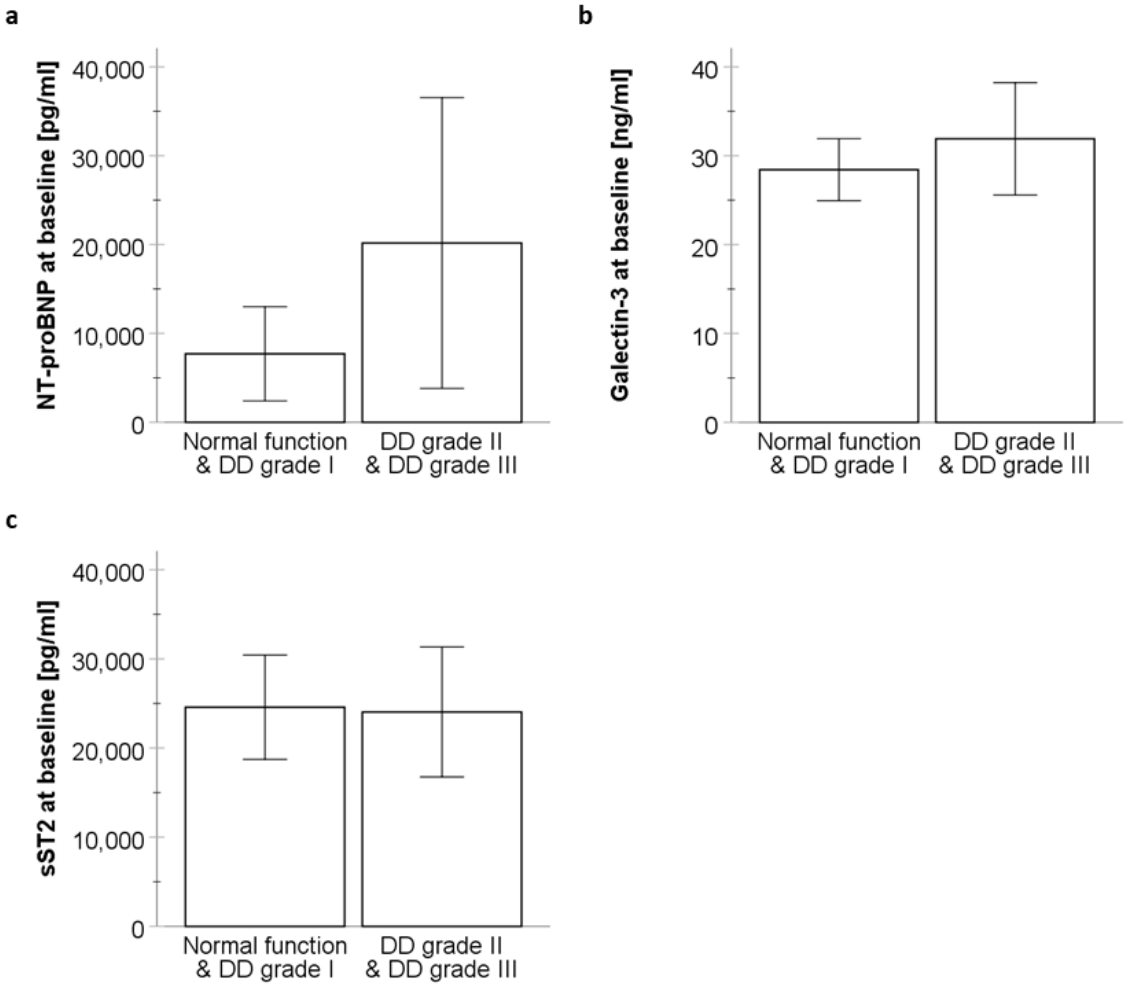

Figure 7: *Serum levels of NT-proBNP (a), Galectin-3 (b) and sST2 (c) compared between grades of diastolic dysfunction*

(a) NT-proBNP serum levels were significantly higher in patients presenting with DD grade II and DD grade III compared to patients with normal diastolic function or DD grade I (Mann-Whitney-U-test: p=0.02); (b) Galectin-3 serum levels did not differ between patients presenting with DD grade II and DD grade III and patients with normal diastolic function or DD grade I (Mann-Whitney-U-test: p=0.19); (c) sST2 serum levels did not differ between patients presenting with DD grade II and DD grade III and patients with normal diastolic function or DD grade I (Mann-Whitney-U-test: p=0.37)

Group size: Normal function & DD grade I: n=58, DD grade II & DD grade III: n=10

Missing data: data from 18 patients are not included in this figure as their diastolic function was indeterminable with the used diagnostic algorithm

DD: Diastolic dysfunction, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

Whiskers represent 95% confidence interval

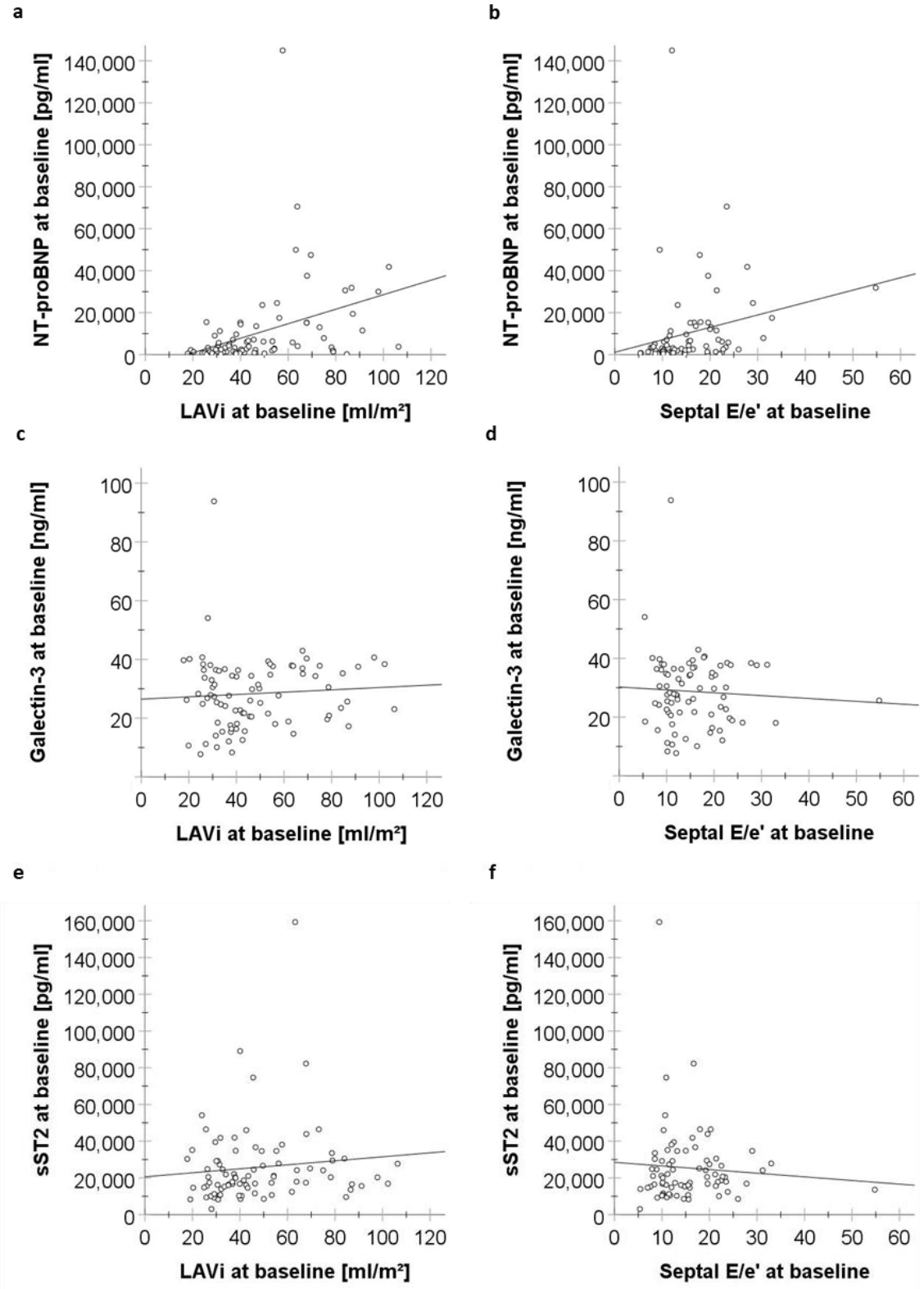

Figure 8: *Correlation between parameters of diastolic function and baseline serum levels of NT-proBNP (a, b) and Galectin-3 (c, d) and sST2 (e, f)*

(a) Baseline NT-proBNP serum levels correlated significantly with LAVi at baseline (Spearman's ρ=0.55, p<0.001); (b) Baseline NT-proBNP serum levels correlated significantly with septal E/e' at baseline

(Spearman's ρ=0.45, p<0.001); (c) Baseline Galectin-3 serum levels did not correlate with LAVi at baseline (Spearman's ρ=0.08, p=0.50); (d) Baseline Galectin-3 serum levels did not correlate with septal E/e' at baseline (Spearman's ρ=-0.02, p=0.85); (e) Baseline sST2 serum levels did correlate mildly with LAVi at baseline without reaching a level of significance (Spearman's ρ=0.18, p=0.10); (f) Baseline sST2 serum levels did not correlate with septal E/e' at baseline (Spearman's ρ=0.08, p=0.49)

LAVi: Left atrial volume index, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

3.3.3 Assessment of correlation of changes in cardiac morphology and functional parameters with changes in biomarker serum levels

Over a 40-week period, parameters of cardiac morphology and function did not change significantly (baseline vs. follow-up (mean ± SD) LVMi: 78.4 ± 23.5 vs. 77.1 ± 22.1 g/m², p=0.37; LAVI: 50.0 ± 21.1 vs. 47.1 ± 21.2 ml/m², p=0.78, septal E/e':15.7 ± 7.7 vs. 16.7 ± 9.4, p=0.27). In individual patients, considerable changes were observed and correlated with changes in biomarker serum levels (Figure 9). Changes of NT-proBNP were connected significantly to changes in LVMi (Spearman's ρ=0.24, p=0.03) and septal E/e' (Spearman's ρ=0.29, p=0.01). Changes in sST2 were mildly correlated with changes in LVMi (Spearman's ρ=0.17, p=0.13). There were no correlations between the remaining constellations of biomarkers and cardiac parameters.

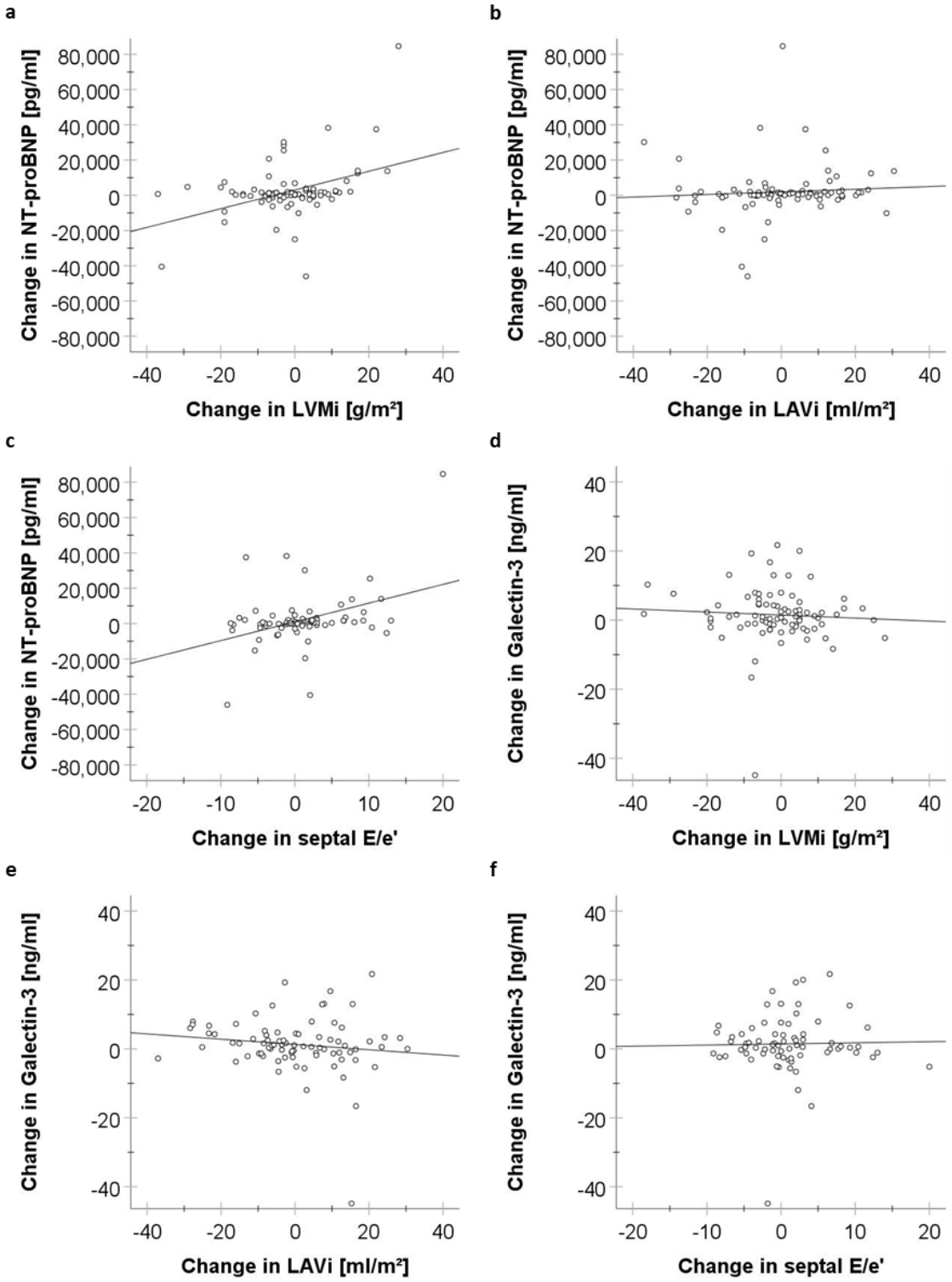

Figure 9: *Correlation of mean change in cardiac morphology and diastolic function parameters with mean change in NT-proBNP (a-c), Galectin-3 (d-f) and sST2 (g-i)*

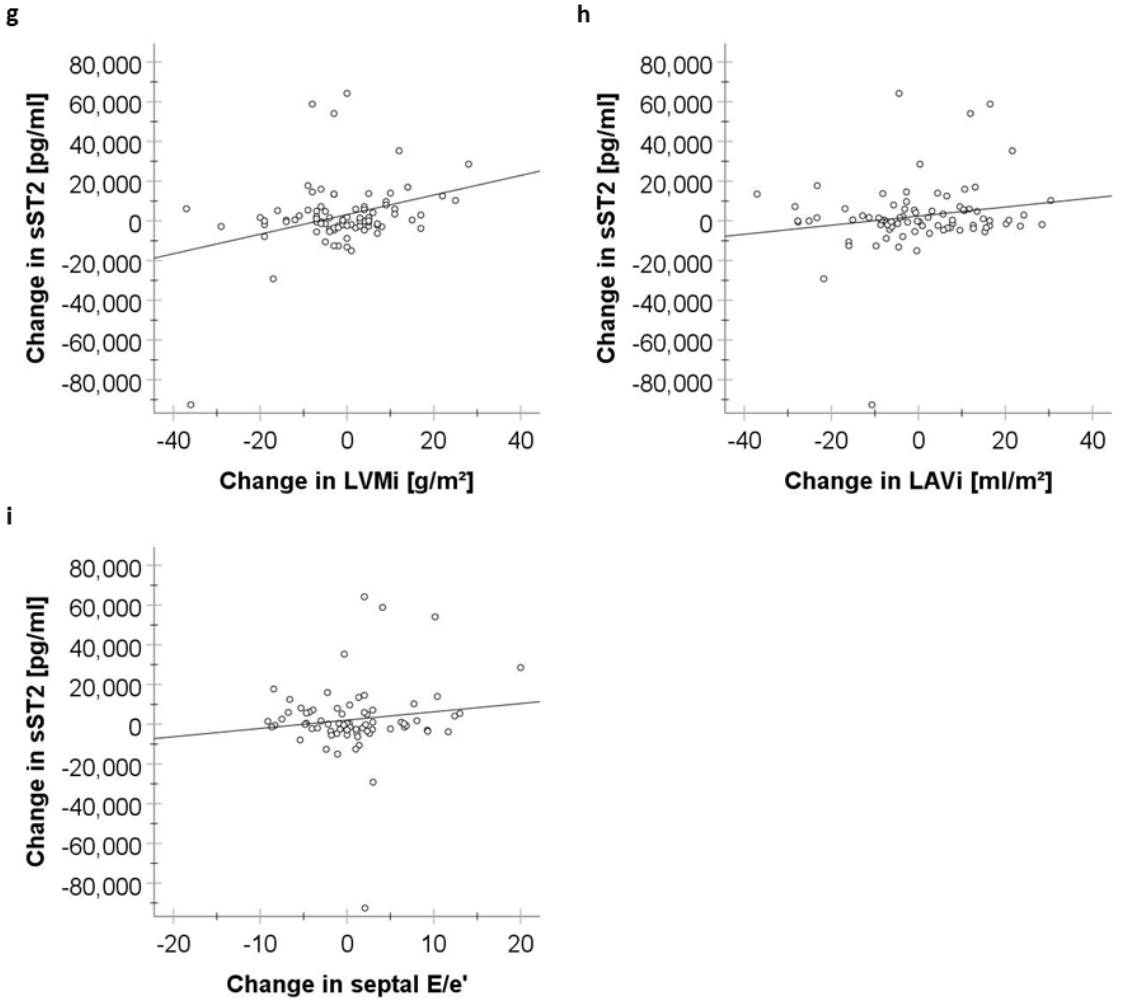

Figure 9 (continued): *Correlation of mean change in cardiac morphology and diastolic function parameters with mean change in NT-proBNP (a-c), Galectin-3 (d-f) and sST2 (g-i)*

(a-c) Mean change in NT-proBNP correlated significantly with mean change in LVMI (Spearman's ρ=0.24, p=0.03) and septal E/e' (Spearman's ρ=0.29, p=0.01), but not with mean change in LAVi (Spearman's ρ=0.07, p=0.54); (d-f) Mean change in Galectin-3 did not correlate with LVMi (Spearman's ρ=-0.10, p=0.35), LAVi (Spearman's ρ=-0.16, p=0.17) or septal E/e' (Spearman's ρ=-0.02, p=0.90); (g-i) mean change in sST2 did not correlate with LVMi (Spearman's ρ=0.17, p=0.13), LAVi (Spearman's ρ=0.11, p=0.35) or septal E/e' (Spearman's ρ<0.01, p=0.99)

LAVi: Left atrial volume index, LVMi: Left ventricular mass index, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

4 Discussion

The main findings of this work are that, firstly, nine months of daily treatment with 50 mg of spironolactone did not affect serum levels of biomarkers including NT-proBNP, Galectin-3 and sST2 in haemodialysis patients compared to placebo. With the exception of serum sodium, which was reduced significantly in the intervention group, no effect of spironolactone was observed on any of the investigated serum parameters.

Secondly, NT-proBNP was the only biomarker to significantly differ between patients with and without LVH or severe DD. NT-proBNP was further the only biomarker to correlate significantly with LVMi, LAVi and septal E/e' at baseline. Changes in LVMi and septal E/e' were strongly correlated with changes in NT-proBNP, whereas changes in LAVi did not show any correlations with the investigated biomarkers. Galectin-3 was not correlated with cardiac structure and function parameters at baseline nor was there a significant interaction between imaging data changes and the biomarker. There was a weak connection between sST2 and LVMi at baseline as well as during the study course that did not reach a level of significance.

4.1 Effect of spironolactone treatment on serum parameters

4.1.1 Impact of spironolactone treatment on biomarkers of heart failure, fibrosis and inflammation

MRA treatment is one of the pillars of treatment in patients with different forms of heart failure, but its effectiveness in haemodialysis patients remains doubtful.[40,43,110,117] Investigating the impact of MRA treatment on biomarkers connected to disease progression and severity of heart failure sheds light on another facet of this relationship. There is limited data on the effectiveness of MRA treatment in lowering serum NT-proBNP levels. Further, only very few studies investigated the impact of MRA treatment on Galectin-3 and sST2 or the connection between their serum levels and MRA treatment efficacy to date. Nielsen *et al.* found that two months of treatment with 25 mg of spironolactone had a borderline significant effect on serum levels of NT-proBNP in 69 patients with diabetic nephropathy in a randomized-controlled study.[118] There was

no effect observed on different biomarkers of inflammation including CRP. Cleland *et al.* reported that 50 mg of spironolactone reduced NT-proBNP and Galectin-3 serum levels in 527 patients randomized one to one to treatment and placebo over a period of up to nine months.[119] Recently, data from 247 participants of the TOPCAT study were released in a biorepository substudy. Over a period of more than three years, different dosages of spironolactone (ranging from 14 – 45 mg) reduced NT-proBNP in ambulatory patients with stable chronic HFpEF.[62] Neither Troponin I nor CRP serum levels were influenced. Also based on data from the TOPCAT study, Anand *et al.* observed a relatively stronger benefit of spironolactone intake with regard to cardiovascular mortality and hospitalisation in patients with NT-proBNP levels within the lowest tertial.[120] At the same time, data from the ATHENA-HF trial suggested no added value of a high dosage of spironolactone (100 mg) inside the first 96 hours of hospital administration.[121] Even though in a mouse model, the absence of Galectin-3, achieved through gene knock-out, interacted strongly with the effect of aldosterone on vascular fibrosis, no clear evidence of an interaction between MRA treatment of human patients and serum Galectin-3 was published to date.[71] In the HF-ACTION study by Fiuzat *et al.*, treatment effect of MRA was independent from Galectin-3 serum levels in 895 ambulatory heart failure patients.[90] Additionally, Koukoui *et al.* reported no interaction between MRA treatment effect and serum levels of Galectin-3 in a subset of 200 patients from the IBLOMAVED study over a three-year follow-up period.[91] In the Aldo-DHF trial, 25 mg of spironolactone over one year could not influence Galectin-3 serum levels in HFpEF patients.[122] Barutaut *et al.* did not see evidence of MRA treatment influencing the prognostic capacity of sST2.[108]

In line with current literature, Galectin-3 and sST2 serum levels appeared to be independent from spironolactone intake in the MiREnDa study. However, there was also no reduction in NT-proBNP serum levels induced by spironolactone compared to placebo in the MiREnDa trial. These findings are in line with those of an echocardiographic substudy of the MiREnDa trial, in which Hauser *et al.* did not observe 50 mg of spironolactone to influence serum levels of NT-proBNP based on a smaller subset of MiREnDa patients.[111] Since a close correlation between cardiac parameters

and biomarkers is suggested, the here reported biomarker findings also well reflect previously published data from the MiREnDa primary and secondary endpoints. As reported by Hammer *et al.*, spironolactone failed to reduce LVMi significantly in 85 haemodialysis patients.[110] Furthermore, there was no significant effect of spironolactone treatment observed on any other functional or morphological echocardiographic parameter.[111] The discrepancy between current literature on the effect of MRA intake on NT-proBNP levels and data from the MiREnDa trial must be assessed with the caveat that, apart from the MiREnDa study, haemodialysis patients were not eligible for trial participation. This MiREnDa substudy appears to be the first randomized-controlled trial to explore the impact of MRA on biomarker levels exclusively in haemodialysis patients. The seen absence of treatment effect on humoral parameters casts doubts on whether the administration of spironolactone in haemodialysis patients can have a positive effect and be justified accordingly. However, as mean levels of NT-proBNP and Galectin-3 are markedly elevated in haemodialysis for various reasons (e.g., recurring volume overload), transferability and comparability might be limited and the impact of spironolactone treatment especially on NT-proBNP might be disguised.[67]

4.1.2 Impact of spironolactone treatment on sodium and potassium serum levels

Aldosterone inhibition leads to an elevation in serum potassium whilst reducing serum sodium levels.[42] As the renal system is of vital importance to maintain balanced electrolyte levels, medicines interfering with electrolyte secretion and retention are prone to cause undesired effects. A variety of studies investigated the safety and efficacy of spironolactone in haemodialysis patients with particular reference to the frequency of critical hyperkaliaemia. They concluded that administration of MRA can be viewed as relatively safe in haemodialysis patients even though a non-critical increase of serum potassium was frequently observed.[123,124] These findings were underlined by recently published data from the SPin-D trial in which dosages up to 25 mg of spironolactone appeared to be reasonably safe in a monitored haemodialysis setup.[40] However, some critical voices continue to raise concerns regarding the risk-benefit assessment of MRA

in the context of ESKD. For instance, upon reviewing MRA administration in CKD with regard to drug safety and efficacy, Cosimato *et al.* find that although MRA might be of additional value in patients with moderately impaired kidney function, a clear benefit of MRA intake is yet to be observed in ESKD.[50] They therefore suggest to avoid MRA in haemodialysis patients.

The here reported data on the impact of spironolactone on serum electrolytes also point in the direction of spironolactone administration being relatively safe in haemodialysis patients. This view reflects previously published data from the MiREnDa study when Hammer *et al.* demonstrated that 50 mg of spironolactone may cause mild hyperkalaemia but did not observe severe hyperkalaemia (>6.5 mmol/l) more frequently compared to placebo.[110] The lack of difference in potassium serum levels between the spironolactone and placebo group as observed in this work therefore appears reasonable. At the same time, the significant reduction of sodium levels in the spironolactone arm as seen in the MiREnDa trial can be interpreted as undesired effect of spironolactone. Even though the absolute difference between both study arms was minimal and there was no report of frequently observed hyponatraemia throughout the study course, the lack of treatment effect puts the necessity of using spironolactone in haemodialysis patients into doubt, especially in view of severe side effects that cannot be ruled out.

4.2 Distribution, prognostic and diagnostic value of NT-proBNP, Galectin-3 and sST2 across left ventricular hypertrophy and diastolic dysfunction in haemodialysis patients

As patients on haemodialysis treatment are highly prone to develop cardiovascular complications, means to early detect patients at risk of deteriorating cardiac function are intensively investigated. Serum biomarkers like NT-proBNP have been established as useful tools in non-ESKD patients but have limitations in maintenance haemodialysis. Novel biomarkers emerged as potential solution to overcome inaccuracies and could facilitate a quick and accurate evaluation of cardiovascular health. The following paragraphs will summarize the current literature on the distribution, prognostic and

diagnostic value of NT-proBNP, Galectin-3 and sST2 in maintenance haemodialysis in comparison to findings from the MiREnDa trial.

4.2.1 N-terminal pro-B-type natriuretic peptide (NT-proBNP)

The importance of NT-proBNP as a gold standard biomarker for the assessment of most forms of heart failure is reflected in current international guidelines.[24,37,54] Although the overall prognostic and diagnostic value of NT-proBNP has been shown in numerous trials, there are some conditions including ESKD that strongly interfere with serum concentrations of this biomarker.[54,65,67,125,126] Consequently, unadjusted cut-off values are of little practical use, but strong correlations remain between serum dynamics of NT-proBNP, disease progression and different clinical outcomes.[127] In the past, different cut-off values have been proposed for haemodialysis patients, but so far ambiguities on their practical accuracy prevented their inclusion into current guidelines.[43,64,128,129] Investigations into the connection between NT-proBNP serum levels and morphological as well as functional cardiac parameters aim to further elucidate the usefulness of NT-proBNP assessment in haemodialysis patients.

Several trials confirm the usefulness of NT-proBNP in CKD and haemodialysis patients.[130,131] By examining 127 ESKD patients being prepared for their initial haemodialysis treatment, Han *et al.* observed current NT-proBNP serum levels to be significantly correlated with LVMi, LAVi and E/e' along with additional echocardiographic parameters.[132] Helal *et al.* confirmed the connection between LVMi and NT-proBNP in 64 patients undergoing haemodialysis and healthy controls.[133] In a small trial of 21 patients, Choi *et al.* saw NT-proBNP being closely associated to LVMi at baseline.[134] Moreover, at two follow-up visits after six and twelve months, a close link could be established between changes in biomarker serum levels and changes in LVMi. Focusing on diastolic dysfunction, Dubin *et al.* reported a strong association of NT-proBNP serum levels and E/e' in 35 haemodialysis patients.[135] Noteworthy, apart from this, patients presenting with diastolic filling patterns compliant with different grades of diastolic dysfunction were not found to have elevated biomarker serum levels compared to patients with physiological diastolic filling patterns. In a slightly bigger trial of 117

haemodialysis patients, Yamazaki *et al.* found NT-proBNP to correlate significantly with LVMi, LAVi and E/e' as seen in echocardiography.[136] A similar connection was observed in the MiREnDa trial. Serum levels of NT-proBNP were significantly elevated in the presence of LVH and more severe forms of diastolic dysfunction. This connection also existed at the level of the individual diagnostic parameters of diastolic dysfunction (septal E/e' or LAVi) when echocardiographic hints for the presence of DD grade II or grade III were reflected in markedly elevated NT-proBNP serum levels. As even large meta-analyses failed to determine accurate cut-off values for NT-proBNP in haemodialysis patients to date, usage of the biomarker in this patient collective is based on the best possible understanding of the connection between the biomarker's serum levels and disease progression and severity.[125,137] Therefore, the here presented findings further strengthen the case for the usefulness of NT-proBNP assessment in chronic haemodialysis patients. Moreover, monitoring changes in NT-proBNP serum levels could be a means of early detection of cardiac health deterioration beyond the implementation of cut-off values. Future research is needed to evaluate the added value of this approach.

4.2.2 Galectin-3

High serum levels of Galectin-3 have repeatedly been associated with poor prognosis in CKD and haemodialysis patients in several large clinical trials. Based on data of more than 3,700 patients from the 4D and LURIC trials, Drechsler *et al.* reported elevated serum levels of Galectin-3 to be connected with the occurrence of several cardiovascular endpoints in patients with impaired kidney function.[85] They moreover saw an increase in Galectin-3 serum levels with deteriorating kidney function and an association of elevated Galectin-3 levels with all-cause mortality and infections. Tuegel *et al.* observed a connection between high Galectin-3 serum levels and mortality in 883 CKD patients without, however, finding an association with specific cardiovascular endpoints.[138] An even stronger predictive value of Galectin-3 was observed in 423 Japanese patients on maintenance haemodialysis.[139] Galectin-3 outperformed NT-proBNP as prognostic biomarker and patients presenting with Galectin-3 serum levels within the highest

tertile were found to be seven times more likely to suffer from a major cardiovascular event. However, median Galectin-3 levels were extraordinarily low compared to the expected range in the setting of haemodialysis. On the contrary, as Galectin-3 was found to be strongly influenced by deteriorating kidney function, several trials determined its diagnostic and prognostic value in haemodialysis to be limited.[67,86,127] Attempted explanations of the strong impact of renal insufficiency on Galectin-3 serum levels included the discovery of impaired renal Galectin-3 handling in heart failure patients, but the precise connections elude scientific understanding to date.[140]

In the MiREnDa trial, serum levels of Galectin-3 were markedly elevated across the entire patient collective. This can most likely be attributed to the impact of deteriorated kidney function on Galectin-3 serum levels.[67] No connection between Galectin-3 serum levels and cardiac disease severity including an association with more severe forms of diastolic dysfunction could be established. Furthermore, Galectin-3 failed to correlate with any of the investigated morphological and functional cardiac parameters. Further, Galectin-3 was only weakly associated with NT-proBNP in the MiREnDa collective, most likely due to the strong influence of renal impairment and haemodialysis on both biomarkers. These results reflect findings by Gopal *et al.* who observed Galectin-3 to be inversely related to renal function regardless of clinical symptoms of heart failure.[141] Also, Zamora *et al.* reported Galectin-3 to rather be connected with renal impairment than with the presence of heart failure.[142] In opposition to these findings, Gurel *et al.* report Galectin-3 to strongly correlate with LAVi and E/e'.[143] Also in this study, mean Galectin-3 serum levels were comparably low across the patient collective. It stands to reason that although Galectin-3 can be strongly influenced by renal impairment and haemodialysis, its significance could highly depend on the degree of observed biomarker elevation at baseline. The simultaneous presence of markedly elevated Galectin-3 levels at baseline across all participants of the MiREnDa trial and the absence of any correlation between biomarker serum levels and echocardiographic parameters support this hypothesis. Further research into this phenomenon could be of high scientific interest. In summary, data from this work do not hint in the direction of any added value from

Galectin-3 measurements in the context of haemodialysis neither with regard to improved diagnostics nor prognostics.

4.2.3 Soluble source of tumorigenicity 2 (sST2)

In many aspects, the prognostic properties of Galectin-3 and sST2 are of similar significance as both are tightly connected to fibrogenesis and remodelling processes in heart failure.[54] Especially in the context of CKD and haemodialysis, sST2 assessment might be more insightful as in contrast to most other biomarkers, its serum levels are only incrementally influenced by haemodialysis treatment and renal impairment.[67,144] Notably, sST2 serum levels do increase with progressing renal impairment, but, in comparison to natriuretic peptides, sST2 is not influenced by ESKD and haemodialysis.[145] Hence, sST2 is widely regarded as one of the more promising novel biomarkers at the boundary between nephrology and cardiology.[92] Its assessment is suggested by current guidelines; however, cut-off values are yet to be established.[43] Several studies investigated the connection between sST2 serum levels and heart failure in haemodialysis patients. In a study by Obokata *et al.*, sST2 predicted all-cause mortality and cardiovascular outcomes and together with Galectin-3 improved risk stratification in 423 haemodialysis patients over a period of more than two years.[139] Kim *et al.* observed haemodialysis patients with an elevated sST2 serum level to be at relatively greater risk of mortality, without however describing a link to cardiovascular disease.[87] In another trial, they saw sST2 to be connected with CKD progression and adverse events in 352 CKD patients.[88] Beyond that, after confirming the independency of sST2 serum levels from renal impairment and haemodialysis, Homsak *et al.* found sST2 to be of independent use as a biomarker for cardiovascular risk stratification.[146] They also investigated a sST2 cut-off value of 35,000 pg/ml with good results.

Despite the above findings, some critical voices remain casting doubts on the usability of sST2 in a broader population and its added value above NT-proBNP.[105,106] In the MiREnDa trial, sST2 serum levels were indistinguishable between patients presenting with sings of DD or LVH. Also, sST2 was inferior to NT-proBNP regarding the biomarkers' connection to the assessed imaging parameters. The discrepancy could potentially be

explained by the study focusing on changes of functional and morphological parameters rather than on cardiovascular or clinical outcomes. The borderline significant correlation of sST2 and LVMi could hence be viewed as a low-threshold sign that there might have been a relationship between sST2 serum levels and cardiovascular mortality in the MiREnDa trial, provided a sufficiently long follow-up period. Underlining findings from the MiREnDa trial, Wang *et al.* also found NT-proBNP to better correlate with E/e' than sST2 in 107 hypertensive HFpEF patients.[102] Further, Najjar *et al.* reported sST2 to not correlate with LVMi and E/e' (but notably with LAVi) in 86 HFpEF patients. At the same time, they observed a close connection between serum levels of NT-proBNP and sST2 as also seen in data from the MiREnDa trial.[147] In synopsis, whereas there appears to be a well-established connection between elevated sST2 serum levels and mortality in haemodialysis patients, the interplay between the biomarker and individual functional or morphological cardiac parameters appears much more difficult to grasp. In this context, data from the MiREnDa study suggest that exclusive assessment of sST2 might not be suitable to represent pathological changes in the heart. The combination of multiple biomarkers including NT-proBNP and sST2 might pose a potential solution.

4.3 Limitations and strengths of the MiREnDa trial in relation to the presented data

4.3.1 Limitations of the MiREnDa trial

There are some limitations to the MiREnDa study that need to be considered when interpreting the presented data. Due to the follow-up interval of only nine months, long-term effects of spironolactone treatment could have been missed. Albeit, as some trials report a treatment effect on NT-proBNP serum levels after a similar or even shorter time to follow-up, the observed absence of treatment effect is likely to be genuine.[118,119] As biomarker levels were only measured at two time points, trends and changes during the course of the trial could not be recorded. Therefore, minor and temporary effects of treatment might have been disguised. However, comparison of the observed effect of spironolactone on potassium serum levels with the previously published continuous potassium measurements for drug safety evaluation suggest that a transient treatment effect can be assumed to be rather unlikely.[110] FGF-23 data was not available in some

participants due to biomarker serum levels outside the kit measurement range. As there was no hint of a treatment effect, inclusion of few supposedly extreme outliers is unlikely to add to the significance of the presented results. The use of two-dimensional echocardiography might have been a source of inaccuracy when compared to three-dimensional methods, although its use is suggested by current guidelines.[23,24] Lateral E/e' was not broadly available. Therefore, septal E/e' measurements were used for all analyses. As septal E/e' was demonstrated to be especially robust regarding volume alterations in the haemodialysis setting, this is unlikely to have negatively influenced the significance of the conducted analyses.[148] Given that a history of heart failure or diastolic dysfunction were no defined inclusion criteria, the significance of the here presented results are based on few patients with severe forms of heart failure as by NYHA functional class assessment. This is also reflected in the low percentage of participants that fulfil the diagnostic criteria of DD grade II or grade III as proposed by Nagueh *et al.*[23] There is room to speculate that more or more defined connections between the investigated biomarkers and selected functional and morphological cardiac parameters could have been observed in patients with more severe forms of cardiac impairment. Since new and improved guidelines for the classification of DD were released in the course of the MiREnDa study, the originally intended diagnostic algorithms were replaced. As part of the new guidelines, LAVi assessment by echocardiography was introduced to the diagnostic algorithm. In the MiREnDa trial, LAVi measurements were conducted by CMR imaging instead of echocardiography. Therefore, the proposed cut-off values had to be adjusted accordingly and might constitute a source of inaccuracy. To account for the difference in imaging technique, CMR specific cut-off values were implemented based on work by Khan *et al*.[116] Several of the evaluated imaging as well as serum parameters are susceptible to changes in volume status. Even though rigorous attention was directed to examining participants 24 hours after the previous haemodialysis session, it cannot be excluded that interindividual differences in volume status affected the conducted analyses. Non-white ethnicities and female patients were underrepresented in the MiREnDa population.

4.3.2 Strengths of the MiREnDa trial

Several aspects of the MiREnDa trial underline the validity of the presented results. Being set up as a randomised-controlled and double-blind trial, the observed treatment effects adhere to the highest scientific standards. The administered dose of 50 mg of spironolactone represents the suggested target dosage in heart failure patients.[37] Hence, it can be assumed that the MiREnDa trial was able to show the majority of expected drug impact, especially when considering findings by Butler *et al.* who observed no added value when administering a higher dosage of 100 mg of spironolactone in a randomised-controlled trial.[121] To warrant for good data quality and reproducibility, biomarker analyses were carried out by expert laboratory staff and in case of hints for suboptimal measurement results, multiple analyses were carried out. Imaging data was evaluated in the MiREnDa core laboratory by readers blinded to treatment. Given difficult echocardiographic conditions or suboptimal imaging quality, multiple imaging runs were conducted. A random sample of the acquired CMR data was re-examined externally confirming primary reading results.

4.4 Conclusion and potential for future research

In conclusion, treatment with 50 mg of spironolactone over 40 weeks had no impact on any of the investigated biomarkers of heart failure, fibrosis and inflammation in the MiREnDa trial. NT-proBNP serum levels were significantly higher in patients with LVH and DD and showed a correlation with morphological and functional cardiac parameters. Galectin-3 was independent from all examined parameters. sST2 showed a weak correlation with LVMi without however reaching a level of significance.

Regarding the usefulness of MRA treatment in haemodialysis patients, data from the MiREnDa trial together with recent findings contrast with hopes associated with the earlier reported efficacy of spironolactone in preventing remodelling processes in haemodialysis. Albeit, novel non-steroid MRAs like Finerenon show good results in diabetic nephropathy and might be a fruitful field to be investigated in the context of heart failure in haemodialysis patients.[149] Furthermore, another large trial, ALCHEMIST,

investigating the efficacy of MRA intake in haemodialysis patients is underway and promises further insights.[50]

The findings of the MiREnDa study moreover illustrate that the connection of novel and established biomarkers of heart failure and fibrosis is more difficult to grasp in haemodialysis patients in comparison to patients with normal kidney function. Connections cannot be as strongly established and unadjusted cut-off values hardly apply. Still, a review of current literature together with the results of this study suggest that both NT-proBNP and sST2 have their merit with regard to diagnostics and prognostics of heart failure in haemodialysis patients.[150] To evaluate the possibilities for their future usage in this patient collective, large trials are needed. Adequate cut-off values for NT-proBNP in the context of haemodialysis are required. In addition, as biomarker dynamics of NT-proBNP appear to possibly have more significance than the exceeding of any particular serum level, the added value of continuous monitoring of this biomarker in maintenance haemodialysis might also be a rewarding field for future research. Since sST2 is to a great extend independent from ESKD, definitive cut-off values could proof to become a valid tool to stratify for a patient's risk of mortality. Combined scores of different biomarkers are currently under investigation. The connected assessment of NT-proBNP and sST2 could indeed be of benefit to enable better and earlier reactions to cardiac complications of ESKD by combining the advantages of both biomarkers. Ultimately, the use of such a score could make a sustainable contribution to better outcomes in haemodialysis patients and pave the way for a more personalised treatment approach.

5 Summary / Zusammenfassung

Patients on haemodialysis are highly susceptible to different forms of heart failure. To date, the benefit of Mineralocorticoid-receptor antagonist (MRA) administration in haemodialysis patients remains subject to discussion. Biomarkers play an important role in therapy guidance and pose a promising tool to detect pathological processes of heart failure in an earlier stage. The randomised-controlled Mineralocorticoid-Receptor Antagonists in End-Stage Renal Disease (MiREnDa) trial was set up to investigate the effect of 50 mg of spironolactone once daily on left ventricular mass index in haemodialysis patients and several secondary endpoints. This dissertation reports findings from the MiREnDa trial on (a) the efficacy of spironolactone to influence serum levels of biomarkers of heart failure, fibrosis and inflammation as well as electrolytes and (b) the ability of N-terminal pro-B-type natriuretic peptide (NT-proBNP), Galectin-3 and soluble source of tumorigenicity 2 (sST2) to reflect left ventricular hypertrophy and diastolic dysfunction assessed by key imaging characteristics. Treatment of spironolactone over a period of 40 weeks did not alter serum levels of biomarkers of heart failure, fibrosis and inflammation including NT-proBNP, Galectin-3 and sST2. A small but significant effect on serum sodium but not potassium was observed. Only NT-proBNP was significantly different in the presence or absence of left ventricular hypertrophy (LVH) (normal vs. LVH (median [IQR]): 2,120 [810; 5,040] vs. 6,340 [2,410; 15,360] pg/ml, $p<0.01$) or moderate and severe diastolic dysfunction (DD) (normal diastolic function and DD grade I vs. DD grade II and DD grade III: 2,300 [850; 6,050] vs. 12,260 [3,340; 34,830] pg/ml, $p=0.02$). NT-proBNP further showed a significant correlation at baseline with LVMi (Spearman's $\rho=0.41$, $p<0.001$), LAVi (Spearman's $\rho=0.55$, $p<0.001$) and septal E/e' (Spearman's $\rho=0.45$, $p<0.001$). No correlation could be observed between Galectin-3 and the investigated functional and morphological parameters. sST2 was mildly correlated to LVMi at baseline (Spearman's $\rho=0.21$, $p=0.05$) and NT-proBNP at baseline (Spearman's $\rho=0.37$, $p<0.001$). In conclusion, spironolactone did not relevantly affect any of the investigated parameters and only NT-proBNP proved to be significantly correlated to cardiac imaging measurements.

- German version -

Dialysepatienten erkranken häufig an verschiedenen Formen der Herzinsuffizienz. Zugleich ist der Nutzen von Mineralkortikoidrezeptorantagonisten bei Dialysepatienten bis heute umstritten. Biomarkermessungen ermöglichen es, pathologische Prozesse am Herzen in einem möglichst frühen Stadium zu erkennen. In der randomisiert-kontrollierten "Mineralocorticoid-Receptor Antagonists in End-Stage Renal Disease" (MiREnDa) Studie wurde der Effekt der täglichen Einnahme von 50 mg Spironolacton auf den linksventrikulären Massenindex bei Dialysepatienten zusammen mit verschiedenen sekundären Endpunkten untersucht. Diese Arbeit beleuchtet Ergebnisse der MiREnDa-Studie zur Wirkung von Spironolacton auf Serumspiegel von Biomarkern für Herzinsuffizienz, Fibrose und Entzündung sowie von Elektrolyten. Darüber hinaus wird der Zusammenhang zwischen N-terminalen natriuretischen Peptid Typ B (NT-proBNP), Galectin-3 und Soluble source of tumorigenicity 2 (sST2) und Veränderungen in den wichtigsten bildgebenden Merkmalen linksventrikulärer Hypertrophie und diastolischer Dysfunktion beschrieben. Die Einnahme von Spironolacton über 40 Wochen hatte keinen Effekt auf Biomarker für Herzinsuffizienz, Fibrose und Entzündung wie NT-proBNP, Galectin-3 und sST2. Lediglich die Natriumspiegel, nicht aber die Kaliumspiegel, wurden signifikant beeinflusst. NT-proBNP unterschied sich signifikant zwischen Patient*innen mit und ohne links-ventrikulärer Hypertrophie (LVH) (normal vs. LVH (Median [IQR]): 2.120 [810; 5.040] vs. 6.340 [2.410; 15.360] pg/ml, $p<0,01$) beziehungsweise mit und ohne relevanter diastolischer Dysfunktion (DD) (normale diastolische Funktion und DD Grad I vs. DD Grad II und DD Grad III: 2.300 [850; 6.050] vs. 12.260 [3.340; 34.830] pg/ml, $p=0,02$). NT-proBNP korrelierte außerdem signifikant mit LVMi (Spearman's $\rho=0,41$, $p<0,001$), LAVi (Spearman's $\rho=0,55$, $p<0,001$) und E/e' (Spearman's $\rho=0,45$, $p<0,001$). Galectin-3 war unabhängig von allen untersuchten Parametern. sST2 korrelierte mäßig mit LVMi (Spearman's $\rho=0,21$, $p=0,05$) und deutlich mit NT-proBNP (Spearman's $\rho=0,37$, $p<0,001$). Zusammenfassend beeinflusste Spironolacton keinen der untersuchten Parameter relevant und lediglich NT-proBNP wies eine signifikante Korrelation zu kardialen Bildgebungsparameters auf.

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Appendix

I Abbreviations

Abbreviation	Full text
25H-VitD	25-Hydroxy-Vitamin D
A	Late peak trans-mitral flow rate
ACE	Angiotensin-converting enzyme
ACEi	Angiotensin-converting enzyme inhibitor
ANCOVA	Analysis of covariance
ARB	Angiotensin receptor blocker
ASE	American Society of Echocardiography
BMI	Body mass index
BNP	Brain natriuretic peptide
BP	Blood pressure
CKD	Chronic kidney disease
CMR	Cardiac magnet resonance
CRP	C-reactive protein
DD	Diastolic dysfunction
E	Early peak trans-mitral flow rate
E/A	Ratio of E to A
E/e’	Ratio of E to e’
e’	Early peak mitral annular tissue velocity
EDTA	Ethylenediamine-tetraacetic acid
ELISA	Enzyme-linked immunosorbent assays
ERA-EDTA	European renal association – European dialysis and transplant association
ESC	European Society of Cardiology
ESKD	End stage kidney disease
et al.	Et alii
FGF-23	Fibroblast growth factor 23
Gal-3	Galectin-3
GFR	Glomerular filtration rate
HD	Haemodialysis
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction

Abbreviation	Full text
HQ	Headquarter
IL	Interleukin
iPTH	Intact parathyroid hormone
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
LAV	Left atrial volume
LAVi	LAV index
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMi	LVM index
MiREnDa	Mineralocorticoid Receptor Antagonists in End-Stage Renal Disease
MRA	Mineralocorticoid-receptor antagonist
n	Number
NT-proBNP	N-terminal pro B-type natriuretic peptide
NYHA	New York Heart Association
p	p-value
pw	Pulsed wave
SD	Standard deviation
sST2	Soluble source of tumorigenicity 2
TropT	Troponin T
TR-velocity	Tricuspid regurgitation peak velocity

II Figures

III Tables

IV Acknowledgements

Since I first approached Prof. Wanner in 2016, it has been an ongoing challenge to discover new aspects and approach the idea for this thesis from different perspectives.

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V Curriculum vitae

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VI Publications

Research articles

Hauser T, Dornberger V, Malzahn U, *et al.*: The effect of spironolactone on diastolic function in haemodialysis patients. Int J Cardiovasc Imaging 2021.

Fabian Hammer, Salvatore S. Buehling, Jaber Masyout, […], Tobias Hauser, *et.al.*: Protective effects of spironolactone on vascular calcification in chronic kidney disease (submitted).

Poster presentations and congress attendances

Wanner C, Malzahn U, Donhauser J, […] Hauser T, *et al.*: The effect of spironolactone on left ventricular mass in hemodialysis patients: the MiREnDa study – a randomized controlled trial. ASN Kidney Week 2017.

Hauser T, Malzahn U, Betz C, *et al.*: Mineralocorticoid hormone antagonism and its metabolic consequences in hemodialysis patients – The MiREnDa study. Synlab Symposium "Biomarkers of the Cardiorenal Axis" 2018.

Hauser T, Malzahn U, Betz C, *et al.*: The prognostic value of nephro-cardiac biomarkers in hemodialysis patients – The randomized-controlled MiREnDa trial. 55th ERA-EDTA Congress 2018.

Hauser T, Dornberger V, Malzahn U, *et al.*: Cardiovascular biomarkers in relation to diastolic dysfunction in hemodialysis patients. Synlab Symposium "Biomarkers of the Cardiorenal Axis" 2019.

Hauser T, Dornberger V, Malzahn U, *et al.*: The impact of spironolactone on diastolic function in hemodialysis patients – Data from the MiREnDa RCT. 57th ERA-EDTA Congress 2020.

VII Supplements

The data generated during the MiREnDa trial are not publicly available due to data protection and privacy. The datasets including standard operating procedures can be made available on request.

Supplemental table 1: *Participating institutions*

DZ: dialysis centre, KfH: Kuratorium für Dialyse und Nierentransplantation e.V., PHV: Patientenheimversorgung

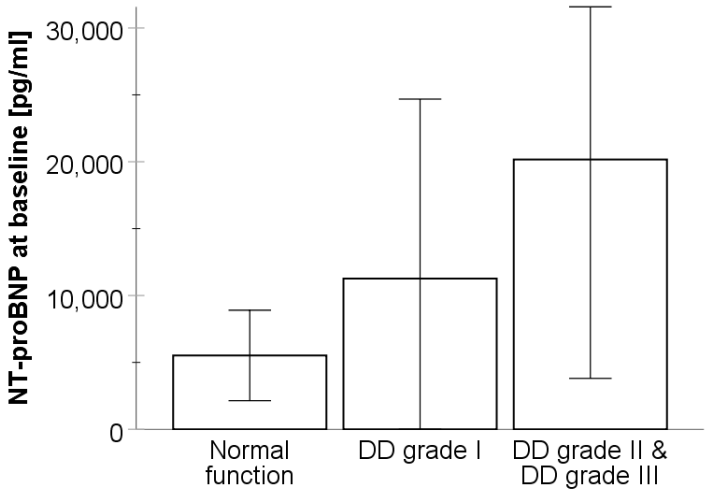
Type of facility	**Name and location**
University hospital trial centres	University Hospital Würzburg, Würzburg, Germany (Headquarter)
	University Hospital Erlangen-Nürnberg, Erlangen, Germany
	University Hospital Frankfurt, Frankfurt/Main, Germany
Trial coordination office	Centre for clinical trials at the University Hospital Würzburg, Würzburg, Germany
Medical laboratory	University Medicine Greifswald, Greifswald, Germany
Dialysis centres	DZ Bad Windsheim, Bad Windsheim, Germany
	DZ Karlstadt, Karlstadt, Germany
	DZ Langen, Langen, Germany
	DZ Mainz, Mainz, Germany
	DZ Marburg/PHV Marburg, Marburg, Germany
	DZ Schweinfurt, Schweinfurt, Germany
	DZ Würzburg, Würzburg, Germany
	KfH Bad Kreuznach, Bad Kreuznach, Germany
	KfH Bamberg, Bamberg, Germany
	KfH Coburg, Coburg, Germany
	KfH Frankfurt/Main-Ginnheim, Frankfurt/Main, Germany
	KfH Fürth, Fürth, Germany
	KfH Kronach, Kronach, Germany
	KfH Lichtenfels, Lichtenfels, Germany
	KfH Lohr, Lohr/Main, Germany
	KfH Nürnberg, Nürnberg, Germany
	KfH Ochsenfurt, Ochsenfurt, Germany
	KfH Zirndorf, Zirndorf, Germany
	Sozialstift Bamberg, Bamberg, Germany
	University Hospital Frankfurt, Frankfurt/Main, Germany

Supplemental table 2: *Used laboratory analysis systems sorted by substance*

CRP: C-reactive protein, FGF-23: Fibroblast growth factor-23, iPTH: Intact parathyroid hormone, NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2

Substance	**Analysis systems**
25-OH-Vitamin-D	Elecsys Vitamin D_3 (25-OH) ECLIA Test, Ref. 03314847 190, Roche Diagnostics GmbH, Germany
Albumin	ALB Flex® reagent cartridge, Cat. No. K1013, Siemens Healthcare Diagnostics Ltd., UK [a]
Aldosterone	Radioimmunoassay RIA ALDOSTERONE, Ref. No. IM1664, Beckman Coulter, Inc., Brazil
Calcium	CA Flex® reagent cartridge, Cat. No. K1023, Siemens Healthcare Diagnostics Ltd., UK [a]
CRP	CardioPhase® hsCRP Flex® reagent cartridge, Cat No. K7046, Siemens Healthcare Diagnostics Products GmbH, Germany [a]
FGF-23	multi-matrix FGF23 (C-terminal) ELISA Cat. No. BI-20702, Biomedica Medizinprodukte GmbH & Co KG, Austria
Galectin-3	Quantikine® ELISA Human Galectin-3 Immunoassay, Cat No. DGAL30/SGAL30/PDGAL30, R&D Systems Europe, Ltd., UK
iPTH	Elecsys PTH STAT ECLIA Test, Ref. 04892470 190, Roche Diagnostics GmbH, Germany
NT-proBNP	PBNP Flex® reagent cartridge, Cat. No. K6423A, Siemens Healthcare Diagnostics Ltd., UK [a]
Phosphate	PHOS Flex® reagent cartridge, Cat. No. K1061, Siemens Healthcare Diagnostics Ltd., UK [a]
Potassium	V-LYTE® Integrated Multisensor, Cat No. K800A, Siemens Healthcare Diagnostics Ltd., UK [a]
Sodium	V-LYTE® Integrated Multisensor, Cat No. K800A, Siemens Healthcare Diagnostics Ltd., UK [a]
sST2	Quantikine® ELISA Human ST2/IL-33 R Immunoassay, Cat No. DST200, R&D Systems Europe, Ltd., UK
Troponin T	Elecsys Troponin T hs ECLIA Test, Ref. 05092744 190, Roche Diagnostics GmbH, Germany
Urea	BUN Flex® reagent cartridge, Cat. No. K1021, Siemens Healthcare Diagnostics Ltd., UK [a]

[a] *utilising the 'SIEMENS Dimension Vista® System' platform*



Supplemental figure 1: *Comparison of NT-proBNP serum levels between patients with normal diastolic function, diastolic dysfunction (DD) grade I and DD grade II and III*

NT-proBNP serum levels were significantly higher in patients presenting with DD grade II and DD grade III compared to patients with normal diastolic function (Kruskal-Wallis-Test: p=0.04); there were no further significant differences between the three groups*

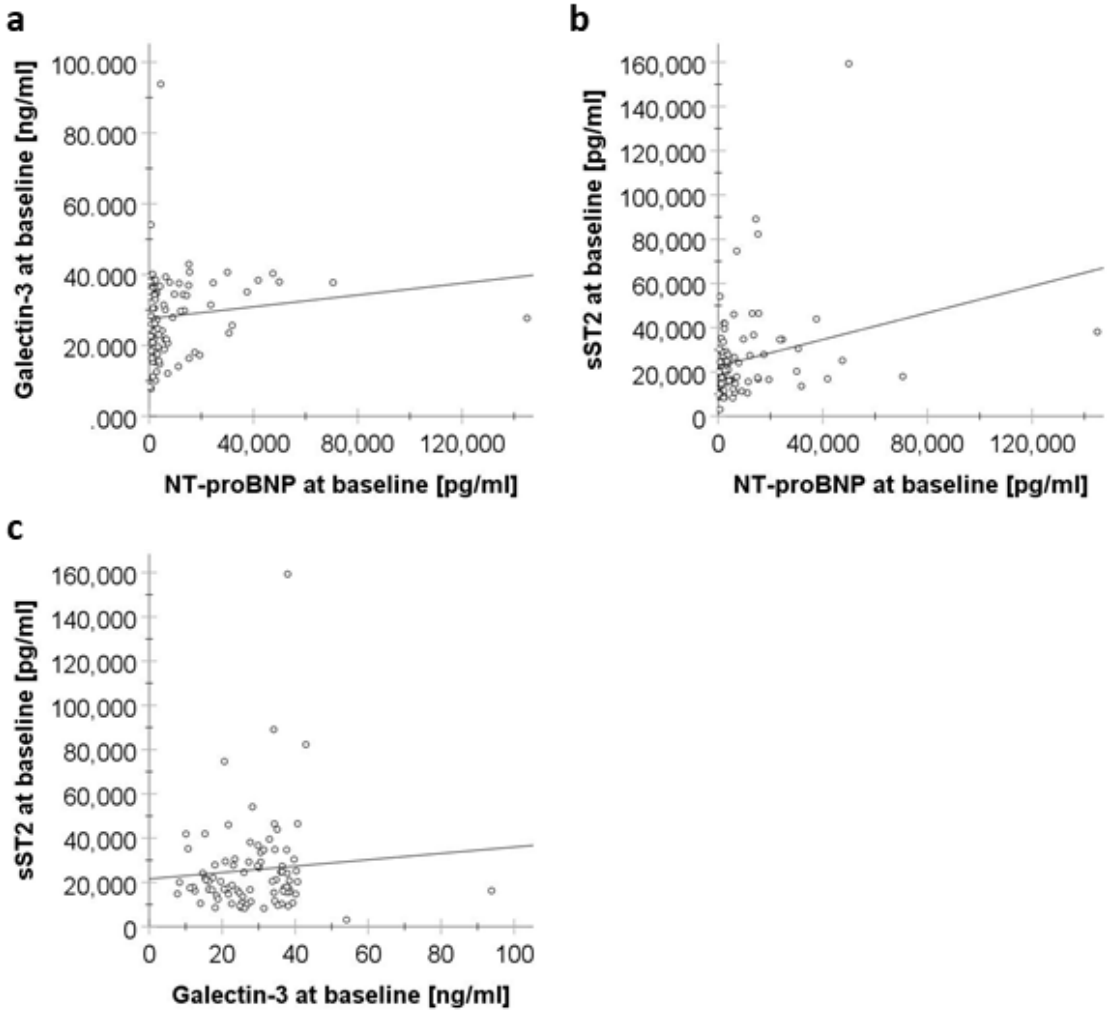
Group size: Normal function: n=36, DD grade I: n=22, DD grade II & DD grade III: n=10

Missing data: data from 18 patients are not included in this figure as their diastolic function was indeterminable with the used diagnostic algorithm

** Bonferroni corrected for multiple comparisons*

DD: Diastolic dysfunction, NT-proBNP: N-terminal pro B-type natriuretic peptide

Whiskers represent 95% confidence interval



Supplemental figure 2: *Biomarker correlation to each other at baseline*

(a) Baseline NT-proBNP serum levels did correlate mildly with Galectin-3 at baseline without reaching a level of significance (Spearman's ρ=0.19, p=0.08); (b) Baseline NT-proBNP serum levels correlated significantly with sST2 at baseline (Spearman's ρ=0.37, p<0.001); (c) Baseline Galectin-3 serum levels did not correlate with sST2 at baseline (Spearman's ρ=0.05, p=0.68)

NT-proBNP: N-terminal pro B-type natriuretic peptide, sST2: Soluble source of tumorigenicity 2