Using Repeated Measures of Blood Biomarkers and Physical Biomeasures to Define Changes inVolume Status in Patients with Decompensated Heart Failure, Normal Volunteers and Patients with Stable Left Ventricular Systolic Dysfunction

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Abstract

Background

The non-invasive assessment of volume status in left ventricular systolic dysfunction (LVSD) is challenging. The main thesis objective was to establish the feasibility and potential clinical utility of repeated measures assessment of non-invasive biomarkers in defining changes in volume status within individual volunteers.

Methods

Differential volume manipulation protocols were achieved in a three-staged plan of investigation, firstly, in patients with decompensated heart failure receiving intravenous furosemide, secondly, in normal volunteers receiving acute loads of oral water or intravenous saline, and thirdly, in stable LVSD patients on chronic furosemide dosing undergoing staged diuretic withdrawal and resumption. Repeated measures of biomarkers relevant to volume status including blood and urine biomarkers, echocardiographic and bioimepdance measures were performed to assess their sensitivity to the induced volume changes.

Results Summary

I demonstrated the smallest variance for bioimpedance measures, and the largest variance for urine biomarkers. In the patients with decompensated heart failure, none of the biomarkers studied showed potential clinical utility at tracking acute volume response to intravenous furosemide. In the normal volunteers, a significant change in estimated blood volume was observed following intravenous saline, but not with acute oral water ingestion. Only whole-body and trunk bioimpedance measures, and to a lesser extent, mitral valve early peak

velocity with and without the Valsalva manoeuvre, appeared sensitive enough to map these changes in volume status. In the stable LVSD patients, statistically significant increases in B-type natriuretic peptide, urinary creatinine, urinary kidney injury molecule 1, and in bioimpedance-estimated body water composition were observed with diuretic withdrawal, with levels of these markers reducing following diuretic resumption. However, the amplitude of the changes observed was either lower than the respective within-subject variance or too small to be potentially useful as a marker of volume change. This would thus limit the clinical utility of these biomarkers in the routine monitoring of volume status in LVSD.

Conclusion

The repeated measures of biomarkers studied in response to different volume manipulations were interpreted in the context of their within-subject normal variance. None of the biomarkers studied appeared to have the ideal characteristics clinically for the monitoring of subclinical changes in volume status in stable LVSD or in response to acute diuresis in decompensated heart failure. The significant increases in urine biomarkers following diuretic withdrawal in stable LVSD suggested potentially beneficial renal effects of furosemide in stable LVSD.

For

My family

Declaration

I, Jennyfer Ng Kam Chuen, declare that this thesis for the degree of Doctor of Medicine has been composed by myself and has not been accepted in any previous application for a degree. I have collaborated with a number of colleagues in the University of Birmingham Centre for Cardiovascular Sciences, City Hospital, Birmingham in order to recruit patients and perform some of the clinical investigations and have recognised their contribution under acknowledgements. I have made every effort to reference previously published work.

The studies presented in this thesis were all approved individually by the Sandwell and West Birmingham Local Research Ethics Committee. Written informed consent was obtained formally from every volunteer that was recruited.

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Abbreviations used in this Thesis

ACE	Angiotensin converting enzyme
ADH	Antidiuretic hormone
ANP	Atrial natriuretic peptide
BIA	Bioimpedance analysis
d	*Distal (whole-body) electrode placement*
p	*Proximal (trunk) electrode placement*
ap	*Anterior-posterior electrode placement for right-lung BIA*
sr	*Sternum-rib electrode placement for right-lung BIA*
BNP	B-type natriuretic peptide
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVa	Analytical variance
CVo	Intra-observer/intra-device variability
CVt_{ws}	Within-subject normal variance (within-subject total variance)
CVt_{bs}	Between-subject variance
dP/dt	Rate of rise of ventricular pressure
ECW	Extracellular water
E/Ea(l)	Ratio of mitral peak early velocity to tissue Doppler early diastolic mitral annulus velocity at lateral wall
E/Ea(s)	Ratio of mitral peak early velocity to tissue Doppler early diastolic mitral annulus velocity at septal wall
*e*PAD	Estimated pulmonary arterial diastolic pressure
GFR	Glomerular filtration rate
Hb	Haemoglobin
Hct	Haematocrit
HF	Heart failure
IMA	Ischaemia-modified albumin
ICW	Intracellular water
IVC	Inferior vena cava
L	*Long axis view*
S	*Short axis view*
e	*Expiration phase*
i	*Inspiration phase*
mi	*Maximal inspiration phase*
IVCCI	IVC collapsibility index
IVCD	IVC corrected for body surface area
LV	Left ventricle
LVEDP	Left ventricular end diastolic pressure
LVEDV	Left ventricular end diastolic volume
LVEF	Left ventricular ejection fraction
LVSD	Left ventricular systolic dysfunction
mPAP	Pulmary artery mean pressure
MVA	Mitral Valve late peak velocity
MVDT	Mitral Valve early deceleration time
MVE	Mitral Valve early peak velocity
MVE/A	Ratio of mitral valve early peak to late peak velocities

NP	Natriuretic peptides
NTproANP	N-terminal pro-atrial natriuretic peptide
NTproBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association functional class
PCWP	Pulmonary capillary wedge pressure
PADP	Pulmonary artery diastolic pressure
PAOP	Pulmonary artery occlusion pressure
PASP	Pulmonary artery systolic pressure
RAAS	Renin angiotensin aldosterone system
RCV	Reference change value
sNGAL	Serum N-acetyl beta-d-glucosaminidase
SNS	Sympathetic nervous system
TBW	Total body water
uKIM-1	Urinary kidney injury molecule-1
uNAG	Urinary neutrophil gelatinase associated lipocalin
uNGAL	Urinary N-acetyl beta-d-glucosaminidase
vMVDT	Mitral valve early deceleration time following Valsalva manoeuvre
vMVE	Mitral valve early peak velocity following Valsalva manoeuvre
vMVE/A	Ratio of mitral valve early peak to late peak velocities following Valsalva manoeuvre
Z	Impedance

Publications

Papers

Chapter 2

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Repeated assessment of physical biomeasures or blood biomarkers for the definition of blood volume and central cardiac loading in LVSD. Biomarkers in Medicine 2007; 1 (3): 355-374.

Chapter 5

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Performing repeated non-invasive bedside measures of volume response to intravenous furosemide in acute pulmonary oedema: a feasibility assessment. Cardiovasc Ther 2009; 27 (2): 89-95.

Chapter 7

Damman K, Ng Kam Chuen MJ, MacFadyen RJ, Lip GYH, Gaze D, Collinson PO, Hillege HL, van Oeveren W, Voors AA, van Veldhuisen DJ. Volume status and diuretic therapy in systolic heart failure, and the detection of early abnormalities in renal and tubular function. Accepted for publication by J Am Coll Cardiol 2011; 57(22):2233-41.

Abstracts

Chapter 5

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Comparing the sensitivity of non-invasive markers of volume response to intravenous furosemide in patients with acute pulmonary oedema. Eur J Heart Fail. Suppl 2007; 6 (1): 111-112.

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Comparing the sensitivity of non-invasive markers of volume response to intravenous furosemide in patients with acute pulmonary oedema. Br J Clin Pharmacol 2007; 63 (6): 767-791.

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Whole body and right lung bioimpedance are more sensitive than proximal bioimpedance at predicting response to intravenous furosemide in patients with acute pulmonary oedema. Br J Clin Pharmacol 2007; 63 (6): 767-791.

Chapter 6

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Haematocrit and urine volume response to acute intravenous saline and oral water loading in normal human volunteers. FASEB J. 2008 22:1159.2.

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Short axis inferior vena cava measures are more sensitive than long axis measures in defining acute volume loading in normal volunteers. FASEB J. 2008 22:970.3

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. The sensitivity of echocardiographic parameters and bioimpedance analysis in defining response to an acute volume load in normal volunteers. Heart Suppl II. 2008; 94: A72-A73

Chapter 7

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Whole body bioimpedance is more sensitive than regional bioimpedance at mapping volume response to diuresis in left ventricular systolic dysfunction. Eur Heart J 2008; 29 (Abstract Suppl.): 557.

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Bioimpedance analysis is more sensitive than body weight in detecting asymptomatic diuretic-induced volume changes in stable patients with LVSD. Heart Suppl II. 2008; 94: A108.

Ng Kam Chuen MJ, Lip GYH, MacFadyen RJ. Echocardiographic parameters as a non-invasive means of volume response to asymptomatic, diuretic-induced volume changes in stable left ventricular systolic dysfunction. Eur Heart J 2009; 30 (Abstract Suppl): 569.

Presentations

BPS: British Pharmacological Society
MRS: Medical Research Society
ACC: American College of Cardiology
FASEB: Federation of the American Society of Experimental Biology
ESC: European Society of Cardiology
ESC HF: European Society of Cardiology Heart Failure Congress

	Type	Meeting
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volume response to intravenous furosemide in patients with acute pulmonary oedema.	Poster	ESC HF Congress 2007
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Short-axis vena cava measures are more sensitive than long-axis measures in defining acute volume loading in	Poster	MRS 2008
normal volunteers.	Poster	FASEB 2008
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Right lung impedance determined by sternum rib electrode position outperforms right lung impedance determined by anterior posterior electrode position in defining acute volume changes in normal volunteers and heart failure patients.	Poster	MRS 2008
The sensitivity of echocardiographic parameters and bioimpedance analysis in defining response to an acute volume load in normal volunteers.	Poster	BCS 2008

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Chapter 7		
Comparing the sensitivity of bioimpedance analysis and body weight to detect asymptomatic diuretic-induced volume changes in stable patients with left ventricular systolic dysfunction.	Poster Poster	MRS 2008 ACC 2008
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Chapter One

Introduction

Volume Status in Left Ventricular Systolic Dysfunction: Clinical Relevance, Determinants, Pathophysiology and Therapeutic Manipulation

1.1 Introduction

Heart failure (HF) is a syndrome characterised by a broad spectrum of heterogeneous symptoms and signs, typically dyspneoa, lethargy and signs of fluid retention, in the presence of objective evidence of a functional or structural abnormality of the heart [Dickstein et al., 2008]. The leading cause of the significant health burden of HF is acute decompensation, with 80 % of acute HF presentations occurring (often recurrently) in patients with a documented diagnosis of HF [Cleland et al., 2003]. Defined by a coherent reduction in left ventricular (LV) systolic function, left ventricular systolic dysfunction (LVSD) is a readily identifiable type of HF which is present in approximately half of patients with a diagnosis of chronic HF, and in between half and two-thirds of acute HF presentations [Adams et al., 2005; Nieminen et al., 2006]. Despite a number of therapeutic strategies with proven benefits based on large scale clinical trials contributing to improved 0prognosis in LVSD, an admission with decompensated HF in patients with LVSD still conveys a poor prognosis [Gheorghiade et al., 2005]. Thus, LVSD patients constitute a significant subset of the HF population in whom the prediction of decompensation is an important clinical goal.

1.2 Clinical relevance of abnormal volume status in LVSD

1.2.1 Abnormal volume status as a cause for decompensation

Acute decompensation in HF is defined as the presence of signs and symptoms requiring urgent treatment or hospitalisation. Whereas factors such as sepsis, worsening renal failure, ischaemia, paroxysmal arrhythmia, uncontrolled hypertension and non-adherence to diet and medication can precipitate decompensation [Fonarow et al., 2008; Tsuyuki et al., 2001; Nieminen et al., 2006], the role of abnormal volume status (whether hypervolaemia, hypovolaemia or volume redistribution) as the final mechanism, and thus as the cause for

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acute decompensation, has not been extensively studied. Registry data suggests that abnormal volume status is a significant contributor to HF hospitalisations, with approximately 66% of patients having auscultatory rales and peripheral oedema, and 74% of patients having evidence of lung congestion on admission chest radiographs [Fonarow et al., 2007].The study by Zile et al. [2008] is pivotal in supporting the fact that abnormal volume status (as evidenced by significant changes in heamodynamic measures) indeed plays a significant, if not the final, mechanistic role in decompensated HF. These authors used continuous invasive haemodynamic monitoring of stable HF patients in New York Heart Association (NYHA) functional class III-IV to determine changes in estimated pulmonary capillary wedge pressure (PCWP) in the transition from symptomatically stable HF to their end point of a HF-related event, defined as an acute unexpected presentation requiring intravenous therapy. Hypervolaemia, as adjudicated by an independent and blinded Clinical Events Review Committee, was present in 181 of the 197 acute HF-related events. Hypervolaemic presentations were associated with significant increases in estimated PCWP, and subsequent reductions in cardiac pressures occurred after treatment. The remaining 16 acute HF-related events were adjudicated to be due to hypovolaemia, hypotension and/or prerenal azotaemia. Hypovolaemic presentations were associated with reduced estimated PCWP. Patients who did not have a HF-related event did not have significant changes in their central cardiac pressures.

1.2.2   Volume status as a mechanistic target for prognostic treatment

Furthermore, the presence of abnormal volume status in LVSD is also associated with an independently poor prognosis. The presence of symptoms and clinical signs of volume overload, raised cardiac filling pressures and increased blood/plasma volume, are all

predictors of increased morbidity and mortality in LVSD [Androne et al., 2004; Drazner et al., 2001]. On the other hand, hypovolaemia, mainly in the context of diuretic overuse, is associated with detrimental neurohormonal activation and poorer renal function with increasing diuretic dosage, these also being adverse prognostic markers in LVSD [Eshaghian et al., 2006; Dries et al., 2000].

The mechanisms by which therapeutic strategies in LVSD improve prognosis range from effects on cardiac remodelling, prevention of progression of atheroslecrosis, blocking detrimental neurohormonal activation and reduction of oxidative stress and inflammation [Pfeffer et al., 2006; Francis et al., 1995; Trachtenberg et al., 2009; Brauwnwald, 2008]. However, the complex mechanism of abnormal volume status in LVSD remains poorly understood and the prevention of abnormal volume status as a prognostic target has not been fully evaluated to date. Given the significant role of abnormal volume status in decompensated HF, specific treatments aimed at maintaining optimal volume status could result in reduced morbidity and mortality.

1.3   Determinants of volume status

1.3.1   Volume compartments of relevance in LVSD

In the context of LVSD, *volume status* is a term used to describe whether a patient is hypervolaemic, hypovolaemic or euvolaemic. Thus hypervolaemia or volume overload tends to describe a state of excessive salt and water retention manifesting as increased jugular venous pressure (increased circulatory volume) and/or dyspnoea, rales, dependent oedema and ascites (increased interstitial space volume). However, each of the above states of volume

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can occur with or without redistribution of fluid between volume compartments secondary to, for example, changes in vascular tone, such that an acute presentation with signs and symptoms of volume overload may not necessarily be secondary to excessive salt and water retention, but may be due to *volume redistribution* between the interstitial and intravascular spaces [Cotter et al., 2008]. Thus, an understanding of the dynamic interplay between the volume compartments of body water and the factors that influence them is crucial in order to gain insight into the determinants of abnormal volume status in LVSD.

The composition of body water is illustrated in Figure 1.1, with the compartments of most relevance in LVSD highlighted. Determinants of stroke volume include cardiac preload, afterload and contractility. Extracellular volume constitutes of intravascular volume [also termed *blood volume*, *circulatory volume*, or *plasma volume* (non-cellular component of intravascular volume)] and interstitial volume. The exchange of fluid across the vascular capillary bed between intravascular volume and the interstitial space is under the influence of Starling's law, the balance between hydrostatic pressure (determined by *systemic vascular resistance* or *cardiac afterload*) and colloid oncotic pressure. The majority of intravascular volume (70-85%) lies within the venous compartment due to the larger capacitance of this compartment compared to the arterial compartment. Fluid volume within the venous compartment contributes to the venous return to the heart or the cardiac preload, measured as LV filling volume or LV filling pressure at the end of diastole [LV end diastolic volume (LVEDV) and LV end diastolic pressure (LVEDP) respectively]. LVEDP is equivalent to left atrial pressure in the absence of mitral valve disease; this can in turn be estimated by the PCWP. Fluid volume within the arterial compartment is responsible for perfusion of peripheral organs. Another relevant compartment in LVSD is *red cell volume* which is

relatively reduced in the presence of increased plasma volume resulting in haemodilutional anaemia, a predictor of poor outcome in LVSD [Androne et al., 2003].

Figure 1.1 Volume compartments of relevance in LVSD.
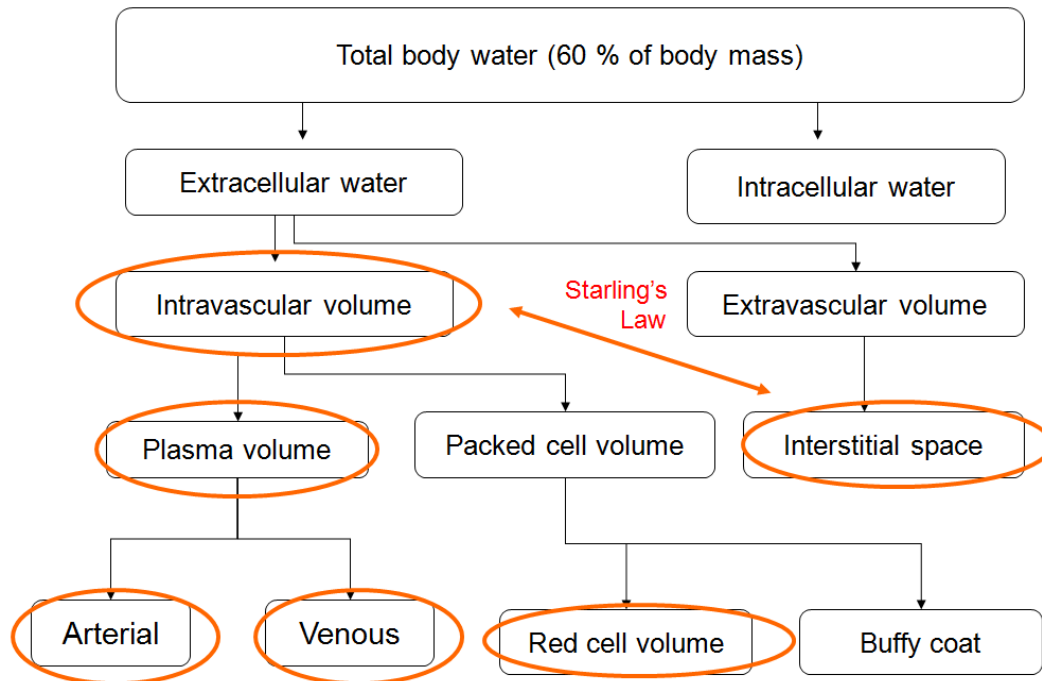
Under normal physiological conditions, extracellular water constitutes one-third of total body water (about 15 L), of which 4/5 is interstitial fluid and 1/5 is intravascular fluid. [Guyton, 2000; Gelman et al., 2008; Braunwald et al., 2004; Adlbrecht et al., 2008].

1.3.2   Pathophysiology of abnormal volume status in LVSD

The active reabsorption of sodium and water by the kidneys is determined mainly by volume alterations in the *arterial component* of circulatory volume [Schrier, 1990]. A drop in cardiac output, such as occurs in LVSD, is sensed by baroreceptors and results in activation of the integrated neurohormonal response. Sympathetic nervous system (SNS) activation results in vasoconstriction and increased heart rate and cardiac contractility, and subsequent activation of the renin angiotensin aldosterone system (RAAS), responsible for increased renal reabsorption of salt and water and further vasoconstriction. Antidiuretic hormone (ADH)

secretion is induced by osmotic and non-osmotic (SNS) stimuli causing renal retention of water independent of sodium reabsorption. The natriuretic system modulates the effects of the other hormones by causing vasodilatation and natriuresis. The net effect of neurohormonal activation is increased sodium and water retention in order to maintain cardiac output and maintain arterial pressure. At this stage, LVSD patients are compensated, and have either no symptoms, or have stable symptoms.

1.3.2.1   *Haemodynamic disturbances*

These compensatory mechanisms only have a finite capacity to sustain cardiac output in the presence of chronic systolic pump failure, such that decompensation eventually ensues. Chronic retention of salt and water results in firstly, elevation of LVEDV and LVEDP, manifesting clinically as elevated jugular venous pressure and hepatomegaly; and secondly to increased capillary hydrostatic pressure resulting in exudation of fluid into the interstitial space, thus producing dependent oedema, pleural effusion, ascites and pulmonary oedema. Furthermore, an inappropriate or sustained increase in vascular resistance may lead to both reduced venous capacitance in the large veins and increased arterial resistance. The former leads to increased venous return or cardiac preload, coupled with net movement of fluid into the interstitial space, and the latter to increased afterload, both potentially detrimental to the failing LV.

The haemodynamic sequelae of decompensation are illustrated in the small early study by Anand et al. [1989], who compared six patients with *untreated* LVSD with clinical signs of volume overload and 11 healthy controls. They demonstrated increased right atrial pressure

and PCWP, and increased systemic and pulmonary arterial resistances in the decompensated patients. The excess *total body water* (TBW) determined by radionuclide techniques in the decompensated patients was 16% above controls (about 4L per patient). This excess TBW was almost entirely in the extracellular space (ECW), with both extravascular and intravascular components increasing proportionately (total extravascular volume rose by 33% and plasma volume rose by 34%).

Similarly, in more contemporary treated patients with severe LVSD who were in cardiogenic shock and awaiting cardiac transplantation, James et al. [1996] found a mean increase in plasma volume of 23% above normal reference values; this was accompanied by similarly increased mean right and left atrial pressures, and reduced cardiac index. In response to treatment interventions, plasma volume reduced to an excess of around 15% of normal values. Much earlier, Davidov et al. [1967] also demonstrated response of plasma volume to treatment in the form of a 25% reduction from baseline in plasma volume following intravenous administration of furosemide in decompensated LVSD patients. Interstital space volume was not assessed in these studies.

On the other hand, Henning et al. [1978] showed that acute cardiogenic pulmonary oedema was associated with increased LVEDP and PCWP in combination with either normal or reduced plasma volume and increased interstitial volume determined by radionuclide techniques. After treatment with the peripheral arterial vasodilator phentolamine, plasma volume increased rather than decreased, with fluid shift from the interstitial space into the intravascular space. Shuster et al.[1994] also showed that there was no significant change in plasma volume in critically ill patients with pulmonary oedema being treated with intravenous

diuretics and who experienced marked diuresis, suggesting that plasma volume expanded (due to fluid shift from the interstitial space as a result of vasodilatation) at a rate approximating the volume removed by diuresis. In patients who failed to respond to diuresis, plasma volume continued to expand despite intravenous diuretic, suggesting movement of fluid from the interstitial space into the intravascular space, which was not then removed by the kidneys.

The above data therefore support the fact that acute cardiogenic pulmonary oedema is associated with the extravasation of large quantities of plasma from the intravascular compartment into the interstitial compartment and subsequent contraction of the intravascular plasma volume. The treatment of acute cardiogenic pulmonary oedema is then associated with the return of fluid from the interstitial compartment back into the intravascular compartment with expansion of plasma volume and reduction of colloid osmotic pressure. Thus, the disagreement between the changes in plasma volume as a result of decompensation and treatment observed in the above studies may simply reflect the dynamic interplay between volume compartments which is involved in volume redistribution; different cohorts of patients may have simply been at different stages of decompensation or improvement with potentially differing renal responses and capacity to effectively excrete excessive salt and water.

1.3.2.2 *Neurohormonal abnormalities*

The baseline neurohormonal tone in decompensated and compensated (with or without baseline angiotensin converting enzyme (ACE) inhibitor treatment) patients is higher than in normal individuals, and the effects of the RAAS predominate over the effects of the

natriuretic system [Volpe et al., 1992; Anand et al., 1989]. Furthermore, LVSD patients fail to escape from the sodium-retaining effect of aldosterone compared to normal subjects despite normalisation of sodium balance [Hensen et al., 1991] due to reduced sodium delivery to the distal tubule and collecting ducts as a result of increased sodium reabsorption in the proximal tubule [Boerrigter et al., 2004; Chen, 2007]. Baroreceptor-mediated non-osmotic release of ADH overrides the osmotic regulation of ADH in LVSD patients [Francis et al., 1990], such that the resulting high baseline ADH levels further enhance water retention [Szatalawicz et al., 1981]. The neurohormonal consequence in LVSD is thus a paradoxical state whereby the kidneys, even with apparently normal function, continue to retain sodium and water despite an increased plasma volume thus spiralling into worsening of the clinical syndrome of HF.

1.3.2.3   Cardiorenal syndrome

The kidney receives 20-25% of total cardiac output, this constituting the highest blood flow per gramme of body weight in the human body. This is not mainly for the metabolic needs of the kidney but in order to maintain glomerular filtration rate (GFR). It is therefore the organ the most likely to be compromised by a true or relative reduction in perfusion pressure which results from the haemodynamic consequences of LVSD.

Renal dysfunction is common in patients with LVSD. Only 26% of a cohort of stable LVSD patients had normal renal function as defined by a GFR of >90 mL/min/1.73 m^2; the majority of these patients (44%) had mild renal impairment (GFR 60-90 mL/min/1.73 m^2), and the remaining 30% had moderate to severe renal impairment (GFR <60 mL/min/1.73 m^2) [Reis et al., 2009]. The incidence of renal impairment in other groups of LVSD patients has varied

depending on criteria used for the definition of renal impairment, but has nevertheless been consistently high [Silverberg et al., 2000; Smith et al., 2006]. There is also evidence of subclinical kidney injury in the form of elevated renal tubular markers even in the presence of normal renal function as estimated by conventional measures of renal function such as serum urea and creatinine (sCreatinine) and GFR [Damman et al., 2008]. The incidence of renal impairment appears higher in hospitalised HF patients compared to outpatients, with 32% of hospitalised patients having moderate to severe renal impairment compared to only 10% of outpatients in one cohort [Smith et al., 2006].

The presence of significant renal impairment confers a poor prognosis in HF in both the acute and chronic settings [Silverberg et al., 2000; Heywood et al., 2007]. One-year mortality of LVSD patients with any renal impairment is around 38%, reaching 51% for patients with moderate to severe renal impairment. The degree of renal impairment also worsens with LV ejection fraction (LVEF) and NYHA class. Worsening renal failure also results in diuretic resistance and is a poor prognostic marker in both acute and chronic HF [Hillege et al., 2000; Metra et al., 2008].

This close relation between renal impairment and LVSD has been termed the cardiorenal syndrome [Ronco et al., 2008]. The most obvious mechanism for renal dysfunction involves reduced renal perfusion [Damman et al., 2007] secondary to reduced cardiac output and/or the impact of cardiorenal drug therapy. Renal blood flow is the most important determinant of GFR. With very low systemic blood pressure, GFR is no longer able to be maintained by autoregulatory mechanisms [Smilde et al., 2009], resulting in sodium and water retention. Other mechanisms that may precipitate renal dysfunction in LVSD include renal hypoxia;

activation of the neurohormonal system with ensuing vasoconstriction, sodium and water retention, and increased venous pressure; immune-mediated damage through endothelial dysfunction and inflammation; the presence and progression of acquired risk factors such as atherosclerosis and hypertension; and anaemia [Damman et al., 2009; Vaidya et al., 2008]. Consequently, a vicious circle of worsening renal and cardiac function ensues, regardless of the initial mechanism, where the result is the same: critically reduced GFR and deranged volume homeostasis.

1.4 Therapeutic manipulation of volume status in LVSD

Given the number of symptomatic and prognostic interventions currently available or under investigation for the treatment of LVSD, it is foreseeable that specific treatments may be tailored for each individual patient in order to achieve optimal volume status. While RAAS blockade impacts on volume status by modulating neurohormonal status [Pfeffer et al., 2006; Dickstein et al., 1995; Shyu, 2005; Pitt et al., 1999; Pitt et al., 2003], other treatments have a more direct impact on volume status, such as by their effects on the kidneys and haemodynamic tone, and will be discussed below.

1.4.1 Diuretics

The thiazide, loop and potassium-sparing diuretics act on different sites of the neprhon and by varying mechanisms to increase sodium and water excretion [Jentzer et al., 2010]. The loop diuretic furosemide, is actively secreted by the proximal tubules into the urine, and acts on the ascending limb of the loop of Henle by inhibiting the NA-K-Cl$_2$ transporters. Loop diuretics

are a mainstay of treatment in LVSD guidelines, having the most perceived impact on symptoms of acute and chronic volume overload [Dickstein et al., 2008]. 90% of patients admitted with decompensated HF are treated with loop diuretics, and the dose of loop diuretics is frequently titrated against clinical signs and symptoms of congestion in chronic HF patients [Peacock et al., 2009]. In the congested LVSD patient, administration of intravenous furosemide results in rapid reduction in LV filling pressures and improvement in cardiac output, as well as an almost immediate relief of symptoms of decompensation [Dikshit et al., 1973; Verma et al., 1987]. These effects are secondary to a rapid venodilatory effect resulting in net movement of fluid from the interstitial space (most critically that involved in compromising gas exchange in the alveoli) back into the intravascular compartment [Pickkers et al., 1997]. The resulting increase in overall plasma volume and relief of hypoxic renal vasoconstriction can then contribute to increased natriuresis and diuresis, which occurs within 30 minutes of intravenous administration, and peaks at 1.5h [McMurray et al., 2009].

There is no doubt that background diuretic treatment is paramount to long term care of a substantial proportion of LVSD patients. Braunschweig et al. [2002], using continuous haemodynamic monitoring in a small cohort of stable LVSD patients in whom oral diuretics were withdrawn for six weeks, showed significant deterioration of haemodynamic parameters and worsening of symptoms. These returned to baseline values with reinstitution of chronic oral diuretic therapy.

Combination therapies are routinely applied in clinical practice in the congested patient [Jentzer et al., 2010]. However, administration of incremental doses or combinations of

diuretics in non-congested LVSD patients who are already on maintenance oral diuretic can result in reduced intravascular volume. Thus, while plasma volume is fundamentally expanded in untreated LVSD patients, and for an unknown proportion of symptomatically decompensated LVSD patients, Feigenbaum et al. [2000] determined that plasma volume in clinically stable LVSD patients on chronic diuretic therapy was in fact contracted by approximately 23% when compared to known euvolaemic controls. Although Bonfils et al. [2010] demonstrated no significant difference between plasma volume and extracellular volume in stable LVSD patients compared to healthy volunteers, they showed a significant tendency towards a contraction in plasma volume with increasing furosemide dose in their LVSD patients.

Diuretic-induced intravascular contraction results in inappropriate RAAS and SNS activation over and above that which can be suppressed by routine neurohormonal antagonist therapies resulting in further deterioration of cardiac performance [Francis et al., 1985]. Volume independent mechanisms can also play a role in this inappropriate neurohormonal activation, such as direct stimulation of renin release by blocking sodium uptake at the macula densa and upregulation of renin gene expression in the kidney, thus blocking the tubuloglomerular feedback [Ellison et al., 2001].

Intravascular volume contraction can also be linked to declining renal perfusion secondary to reduced renal blood flow and GFR, such that the emergent cardiorenal syndrome in fact can be due to the therapy as much as the disease. In the SOLVD study, patients taking diuretics had higher sCreatinine levels compared to patients not taking diuretics [Dries et al., 2000]. Furthermore, higher doses of loop diuretic are linked to worse renal function in LVSD

patients, patients with pre-existing renal dysfunction requiring higher doses of loop diuretic to achieve adequate trans glomerular concentration of drug in the urine in order to reach their site of action in the kidney [Neuberg et al., 2002; Gottlieb et al., 2002]. The recognition and definition of mechanism of this renal resistance to diuretic treatment is a major problem in LVSD management [MacFadyen et al., 2003; Brater, 1994].

Loop diuretic therapy has no controlled trial evidence of prognostic benefit in LVSD patients. Whether loop diuretics may improve prognosis in LVSD by their mode of action on abnormal volume status thus remains unclear. Indirectly, there is evidence that patients taking non-potassium sparing diuretics have a worse prognosis compared to patients who are not treated with diuretics or patients who are on a potassium-sparing diuretic [Domasnki et al., 2003; Domanski et al., 2006]. There is a dose-related relationship with increasing mortality, and patients with the most altered doses of diuretics are also most likely to die [Eshaghian et al., 2006]. These of course can be patients with inadequate treatment or those whose treatment is uptitrated due to worsening of their underlying HF; either can play a variable role in emergent mortality.

1.4.2   Vasodilators

Despite being used as cornerstones in the treatment of acute decompensated LVSD, the use of vasodilators is based on evidence from small studies focusing mainly on their acute haemodynamic effects and symptomatic benefit [Metra et al., 2009; Dickstein et al., 2008]. In patients with acute pulmonary oedema, vasodilators produce almost immediate symptomatic

benefit and improvement in central haemodymanic indices by volume compartment redistribution [Jugdutt, 1994].

The use of vasoldilators in chronic LVSD, particularly in combination with hydralazine, although resuling in improved symptomatic benefit and increased exercise tolerance and LVEF compared to ACE inhibitors, does not confer the same mortality benefit as ACE inhibitors [Pfeffer et al., 2003; Cohn et al., 1991]. Thus the vasodilator combination is reserved for patients who are still symptomatic on ACE inhibitors, who are intolerant to ACE inhibitors and ARB, and in Afro-Caribbean patients where haemodynamic responses to both beta-blockers and ACE inhibition are attenuated [Taylor et al., 2004].

1.4.3   Ultrafiltration (UF)

UF filters plasma directly across a semi-permeable membrane in response to a transmembrane pressure gradient, resulting in ultrafiltrate that is isoosmotic compared with plasma. Its use is advocated for diuretic-resistant fluid overloaded patients in the acute setting in whom it produces greater weight and fluid loss, reduces hospital stay and readmission rates for HF compared to optimal doses of intravenous diuretics [Costanzo et al., 2008; Costanzo et al., 2007]. During acute UF, mean right atrial pressure and PCWP reduce progressively resulting in increased cardiac output in the short-term. Overall intravascular volume remains stable throughout UF with no suggested haemodynamic instability, suggesting movement of fluid from a congested interstitial space into the circulatory space [Marenzi et al., 2001; Marenzi et al., 1998]. Limited data also suggests that UF is able to remove more sodium and less

potassium for an equivalent amount of fluid removal when compared to traditional high-dose diuretic therapy.

In the chronic setting, moderate LVSD patients without fluid overload have been randomised to either traditional diuretic based fluid management strategy or had in addition one single session of UF. The latter patients showed predictable marked reduction in right atrial pressure soon after UF, and had significant improvements in clinical and functional status at 6 months compared to the control group [Agostoni et al., 1993]. Further studies are needed to establish the clinical applicability of UF beyond the acute setting, especially due to its high financial costs, and the fact that it may not have long-term reno-protective effects and indeed could exacerbate renal injury by abrupt fluid flux.

1.4.4 Therapies under investigation

The *vasopressin antagonists* conivaptan (V1/V2-receptor antagonist) and tolvaptan (specific V2-receptor antagonist) are currently being evaluated for their use in LVSD in view of their ability to increase net water loss and reduce hyponatraemia [Russell et al., 2003]. So far, they have shown significant reduction in cardiac preload and body weight, with increased urine output and consequent improvement in oedema [Udelson et al., 2008]. Increased serum sodium levels in conjunction with a reduced urinary osmolality and increased net water loss have also been demonstrated [Rossi et al., 2007].

A1 adenosine-receptor antagonists have the potential to block the vasoconstrictive effect of A1-receptor activation on the afferent renal arteriole. This should in theory increase renal

blood flow and GFR, thus reducing sodium reabsorption by the proximal tubule. So far, they have been shown to produce a potassium-neutral diuresis while maintaining renal function. Furthermore, their use may potentiate the effect of diuretics [Cotter et al., 2008]. Unfortunately the net effect of the primary agent in this group, rolofylline, has been discontinued due to lack of efficacy and adverse effects in blinded acute studies. Although several other agents in this class are available, the clinical outlook for these agents in LVSD with concomitant renal impairment seems poor [Massie et al., 2010].

1.5 Conclusion

Predicting acute HF decompensation is an important clinical goal in LVSD, and abnormal volume status is an important determinant of decompensation. However, volume homeostasis has not been fully evaluated as a prognostic marker in LVSD nor has it yet been shown to be a target for prognostic treatment. Further studies are needed to explore the value of current and future therapies that impact directly or indirectly on volume status as a means of prolonging life and preventing decompensation in patients with LVSD.

Chapter Two

Current Means of Defining Volume Status in Left Ventricular Systolic Dysfunction

2.1 Introduction

In an attempt to prevent acute decompensation in LVSD, it is vital for the clinician to be able to assess the volume status of each individual patient accurately. In current clinical practice, the assessment of volume status in LVSD is challenging. Furthermore, progressive abnormalities in volume status can occur prior to symptomatic or clinical recognition, such that the assessment of patients' volume status encouraged through patterns of self-monitoring or regular clinical review is limited [Dickstein et al., 2008]. This Chapter will discuss currently available means that can potentially enable the clinician to assess volume status. A number of these means of volume assessment can be used routinely during inpatient and outpatient monitoring, while others have not made their way into routine clinical practice due to factors such as the complexity of their measurement, their costs, and risks of complications. In general, the routine non-invasive means of volume assessment tend to be cheap and more accessible, but have low specificity and/or low sensitivity, whereas the more complex and invasive means tend to be more accurate but may be associated with more complications.

2.2 Clinical symptoms

The sensitivity and specificity of symptoms at estimating fluid status in LVSD are in general poorly defined. Perceived exertional symptoms or scores (e.g., as assessed by NYHA functional class) poorly reflect exercise capacity, which may be regarded as a surrogate measure of cardiac output and thus of volume status. In patients with reduced LVEF, there is only a weak association between peak exercise VO_2 and NYHA functional class [Smith et al., 1993], and no correlation of perceived exercise intolerance and peak VO_2 or PCWP [Wilson et al., 1995].

2.3 Clinical signs

Routine assessment of both acute and chronic LVSD includes a range of clinical signs on physical examination, documentation of which is highly dependent on the skill and technique of the operator. While signs of hypervolaemia particularly during acute decompensation have some prognostic association [Drazner et al., 2003], the sensitivity of these signs at detecting abnormal volume status in chronic stable patients is low. On the basis of the pathophysiological model of dynamic volume redistribution in HF, it is not surprising that only 24% of stable LVSD patients with PCWP ≥18 mmHg had pulmonary rales in one study [Butman et al., 1993]. In a series of 52 HF patients referred for cardiac transplantation assessment, radiographic pulmonary congestion was absent in 53% patients with PCWP of 16-29mmHg, and in 39% of patients with PCWP ≥30 mHg [Chakko et al., 1991]. Stevenson et al. [1989] found that the combination of rales, dependent oedema and elevated JVP only had a 58% sensitivity but a specificity of 100% in predicting PCWP ≥22 mmHg.

2.4 Blood Volume Analysis

Blood volume analysis forms the main basis of our understanding of the pathophysiological mechanisms involved in abnormal volume status in LVSD. It constitutes a gold standard against which other means of volume assessment are compared in multiple clinical studies [Anand et al., 1989; Henning et al., 1978; Feigenbaum et al., 2008]. The volumes of each body water compartment as described in Figure 1.1 can be calculated based on dilution and equilibrium principles using appropriate radioactive or non-radioactive tracers. These are distributed and diluted in the various fluid compartments, and their radioactive activity or

concentration levels can be measured at specific times so that volume of specific compartments can be determined by extrapolation [Veall et al., 1980]. Thus specific tracer characteristics are applied to measure a specific volume compartment, e.g., a tracer to measure ECW should diffuse freely across the capillary membrane, should not penetrate the cellular membrane barrier and should have a relatively slow rate of elimination from the system [Albert et al., 1968]. Single or multiple tracers can be used at the same time for simultaneous measurement of different volume compartments.

Thus, the standard method to determine plasma volume is iodine-labelled human serum albumin (^{125}I-HSA) [International Committee for Standardisation in Haematology, 1980]. Alternative non-radioactive methods to determine plasma volume are less accurate and include Evans Blue dye dilution, Indocyanine green dilution, high molecular weight dextran and an extrapolated calculation of blood volume based on concomitant haemoglobin and Hct concentrations [Kalra et al., 2002]. For red cell volume estimation, use of sodium radiochromate (^{51}Cr) or sodium pertechnetate (Tc-99m) is advocated [International Committee for Standardisation in Haematology, 1980]. The performance of radiosulfate (^{35}S) is reliable for ECW estimation [Albert et al., 1968], and with simultaneous estimation of blood volume, allows the determination of interstitial fluid. TBW can be determined by using tritiated water.

While sensitive and specific for volume status in LVSD, and useful as a standard against which other methods can be compared, blood volume analysis is not routinely applied in the care of LVSD patients due to the complexity of its measurement and necessary exposure to radioisotopes [Brown et al., 1992].

2.5   Body weight monitoring

HF guidelines routinely advocate daily weight monitoring in LVSD patients as an index of volume status [Dickstein et al., 2008]. Patients are in general advised to contact medical or nursing personnel or to temporarily increase their diuretic dosing if they develop a weight gain of 2 Kg over stable body weight over a period of 48-72h. While this practice potentially motivates and empowers individual patients to take control of their own disease management, and is linked to reduced mortality, data linking changes in body weight to volume status is fundamentally lacking, and can only be interpolated from studies linking serial weight gain and acute decompensation [Chaudhry et al., 2007].

In a multi-centre randomised controlled trial of a technology-based automated daily weight and symptom-monitoring system of advanced LVSD patients, there was no difference in the primary end-point of re-hospitalisation between the intervention group and the control group. However, mortality at 6 months was lower by 56% in the overall intervention group [Goldberg et al., 2003]. Similarly, in the larger TEN-HMS study, telemonitoring of body weight and other variables did not improve the hospitalisation end-point but was associated with improved survival [Cleland et al., 2005]. Lewin et al. [2005] demonstrated that the commonly used ≥2 Kg weight gain over 48-72h has a very low sensitivity at detecting clinical deterioration (only 9%), however, in common with many measures in proven LVSD it has a high specificity (97%). Thus, weight gain, however defined, is insensitive to acute decompensation, and patients also have to be reminded that lack of weight change does not exclude impending clinical deterioration.

2.6 Invasive haemodynamic monitoring

2.6.1 Non-ambulatory invasive haemodynamic monitoring

Invasive haemodynamic monitoring by right heart or pulmonary artery catheterisation is commonly used in acute decompensated HF to estimate cardiac preload, cardiac ouput and systemic vascular resistance in order to guide treatment. Surrogate measures of cardiac preload include LVEDP, LVEDV, left atrial pressure and PCWP [also known as pulmonary artery occlusion pressure (PAOP)]. These left sided haemodynamic measures are closely related to right-sided filling pressures, i.e., central venous pressure or right atrial pressure, pulmonary artery systolic pressure (PASP) and pulmonary artery mean pressure (mPAP)[Drazner et al., 1999, Rhode et al., 2002], right-sided pressures being more readily measured clinically.

There is also fair correlation between haemodynamic variables and blood volume. In one small open study of 17 stable LVSD subjects [Androne et al., 2004], a PCWP of ≥15mmHg had a sensitivity and specificity of 0.85 and 0.75 respectively for predicting a total blood volume of > 8% from predicted normal value. However, blood volume estimates reflect indices of cardiac function more accurately than either left or right-sided filling pressures in mechanically ventilated patients or in patients with potentially large fluid volume shifts (iatrogenic or pathophysiological) [Brock et al., 2002, Sakka et al., 1999].

Catheter-titrated intravenous diuretic and vasodilator therapy in refractory HF enabled the achievement of low to normal LV filling pressures while maintaining low peripheral systemic resistance [Rohde et al., 2002], resulting in improved cardiac output and functional class. Steimle et al. [1997] showed similar haemodynamic and sustained clinical improvement in

patients referred for cardiac transplantation. Repeat right heart catheterisation performed at 8 ±6 months in survivors showed sustained increase in stroke volume on unchanged chronic therapy. LVEF improved and the severity of mitral regurgitation fell. Similarly, in the ESCAPE trial, treatment guided by pulmonary artery catheterisation compared to clinical assessment in decompensated LVSD patients resulted in a trend towards significant improvement in quality of life and exercise capacity both in-hospital and at one month post discharge [Binanay et al., 2005]. However, use of pulmonary artery catheterisation did not improve mortality figures and rates of hospitalisation, and was associated with predictably higher incidence of catheter-related adverse events such as bleeding and infection. Thus, the use of invasive non-ambulatory haemodynamic monitoring is limited by the need for intensive observation, additional costs and an association with increased adverse events. It is also impractical in the follow up of ambulatory chronic LVSD patients.

2.6.2 Ambulatory invasive haemodynamic monitoring

Ambulatory monitoring of right ventricular pressures is feasible and safe by means of implantable haemodynamic monitors, whose use currently is still at an investigational stage [Zile et al., 2008]. This method provides continuous haemodynamic data that is potentially more reliable than isolated static readings from a right heart catheter. Current implantable haemodynamic monitors consist of a programmable device that processes and stores information and is similar in appearance to a pacemaker pulse generator, and a transvenous lead with a sensor near its tip to measure intracardiac pressure. Variables such as heart rate, body temperature, patient activity, right ventricular systolic and diastolic pressure, rate of rise of ventricular pressure (dP/dt), and estimated pulmonary artery diastolic pressure (ePAD) can be continuously monitored and stored [Bourge et al., 2008]. The ePAD is defined as the right

ventricular pressure at the time of pulmonary valve opening, which occurs at the time of maximal dP/dt [Reynolds et al., 1995]. A strong correlation exists between ePAD and actual pulmonary artery pressures measured under a variety of physiological conditions [Magalski et al., 2002; Ohlsson et al., 1995; Chuang et al., 1996].

Braunschweig et al. [2002] demonstrated a potential clinical utility of an implantable haemodynamic monitor in adjusting diuretic dose in patients with HF, linking right ventricular pressure to volume changes induced by diuretic manipulation. During haemodialysis in 5 patients with systolic or diastolic dysfunction (EF 20-50%), ePAD dropped by a mean 50 % , with the lowest pressures recorded during the first 90 minutes of dialysis[Braunschweig et al., 2006]. Long-term haemodynamic monitoring unmasked severe volume overload in one patient, in whom dry weight was kept constant despite a decrease in lean body mass. A study by Adamson et al. [2003] showed that continuous haemodynamic monitoring in 32 patients with LVSD revealed increased right ventricular pressures 4 ±2 days prior to exacerbations requiring hospitalisation in 9 out of 12 cases.

On a larger scale, however, the use of implantable haemodynamic monitor-guided management compared to conventional management in 277 stable patients with severe HF, did not meet the primary efficacy end-point of reduction of HF-related events, defined as hospitalisations and emergency or urgent care visits requiring intravenous therapy [Bourge et al., 2008]. However, further analysis showed that there was a non-significant 21% reduction in total event rates and a non-significant 36% reduction in the relative risk of a first HF-related hospitalisation. The study may have been underpowered to show significant difference in event rates, and event rates in the control group may have been much lower than

expected for conventionally treated patients due to intensification of their contact schedule to match that of the implantable monitor-guided group.

2.7 Bioimpedance analysis

2.7.1 Definition and techniques

Impedance (Z) is a measure of overall resistance of a body to an alternating current. Several techniques enable the estimation of measures relevant to volume status by integrating impedance values into regression equations validated against gold standards such as radioisotope techniques, densitometry, dual-energy absorbtiometry, thermodilution studies or the Fick principle [Sodolski et al., 2007]. Bioimpedance analysis (BIA) is a technique which applies the difference in impedance to different current frequencies to estimate body water composition based on the principle that electrolyte-containing water (a better conductor) has lower impedance than fat (a worse conductor). Low frequency currents are unable to penetrate cell membranes, thus they enable approximation of ECW, whereas higher frequency currents are able to penetrate cell membranes, and give an approximation for total body water [Kyle et al., 2004]. Intracellular water (ICW) can then be estimated from subtracting ECW from TBW.

Impedance cardiography and impedance tomography are techniques utilising the change in impedance with every cardiac cycle secondary to a change in intravascular volume to estimate haemodynamic variables. Common haemodynamic variables derived include cardiac output, stroke volume, systemic vascular resistance, thoracic fluid content, velocity index, pre-ejection period, LV ejection time and heart rate. Furthermore, bioimpedance variables can be

derived either across the whole body or across a specific area of interest, so called segmental bioimpedance. In addition, although most impedance analysis is performed non-invasively via surface electrodes (current electrodes delivering the current and voltage electrodes detecting the temporary changes in voltage), impedance-derived information can also be obtained invasively by integration of current fields into implantable cardiac devices. A device will typically measure the impedance between the right ventricular lead and the generator, thus giving regional information about lung and cardiac impedance.

In LVSD, bioimpedance has been applied in an attempt to determine body water composition using BIA, or to provide estimates of haemodynamic variables using impedance cardiography (either non-invasively by means of surface electrodes across the chest, or invasively by means of cardiac devices).

2.7.2   Diagnosis of systolic HF and prediction of acute decompensation

The earliest potential application of bioimpedance was to diagnose systolic HF. Using electrical impedance tomography in a group of 20 patients with acute pulmonary oedema, Noble et al. [1999] showed that lung impedance was lower compared to controls, and increased in response to treatment during the admission, at the same time as radiographic improvement on serial chest radiographs.

Impedance reduces for some time prior to the onset of clinical pulmonary oedema, and thus may predict acute decompensation. Repeated non-invasive thoracic impedance was performed in 328 patients admitted with an acute coronary syndrome without acute

pulmonary oedema [Shochat et al., 2006]. In 37 patients who subsequently developed cardiogenic pulmonary oedema, impedance decreased by more than 12% at least 30 minutes prior to the development of symptomatic cardiogenic pulmonary oedema. Similarly, Charach et al. [2001] demonstrated a significant decrease in non-invasive thoracic impedance in 30 patients developing cardiogenic pulmonary oedema compared to patients who did not develop cardiogenic pulmonary oedema in a critical care setting. The decrease in impedance values preceded the appearance of symptoms and signs by approximately 1h, and impedance values started to rise 1h prior to the resolution of clinical pulmonary oedema.

In the chronic ambulatory setting, Yu et al. [2005] performed the first study to demonstrate the feasibility and potential clinical usefulness of intra-thoracic impedance monitoring in stable LVSD. 33 patients were implanted with an implantable cardioverter-defibrillator device which was also capable of measuring intrathoracic impedance. Of the 10 patients hospitalised for HF decompensation, intrathoracic impedance decreased by an average of 12.3% over an average of 18.3 days prior to the admission. Reduction in the mean value of impedance values started on average 15.3 days prior to the onset of symptoms. Similarly, a small scale study of 212 stable LVSD patients showed that higher composite scores for non-invasively determined impedance parameters predicted the likelihood of a related cardiac event (death or a clinical HF presentation) within the next 14 days [Packer et al., 2006]. Currently, invasive intra-thoracic impedance monitoring can be performed using the Medtronic OptiVol® which is integrated within implantable cardiac devices. A reference impedance line is derived from the individual patients' continuous data 34 days after implant to allow for pocket oedema to settle and for lead maturation to take place. A combination of deviation from this reference line and the duration of this deviation in days constitutes the

fluid index measure, above which an audible alert will be triggered. The fluid index is physician-programmable and can be tailored to individual patients. Ypenburg et al. [2007] showed that an impedance index threshold of 120 Ohm.day had a sensitivity of 60% and specificity of 73% for decompensated HF.

2.7.3 Correlation with invasive haemodynamic indices

The correlation of bioimpedance measures with gold standard measures is variable. In 25 healthy volunteers, a weak correlation was seen between cardiac output estimated by non-invasive impedance cardiography and that estimated by echocardiography when the volunteers were at rest, with a percentage error of 53% between the two readings [Fellahi et al., 2009]. Following haemodynamic load challenge, there was no statistically significant relationship between percentage change in cardiac output determined by the two methods. Similarly, in 13 patients post cardiac surgery [Simon et al., 2009], good correlation was demonstrated between cardiac output determined by impedance cardiography and pulmonary artery catheterisation, however, the percentage error between the 2 methods was 49%. In patients hospitalised with decompensated HF, haemodynamic measures using non-invasive impedance cardiography had poor correlation with invasively measured PCWP and cardiac output [Kamath et al., 2009]. On the other hand, during acute decompensation, an inverse correlation was found between intrathoracic impedance determined from an implantable cardioverter-defibrillator device and invasively measured PCWP, and between intrathoracic impedance and net fluid loss during hospitalisation [Yu et al., 2005].

This variability between bioimpedance systems may stem from the use of widely varying regression equations, calibration against differing gold standards, definitions made in widely variable reference populations, the use of whole-body versus segmental measurements, and whether algorithms are used to subtract the greater skin-electrode impedance values in non-invasive systems [Yamamoto et al., 1976; Smye et al., 1993]. Furthermore, other confounders can cause changes in routine impedance values, such as infection, non-specific pyrexial illnesses, electrolyte abnormalities, conduction of current by sweat and electrode paste, electrode application errors and/or pocket infection in device-based estimates, significant aortic regurgitation, septic shock, arterial hypertension, extremes of body dimensions (height and weight), use of an intra-aortic balloon pump and some arrhythmias [Sodolski et al., 2007, Yamamoto et al., 1986].

Despite these limitations, in a small prospective observational study, clinicians felt that impedance cardiography helped them with clinical decisions and enabled them to avoid the use of invasive haemodynamic parameters in decompensated HF [Silver et al., 2004]. This highlights the fact that interpretation of trends rather than absolute values of derived or raw variables may be more clinically relevant. The DOT-HF trial is currently in progress to address the impact of continuous intrathoracic impedance measured from an implanted device to detect increases in pulmonary fluid retention early and to facilitate timely treatment interventions. The primary end-point will be all-cause mortality and hospitalisation. Secondary end-points will also include quality of life and cost-effectiveness in this study aiming to involve 2400 patients [Braunschweig et al., 2008].

2.8   Echocardiography

The primary aim of echocardiography in the routine assessment of patients with LVSD is to establish LV size and function and to determine the aetiology or classification of LV impairment. However, despite not being used routinely for the purposes of volume assessment, some echocardiographic indices can provide estimates of cardiac preload and of cardiac performance.

2.8.1   Pulsed wave Doppler and Tissue Doppler indices

Pulsed wave mitral inflow indices correlate significantly with invasive estimates of LV filling pressures such as PCWP in LVSD [Bartel et al., 1996]. In a study comparing the relation of mitral flow indices to left atrial pressure in patients with LVSD and patients with hypertrophic cardiomyopathy, left atrial pressure determined by angiography was directly related to the ratio of the early transmitral velocity (MVE) to the late transmitral velocity (MVA) (MVE/A), and inversely related to the early deceleration time (MVDT) in the LVSD group [Nishimura et al., 1996]. The sensitivity and specificity of an MVDT <180m/s which indicated a mean left atrial pressure of $\geq$ 20mmHg in this group were both 100%. However, there was no significant correlation between mean left atrial pressure and MVDT in patients with hypertrophic obstructive cardiomyopathy, suggesting this correlation may only be valid in patients with LVSD. This may be secondary to impaired myocardial relaxation in patients with hypetrophic obstructive cardiomyopathy. Similarly, the use of pulsed wave Doppler mitral inflow indices also provides only limited haemodynamic information in patients with sinus tachycardia, a prolonged PR interval, diastolic HF and primary abnormal left atrial dysfunction [Pozzoli et al., 2000]. The clinical utility of a combination of serial weekly

measures of mitral inflow indices (MVE/A, MVDT, and PASP) used in conjunction with raised B-type natriuretic peptide (BNP) levels and high NYHA functional class was demonstrated by Ben Driss et al. [2007], with a 100% sensitivity in retrospectively predicting acute decompensation in LVSD patients during beta-blocker initiation or up titration.

Tissue Doppler imaging assesses the regional LV myocardial tissue velocities during diastole. The early diastolic mitral annular velocity (Ea) is a relatively load-independent measure of myocardial relaxation in patients with cardiac disease and does not correlate with mean PCWP. However its combination with MVE (E/Ea ratio) correlates well to invasively measured PCWP both in patients with normal and reduced LVEF [Dokainish et al., 2004; Nagueh et al., 1995]. Varying degrees of correlation have been found for E/Ea measured at the septal (E/Ea(s) and lateral (E/Ea(l) walls, and for the average of these two measurements. Furthermore, changes in PCWP in critically ill patients following treatment with intravenous inotropes and diuretics correlated well with changes in E/Ea [Dokainish et al., 2004]. However, the accuracy of E/Ea as a measure of LV filling pressure appears to be limited in patients with restrictive heart disease; chronic resynchronisation therapy, significant mitral regurgitation, or in advanced LVSD with extensive LV remodelling [Mullens et al., 2009]. The presence of regional wall motion abnormalities is also problematic, especially when measuring E/Ea(s). E/Ea (s) did not correlate to LV filling pressures in patients with basal septal wall abnormalities following a myocardial infarction, whereas E/Ea(l) correlated with LV filling pressure, regardless of the location of the regional wall motion abnormality [Lim et al., 2009]. In patients with normal to low-normal LVEF, however, some authors have demonstrated better correlation of E/Ea(s) to PCWP [Arteaga et al., 2008; Drighil et al.,

2008], whereas others have shown that E/Ea(l) performs better at predicting LV filling pressures [Rivas-Gotz et al., 2003].

Pulsed wave Doppler assessment of pulmonary vein flow provides yet another potentially useful estimate of LV filling pressure. The pulmonary vein Doppler spectrum reflects the phasic change of left atrial filling and is highly load dependent [Tabata et al., 2003]. Several studies have found close correlations between the pulmonary systolic velocity /pulmonary diastolic flow velocity ratio of the pulmonary vein Doppler spectrum and LV filling pressure in the perioperative setting and in patients undergoing elective cardiac catheterisation [Kuecherer et al., 1990; Rossvol et al., 1993]. In patients undergoing haemodialysis, this ratio outperformed other mitral inflow indices in its correlation with changes in ECW as assessed by BIA [Wu et al., 2004]. Confounding factors between fluid status and pulmonary vein Doppler parameters are age, sex, body surface area and LV diastolic function [Gentile et al., 1997].

2.8.2   Inferior vena cava indices

The inferior vena cava (IVC) is easily imaged and is a highly compliant vessel whose size and dynamics change with changes in central venous pressure and volume, and with the respiratory cycle. The retrohepatic or superior part of the IVC is most commonly imaged, with good correlation between measurements using 2-D and M-mode imaging in the long and short-axis, and with little intra and inter-observer variability [Moreno et al., 1984].

IVC diameter and percentage collapse is recommended in echocardiography guidelines for the routine estimation of right atrial pressure [Lang et al., 2006], using a variety of equations with

differing cut-off values for IVC diameters to estimate right atrial pressure. Several studies

have demonstrated varying degrees of correlations of IVC indices to invasively measured

haemodynamic variables. IVC diameter has a curvilinear correlation with pulmonary artery or

right atrial pressures measured during cardiac catheterisation in varied groups of normal

volunteers, patients with predominantly right-heart disease, and patients with left-sided

cardiac disease [Moreno et al., 1984]. This may be due to the reduced distensibility of the

vein at larger diameters. IVC collapsibility index (IVCCI), defined as [(IVC diameter on

expiration – IVC diameter on inspiration) / IVC diameter on expiration] × 100, also correlated

well with these invasive haemodynamic parameters, supporting the recommendations for the

use of this variable as an estimate of right atrial pressure [Natori et al., 1979]. In view of the

large variation in body weight and composition of patients with varying pathologies, IVC

diameters are commonly indexed to increase their accuracy at estimating haemodynamic

variables or blood volume. Thus, IVC diameter corrected for body surface area (IVCD)

correlated well with both invasively-measured mean right atrial pressure and blood volume in

haemodialysis patients without cardiac or pulmonary disease [Cheriex et al., 1989]. There

was also good correlation between changes in IVCD and changes in blood volume. Although

there was also good correlation between IVCCI and mean right atrial pressure in this group of

patients, IVCCI did not correlate to blood volume, suggesting that IVCD is pressure and

volume-related, whereas IVCCI is pressure-related only. Similarly, in a cohort of 100 dilated

cardiomyopathy patients, IVC diameters on inspiration and expiration, and IVCCI correlated

with right atrial pressure before and after loading manipulations. Furthermore, the increase in

IVC diameter from baseline in patients with acute decompensated HF reflected the increase in

body weight from baseline to that on admission [Sasaki et al., 2001]. In an attempt to ensure

that the assumption that IVC indices are indeed indicative of LV filling pressures, as studies

up to then had only compared IVC indices to right-sided pressures, Blair et al. [2009]

evaluated the ability of IVC diameter and IVCCI to predict an increased PCWP. The

respective sensitivities for this prediction were 75% for an IVC diameter of >2.0 cm, and 83%

for an IVCCI of <45%.

IVC enlargement alone can be a non-specific finding, with a degree of overlap between

normal subjects, patients with right-sided and left-sided HF [Rein et al., 1982]. However,

increased IVC diameter on admission and at discharge for acute HF was a reasonable

predictor of subsequent adverse events [Goonewardena et al., 2008]. Similarly, in stable

patients with varying comorbidities attending routinely for an echocardiogram, larger IVC

diameter with reduced collapsibility was a predictor of poorer outcome. A reduced IVCCI

had 92% sensitivity and 84% specificity for the diagnosis of HF in patients who presented to

hospital with shortness of breath [Blehar et al., 2009].

2.9 Blood biomarkers

2.9.1 Natriuretic peptides (NP)

B-type natriuretic peptide (BNP) and its inactive form N-terminal pro-BNP (NTproBNP), the

B-type NP, and to a lesser extent, atrial natiuretic peptide (ANP) and its inactive form N-

terminal pro-ANP (NTproANP), the A-type NP, have been widely studied in terms of their

sensitivity as indices in both the diagnosis and prognosis of LVSD, in addition to their

potential utility in mapping response to treatment [Maisel et al., 2007]. The routine use of

BNP and NTproBNP, for which there are commercially available assays for point of care

measurement, is endorsed in HF guidelines as adjuncts in HF diagnosis [Dickstein et al.,

2008].

BNP and NTproBNP have a useful role in the diagnosis of LVSD in unselected patients presenting with breathlessness [Januzzi et al., 2005, Mueller et al., 2006; Moe et al., 2007]. Furthermore, admission and discharge BNP and NTproBNP levels have good prognostic value for future adverse events [Maisel et al., 2004; Fonarow et al., 2007; Dokainish et al., 2005; Logeart et al., 2002]. BNP and NTproBNP seem to have more prognostic power in the short term compared to ANP and NTproANP [Yan et al., 2005].

Data on the use of NP to diagnose acute decompensation of chronic LVSD is less robust than its utility during an index presentation of acute LVSD [Maisel et al., 2008]. Although NP levels correlate well to left atrial and LV dimensions, LVEF and symptoms [Pfisterer et al., 2009; Iwanaga et al., 2006], data regarding the correlation of NP levels with invasive estimates of LV filling pressures are inconsistent. In acute decompensation, point measurements of BNP do not correlate to right atrial pressure or PCWP, although BNP and NTproBNP levels tend to be increased in patients who have high LV filling pressures [O'Neill et al., 2005; Dokainish et al., 2004; Shah et al., 2007]. Serial BNP levels then decrease significantly following HF treatments, similarly to invasive haemodynamic indices, however, changes in levels of the respective variables do not correlate well with each other. BNP decreases in response to effective diuresis in refractory LVSD within 6 days [Salvatore et al., 2005], but not within 24h of acute treatment where it correlates poorly to either blood volume or haemodynamic measurements [James et al., 2005]. BNP levels, however, seem more responsive to rapid haemodynamic changes in acute HF compared to NTproBNP [Mair et al., 2007]. Thus, BNP and NTproBNP measures may be better measures of medium to longer-term haemodynamic changes [Braunschweig et al., 2006].

ANP and NTpro-ANP appear more sensitive to *acute* volume loading in both normal volunteers and haemodialysis patients with normal LV function [Corboy et al., 1994; Herringlake et al., 2004]. In these studies ANP and NTproANP are both more sensitive in terms of onset of response, time for achieving peak levels and time for levels to return to baseline compared to both BNP and NTpro-BNP. In LVSD patients, data regarding this comparative responsiveness is more limited. In small scale studies, acute volume load in stable HF was accompanied by acute increases in ANP levels [Uretsky et al., 1990; Rodeheffer et al., 1991; Gabrielsen et al., 2001]. Similarly, in LVSD patients receiving vasodilator therapy, ANP levels reduced quickly, with subsequent modest increase over a 24h period (mirroring PCWP), whereas BNP levels reduced more slowly and correlated better with systemic vascular resistance [Larsen et al., 2006].

This difference in sensitivity of the A-type and B-type NP to acute volume changes may be due to differing dynamic responsiveness and release, and metabolism and excretion of each NP [Yoshimura et al., 1993]. ANP is present in the atria and to a lesser extent in the ventricles and is released in response to both atrial and ventricular stretch [Yasue et al., 1994]. BNP on the other hand is present in the ventricles only and is released in response to ventricular stretch, but may also be released in response to ventricular damage or injury due to the cardiotoxicity of other processes such as other neurohormones or production by cardiac fibroblasts [Maeda et al. 1998]. Furthermore, the A-type NP are released from granlues in the atrial myocardium in a rapid process, whereas only small amounts of B-type NP are prestored in the ventricles. The response of B-type NP is thus dependent on upregulation at the level of gene activation for secretory adjustments of BNP, which would take longer to occur [Yasue et al., 1994]. It is also plausible that the atria, being more distensible than the ventricles, are

stretched to a larger extent by the same mechanical or pressure stimulus compared to the less distensible ventricles. This results in faster and proportionally larger release of A-type NP compared to B-type NP [Ogawa et al., 1996].Furthermore, whereas a difference between the renal clearance of A-type and B-type NP remains to be clarified, there is a difference in the circulatory metabolism and pulmonary clearance of the NP [Smith et al., 2000; Yoshimura et al., 2000]. B-type NP are more resistant to clearance by peptidase in the ciruclation, and their metabolic clearance in the pulmonary circulation occurs to a lesser extent than for A-type NP.

There is conflicting evidence that BNP-guided or NTproBNP-guided treatment of HF on an outpatient basis compared to treatment guided by clinical assessment alone produces better outcome, and whether this may be secondary to optimisation of volume status. In the TIME-CHF study where NTproBNP-guided treatment was compared to standard symptom-guided management and therapy in a randomised controlled trial, no significant differences were found for the primary outcomes of survival free of hospitalisations and quality of life over a follow up period of 18 months [Pfisterer et al., 2009]. Doses of drugs with proven prognostic efficacy were up titrated to a significantly greater extent in the NTproBNP-guided group, this being mainly driven by the significantly higher use of aldosterone receptor blockers and number of dose increases of beta-blockers. Changes in diuretic, ACE inhibitor and angiotensin receptor blocker use did not differ between the groups. Levels of NTproBNP and symptoms were also not significantly different between the two groups. A similar pattern of drug changes was found in the STARS-BNP study [Jourdain et al., 2007], whereas Troughton et al. [2000] demonstrated significantly higher titration of ACE inhibitors, aldosterone antagonists and diuretics only to achieve very similar levels of BNP in both BNP-guided and standard management groups. Both studies however, showed positive outcomes

with BNP-guided treatment versus standard management. Thus, it is still not clear which specific treatment would confer benefit guided by NP levels.

2.9.2   Haematocrit (Hct)

Hct (red cell volume) is the percentage of red blood cells as a proportion of total blood volume. This is perhaps the simplest of blood biomarkers applicable to variations in blood volume, and it can be simply measured by automated analysers [Lewis et al., 2001]. Hct levels can be an indirect measure of blood volume in patients with HF, thus increasing due to haemoconcentration in hypovolaemia, and reducing due to haemodilution secondary to circulatory volume expansion. Strauss et al. [1951] proposed the use of Hct and haemoglobin levels before and after an intervention to alter plasma volume in order to estimate the percentage change in plasma volume. Thus, percentage change in plasma volume was calculated as 100 × [Haemoglobin (before)/Haemoglobin (after)] × [1-Hct (after)/1-Hct (before)] −100.

However, a low Hct in patients with LVSD can be due to either an increased plasma volume (haemodilution), and/or from reduced red blood cell volume (true anaemia) [Ng Kam Chuen et al. 2007]. Determination of red cell and plasma volume is important in this circumstance to differentiate between the two. Anaemia, defined as a low Hct level, is relatively common in patients with chronic LVSD, and is a strong predictor of adverse outcome [Kosiborod et al., 2005]. Anaemia in chronic LVSD may be due to chronic disease, bone marrow depression from excessive cytokine production, malnutrition, concomitant or related renal disease, and/or

drug therapy. This complicates the use of Hct as a practical measure of volume status, and makes it non-specific.

When isolated point measured samples are compared, haemodilution can be present in up to 46% of anaemic patients with LVSD [Androne et al., 2003]. Patients with low Hct due to haemodilution have poorer outcome compared to patients with a true anaemia. Similarly acute HF patients who are subsequently stabilised show lower BNP and NTproBNP levels which inversely correlate with haemoglobin and Hct. The association between simple Hct and progression of disease from asymptomatic LVSD to symptomatic HF in 2,281 patients from the SOLVD trial showed that patients with the lowest Hct quartile were at increased risk for the development of HF symptoms, first hospitalisation due to HF, and death compared with patients in the highest Hct quartile [Das et al., 2005].

Boyle et al. [2006] proposed that haemoconcentration could be used as a surrogate marker to indicate that plasma refill rate has been exceeded by the rate of fluid removal during UF in the treatment of acute decompensated HF. Hct can be easily and continuously measured by using an on-line Hct sensor during UF therapy. Thus, the rate of volume extraction could be adjusted to approximate plasma refill rate so that complete decongestion can be reached when haemoconcentration is observed at minimal rates of volume extraction. On-line Hct monitoring has enabled the above principle to be applied to haemodialysis patients prone to dialysis-related hypotension with some evidence of efficacy [Schroeder et al., 2004]. One potential advantage of Hct measures is that while low Hct levels would suggest developing hypervolaemia, alternatively a high Hct might indicate dehydration thus allowing a potentially damaging therapy to be balanced. As with other biomarkers the change in measured value

and direction of change may be the key in clinical application. The best setting for Hct measuresmay lie in repeatedly monitoring acute volume changes, as many other factors may affect Hct levels in the longer term.

2.10   Summary

In the management of individual LVSD patients, the accurate non-invasive definition of volume status remains severely problematic. Despite large resources being placed in systems of care designed to optimise therapy and prevent decompensation, a suitably sensitive and specific non-invasive biomarker for detecting changes in volume status, whether at a clinical or preclinical stage in LVSD has not yet been defined.

Chapter Three

Defining the Source of Variance of Blood Biomarkers Relevant to the Pathophysiology of Abnormal Volume Status in Left Ventricular Systolic Dysfunction

3.1 Characteristics of a clinically useful biomarker for the monitoring of volume status in LVSD

A biomarker is an objectively measured indicator of normal biological or pathogenic processes, or response to a therapeutic intervention, and can consist of blood, urine or tissue constituents or physical biomeasures [Biomarkers Definitions Working Group, 2001]. In utilising biomarkers to track changes in volume status in LVSD, the clinician needs to be aware of the characteristics required of clinically useful biomarkers for this purpose, the sources of their measurement variance, and principles to minimise this. These issues will be discussed in this Chapter, with particular emphasis on the specific blood biomarkers which will be studied in this thesis. Despite this Chapter focusing on blood biomarkers, the principles described here will also apply to the physical biomeasures studied in this thesis.

In general, a candidate biomarker will only have clinical utility if it can be measured by the clinician, if it adds new information, and if it helps the clinician to manage the patient, over and above any tests currently available and in addition to clinical acumen [Morrow et al., 2007]. To be readily measured by the clinician, its measurement must be accurate, reproducible, rapid and cost-effective. The results must be easy to interpret, and undergoing the test must be acceptable to the patient [Malino et al., 2003]. The properties of biomarkers will vary with the sample frame of their intended use [Vasan et al., 2006]. In the context of monitoring of volume status in LVSD, not only do the biomarker levels need to correlate to spot measures of gold standards for volume status, more importantly, changes in their levels need to have high sensitivity and specificity for changes in volume status. Thus, their levels need to change rapidly and to an appreciable extent with changes in volume status, unlike for diagnostic biomarkers, where sustained elevated levels are an advantage. Narrow within-

subject variability is required, although between-subject variance is less important as each patient will serve as his/her own control. Because biomarker levels for monitoring need to be performed on a regular basis, tolerability and cost effectiveness are also important features.

3.2   Sources of variance of blood biomarkers

When using a biomarker to guide patient management, clinicians must appreciate that a point measurement of the biomarker is an estimate of its absolute value; this estimate needs to be interpreted in the context of the normal variance both within an individual and between individuals [MacFadyen, 2006]. The *normal variance* of a biomarker is a measure of the range of measured values from the absolute value, and is also termed the *total variance* of that biomarker (CVt). Thus, for blood, urine or tissue biomarkers, normal variance is affected by pre-analytical, analytical and post-analytical factors, as outlined in Figure 3.1. Similar principles apply to physical biomeasures, with intra and inter-observer variability determing their analytical and post-analytical variance.

Pre-analytical variance refers to inherent *biological variability* (CVi) and sample handling, in other words the stability of the biomarker depending on conditions under which it is sampled and stored. While the latter component of pre-analytical variance can be minimised to non-significant values by taking steps such as ensuring that steady state is reached prior to sampling, good training of staff and good adherence to standard operating procedures, biological variability can be very large for some biomarkers, and generally constitutes the largest source of variance [Fraser, 2009]. Biological variability is determined by several factors as outlined in Figure 3.1. Within an individual, it can be considered as a random

fluctuation around a homeostatic set point, which tends to be constant over time, geography, methodology and in health and chronic disease. This is termed the *within-subject biological variation*. However, individual homeostatic set points vary between individuals; this difference between individuals is termed the *between-subject biological variation*. The former is generally much smaller than the latter for most biomarkers.

Figure 3.1. Potential sources of pre-analytical, analytical and post-analytical variance of blood biomarkers [Vasan 2006; Fraser, 2009].
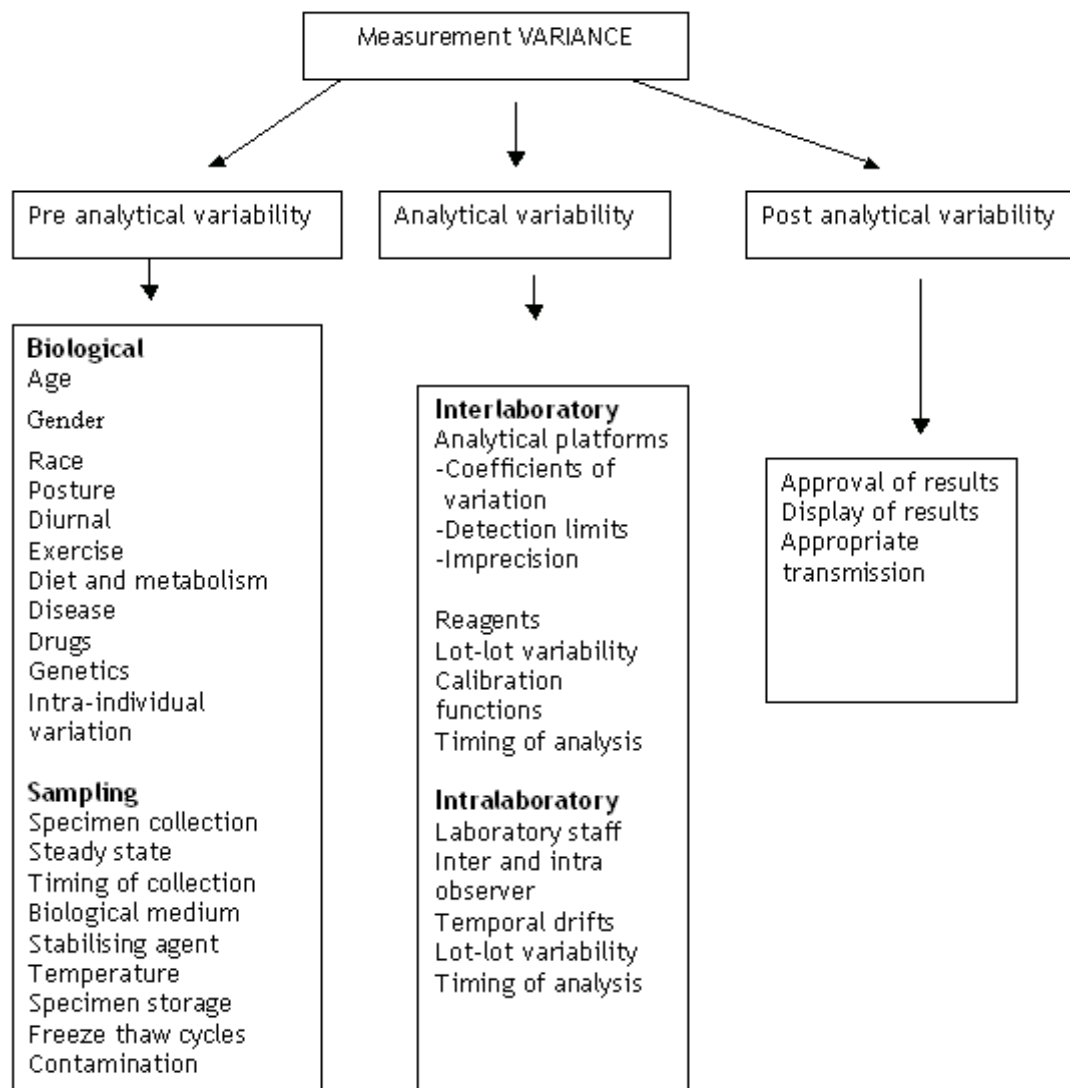
Analytical variability relates to the assay performance in the laboratory, and post- analytical factors include the processes of approval and transmission and the appropriate display of test results with the use of the laboratory's information management systems. Post-analytical sources of variance can also in practice be minimised to non-significant levels.

Thus, the main determinants of the total variance (normal variance) of a biomarker within an individual (CVt_{ws}) consist of the within-subject biological variance (CVi_{ws}) and analytical variance (CVa) [Fraser, 2001]. CVt_{ws} of a biomarker over a specific period of time can be calculated from several measured values of the biomarker over that specific time period by determining the coefficient of variation [(standard deviation/mean) × 100] of values measured over that time period within an individual. The longer the time period over which values are measured, the larger the CVt_{ws} will tend to be. Analytical variance can be calculated generally by determining the coefficient of variation of several measurements of quality control specimens with pre-determined values using one assay (intra-assay CVa) or using different assays (inter-assay CVa). The within-subject biological variability (CVi_{ws}) can then be determined by using the equation $CVi_{ws} = \sqrt{(CVt_{ws}^2 - CVa^2)}$ [Bruins et al., 2004]. The between-subject total variance (CVt_{bs}) can also be calculated using the same equation as for the determination of CVt_{ws} [(standard deviation/mean) × 100], but applying population means and standard deviations for the calculation of the CVt_{bs}. CVi_{bs} can thus be derived in the same way to CVi_{ws}, by applying the equation $CVi_{bs} = \sqrt{(CVt_{bs}^2 - CVa^2)}$.

To the clinician, whose role it is to diagnose and monitor disease or response to treatment, it is the within-subject and between-subject total variance that are of most relevance in the context of interpreting biomarker levels released by the laboratory and relating this to the

clinical state of the individual patient. The between-subject total variance can be very large for some biomarkers, such that a reported biomarker level may be highly unusual for a patient, but may still lie below conventional cut-off points or within conventional population-based reference ranges. This may explain the controversies surrounding for example, the use of NP, namely the false positive and false negative rates for diagnosis of HF [Tang et al., 2003], and the ongoing debate about whether biomarker-guided treatment is beneficial over and above treatment guided by clinical assessment [Berger et al., 2010].

In an attempt to solve this problem, some authors advocate the use of reference change values (RCV) [Harris, 1979]. The RCV of a biomarker is defined as that measured value which indicates a difference between two consecutive measures that is statistically significant and links to a significant change in a patient's health status; it is usually expressed as a percentage of a baseline value. It can be calculated simply by using the following equation:

$RCV = \sqrt{2} \times Z \times \sqrt{(CVa^2 + CVi^2)}$, or $RCV = \sqrt{2} \times Z \times CVt$, where Z is the number of standard deviations appropriate to the desired probability, e.g., 1.96 for $p < 0.05$ and 2.58 for $p < 0.01$. However, there is still current debate around the clinical application of this principle, and the statistical calculation of RCV for non-normally distributed data, which is the case for a large number of biomarkers relevant to LVSD [Fokkema et al., 2006; Omar et al., 2008]. Some authors advocate using a log-normal approach , producing two different RCV for increasing and decreasing values, e.g., RCV for BNP: +198% and –66% from baseline measure [Wu et al., 2009].

An alternative way of using the principle of using a difference between two measured values within an individual for detecting a significant change in that individual's clinical status

involves mapping the within-subject normal variance at times of clinical stability by means of repeated measures assessment. With continuous/intermittent monitoring, the onset of acute exacerbation of disease would be identified as a *significant* and *sustained* deviation from this within-subject normal variance, as illustrated in Figure 3.2. This principle is currently employed in implantable haemodynamic monitors or bioimpedance sensors within implantable cardiac devices; the clinical validity of its use still being under investigation [Braunschweig et al., 2006]. Importantly, these principles potentially enable the identification of the subclinical phase of disease, such that in theory, treatment can be intensified and hospital admissions avoided.

Figure 3.2. Potential patterns of deviation from within-subject normal variance for the prediction of a clinical event.
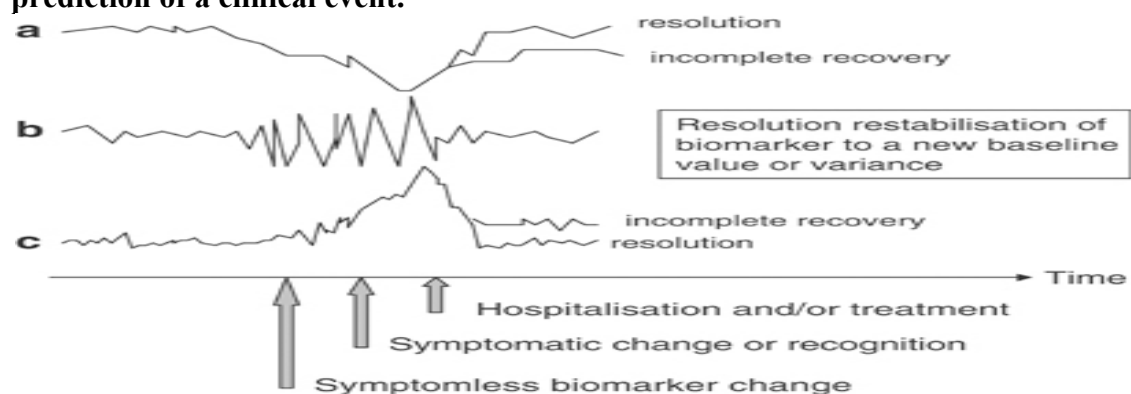
Reproduced with the author's permission from J Human Hypertension 2006; 20: 383-6. [MacFadyen, 2006].

3.3   Blood biomarkers relevant to LVSD to be studied in this thesis

The panel of blood biomarkers to be studied in this thesis was selected based on their current availability and ongoing application in routine clinical practice, and includes NP, cardiac troponin peptides, ischaemia modified albumin and Hct. The next section will discuss the

sources of variance of these blood biomarkers which can be directly controlled, and thus

mininised by the clinician, namely pre-analytical variance.

3.3.1 Natriuretic peptides

3.3.1.1 Biological variance

NP levels are affected by a number of physiological and non-physiological conditions (Table

3.1), which if present concurrently with LVSD may confound their monitoring capacity.

Table 3.1. Physiological and non-physiological states associated with changes in natriuretic peptide levels [Maisel et al., 2008; McDonagh et al., 2007].

Physiological states	
Increased	
• Children	• Left atrial size
• Old age	• Pulse pressure
• Female	• Genetic
Decreased	
• Increasing body mass index	
Non-physiological states	
Cardiac causes	
Increased	
• Systolic heart failure	• Hypertension
• Diastolic heart failure	-with or without LVH
• Left ventricular hypertrophy	• Valvular heart disease
• Coronary artery disease	• Cardiac allograft rejection
• Right ventricular dysfunction	• Hypertrophic obstructive cardiomyopathy
Non cardiac causes	
Increased	
• Pregnancy-related hypertension	• Primary pulmonary hypertension
• Chronic renal impairment	
Decreased	
• Uncomplicated diabetes	• Pulmonary embolism
• Obesity	• Subarachnoid haemorrhage
Effect of treatment	
Increased	
• Beta-blockers (short-term)	• Digoxin
Decreased	
• Beta-blockers (long-term)	• Angiotensin II receptor blockers
• Diuretics	• Spironolactone
• ACE inhibitors	• Cardiac resynchronisation therapy

Table 3.2. Within-subject total variance (CVt$_{ws}$), analytical variance (CVa), within-subject biological variance (CVi$_{ws}$) and reference change values (RCV) of natriuretic peptides.

Population	Biomarker	Time period	CVt$_{ws}$ (%)	CVa (%)	CVi$_{ws}$ (%)	RCV (%)
Bruins et al., 2004	BNP	2° for 8h	12.0	8.4	8.2	32.0
Stable HF (n=43)		Daily for 5 days	27.0	8.4	25.0	74.0
		Weekly for 6 wks	41.0	8.4	40.0	113.0
	NTproBNP	2° for 8h	9.1	3.0	8.6	25.0
		Daily for 5 days	20.0	3.0	20	55.0
		Weekly for 6 wks	35.0	3.0	35	98.0
Wu et al., 2003						
NV (n=12)	BNP	4 samples every	NA	8.6	43.6	NA
	NTproBNP	alternalte week	NA	1.6	33.3	NA
Stable HF (n=5)	BNP	2° for 24 h	NA	8.6	24.0	NA
Unstable HF (n=11)	BNP	Daily during acute	NA	8.6	NA	130.0
	NTproBNP	admission	NA	1.6	NA	90.0
O'Hanlon et al., 2007	BNP	1 hour apart	14.6	13.7	5.0	34.0
Stable HF		1 week apart	28.4	13.7	24.8	66.2
(n=45)	NTproBNP	1 hour apart	6.9	2.8	6.3	16.1
		1 week apart	21.1	2.8	20.9	49.2
McDowell et al.,2002						
NV (n=10)	ANP	Every 2 min for 90	65.0	8.0	NA	NA
	NTproANP	min	8.9	5.9	NA	NA
Stable HF	ANP	Every 2 min for 90	51.0	8.0	NA	NA
(n=10)	NTproANP	min	3.6	5.9	NA	NA

NV: normal volunteers; NA: not documented by authors.

NP have large within-subject and between-subject normal variance. In a cohort of 558 LVSD patients, up to one-fifth of *symptomatic* but clinically stable patients (NYHA class II-III) had BNP levels of <100pg/mL, whereas *asymptomatic* patients' BNP levels ranged from 5-572 pg/mL, with a median value of 147pg/mL [Tang et al., 2003]. The large within-subject normal variance of NP has been demonstrated in a number of studies in a variety of populations, as

shown in Table 3.2. The amplitude of this variance has not been consistent in different studies, such that considerable uncertainty remains regarding these estimates. This within-subject normal variance has been shown to be independent of factors such as renal function, age or gender [O'Hanlon et al., 2007]. NP levels in both diastolic and systolic HF have reduced within-subject normal variance compared to levels measured in healthy individuals [Kjaer et al., 2005]. Furthermore, within-subject normal variance for NP reduces with advancing severity of HF and increasing NP levels [Araújo et al., 2006].

Thus, current consensus recommendations are that NP levels are interpreted as a continuous variable so that the higher the NP value, the higher the likelihood of the presence of HF, and that two cut-off values are used to rule in and rule out HF [Maisel et al., 2008]. For BNP, cut-off values of <100 pg/mL and >400 pg/mL have high sensitivity and specificity at ruling out and in a diagnosis of HF respectively [Mueller et al., 2004]. For NTproBNP, the recommended cut-off point to rule out a diagnosis of HF is <300 pg/mL, whereas different age-dependent cut-off values for ruling in HF include >450 pg/mL (<50 years), >900 pg/mL (50-75 years) and >1800pg/mL (>75 years) [Januzzi et al., 2005; McDonagh et al., 2004]. Nevertheless, considerable error may be encountered when trying to diagnose HF in a particular individual while trying to apply this population-generated approach.

The large within-subject and between-subject total variance of NP makes their measured point values difficult to interpret in the context of diagnosing an acute decompensation of LVSD; some authors recommend the use of RCV, which can be as high as 130% in the case of BNP. This makes the interpretation of results from studies implying significant treatment benefit with reductions of NTproBNP and BNP levels of *up to* 35% and 50% respectively, difficult.

Furthermore, in clinical practice, total variability of NP levels is likely to be much more than that established in selective clinical studies, as many precautions taken for granted in the former setting to actively minimise biological and analytical error are not followed in routine practice. Currently, some opinion advocates that a rise of >50% of NP values should draw the clinician to consider increasing the patient's diuretic dose, in the absence of symptoms of volume overload [Maisel et al., 2008], a practice which would be entirely flawed in the light of the above discussion.

3.3.1.2   *Sampling and storage*

The half-lives of ANP, NTproANP, BNP and NTproBNP are 2-3 minutes, 16-24 minutes, 20 minutes and 70 minutes respectively. Thus, ideally, sampling should take place after steady state is reached (5-6 half-lives) [Fraser, 2004], which for BNP would require 100-120 minutes. Most clinical studies take blood for measurement after resting supine for 30 minutes, and in busy outpatient departments, patients are very rarely rested and supine before drawing blood.

NP in plasma or serum is degraded by proteolysis, occurring within 24h of separation of plasma or serum from whole blood, progressing on refrigeration or freezing. Stabilisation of NP in vitro can be achieved by collection into EDTA tubes [Belenky et al., 2004, Dupuy et al., 2006; van der Merwe et al., 2004; Sokoll et al., 2002], and by adding protease inhibitors such as aprotinin, benzamidine and D-Phe-Phe-Arg-chloromethylketone to reduce ex-vivo degradation of NP [Belenky et al., 2004]. The latter seems the most effective, whereas aprotinin, which has been used extensively in many studies, is more widely available.

In general, quick centrifugation of NP samples is recommended, with storage of whole blood at 4°C until this takes place. The separated sample should then be stored at temperatures of around −70°C. Furthermore, repeat freeze-thaw cycles should be avoided; this can result in BNP loss ranging from 10-36%, independent of the type of assay used [Mueller et al., 2004].

3.3.2   Cardiac troponin peptides (cTn)

The role of cTn in the diagnosis of acute coronary syndromes is well established; both troponin I (cTnI) and troponin T (cTnT) are very sensitive and specific markers of myocardial damage [Alpert et al., 2009]. Disease progression in LVSD even in the absence of overt ischaemia is associated with ventricular remodelling and myocyte loss as illustrated by indium anti-myosin antibody imaging in patients with dilated cardiomyopathy [Cohn et al., 2000; Matusmori et al., 1993]. In theory, the cTn, in ischaemic cardiomyopathy at least, could map disease status, response to treatment and prognosis by providing a measure of myocyte injury secondary to LV stretch or dilatation as a result of ventricular remodelling in response to abnormal volume status. Other mechanisms leading to myocyte injury could include inflammatory, oxidative stress and neurohormones [Braunwald, 2008].

In stable LVSD, modest elevations of cTn are found even in the *absence* of ischaemia or acute symptomatic decompensation in around half of patients being studied [La Vecchia et al., 1997; Horwich et al., 2003]. Incremental increases in, and higher cTn levels are linked to increasing mortality, rates of hospitalisation, declining LVEF, worse haemodynamic variables and higher BNP levels [Miller et al., 2007; Miller et al., 2009]. Latini et al. [2007] showed

that using a high sensitivity-assay, a higher proportion (92%) of stable HF patients had elevated cTnT, compared to only 10.4% of patients identified by a standard cTnT assay. After adjustment for baseline variables and BNP level, the detection of cTnT by means of the high-sensitivity assay was still associated with an increased risk of death.

Similarly, increased cTnT levels were found in 9.2% of patients being admitted with acute decompensated HF in whom there was no evidence of ischaemia or coronary disease [Peacock et al., 2008]. Patients who had positive cTnT levels had lower LVEF and higher in-hospital mortality. Serial cTn measures could provide useful information regarding clinical improvement in acute decompensation. 39 decompensated LVSD patients had elevated cTn levels on admission; these levels then fell at the time of hospital discharge to their values during a compensated state, and were maintained at several weeks after discharge [Biolo et al., 2009]. Gheorghiade et al. [2005] established prospectively the pattern of cTn variance in patients with acute decompensated ischaemic LVSD where decompensation was not due to ischaemia. During periods of stable disease, 73.9% of patients had detectable levels of cTnI, and 43.5% had detectable cTnT levels. Median concentrations of both cTn remained unchanged 0-32h from decompensation but rose between 32-56h. cTnT levels eventually plateaued until discharge, whereas cTnI levels decreased back to baseline values by 80h. Chen et al. [1999] also demonstrated raised cTnI levels in 89% of patients with acute decompensation. cTnI levels fell in patients who improved clinically, while a deteriorating clinical profile was associated with increasing cTnI. However, only cTnI drawn at the time of first haemodynamic measurements correlated weakly with right atrial pressure in decompensated HF [Shah et al., 2007].

3.3.2.1   Biological variance

Table 3.3 summarises a number of physiological and pathological conditions in which cTn can be elevated. The first study to establish the normal variance of cTnI in healthy volunteers was performed by Wu et al. [2009], who collected repeated blood samples hourly over 4h, and on alternate weeks over 8 weeks. Using a high-sensitivity assay, they found within-hour within-subject and between-subject normal variance to be 9.7% and 57% respectively, and between-day within-subject and between-subject normal variance to be 14% and 63% respectively. RCV were calculated using a lognormal approach as the cTnI values were skewed to the right. Lognormal within-day values were +46% and −32%, between-day values were +81 and −45%. The normal variance of cTn in LVSD patients has not been defined.

Table 3.3. Physiological and non-physiological states associated with elevated cardiac troponin [Kelly et al., 2009; Ammann et al., 2004].

Physiological	
• Strenuous exercise	- Amateur and professional
Cardiac diseases	
• Acute coronary syndromes	• Valvular heart disease
• Heart failure (systolic and diastolic)	• Congenital heart disease
• Myocarditis	• Coronary vasospasm
• Pericarditis	• Cardiac amyloidosis
• Arrhythmias	
Non cardiac diseases	
• Critically unwell patients	• Pulmonary hypertension
• Renal failure	• Subarachnoid haemorrhage
• Sepsis	• Stroke
• Pulmonary embolism	• High dose chemotherapy
Cardiac interventions	
• Percutaneous coronary intervention	• Closure of atrial septal defects
• Cardiac surgery	• Cardiac transplantation
• DC cardioversion	• Radiofrequency ablation
• ICD implantation	

3.3.2.2 *Sampling and storage*

The half-life of cTnI is much shorter than that of cTnT, and is around 24h in patients with normal renal function. Collection of blood samples in heparin tubes, although allowing more rapid analysis of cTn, results in reductions of up to 15% in measured cTn values compared to serum tubes [Gerhardht et al., 2000]. Measured concentrations of cTn also decreases with increasing heparin concentrations added to sera [Speth et al., 2002]. Similar tubes should thus be used for repeated cTn analysis in an individual patient. cTn samples should also either be analysed immediately or stored in serum at −70°C and analysed within a year. There were no significant changes in cTnT levels stored up to 12 months, whereas at 24 months, there was a significant decrease from baseline levels [Basit et al., 2007].

3.3.3 Ischaemia modified albumin (IMA)

IMA is a marker of acute ischaemia whose levels rise within minutes of the onset of ischaemic chest pain [Sinha et al., 2004]. Of the large number of potential biomarkers of ischaemia, it is the only biomarker in addition to the cTn to have reached the clinical validation stage [Var-Or et al., 2000]. Percutaneous coronary intervention, as a model of transient reversible ischaemia [Sinha et al., 2003], reveals an IMA response in 18 out of 19 patients sampled immediately and 30 minutes post procedure, with levels returning to baseline by 12h. This occurs in the absence of cTn elevation. IMA levels correlate with the severity of ischaemia, being higher in patients with more balloon inflations, higher pressure inflations, and longer inflation times [Quiles et al., 2003], and lower in patients with collateral circulation [Garrido et al., 2004].

IMA thus appears to be a more sensitive marker of *reversible* ischaemia in the absence of myocardial necrosis in this respect, distinguished from cTn which is a better marker of micro necrosis but a poorer marker of true reversible ischaemia. IMA was more sensitive at ruling out a non-ischaemic cardiac cause in patients presenting with symptoms of acute chest pain, although it fared poorer as a discriminator of ischaemia with and without infarct [Bhagavan et al., 2003]. IMA levels performed within 3h of the onset of chest pain had a sensitivity of 82% for a final diagnosis of ischaemic chest pain, compared to a markedly poorer sensitivity of 45% and 20% for admission ECG and cTnT respectively [Sinha et al., 2004]. Furthermore, IMA levels, used in conjunction with cTnI levels were found to be useful in the triage of patients presenting with undifferentiated chest pain [Collinson et al., 2006]. Admission IMA levels in patients presenting within 3h of the onset of chest pain suggestive of cardiac ischaemia were also predictive of poor short and medium-term prognosis [Consuegra-Sanchez et al., 2008].

With respect to biomarker utility in LVSD, IMA may only be relevant in those individuals with underlying reversible ischaemia. While recurrent ischaemia is a potential mechanism of worsening systolic function, it is rarely well defined in routine clinical practice. However, it may also reflect the process of relative myocyte ischaemia as part of the process of cardiac remodelling (regardless of epicoronary stenosis as a cause of LVSD). In the same respect that cTn changes might sensitively define the cardiac remodelling process and thus might indicate abnormal volume status compromising myocyte stretch, IMA, if suitably sensitive, might also reflect the impact of abnormal volume status as sub-clinical ischaemia via increased wall stress. Alternatively, it might reflect the other processes involved in the pathogenesis of HF, such as oxidative stress, inflammation or neurohormonal activation acting detrimentally on

the myocyte [Braunwald, 2008; Sbarouni et al., 2008]. These issues have not been examined thus far.

In end-stage renal failure, raised IMA levels associate with larger LV, lower LVEF, higher echocardiographic estimates of LV filling pressures and objective evidence of reversible ischaemia as evidenced by stress echocardiography and coronary angiography, as well as a worse prognosis during a follow up period of 2.25 years [Sharma et al., 2006]. In patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention, serum IMA concentrations measured 15 minutes post-procedure are significantly related to LVEF, and represent early markers of the development of clinical HF during the admission [Dominguez-Rodriguez et al., 2008]. Similarly, in stable dilated cardiomyopathy, IMA levels were found to be significantly negatively correlated to LVEF, and levels differed significantly between NYHA functional classes [Sbarouni et al., 2009]. However, IMA levels in these stable patients were no different compared to age-matched healthy volunteers. On the other hand, in stable LVSD patients about to undergo chronic resynchronisation therapy, IMA levels were approximately 24% higher compared to age-matched healthy volunteers, and were not significantly different between patients with ischaemic cardiomyopathy and dilated cardiomyopathy [Franceschi et al., 2009]. Furthermore, IMA levels correlated with BNP levels, and could thus be a potential biomarker of volume status. The difference in observed levels of IMA in the latter two studies may be due to varying severity of LV dysfunction, and suggests that in stable patients with a non-ischaemic aetiology for LVSD and with milder degrees of LV dysfunction, lower levels of IMA may prevail in the same way that only modest elevations of cTn are present in a large proportion of stable LVSD patients with no evidence of ongoing ischaemia [Latini et al., 2007]. Further studies are needed to establish

prognostic utility in HF as well as relationships to other measures of HF progression such as volume status.

3.3.3.1 Biological variance

Measured IMA levels are not cardiospecific; Table 3.4 shows a number of physiological and non-physiological conditions that can affect IMA levels. Only one study so far has established the normal variance of IMA levels in normal volunteers. Blood samples were taken weekly over a 5-week period at the same time and by the same phlebotomist [Govender et al., 2008]. Within-subject and between-subject normal variance were 2.89% and 6.76% respectively.

Table 3.4 Physiological and non-physiological conditions affecting ischaemia modified albumin levels [Sbarouni et al., 2008; Sbarouni et al., 2009; Kaefer et al., 2009; Haklıgör et al., 2009; Turedi et al., 2009; Gundez et al., 2009; Guven et al., 2009].

Physiological	
Increased	
•Increasing age	•Strenuous exercise
Reduced	
•Increasing albumin	•Increasing lactate
Non-physiological	
Increased	
•Cardiac ischaemia	
•Ischaemic stroke	•End stage renal failure
•Skeletal muscle ischaemia	•Peripheral vascular disease
•Intra uterine ischaemia	•Gastrointestinal ischaemia
•Right ventricular dysfunction	•Pulmonary embolus
•Pulmonary hypertension	•Diabetes mellitus
Cardiac interventions (increased)	
•Percutaneous coronary intervention	•Radiofrequency ablation
•Permanent pacemaker intervention	•Internal cardio defibrillator implantation

3.3.3.2   *Sampling and storage*

Blood has been collected in serum or heparinised tubes in several studies [Christenson et al., 2001; Bhagavan et al., 2003]. Gidenne et al. [2004] observed that paired samples of heparinised plasma or serum were not equivalent, but only reported results for serum specimens as the assay they used was validated for commercial use for serum samples only. Limited studies have revealed small but significant differences in IMA dependent on specimen storage, real time analysis or analysis after freezing [Christenson et al., 2001]. Samples stored at -20°C show minimal variation in IMA (-2.7 to +4.7%) from immediate analysis provided frozen tubes are thawed at 4°C for 20 minutes, whereas thawing at room temperature for 20 minutes increased measured IMA by 15% [Gidenne et al., 2004] .

3.3.4   **Haematocrit**

3.3.4.1   *Biological variance*

Hct levels increase with age and in association with male gender [Cheng et al., 2004]. They vary with ethnicity, genetics, exercise training, living at high altitude for prolonged periods and other environmental factors [Lugada et al., 2004]. Hct shows small seasonal within-subject normal variance of 3-7%. From 12 studies of 638 normal healthy adults, sampling between daily to 2 months gave a within-subject normal variance of Hct of about 3% [Thirup, 2003].

3.3.4.2   Sampling and storage

Hct values are significantly higher if collected into sodium EDTA tubes compared to potassium EDTA tubes [Chen et al., 1999]. Under-filling of tubes results in lower estimates for Hct, with values of Hct determined on 2mL sample volume significantly lower than those from a 5mL sample volume. Sequential rise in Hct occurs between 1, 8 and 12h from collection. Storage of samples at room temperature results in a gradual increase in Hct up to 7 days after collection [Gulati et al., 2002]. Thus, collection of serial blood samples for Hct measures should be in similar tubes, and analysis should take place within the same time frame for all measurements.

3.4   Summary

In this chapter, a range of biologically plausible blood biomarkers with potential for monitoring volume status in LVSD have been highlighted. They all have potential clinical application in the routine management of LVSD in terms of their current availability to the physician on a routine basis. However, it is essential that clinicians understand the potential sources of variance and take steps to minimise them in order to successfully utilise these biomarkers in routine patient care.

Chapter Four

Hypotheses, Experimental Design, Rationale for Selection of Biomarkers and Pre-analytical and Analytical Methods Outline

4.1   Introduction

The main aim of this thesis was to investigate the potential utility of repeated measures assessment of relevant biomarkers as non-invasive means of defining changes in volume status in different volunteer groups with a view to applying the principles tested to the situation in LVSD. Changes in each biomarker studied will be considered as potentially reflecting changes within specific compartments of volume status or depicting a specific pathophysiological step in LVSD, and will be interpreted in the context of its within-subject and between-subject normal variance, a concept which is rarely applied in routine clinical practice. 'Biomarkers' will be the collective term used for all biomarkers studied, which will include blood and urine biomarkers, and physical biomeasures.

4.2   Hypotheses (H1)

My general hypotheses (H1) were:

- Repeated measures of relevant blood and urine biomarkers, and physical biomeasures might provide a non-invasive means of assessment of changes in volume status by their response to induced volume changes.

- Changes in specific biomarkers might reflect changes in specific components of volume status, such as specific volume compartments, haemodynamic and renal variables.

- Changes in repeated measures of biomarker levels as a result of volume changes might be described above the within-subject normal variance for these biomarkers.

- Changes in repeated measures of biomarker levels in response to volume changes may occur without the development of symptoms (these may precede the development of symptoms or occur subclinically), and could thus have the potential to predict decompensation in LVSD.

- The mechanism for ongoing cardiac ischaemia or injury in LVSD may be secondary to volume overload. This might be reflected by an increase in biomarker measures of ischaemia in LVSD patients in response to volume expansion.

- The mechanism of renal tubular dysfunction in LVSD may be secondary to abnormal volume status. This might be reflected by changes in markers of renal tubular function following induced volume changes in LVSD patients.

4.3   Aims and experimental design

While mainly interested in the clinical application of repeated measures of non-invasive biomarkers to predict changes in volume status in stable LVSD patients, this group of patients would be unlikely to tolerate large changes in volume without the risk of decompensation. It was therefore possible that some of the less sensitive biomarkers being studied here may not show significant response to the small asymptomatic volume changes that were planned in this group of patients in an outpatient setting. In order to still be able to demonstrate the principle that a suitable biomarker of volume change has to show significant deviation from its within-subject normal variance, a principle that could then be applied to other biomarkers in future studies, protocols to study larger induced volume changes in decompensated HF and normal volunteers were also devised. Furthermore, the principle of significant deviation from within-subject normal variance in response to induced volume changes would in theory, be

more readily demonstrated in normal volunteers due to the likelihood of a more stable steady state with the distribution of most biomarkers likely to be non-skewed, and more control of the start of volume stimulus. Physiological responses to induced volume changes are different in LVSD patients compared to normal volunteers [Volpe et al., 1995], as are patterns for within-subject normal variance [Wu et al., 2003], such that induced volume changes in normal volunteers could not be directly compared to the situation in LVSD. However, studies in normal volunteers could potentially highlight abnormal responses in LVSD patients.

A three-stage plan of investigation was therefore devised, consisting of an observational study of repeated measures of physical biomeasures in patients with decompensated HF receiving intravenous furosemide (Chapter Five); repeated measures assessment of blood biomarkers and physical biomeasures in normal volunteers receiving controlled acute volume loading (Chapter Six); and repeated measures assessment of blood and urine biomarkers, and physical biomeasures in stable chronic LVSD patients who underwent temporary diuretic-induced volume changes (Chapter Seven). Respective biomarkers studied will be outlined in more detail in the latter sections of this chapter, and more detailed description of experimental protocols will be outlined in each respective chapter.

4.3.1   Studies in patients with decompensated heart failure

Pilot studies of repeated measures of physical biomeasures were completed in volunteers who were in decompensated HF. These were individuals who were admitted with acute cardiogenic pulmonary oedema being treated with high-dose intravenous furosemide (ivF) and they were predicted to have significant drug-induced changes in volume status. The main aim of this observational study was to establish the feasibility of performing repeated timed

physical biomeasurements in a group of unstable patients in a set pattern or systematic way in an attempt to reduce measurement error of physical biomeasures, to assess whether these measures were well tolerated by patients, and to define the response pattern of physical biomeasures to acute drug-induced volume changes. This experiment did not involve blood or urine biomarker analyses. Physical biomeasures measured at different time points consisted of:

Echocardiographic measures

- Mitral inflow indices

- Tricuspid inflow indices

- IVC indices

BIA measures

- Whole-body (distal) BIA

- Trunk (proximal) BIA

- Right-lung BIA (anterior-posterior electrode placement)

4.3.2   Studies in normal volunteers

Normal volunteers received differential acute volume loading protocols from a stable baseline under controlled conditions. The main aim of this stage of investigation was to establish the within-subject and between-subject normal variance of selected blood biomarkers and physical biomeasures in normal volunteers, and to define their response patterns to differential volume loading protocols as a deviation from their within-subject normal variance pattern.

Biomarkers determined in this study were:

Estimated blood volume

Blood biomarkers

- NP (ANP and BNP)

- Hct

Echocardiographic measures

- Mitral Doppler indices

- IVC indices

BIA measures

- Whole-body (distal) BIA

- Trunk (proximal) BIA

- Right-lung BIA (anterior-posterior and sternum-rib electrode placements)

4.3.3 Studies in stable chronic LVSD patients

Stable LVSD patients on chronic oral diuretic treatment underwent controlled manipulation of volume status by changes to their stable diuretic therapy over a 7-day period. Patients took their usual oral diuretic on Day 1, omitted their diuretic on Days 2, 3 and 4 (diuretic withdrawal phase), and resumed their oral diuretic on Days 5, 6 and 7 (diuretic resumption phase).The longitudinal response of bioamarkers could thus be established as a result of volume changes induced by diuretic manipulation. Furthermore, the normal variance of bioamakers could be established over an 8h monitoring period on Day 1. On Day 4 of the protocol, after 0h measures, a bolus of ivF was given to re-equilibrate volume status following maximal volume expansion associated with diuretic withdrawal, following which they were monitored under controlled conditions for 8h.

The variables assessed by repeated measures were:

Blood biomarkers

- NP (ANP and BNP)

- Hct

- Markers of ischaemia (cTnI and IMA)

- Markers of renal function [serum creatinine (sCreatinine) and serum NGAL (sNGAL)]

Urine biomarkers of renal tubular function

- Kidney injury molecule-1 (uKIM-1)

- Neutrophil gelatinase-associated lipocalin (uNGAL)

- N-acetyl-beta-D-glucosaminidase (uNAG)

- Urinary creatinine (uCreatinine)

Echocardiographic measures

- Mitral Doppler indices

- IVC indices

BIA measures

- Whole-body (distal) BIA

- Trunk (proximal) BIA

- Right-lung (anterior-posterior and sternum-rib electrode placement) BIA

4.4 Rationale for selection of biomarkers to be studied

A panel of blood and urine biomarkers and physical biomeasures was selected on the basis of the relevance in the potential depiction of different components of volume status and on the

background of previously documented sensitivities to volume change. Furthermore, the ease of availability of their measurement during both my study protocol and in routine clinical practice in terms of accessibility, reliability and practicality was a deciding factor. The emphasis was to study parameters with potential relevance for current clinical utility as opposed to biomarkers which were still at an experimental stage.

4.5   Blood and urine biomarkers

4.5.1   Indicators of intravascular volume change through haemoconcentration or haemodilution

Hct is potentially the simplest measure of haemoconcentration or haemodilution if normovolaemic Hct values are known [Androne et al., 2003]. As discussed in Chapter Three, the most useful setting for applying changes in Hct levels to track intravascular volume changes would be in the short-term monitoring of either acute or chronic LVSD [Valeri et al., 2006]. This is because of its low sensitivity for haemodilution or haemoconcentration in the long term due to the wide range of pathological processes associated with the progression of LVSD [Ng Kam Chuen et al., 2007]. These processes are unlikely to develop over the short time course of this study protocol, and changes in Hct levels in my volunteers are therefore likely to reflect primarily changes in intravascular volume secondary to the experimental volume manipulations. Hct levels are thus expected to fall following IV Saline infusion in the normal volunteers study and during diuretic withdrawal in the stable LVSD study, and to increase following diuretic reinitiation in the stable LVSD study. Furthermore, the within-subject normal variance of Hct will be established in stable volunteers at baseline, and the key element being studied here is the use of repeated measures to detect deviation from normal variance in response to volume manipulation.

Due to its simplicity and potential for in line continuous assessment, Hct has potential utility as it can be routinely and easily measured in clinical practice as part of the full blood count analysis. If repeated measures were sensitive to volume changes, Hct would constitute a cheap biomarker for routine repeated measures use in clinical practice that would accurately reflect changes in intravascular volume.

4.5.1.1 Estimation of blood volume using Hct measures

In the absence of invasive measures of intravascular volume, the regression equation for the calculation of estimated blood volume from Nadler et al. [1962] was used in an attempt to approximate a gold standard to which changes in suitable non-invasive biomarkers to different protocols could be correlated. This equation is based on body weight and height, and has been shown to have good correlation with radioisotope measures of blood volume in normal volunteers.

Thus, in my cohort of normal volunteers, blood volume at Time 0h (BV_0) was estimated using Nadler's formula: BV_0 (L) $= 0.0329 \times BW_0 + 0.3669 \times Ht^3 + 0.6041$, where BW_0 is the body weight in Kg at Time 0h, and Ht is the height in m.

Unlike in normal volunteers, there is no validated equation for the estimation of plasma/blood volume in LVSD patients based on body weight, height and percentage change of Hct. It would be inappropriate to use such equations which have been applied in normal volunteers, especially in a group of HF patients in whom gradual but dynamic fluid shifts would be

induced, and who were on multiple vasoactive treatments. Thus, no estimates of blood volume were calculated for stable LVSD patients.

In normal volunteers, the equations from Lobo et al. [2010] were used to then derive blood volume in litres at each time point by applying percentage changes in Hct values at each respective time point to determine percentage change in blood volume from baseline.

The percentage change in Hct at time t (ΔHct_t) was determined by the following equation:

ΔHct_t (%) = [(Hct_0 - Hct_t) / Hct_0] × 100, where Hct_0 is the Hct at 0h, and Hct_t is the Hct at time t.

Thus, the percentage change in blood volume at time t (ΔBV_t) can be calculated using the following formula: ΔBV_t (%) = {[100 / (100 - ΔHct_t)] × 100} − 100.

Blood volume at time t, BV_t, can thus be calculated by applying the formula:

BV_t (L) = [(BV_0 × (100 + ΔBV_t)] / 100. Blood volume change (in L) from baseline can then be derived as follows: BV_t (L) = BV_t − BV_0.

4.5.2 Indicators of venous compartment of intravascular volume/cardiac preload change through myocyte stretch

As previously discussed, the linkage of a range of NP to cardiac preload and myocyte stretch is well established. Consequently, the repeated measures sensitivity of ANP and BNP was evaluated in the studies in this thesis as potential indicators of cardiac stretch in response to the differential volume manipulation protocols which would induce changes in intravascular volume and thus affect cardiac preload.

ANP and BNP have shorter half-lives than their respective inactive forms, NTproANP and NTproBNP. A shorter half-life incorporates a greater ability to respond to acute changes in volume status, but is reflected in the greater observed variability over time of ANP and BNP compared to their inactive forms [McDowell et al., 2002; Bruins et al., 2004]. While increased variability might reduce the effectiveness of these markers when used simply as one-off diagnostic measures, this need not be a limitation for the use of repeated measures assessment during the short term observations planned during my studies as changes in ANP and BNP response to volume manipulation will be interpreted in the context of their baseline within-subject variance.

While ANP appears to be potentially more responsive to acute intravascular volume changes and haemodynamic changes [Gabrielsen et al., 2000; Herringlake et al., 2004; Maisel et al., 2002; James et al., 2005], BNP is certainly the more widely studied and more easily measured NP biomarker. Thus, ANP might increase in response to IV Saline infusion in the normal volunteers study and during the diuretic withdrawal phase in the stable LVSD study, and might reduce following diuretic reinitiation in the stable LVSD study. On the other hand, BNP was not expected to respond to the acute volume changes in the normal volunteers study. However, its levels might increase during the less acute diuretic withdrawal phase and reduce during diuretic resumption in the stable LVSD patients.

4.5.3 Potential indicators of cardiac preload/cardiac remodelling through myocardial ischaemia or necrosis

As previously outlined, ongoing myocyte injury is part of the cardiac remodelling process in LVSD occurs in both ischaemic and non-ishaemic cardiomyopathy [Matsumori et al., 2993], and is not necessarily linked to overt ischaemia [Sato et al., 2001]. The mechanisms for this process likely involve oxidative stress, inflammation, neurohormonal activation [Braunwald, 2008], but may also involve increased cardiac preload inducing myocyte stretch and subclinical injury.

cTnT and cTnI, as the most robust markers of myocyte death, are the obvious choice of blood biomarkers in an attempt to track these processes in my cohort of stable LVSD patients. However, their long half-lives [Ellis et al., 2001] are likely to explain their lack of responsiveness to acute changes in blood volume and haemodynamic indices in acute HF [Shah et al., 2007]. In this context, cTnI appears to be the more responsive of the two cTn [Gheorghiade et al., 2005], and would therefore be more likely to track small subclinical volume changes in my stable HF volunteers than cTnT. IMA, however, as an early marker of ischaemia whose levels appear within minutes of the onset of cardiac ischaemia with levels normalising rapidly [Sinha et al., 2003], and whose levels correlate with non-invasive measures of LV filling pressure [Sharma et al., 2006] could outperform the cTn as a more sensitive marker of volume changes. Thus, IMA levels might increase in response to diuretic withdrawal and reduce in response to diuretic reinitiation in the stable LVSD patients, whereas cTn levels may not change significantly. These biomarkers were not tested in the setting of volume expansion in normal volunteers as their baseline levels would likely be undetectable using the available assays.

4.5.4 Potential indicators of intravascular volume change through markers of renal tubular function

As discussed in Chapter One, the relationship between cardiac and renal function is critical in the maintenance of volume homeostasis. The potential mechanisms of renal injury as a direct haemodynamic consequence of reduced cardiac output in HF include reduction of renal blood flow resulting in reduced GFR, renal hypoxia, drug-induced dehydration or renal damage; and activation of the neurohormonal axis causing sodium and water retention with increased venous pressure [Ronco et al., 2008]. Increased CVP, right atrial pressure or physical signs of venous congestion are associated with reduced GFR in a range of stable patients with or without impaired LVEF, regardless of cardiac output [Damman et al., 2009; Nohria et al., 2008]. Particularly in the presence of reduced renal blood flow, increased CVP is associated with further reductions in eGFR. Furthermore, increased CVP associates with worsening renal function in acute HF [Mullens et al., 2009]. While the mechanisms for this interaction between venous congestion and renal impairment has not been fully elucidated, it is possible that increased venous congestion causes renal dysfunction by increasing renal venous pressures with subsequent reductions in renal blood flow [Mullens et al., 2009], or that impaired kidneys as a result of multiple pathophysiological mechanisms associated with HF are unable to excrete salt and water appropriately. Regardless of whether the mechanism of renal dysfunction related to increased central pressures is causative, consequential or merely associative, markers of renal damage could potentially also act as markers of changes in central venous pressure, and thus of cardiac preload. Furthermore, markers of renal damage could potentially also act as direct measures of renal perfusion, and thus of reduced cardiac output.

Renal damage in the context of LVSD can occur via a number of cellular mechanisms, namely, tubular dysfunction, cell death by apoptosis and necrosis contributing to intratubular obstruction, and tubulointerstitial inflammation [Vaidya et al., 2008]. The final common pathway for kidney injury may involve renal hypoxia, especially in the proximal tubules which utilise the largest amount of oxygen [Nangaku et al., 2006]. Renal impairment is traditionally defined by reduced GFR, elevated sCreatinine or blood urea nitrogen levels, with changes in these markers occurring hours or days after the inciting event. However, the process of renal impairment is a continuous process as described by the RIFLE criteria, which ranges from initial risk of kidney dysfunction to renal injury through damage of renal structures which eventually results in failure of kidney function, loss of kidney function (reduced GFR) and eventually kidney failure (end-stage kidney disease) [Bellomo et al., 2004]. There is a delicate and dynamic relationship between tissue repair and progression or regression of renal injury, where each of the steps within this continuum from renal injury to failure could be potentially reversible. Thus, whereas measures of reduced GFR would identify the latter stages of this process and reflect *functional* failure of the kidneys, biomarkers identifying *structural* damage at the earlier stages of this process could not only identify early kidney dysfunction at a reversible stage, but also identify the potential sites and severity of the injury, and the mechanism of the injury [Vaidya et al., 2008]. Therefore, while reduced GFR may coexist with raised markers of morphological damage to the kidneys, it is likely that in a large number of patients, there is subclinical renal dysfunction manifesting as increased markers of structural/morphological damage with normal GFR, serum urea and serum creatinine (sCreatinine) levels.

In this respect, a range of candidate renal biomarkers of tubular function have been defined whose levels increase within hours of acute kidney injury and have been shown to have diagnostic and prognostic value for renal damage.

N-actetyl-β-glucosaminidase (uNAG) is a proximal tubule lysozyme which is shed into the urine during subtle alterations in the epithelial cells of the proximal tubules. The amount of enzyme shed can be directly correlated to tubular injury, and has thus been shown to be a sensitive, persistent and robust indicator of tubular injury in several contexts including cardiopulmonary bypass procedures [Vaidya et al., 2008]. uNAG levels are highly sensitive at detecting acute kidney injury in critically ill adult patients, increased levels preceding increases in sCreatinine by 12h to 4 days [Westhuyzen et al., 2003]. Poorer outcome, as determined by death in hospital and requirement for long-term renal replacement therapy, was associated with high levels of uNAG on admission to a renal care unit compared to normal uNAG levels [Chew et al., 1993].

Kidney Injury Molecule-1 (uKIM-1) is a renal tubular glycoprotein whose ectodomain is shed into the urine following proximal tubular kidney injury; this can then be measured by means of an ELISA. Its levels are also elevated within 12h after an initial ischaemic renal insult, prior to the appearance of casts in the urine, and its levels correlate with tubulo-interstitial fibrosis and inflammation. uKIM-1 seems to be most sensitive to ischaemic injury to the proximal tubules, its levels being most elevated in ischaemic acute tubular necrosis compared to other mechanisms of injury [Han et al., 2005]. There is also evidence that its levels are the most elevated compared to other biomarkers following kidney injury. uKIM-1 and uNAG levels in acute kidney injury were significantly associated with the clinical composite end-

point of death or dialysis requirement, even after adjustment for disease severity and co-morbidities [Liangos et al., 2007].

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein initially identified bound to gelatinase in specific granules of the neutrophil. It is normally secreted in small amounts in lungs, kidney, trachea, stomach and colon tissue. Normal adults only have undetectable to low levels of serum and urinary NGAL (sNGAL and uNGAL); however their levels are elevated in various pathological states [Vaidya et al., 2008]. In renal failure, both sNGAL and uNGAL levels rise markedly following the same trends. A prospective study of paediatric patients without pre-existing renal failure undergoing cardiopulmonary bypass found both sNGAL and uNGAL to be powerful early markers of acute kidney injury, levels being raised within 2h after bypass, and preceding any increase in sCreatinine by 1-3 days [Mishra et al., 2005]. A similar study in adult patients showed uNGAL levels at 1, 3 and 18h after cardiac surgery to be significantly higher in patients who went on to develop clinically significant acute kidney injury [Wagener et al., 2006]. Damman et al. [2008] demonstrated for the first time, increased levels of uNGAL in LVSD patients compared to controls, thus indicating the presence of tubular damage in LVSD. Both sCreatinine and eGFR were significantly correlated to uNGAL levels in their cohort of patients. However, it is unclear as to how many patients with normal eGFR and urinary albumin excretion had increased uNGAL. uNAG and uKIM-1 levels have so far not been established in HF populations.

Thus, based on the diuretic-induced volume manipulation protocol in my cohort of LVSD patients, I hypothesised that renal injury might occur in clinically dehydrated patients as a result of chronic diuretic treatment. This might improve following diuretic withdrawal, thus

causing a reduction in levels of the renal tubular markers, and worsen following diuretic resumption, causing an increase in renal tubular marker levels. On the other hand, renal injury might occur secondary to increased central venous pressure and could potentially be reflected by increased levels of these sensitive renal tubular markers in response to diuretic withdrawal.

4.6 Physical biomeasures

4.6.1 Echocardiographic variables: Indicators of intravascular volume/cardiac preload change through estimates of LV filling pressure

As outlined in chapter Two, transthoracic echocardiography is used in HF patients mainly as a diagnostic tool to assess LV function and to determine the aetiology of a HF presentation. Although many individual measures have been linked to a variety of invasively measured haemodynamic indices of cardiac preload [Mansencal et al., 2007], these are not routinely repeated as a measure of volume status to guide treatment decisions. However, as with Hct, potential echocardiographic indicators of volume status have considerable interest as they are freely available as part of a routine comprehensive study.

The utility of echocardiographic measures is limited by their inter-observer and intra-observer variability, and more fundamentally by subject echogenicity. These are perhaps less important in the application of routine echocardiography in a simple diagnostic role to address the presence or absence of LVSD. However, these principles become key aspects of utility where any echocardiographic measure is to be used as a repeated measure of volume status within an individual.

Repeated measures assessment by a single operator has the potential to reduce measurement error and improve sensitivity. Thus, echocardiograms were performed by only one operator (MJNKC), to eliminate inter-observer variability. All patients were screened prior to enrolment to ensure adequate echocardiographic windows and to determine LVEF using the biplane modified Simpson's rule. A GE Vivid 3 portable platform (General Electric Medical Systems, Solingen, Germany) was used for the study in patients with decompensated HF in an in-patient setting; and a GE Ving Med System 5 platform (GE Medical Systems Ltd, Slough, UK) was used for the normal volunteers and stable LVSD studies in the study centre location. A 2.5 MHz transducer probe was used. Relevant echocardiographic windows were stored on video for the former, and on optical disc for the latter, for off-line, anonymised and blinded analysis using the Echopac system. Measurements were made according to the recommendations of the American Society of Echocardiography [Lang et al., 2006; Quinones et al., 2002].

Following literature review of potential echocardiographic measures of LV filling pressure (Chapter Two), markers which are easily obtainable and whose documented time for measurement and intra- and inter-observer variability were small were selected [Khan et al., 2004; Rijsterborgh et al., 1990]. These comprised mitral Doppler indices, tricuspid inflow indices and IVC indices. Pulmonary vein Doppler indices, right atrial volume, LV end diastolic and end systolic dimensions and volume and contractility indices which have also been correlated to changes in cardiac prelaod, were not incorporated into the repeated measures assessment due to time constraints of repeated measurement and volume manipulation, which would compromise the accuracy of their measurement, and also due to large measurement variation even under optimal conditions [Gordon et al., 1993].

Furthermore, these particular indices are affected by accurate wall detection and echogenicity within a subject, again affecting their accuracy. The reported success rates for obtaining echocardiographic measures in 80 patients were 100% for mitral inflow indices compared to only 46-73% for pulmonary vein Doppler indices, the inter-observer variability being reported as 2-5% for mitral inflow indices, increasing slightly to 2-9% after Valsalva manoeuvre, compared to 3-12% without Valsalva manoeuvre for pulmonary vein Doppler indices [Khan et al., 2004]. Furthermore, the time taken to record mitral inflow indices was 29-36 seconds, compared to 81 seconds for pulmonary vein Doppler indices.

Mitral and tricuspid inflow indices were assessed with and without the Valsalava manoeuvre on expiration to minimise their respiratory variation. The strain phase of the Valsalava manoeuvre results in an acute increase in intra-thoracic pressure and decreased stroke volume as a result of decreased venous return [Hamilton et al., 1994]. Thus, during the strain phase, the expected reduction in cardiac preload is likely to attenuate the mitral and tricuspid inflow responses, such that greater sensitivity would be indicated for variables whose levels still change significantly following a particular volume stimulus during the manoeuvre (rather than only changing significantly without the Valsalava manoeuvre). Patients were instructed to perform the Valsalava manoeuvre at the end of a normal inspiratory effort, and the Valsalava manoeuvre was judged adequate if accompanied by the development of florid facies, distended neck veins and increased abdominal wall muscle tone [Zema et al., 1984]. Relevant echocardiographic windows for each variable were optimised before strain; strain was then maintained for the duration of acquisition of images which in general lasted less than 10 seconds.

4.6.1.1   *Mitral Doppler indices*

Mitral inflow pattern, as illustrated in Figure 4.1, consists of MVE, MVDT and MVA, from which MVE/A was calculated. As outlined in Chapter Two, MVE, MVDT and MVE/A correlate to invasive measures of LV filling pressure in patients with depressed LV systolic function, to a lesser extent, in subjects with normal LVEF [Nishimura et al., 1996; Szatajzel et al., 1993; Kinet et al., 1982; Hurrell et al., 1997].

Figure 4.1. Mitral inflow indices.
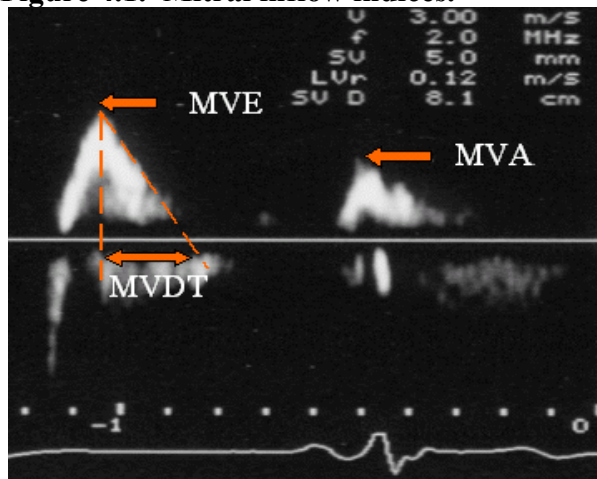
MVE: early peak transmitral velocity; MVA: late peak transmitral velocity; MVDT: transmitral deceleration time.

E/Ea indices correlate to varying extents to PCWP and LVEDP in a wide variety of cardiac conditions and were also used as estimates LV filling pressure [Nagueh et al., 1997; Mullens et al., 2009; Arteaga et al., 2008]. Ea was measured at the lateral and septal walls (Figure 4.2), enabling the calculation of E/Ea(l) and E/Ea(s) respectively.

Figure 4. 2. Tissue Doppler early diastolic mitral annular velocity at the lateral wall [Ea(l)] and at the septal wall [Ea(s)].
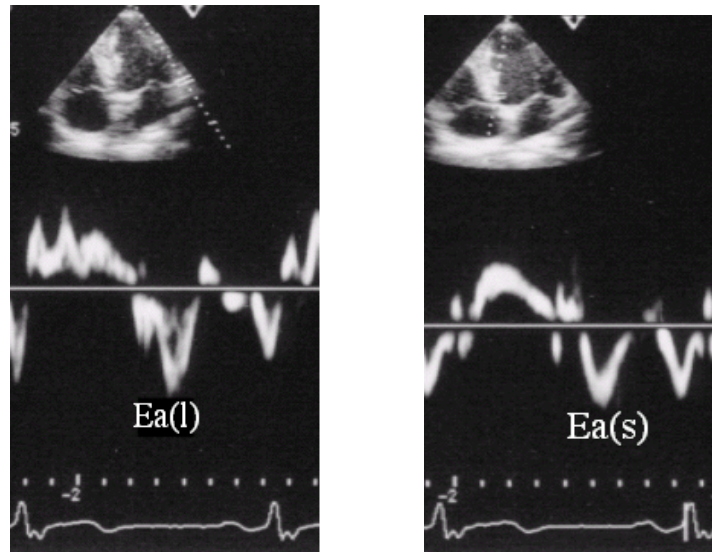
4.6.1.2   *Tricuspid inflow indices*

Tricuspid inflow indices (Figure 4.3) including early peak transmitral velocity (TVE), early deceleration time (TVDT), late peak transmitral velocity (TVA) and the ratio of early to late peak mitral velocities (TVE/A) were also measured to establish whether changes in right ventricular inflow indices would be more sensitive to volume changes, the right ventricle being a much more distensible structure than the LV and thus possibly less likely to be affected by relaxation compared to preload. Changes in tricuspid inflow velocities have not been studied extensively in relation to cardiac preload changes, but TVE has been correlated to right atrial pressure [Harada et al., 1994; Bartel et al., 1996]. In atrial fibrillation, all transtricuspid and transmitral measures were measured three times and averaged.

Figure 4.3. Tricuspid inflow indices.
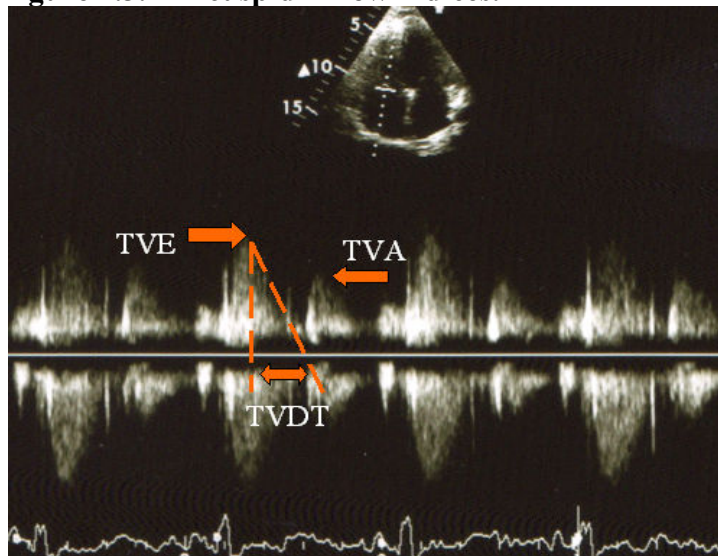
TVE: early peak transtricuspid velocity; MVA: late peak transtricuspid velocity; MVDT: transtricuspid deceleration time.

4.6.1.3   *Inferior vena cava indices*

As described in Chapter Two, several IVC indices correlate to invasive haemodynamic measures of LV filling pressures or blood volume in a number of conditions [Cheriex et al., 1989; Blair et al., 2009; Capomolla et al., 2000]. The difficulty with interpreting and applying these findings to routine clinical practice is that different studies of IVC have employed varying views (long-axis verus short-axis), modes of measurement (2-D versus M-mode) and states of IVC (in expiration, inspiration, deep inspiration or after a sniff) in a wide variety of conditions with varied volume manipulation protocols (haemodialysis patients, patients awaiting cardiac transplant, normal volunteers). Furthermore, the timing of IVC measurement in relation to the cardiac cycle is not standardised. I therefore aimed to systematically measure all IVC indices used in previous studies in order to provide a direct comparison of the potential performance of these markers in mapping changes in volume

status within the venous compartment and LV filling pressure within the same volunteer group.

The 2-D image was firstly optimised in the long-axis view, and the M-mode cursor was placed 2 cm from the junction between the IVC and the right atrium. Following the long-axis M-mode measures, the probe was turned by 90 degrees from the previous 2-D long-axis view in order to produce the short-axis view, as in the study by Brennan et al. 2007. The M-mode cursor was placed in the centre of the IVC image, and further M-mode measures were made. Thus, IVC was measured by M-mode in expiration (e), inspiration (i) and maximal inspiration (mi) in long (Figure 4.4) and short-axis views (Figure 4.5).   IVC was measured before the P wave on the ECG to avoid the A wave, or before the QRS complex in atrial fibrillation. IVC was corrected for body surface area (IVCD) and the IVC collapsibility index (IVCCI) was calculated using the equations: IVCCIi = [(IVCe-IVCi) / IVCe] × 100; IVCCImi= [(IVCe-IVCmi) / IVCe].

E/Ea indices could not be performed in the cohort of patients with decompensated HF as the portable Vivid 3 platform used in this study did not have tissue Doppler modality. Furthermore, tricuspid Doppler indices were not analysed in the normal volunteers and stable LVSD cohort as the tricuspid pulsed wave signals from the study in patients with decompensated HF were not well defined for the majority of volunteers and were therefore felt to significantly affect the accuracy of measures in subsequent studies.

Figure 4.4. Long-axis inferior vena cava diameter in expiration (IVCe), inspiration (IVCi) and maximal inspiration (IVCmi).
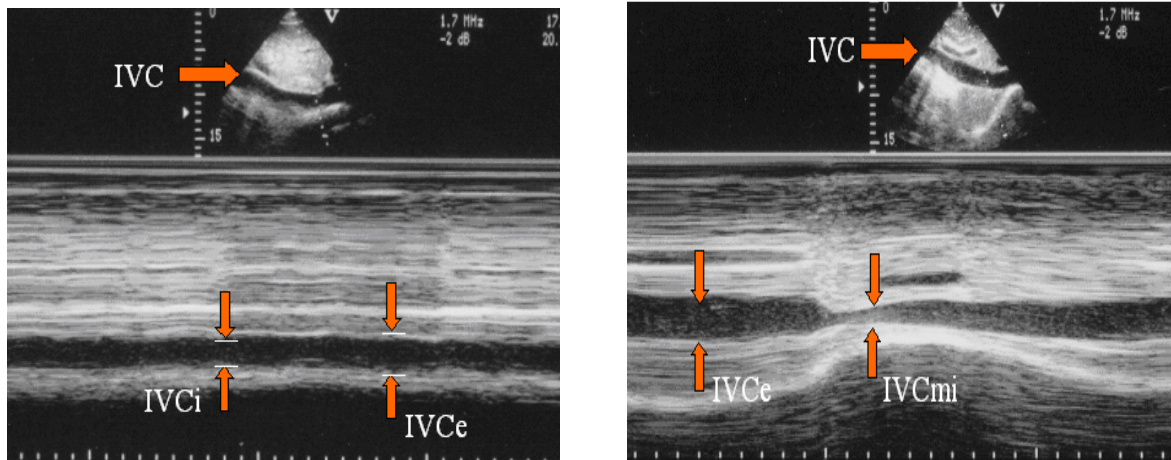
Figure 4.5. Short-axis inferior vena cava diameter in expiration (IVCe), inspiration (IVCi) and maximal inspiration (IVCmi).
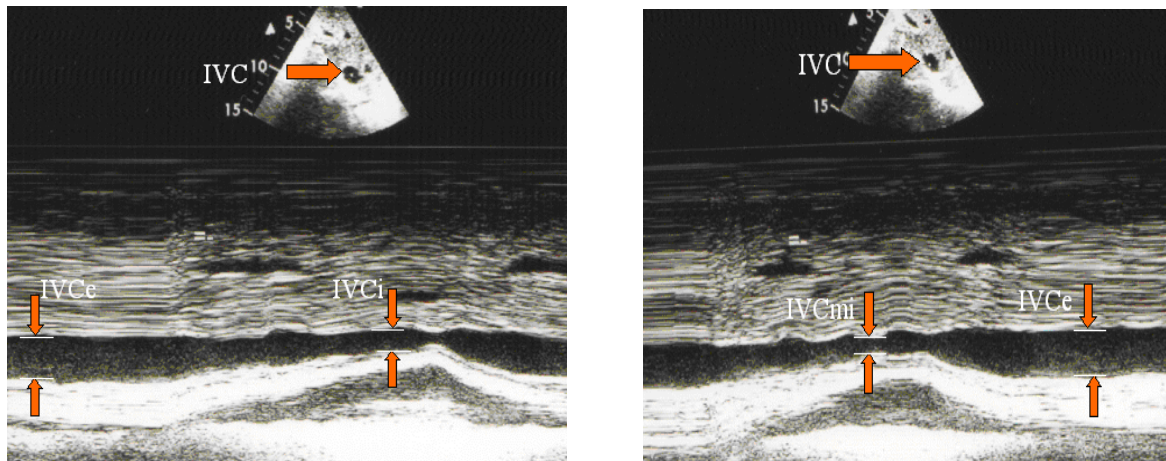
4.6.2   **Bioimpedance analysis: Indicators of volume change through changes in estimates of extracellular volume (intravascular and interstitial) and total body water**

Given increasing commercial interest in bioimpedance, largely without predefined sensitivity and specificity, BIA could potentially be a useful, cheap and non-invasive means of tracking volume changes in the models of volume manipulation defined here. Furthermore, it constituted the only variable of the biomarkers selected for the study which has been shown to change reliably prior to the onset of symptoms of HF, and could thus potentially be the most

sensitive to the planned subclinical volume changes. Although sophisticated and expensive

devices could provide estimates of haemodynamic measures such as cardiac output, these are

all reliant on regression equations validated against gold standards in specific populations

[Sodolski et al., 2007]. The chosen device for my studies would be used in different study

populations, where the regression equations would have variable accuracy. The emphasis

therefore, was on obtaining raw impedance values, with the assumption that impedance would

decrease with increasing volume [Kyle et al., 2004].

A commercially available multi-frequency BIA device (Bodystat Quadscan 4000®, Isle of

Man, UK) was therefore chosen which would measure body water compartments and raw

impedance values (BIA measures) using tetrapolar electrodes. Impedance values to currents

of 5, 50, 100 and 200 kHz (Z_{5kHz}, Z_{50kHz}, Z_{100kHz}, Z_{200kHz} respectively), and estimated body

water composition (TBW, ECW and ICW) were all collectively obtained in one measurement.

The device has been calibrated against under-water weighing for percentage fat, tritium

dilution techniques for TBW estimation, and NaBr dilution technique for estimation of ECW;

ICW is then estimated as the difference between TBW and ECW (personal communication

from manufacturer). According to the product literature, Z_{50kHz} maps ECW (current at low

frequency unable to penetrate cell membrane), and Z_{200kHz} maps TBW (high frequency able to

penetrate cellular membrane). The most useful marker would potentially be impedance at

lower frequency currents as this would be a measure of the ECW (intravascular plus

interstitial spaces), the compartment most likely to be affected by the relatively acute stimuli

generated by volume manipulation.

Whole-body (distal), trunk (proximal) and right-lung BIA measures were documented. However, whereas estimates of body water composition were derived by the device for whole-body and trunk measurements, no derivation equation was available from the manufacturer for use of the right-lung impedance values. In fact, electrode position for right-lung BIA was only recommended by the manufacturer after the study in the patients with decompensated HF was completed.

Whole-body BIA was measured by connecting the electrodes to the right wrist and ankle, trunk BIA by electrode placement to the suprasternal notch and right knee. Right-lung impedance was measured by using the manufacturer-recommended electrode placements to the suprasternal notch and intersection of right anterior axillary line and lower rib (sternum-rib), and by my own devised electrode placement to the anterior fourth and fifth ribs and to the posterior scapula (anterior-posterior), as shown in Figure 4.6.

Figure 4.6. Bioimpedance analysis across whole body (a), trunk (b), right lung with electrodes in anterior-posterior position (c) and sternum-rib position (d).

4.7 Pre-analytical and analytical methods for blood and urine biomarkers

Based on the discussions in Chapter Three, steps to minimise pre-analytical and analytical variance of blood and urine biomarkers are described below.

4.7.1 Blood biomarkers

Blood for Hct was collected into EDTA tubes (1.5mg/mL) and kept at room temperature. Analysis was carried out using the Bayer ADVIA® 120 Haematology System (Siemens Medical Solutions Diagnostics Ltd, Newbury, Berks, UK) at the Centre for Cardiovascular Sciences within 2h of collection. Blood was well mixed prior to analysis. The intra- and inter-assay coefficients of variation were 1.5% and 2.3% respectively.

For ANP and BNP, blood was collected into chilled EDTA and aprotinin (500i.u./mL, Trasylol, Bayer, Newbury, Berks, UK) tubes. Samples were kept on ice until they were centrifuged at 4°C at 1200 rpm for 20 minutes within 2h of collection. Samples were aliquoted into respective plastic tubes and stored at -70°C for subsequent analyses. Samples were gently thawed and vortexed prior to analysis and all samples from the same patient were analysed in the same batch to minimise analytical error. ANP was analysed by ELISA (USCN Life Science Inc., Wuhan, China) at St George's Hospital by Dr David C. Gaze and Dr Paul O. Collinson. Intra-assay coefficient of variation was <5%, inter-assay CV was <14%, the assay range was 0-25 ng/mL. BNP was measured using the ADVIA Centaur^{TM} automated chemiluminescence system (Siemens Medical Solutions Diagnostics, Newbury, Berks, UK). BNP values were converted from pmol/L to pg/mL by dividing by 0.289 [Gabriel et al., 2008;

Kroll et al., 2007] in order to standardise values to previous studies. The inter-assay coefficient of variation was 13.6% for low (4.26 pg/mL), 10.0% for medium (36.9 pg/mL) and 11.2% for high (133.0 pg/mL) BNP values. The intra-assay coefficient of variation was 22.3% for low, 10.9% for medium and 11.6% for high values. The quality control analyses were performed in between the study sample analyses. The sensitivity of the assay was 6.9 pg/mL and the upper detection limit was 17301 pg/mL. A BNP value of >100 pg/mL was considered as elevated (specificity >95% and above the upper confidence interval of 95th percentile of healthy reference population). Normal range of ANP was considered to be 10-70 pg/mL.

Blood was also collected in serum separator tubes at room temperature and centrifuged at 4°C at 1200 rpm for 20 minutes. Samples were aliquoted into respective plastic tubes and stored within 2h at -70°C for cTnI , cTnT and sNGAL, and at -20°C or less for IMA for subsequent analyses. Similar precautions as for ANP and BNP were taken prior to analysis to minimise pre-analytical and analytical error. cTnI was measured using the ADVIA Centaur [TM] automated chemiluminescence system (Siemens Medical Solutions Diagnostics, Newbury, Berks, UK) in the Biochemistry Department at Sandwell Hospital. The lower detection limit was 0.010 ng/mL, and a cTnI value of >0.040 ng/mL was considered as elevated. The intra-assay coefficient of variation was 5.6% for low (0.051 ng/mL), 1.13% for medium (0.894 ng/mL), and 0.47% for high (29.7 ng/mL) quality control values. The inter-assay coefficient of variation was 1.49% for low, 0.78% for medium and 1.13% for high cTnI quality control values.

cTnT was measured by Bayer autoanalyser at Sandwell Biochemistry Laboratory. The detection limit of cTnT was 0.010 ng/mL, with a cTnT value of >0.010 ng/mL considered as elevated. 100 random samples from the cohort of chronic LVSD patients were initially analysed. Only 3 produced detectable results (0.012, 0.020 and 0.011 ng/mL respectively). The remaining samples had undetectable cTnT levels (<0.010 ng/mL). I therefore felt that the majority of LVSD patients would have undetectable levels of cTnT by our available autoanalyser, and no further cTnT analyses were carried out.

IMA was analysed at St George's Hospital by Dr Paul O. Collinson and Dr David C. Gaze using a colorimetric assay, the ACB® (Albumin Cobalt Binding) test (Inverness Medical Innovations, Inc., Massachussets, USA) on the Roche Cobas Mira platform. The normal range was 0-85 U/mL. Intra-assay coefficient of variation at low IMA value (75.9 U/mL) was 2.2% and high IMA value (119.9 U/mL) was 2.1%. Inter-assay coefficient of variation at low IMA value (75.9 U/mL) was 5.4% and high IMA value (119.9 U/mL) was 4.4%.

4.7.2   Urine biomarkers

Urine samples were stored in plastic tubes at room temperature. They were frozen at -70°C within 2h of collection until the time of analysis, at which point they were gently thawed and vortexed. uKIM, uNGAL, sNGAL, uNAG, sCreatinine and uCreatinine were analysed in Groningen by Dr Kevin Damman and Prof Dirk J. van Veldhuisen. NGAL was determined by means of a commercially available ELISA test kit from Antibody Shop (Gentofte, Denmark). Samples were diluted 500 times in dilution buffer supplied with the test kit. uNAG was evaluated using the substrate p-nitrophenyl *N*-acetyl-β-D-glucosaminide (Sigma, St Louis,

MO) in citrate buffer at pH 4.5. After 60 minutes at 37°C, 1 M Na_2CO_3 was added to the mixture to terminate the reaction and to develop a yellow colour released from the converted substrate. Controls were obtained from each sample by addition of Na_2CO_3 at time=0h. The colour was measured at 405 nm by a microtitre plate reader and controls were subtracted. A standard curve was made with *N*-acetyl-β-D-glucosaminidase. uKIM-1 measurements were performed using microsphere-based Luminex xMAP technology with polyclonal antibodies raised against the human KIM-1 ectodomain. For measurements, 30 µl of urine samples were analyzed in duplicate. The lowest limit of detection for this assay is 0.125 ng/ml. The inter- and intra-assay variability for these biomarkers was <20%. uNGAL, uNAG and uKIM-1 levels were corrected for urinary concentration by dividing their values by corresponding uCreatinine concentration, and expressed as /gram urinary creatinine (/gCr).

4.8   Body weight and height

Body weight was recorded to the nearest 0.1 Kg using a Seca 761 mechanical medical scale (Seca Ltd, Birmingham, UK), and height was recorded to the nearest 0.01 m.

4.9   Statistical analysis

Normally distributed data were presented as means (SD), and non-normally distributed data as median (IQR). Mean, median and standard deviation values, as well as histograms and the Kolmogorov-Smirnow test were used to define whether data was normally or non-normally distributed. Specific statistical analyses for each study are discussed in the respective chapters. A p value of ≤0.05 was significant. Data were analysed using SPSS 14.0 for

Windows (SPSS Inc., Chicago, Illinois, USA). Graphs were created using GraphPad Prism

Software (GraphPad Software Inc., San Diego, CA, USA).

4.10 Ethical approval

The Sandwell and West Birmingham Local Research Ethics Committee granted ethical

approval for all studies reported in this thesis. All volunteers gave written informed consent

to study procedures.

Chapter Five

Studies in Patients with Decompensated Heart Failure

Performing Repeated Measures of Non-invasive Physical Biomeasures of Volume Response to Intravenous Furosemide: a Feasibility Study

5.1   Introduction

A study investigating the performance of repeated measures of physical biomeasures in tracking volume changes relies on all repeated measures being collected and analysed under optimal conditions to minimise measurement error, but equally, on it being feasible for all repeated measures to be consistently recorded at all time points. This is in the context of repeated measures having to be performed within the time constraints of the study protocols, with the onset of intervention being staggered between individual patients to allow optimisation of operator use of resources and time.

Thus, only a limited period of time would be available to the operator to collect the planned series of physical biomeasures before either the next series of measures is due (whether in the same volunteer or a different volunteer), or the volume change at a particular time point is missed, for example, the 0.5h measures still being collected at 1h into the protocol. This was particularly relevant for echocardiographic measures which are time consuming and dependent on both the skills of the operator, speed of acquisition and patient factors such as tolerability of the measures, echogenicity, optimal position (e.g., left lateral position for mitral and tricuspid inflow indices, but supine for IVC measures), and compliance with instructions, such as ability to perform the Valsalva manoeuvre and control of respiration. These factors would all contribute to measurement error. Thus, in a similar way to taking steps to minimise the sources of variance of the selected panel of blood biomarkers as outlined in Chapters Three and Four, there was also a need to explore ways in which measurement variance for physical biomeasures could be minimised.

A set pattern of measuring physical biomeasures in a systematic way was therefore devised which would optimise both operator and patient factors as well as time resources. To eliminate inter-observer variability, the physical biomeasures were performed by only one operator (MJNKC). A readily available group of volunteers to perform a pilot study for the feasibility of this pattern of measuring physical biomeasures consisted of hospitalised patients who were admitted with acute cardiogenic pulmonary oedema, and who were undergoing intravenous diuresis for the treatment ofdecompensated HF. Performing the pilot study in this group of patients would not involve any additional attendance or intervention to their routine medical care. These patients would by definition, have abnormal volume status, with at least increased interstitial water as evidenced by the clinical signs of pulmonary oedema, peripheral oedema or ascites, with potentially variable volume status in their intravascular space, this being predefined by their underlying neurohormonal status and current and previous treatments, in particular prior doses of diuretics[Cotter et al.]. Fluid redistribution, and diuresis and natriuresis were expected to occur on a significant scale as a result of intravenous diuretic administration.

5.2   Hypotheses (H1)

I hypothesised that it would be feasible to perform repeated measures of physical biomeasures consistently in a systematic way by a single operator in this potentially unstable group of patients, that the repeated assessments would be well tolerated by the patients, and that practice of and increased familiarity with the set pattern of measurement would reduce the measurement error of echocardiographic measures. Furthermore, I hypothesised that physical biomeasures that changed the most from baseline measures following intravenous diuresis

would potentially be the most sensitive physical biomeasures at tracking volume changes in response to intravenous diuresis in this group of patients.

5.3   Methods

5.3.1   Study population

Patients were recruited from the Coronary Care Unit, Post Coronary Care Unit and the Cardiology ward at City Hospital who had been admitted with a clinical diagnosis of acute cardiogenic pulmonary oedema, defined as the presence of acute symptoms and signs, as well as radiological evidence of lung congestion. In addition, these patients had other symptoms and signs of decompensated HF such as lethargy, raised jugular venous pressure, peripheral oedema, third heart sound and ascites to varying extents. They were all receiving bolus doses of intravenous furosemide (ivF) up to twice daily. Exclusion criteria included patients who were too clinically unstable and unable to cooperate or consent to repeated measures assessment, and patients receiving more than their 10th dose of ivF. Patient demographic details were documented.

5.3.2   Set pattern for collection of repeated measures of physical biomeasures

Physical biomeasures were performed at baseline (0h), then at 0.5, 1, 2 and 3h after ivF. At each time point, BIA measures were performed first, with patients lying supine. Prior to the 0h measures, the relevant areas of skin were prepared by shaving and by applying alcohol wipes, followed by positioning of respective electrodes, as described in Chapter Four. The BIA device was then connected, in the following order, to electrodes placed distally to determine whole-body BIA, then to proximal electrodes to determine trunk BIA and finally,

to electrodes placed in the antero-posterior position to determine right-lung impedance. The latter electrode position was my own devised position, as the manufacturer had not devised their recommended electrode position for right lung BIA measurements at the time of this study. The BIA device was then disconnected, followed by connection of the ECG monitor on the echocardiography platform. IVC measures on expiration, inspiration and maximal inspiration were then performed with the patient still lying supine. The patient was then turned to the left lateral position for the remaining echocardiographic assessment. Mitral inflow indices with and without the Valsalva manoeuvre and tricuspid inflow indices with and without the Valsalva manoeuvre were then performed. BIA pmeasures determined included Z_{5kHz}, Z_{50kHz}, Z_{100kHz}, Z_{200kHz} TBW, ECW and ICW across the whole-body and the trunk. Only impedance values were available from the right-lung measures. Echocardiographic measures that were collected consisted of MVE, MVDT, MVE/A, TVE, TVDT, and TVE/A with and without the Valsalva manoeuvre, and IVC indices on expiration, inspiration and maximal inspiration in both long-axis and short-axis views.

5.3.3   Determination of intra-observer variability for physical biomeasures

The intra-observer variability for echocardiographic measures and the intra-device variability for BIA measures were determined by performing 10 repeated measures on one healthy individual and calculating the coefficient of variation before the start of the study. This process was then repeated for echocardiographic measures after completion of the study to assess whether intra-observer variability would reduce after practice and increased familiarity with the process of repeated measures assessment. The first set of measures was performed using the GE Vivid 3 portable platform, which was used in the current study, and the second

set of measures were performed using the GE VingMed System 5 platform which would be used in the studies in normal volunteers and stable LVSD patients.

5.3.4   Statistical analysis

General linear model repeated measures one-factor ANOVA was used to establish the response of variables to ivF against time, and Bonferroni post-hoc analysis was performed to identify the time points between which the significant changes in biomarker measurement took place.   The Bland-Altman method was used to assess the agreement between the measurement of IVC diameters using the long-axis and short-axis views.There is no precedent to this study investigating the acute effects of ivF on mitral and tricuspid inflow indices, IVC indices or BIA measures, thus data are lacking to estimate power for these markers. However, based on the study by Dikshit et al. [1973], a sample size of 30 patients would give a power of > 85% to detect a 30 % reduction in LV filling pressure in response to ivF.

5.4   Results

Patients were recruited over a 6-month period. Of the 31 patients enrolled, 61% were male. Table 5.1 illustrates their demographic details. The predominant cause for acute cardiogenic pulmonary oedema was cardiac ischaemia. 14 (46%) patients were receiving between their first to third dose of ivF, and 14 (46%) patients were receiving their fourth to sixth dose of ivF. 13 (42%) patients had LVEF < 40%; 9 (29%) patients had LVEF 40-50%, and 9 (29%) patients had LVEF of >50%. 12 (39%) patients had renal dysfunction, as evidenced by a serum creatinine level of > 1.20 mg/dL.

Table 5.1. Demographic details of patients with decompensated heart failure.

Variable	Value
Male (%)	19 (61)
Age (yrs)	72 (12)
Systolic BP (mmHg)	122 (22)
Diastolic BP (mmHg)	71 (11)
Heart rhythm	23 sinus rhythm; 8 atrial fibrillation
Body mass index (Kg/m^2)	28.6 (5.5)
Body surface area (m^2)	1.9 (0.2)
Left ventricular ejection fraction (%)	43 (14)
Haemoglobin (g/dl)	12.2 (2.2)
Haematocrit (%)	36.8 (6.3)
Urea (mmol/L)	9.7 (6.2)
Serum Creatinine (mg/dL)	1.21 (0.92-1.67)
Aetiology, n(%)	
Ischaemia	12 (39)
Valvular heart disease	3 (10)
Arrhythmia	3 (10)
Hypertension	1 (3)
Renal disease	5 (16)
Non ischaemic cardiomyopathy	2 (7)
Others	5 (15)
Dose of IV furosemide given (mg)	40-50 mg: n=14
	80-100 mg: n=17
Total daily dose of IV furosemide (mg)	120 (40); range 50-200
n^{th} dose of IV furosemide	1st-3rd: n=14
	4th-6th: n=14
	7th-10th: n=3

5.4.1   Feasibility and tolerability

Repeated measures of echocardiographic and BIA indices were well tolerated by patients and were feasible at all time points in all patients by applying the devised pattern of measurement. Preparation of the skin and placement of all BIA electrodes took less than one minute. The combined set of BIA results across each region was obtained collectively within 20-30 seconds each, such that the entirety of BIA variables was obtained within three minutes in general. The complete set of echocardiographic measures was collected within 10 minutes.

Overall, each timed collection of measures took an average of 15 minutes. I observed that tricuspid inflow traces, although collected at each time point, were not as well defined as the mitral inflow indices.

5.4.2 Intra-observer/intra-device variability

Table 5.2 shows the intra-observer variability for echocardiographic measures before and after completion of the study in one normal volunteer. Intra-observer variability generally reduced after completion of the study. Table 5.3 shows the intra-device variability of BIA measures, which was much smaller compared to that of the echocardiographic measures.

Table 5.2. Intra-observer variability of echocardiographic variables before and after completion of the study in patients with decompensated HF.

Variable	Intra-observer variability (%)		Variable	Intra-observer variability (%)	
	Before	After		Before	After
MVE (m/s)	5.5	3.4	vTVDT	23.1	19.5
MVDT (ms)	16.5	14.0	vTVA	13.6	9.4
MVA (m/s)	12.6	5.6	E/Ea (l)	NA	5.4
TVE (m/s)	7.7	6.2	E/Ea (s)	NA	7.8
TVDT (ms)	22.6	9.1	LIVCe	5.0	4.2
TVA (m/s)	11.3	5.9	LIVCi	14.5	7.8
vMVE (m/s)	7.1	7.5	LIVCmi	14.5	13.8
vMVDT (ms)	11.1	5.9	SIVCe	6.7	2.7
vMVA (m/s)	15.3	10.1	SIVCi	9.5	7.3
vTVE (m/s)	10.6	7.3	SIVCmi	12.1	5.7

MV: transmitral; TV: transtricuspid; E: early peak velocity; A: late peak velocity; DT: deceleration time; Ea: Tissue Doppler early peak mitral annulus velocity; l: lateral wall; s: septal wall; IVC: inferior vena cava diameter; IVCD: IVC corrected for body surface area; S: short axis; L: long axis; e: expiration; i: inspiration; mi: maximal inspiration; v: Valsalva manoeuvre; NA: not assessed.

Table 5.3. Intra-device variability for bioimpedance measures.

Variable	Intra-device variability (%)	Variable	Intra-device variability (%)
dTBW (%)	0.22	$pZ_{50\,kHz}$ (Ω)	0.47
dECW (%)	0.22	pZ_{100kHz} (Ω)	0.50
dICW (%)	0.36	pZ_{200kHz} (Ω)	0.50
pTBW (%)	0.22	srZ_{5kHz} (Ω)	2.62
pECW (%)	0.35	$srZ_{50\,kHz}$ (Ω)	2.1
pICW (%)	0.51	srZ_{100kHz} (Ω)	2.4
dZ_{5kHz} (Ω)	0.54	srZ_{200kHz} (Ω)	2.85
$dZ_{50\,kHz}$ (Ω)	0.63	apZ_{5kHz} (Ω)	0.82
dZ_{100kHz} (Ω)	0.43	$apZ_{50\,kHz}$ (Ω)	0.95
dZ_{200kHz} (Ω)	0.34	apZ_{100kHz} (Ω)	1.69
pZ_{5kHz} (Ω)	0.74	apZ_{200kHz} (Ω)	1.10

d: whole-body; p: trunk; sr: right lung with sternum-rib electrode position; ap: right-lung with anterior-posterior electrode position; Z: impedance; 5-200 kHz: current frequency; ECW: extracellular water; ICW: intracellular water; TBW: total body water.

5.4.3 Changes in echocardiographic measures in response to ivF

5.4.3.1 Mitral and tricuspid inflow indices

Table 5.4 shows changes in mitral and tricuspid inflow indices that occurred in response to ivF, with the maximal percentage change of these variables from baseline and the time point at which they occurred. Following ivF, there were statistically significant reductions in vMVE and TVE, and statistically significant increase in MVDT , with maximal change for TVE occurring earlier than corresponding left-sided variables. Figure 5.1 illustrates the large between-subject variance not only in baseline measures, but also in response pattern to ivF of individual patients.

Table 5. 4. Changes in mitral and tricuspid inflow indices following ivF. Mean (SD).

Variable	Baseline	Maximal response	Maximal change (% baseline)	Maximal response time (h)	p value
MVE (m/s)	1.15 (0.27)	1.09 (0.30)	5.2 (10.2)	3	*0.105*
vMVE (m/s)	1.10 (0.30)	0.99 (0.25)	8.8 (18.6)	2	***0.012***
MVDT (ms)	204 (75)	245 (94)	20.1 (34.3)	3	***0.030***
vMVDT (ms)	211 (75)	219 (79)	3.8 (6.4)	1	*0.883*
MVE/A	1.88 (0.81)	1.81 (0.94)	3.7 (5.3)	1	*0.611*
vMVE/A	2.02 (0.80)	1.90 (0.73)	5.9 (9.9)	1	*0.870*
TVE (m/s)	0.77 (0.23)	0.68 (0.21)	11.7 (13.9)	1	***0.010***
vTVE (m/s)	0.67 (0.21)	0.61 (0.18)	9.0 (12.0)	1	*0.362*
TVDT (ms)	222 (81)	246 (120)	10.8 (15.3)	3	*0.069*
vTVDT (ms)	215 (59)	235 (94)	9.3 (15.2)	1	*0.250*
TVE/A	1.63 (0.45)	1.55 (0.47)	4.9 (6.5)	1	*0.683*
vTVE/A	1.50 (0.53)	1.45 (0.50)	3.3 (5.4)	0.5	*0.259*

MV: mitral inflow; TV: transtricuspid; DT: Deceleration time; E: early peak velocity; A: late peak velocity; v: Valsalva manoeuvre. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

Figure 5. 1. Changes in mitral valve early peak velocity with Valsalva manoeuvre (vMVE) and tricuspid valve early peak velocity (TVE) following ivF.
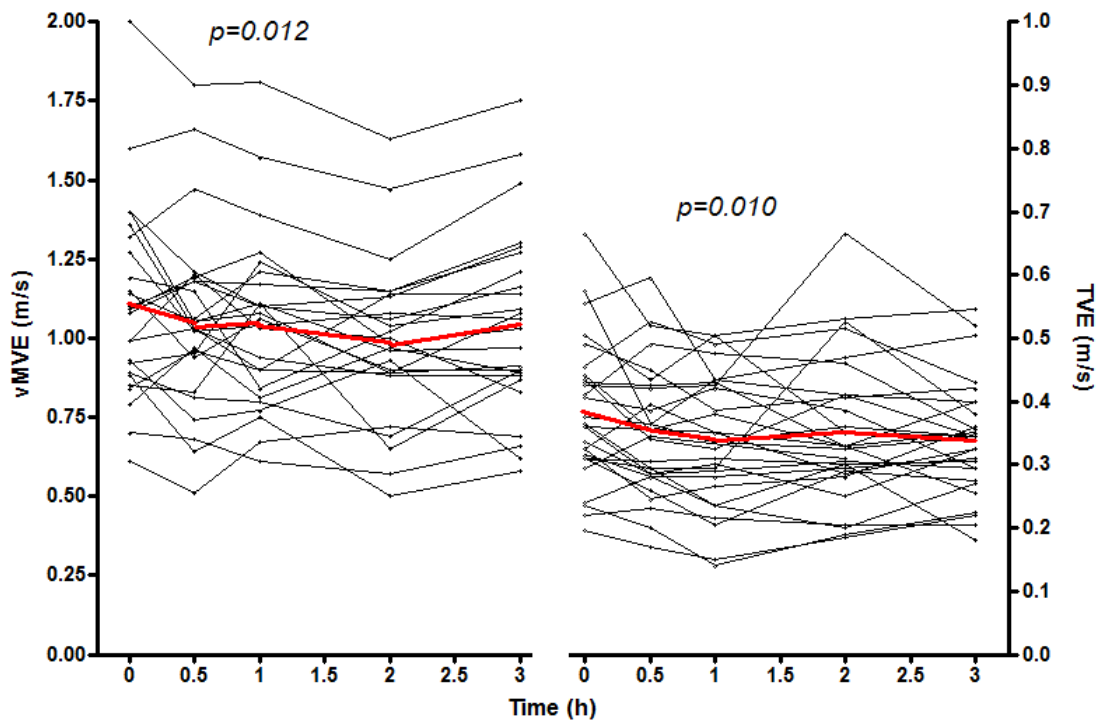
Red line denotes mean change in response to ivF. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

5.4.3.2 *Inferior vena cava indices*

There was a positive bias (mean difference) between IVC diameters measured in the long-axis and short-axis as shown in Figure 5.2. This was particularly large for the measurement of IVCe (bias of 0.19 cm), but small for the measurement of IVCmi (bias of 0.03 cm). However, the variability of measures using both methods appeared consistent regardless of the vessel diameter.

Figure 5.2. Bland-Altman plots of long-axis and short-axis measures of IVC diameters.
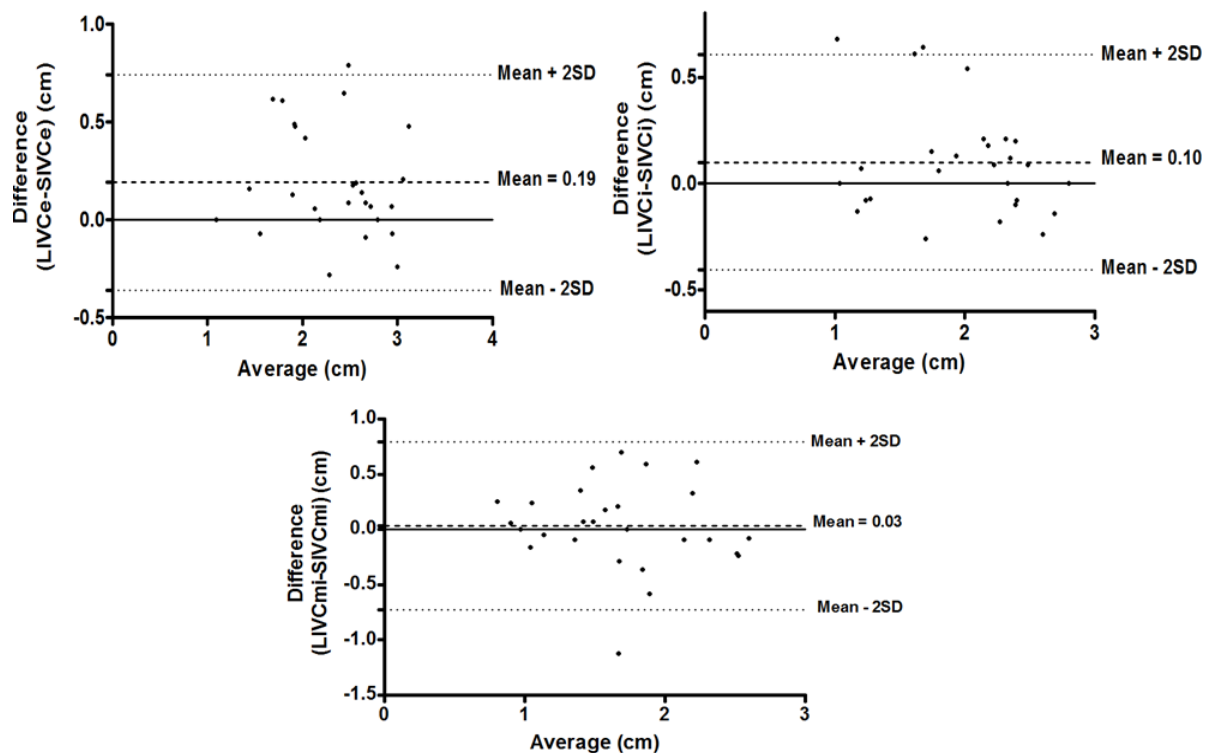
L: long-axis; S: short-axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration; mean: mean difference between LIVC and SIVC measures; SD: standard deviation of the mean.

Table 5.5 shows maximal changes in IVC indices following ivF. For the majority of variables, these maximal changes occurred at the 3h measures. There were statistically significant reductions in SIVCe and SIVCi, and their corresponding indexed measures (SIVCDe and SIVCDi). Figure 5.3 illustrates the SIVCDe and SIVCDi responses of individual patients to ivF.

Table 5.5. Changes in IVC indices following ivF. Mean (SD).

Variable	Baseline	Maximal response	Maximal change (% baseline)	Maximal response time (h)	p value
SIVCe (cm)	2.23 (0.58)	2.08 (0.56)	5.1 (21.5)	3	*0.039*
SIVCi (cm)	1.96 (0.53)	1.78 (0.54)	6.4 (26.6)	3	*0.020*
SIVCmi (cm)	1.69 (0.55)	1.58 (0.47)	6.5 (25.1)	3	*0.306*
SIVCDe (cm/m^2)	1.23 (0.33)	1.14 (0.31)	5.1 (21.5)	3	*0.032*
SIVCDi (cm/m^2)	1.08 (0.30)	0.98 (0.27)	6.4 (26.6)	3	*0.016*
SIVCDmi (cm/m^2)	0.93 (0.32)	0.87 (0.24)	6.5 (24.3)	3	*0.288*
SIVCCIi (%)	12.8 (9.1)	15.5 (6.8)	21.1 (30.2)	2	*0.417*
SIVCCImi (%)	24.8 (13.4)	25.9 (12.9)	4.4 (8.2)	1	*0.783*
LIVCe (cm)	2.43 (0.53)	2.30 (0.54)	5.3 (12.3)	3	*0.172*
LIVCi (cm)	2.06 (0.51)	1.90 (0.50)	7.8 (15.2)	3	*0.111*
LIVCmi (cm)	1.71 (0.51)	1.59 (0.51)	7.0 (13.2)	1	*0.450*
LIVCDe (cm/m^2)	1.34 (0.30)	1.26 (0.28)	6.0 (14.9)	3	*0.121*
LIVCDi cm/m^2)	1.14 (0.31)	1.04 (0.26)	8.8 (15.8)	3	*0.088*
LIVCDmi cm/m^2)	0.95 (0.28)	0.87 (0.28)	8.4 (13.2)	1	*0.472*
LIVCCIi (%)	15.9 (9.7)	17.8 (9.0)	11.9 (23.0)	1	*0.404*
LIVCCImi (%)	29.6 (13.3)	29.9 (13.6)	1.0 (4.6)	3	*0.766*

S: short-axis; L: long-axis; IVC: inferior vena cava diameter; IVCD: IVC corrected for body surface area; IVCCI: IVC collapsibility index; e: expiration; i: inspiration; mi: maximal inspiration. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

Figure 5.3. Changes in short-axis inferior vena cava diameter corrected for body surface area in expiration (SIVCDe) and inspiration (SIVCDi) in response to ivF.
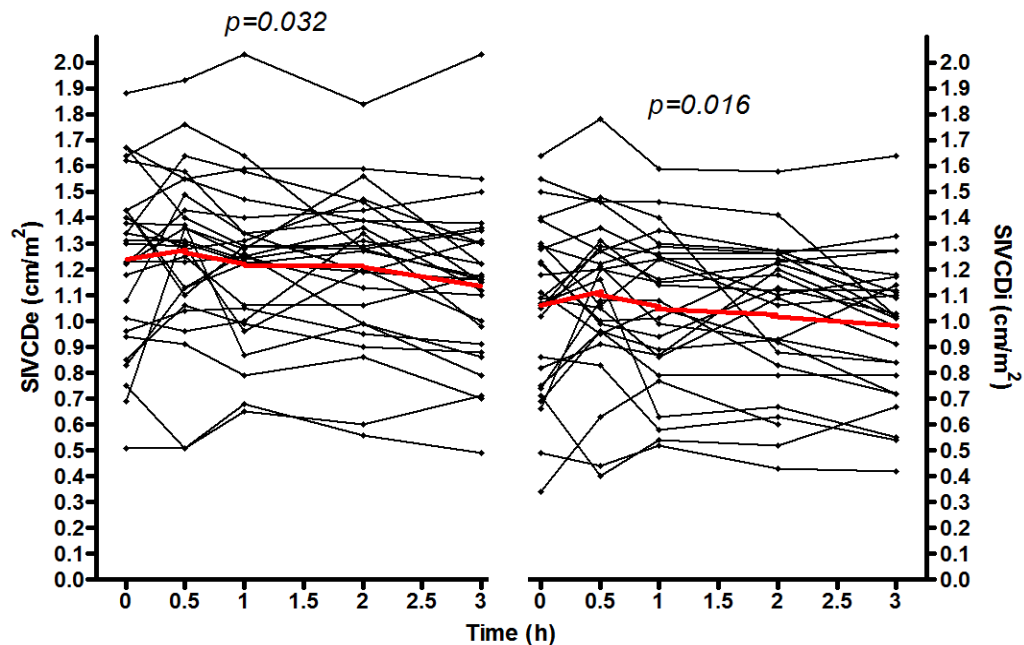
Red line denotes mean changes in response to ivF. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

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5.4.4 **Changes in bioimpedance measures in response to ivF**

Whole-body measures of Z at similar frequency currents were observed to be larger than

trunk measures, which in turn were larger than right-lung measures, as shown in Figure 5.4.

Furthermore, estimated body water composition was generally smaller when measured

proximally compared to distally, e.g., mean baseline ECW estimated using distal electrode

placement was 24.9 (3.6)%, compared to 23.9 (3.8)% when estimated using proximal

electrode placement. For TBW estimation, the difference in readings was >5%.

Figure 5.4. Impedance values at 50kHz frequency current (Z_{50kHz}) measured across the whole body, trunk and right lung: between-subject variability and response of to ivF

Red line denotes mean changes in response to ivF
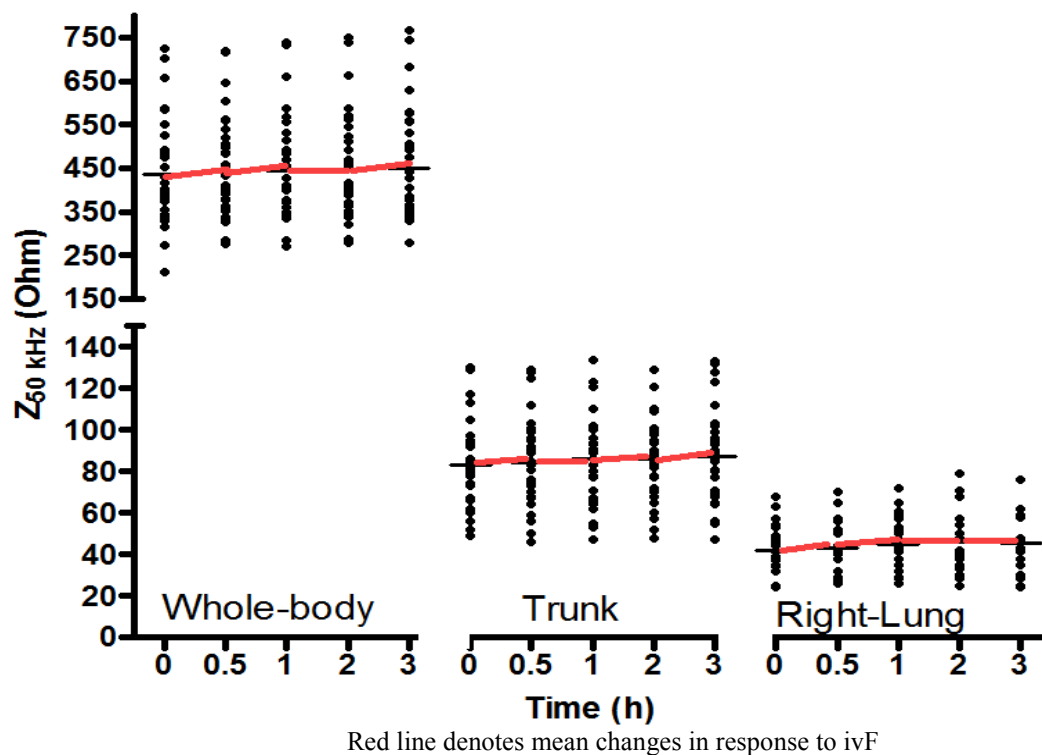
Table 5.6 shows maximal changes in BIA measures following ivF. All components of body

water composition reduced and Z values increased following ivF, reaching maximal change

from baseline at between 2-3h post dosing, although not all changes in variables showed

statistical significance. Figure 5.5 illustrates the changes in whole-body Z_{5kHz} and Z_{50kHz}

following ivF in individual patients, as well as the mean change within the cohort.

Table 5. 6. Changes in bioimpedance measures following ivF. Mean (SD).

Variable	Baseline	Maximal response	Maximal change (% baseline)	Maximal response time (h)	p value
d ECW (%)	24.9 (3.6)	24.4 (3.4)	2.1 (3.0)	3	**0.001**
d ICW (%)	30.2 (4.9)	29.4 (3.8)	2.6 (3.4)	1	0.309
d TBW (%)	55.8 (9.1)	54.8 (8.9)	1.8 (2.3)	3	0.058
d Z_{5kHz} (Ω)	486 (138)	506 (150)	4.2 (6.1)	3	**<0.0001**
d Z_{50kHz} (Ω)	442 (122)	457 (130)	3.7 (7.2)	3	**0.006**
d Z_{100kH} (Ω)	423 (118)	438 (122)	4.4 (9.6)	3	**0.010**
d Z_{200kHz} (Ω)	404 (115)	418 (115)	3.5 (5.3)	3	0.051
p ECW (%)	23.9 (3.8)	23.3 (3.8)	2.3 (5.9)	3	0.052
p ICW (%)	26.6 (4.1)	26.1 (3.3)	1.9 (4.2)	3	0.381
p TBW (%)	50.5 (7.5)	49.3 (7.0)	2.4 (4.9)	3	0.121
p Z_{5kHz} (Ω)	95 (23)	100 (25)	5.3 (7.3)	2	0.115
p Z_{50kHz} (Ω)	85 (20)	89 (22)	4.0 (6.0)	3	**0.039**
p Z_{100kH} (Ω)	81 (19)	84 (20)	3.7 (5.2)	3	0.323
p Z_{200kHz} (Ω)	77 (18)	79 (19)	2.5 (5.1)	3	0.377
apZ_{5kHz} (Ω)	49 (16)	53 (17)	7.8 (13.0)	2	0.054
apZ_{50kHz} (Ω)	42 (13)	45 (13)	10.5 (17.9)	1	**0.016**
apZ_{100kHz} (Ω)	40 (13)	43 (13)	9.7 (19.0)	1	0.068
apZ_{200kHz} (Ω)	38 (12)	41 (13)	10.2 (18.8)	3	**0.036**

d: whole-body; p: trunk; ap: right-lung with anterior-posterior electrode position; Z: impedance; 5-200 kHz: current frequency; ECW: extracellular water; ICW: intracellular water; TBW: total body water. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

Figure 5. 5. Whole-body impedance (Z) at 5 kHz and 50 kHz frequency currents in response to ivF.
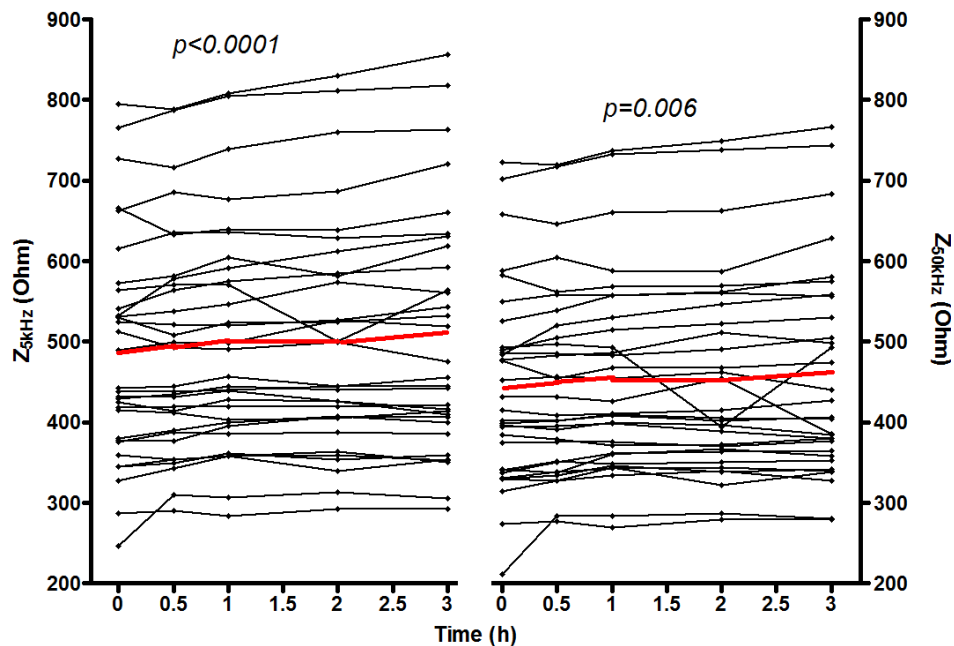
Red line denotes mean changes in response to ivF. p value calculated from repeated measures one-factor ANOVA of variables at 5 time points in response to ivF.

5.5   Discussion

5.5.1   Feasibility and minimisation of measurement error

Determining the feasibility of consistently performing and obtaining physical biomeasures at each time point was a crucial step for the assessment of biomarker sensitivities in the future planned study protocols in normal volunteers and stable LVSD patients. The accurate assessment of biomarker sensitivities would be reliant on the availability of all measures at each specific time point for subsequent analysis. The time scale in which the collection of biomeasures was achieved confirmed that my choice of echocardiographic variables and of BIA device was appropriate. In accordance with previous studies, mitral inflow traces were consistently adequate, and were obtained in a small amount of time [Khan et al., 2004]. IVC indices were also easy to measure, with adequate images in all patients. However, traces for

tricuspid inflow indices were not as consistently clear as the mitral inflow indices and may have contributed to their documented high intra-observer variability.

The tolerability of the repeated measures was the next crucial step as this would be a major factor determining the comfort and compliance of volunteers, thus affecting measurement variance. The fact that the repeated measures assessment was well tolerated in this potentially unstable group of patients implied that normal volunteers and stable LVSD patients would also tolerate repeated measurements using this set pattern well.

A reduction was observed in intra-observer variability for most echocardiographic measures, in some cases by approximately 100%, following practice and increased familiarity with the devised pattern for collecting repeated measures of biomarkers. The fact that a different echocardiographic platform was used before and after completion of the study may be a confounding factor, however, the images obtained were of similar quality with similar settings used to optimise images. Furthermore, the studies in normal volunteers and stable LVSD patients would be performed using the GE VingMed System 5 platform, thus, determining the intra-observer variability using that particular platform was more appropriate to enable accurate interpretation of results for the studies in these volunteer groups.

The intra-observer variability of MVE determined in this Chapter (3.4%) was higher than that documented by Rijtsterborgh et al. [1990] (1%). However, the reported inter-observer variability for MVE in the latter study was 6.5% compared to 2% reported by Khan et al. [2004]. Furthermore, I observed much larger intra-observer variability for MVDT (14%) compared to the inter-observer variability in the study by Khan et al. of 5% (intra-observer

variability not reported). This illustrates the fact that intra- and inter-observer variability will vary depending on a number of factors such as the clinical settings (mitral valve disease or replacement in the former study, unselected population in the latter study), echocardiography platforms and experience of the operator. These factors need to be considered when interpreting results from studies involving echocardiographic measures, or indeed when using echocardiographic measures as a monitoring tool in practice. I observed increased intra-observer variability of mitral and tricuspid inflow indices when measured with the Valsalva manoeuvre compared to without the Valsalva manoeuvre, in accordance with findings from the above studies.

The intra-observer variability of IVC indices is not well documented. I found that in general, it increased the smaller the IVC diameter being measured, i.e., that of IVCe being the smallest and that of IVCmi being the largest. The measurement of IVC diameters using the long-axis and short-axis views in this cohort of patients had a clinically significant bias, particularly when measuring the IVC in expiration. IVC diameter measurement using these two views is thus not interchangeable in this group of patients, and when used in the monitoring of individual patients, should be obtained using the same view each time to allow direct comparison of IVC measures. Although the intra-device variability for BIA measures was very small, it tended to increase the smaller the body segment being studied (largest for right-lung measures), in a similar way to IVC measures. It is thus preferable to measure larger variables in order to minimise measurement error.

5.5.2   **Repeated measures of physical biomeasures in response to ivF**

The patients studied were admitted with acute cardiogenic pulmonary oedema, and had to differing extents other signs and symptoms of decompensated HF. Interstitial space volume would thus be increased, while intravascular volume would be normal, reduced, or increased depending on baseline neurohormonal activation and haemodynamic variables [James et al., 1996; Davidov et al., 1967; Henning et al., 1978]. This statement not only holds for patients who were studied on admission and those receiving their first few doses of ivF, but also for the patients who had received multiple previous doses of ivF, a large proportion of whom still had evidence of pulmonary oedema in the form of rales on lung auscultation on the day of the study despite being able to lie supine for the short duration of physical biomeasurements. Furthermore, these patients were still requiring ivF therapy on clinical grounds in view of their ongoing signs and symptoms of abnormal volume status.

As described in Chapter One, ivF administration in volume overloaded patients reduces cardiac preload secondary to almost immediate venodilatation followed by volume redistribution, natriuresis and diuresis within 30 minutes [Schaper et al., 1997]. The haemodynamic effects of ivF were still evident in a study by Ikram et al. by the end of a 4h-monitoring period, where PCWP was still declining. The net effect of ivF administration in my cohort of patients was therefore expected to be one of overall reduced ECW (likely to involve reduction in at least the interstitial space, and possibly in intravascular volume) secondary to venodilatation, diuresis and natriuresis, with possible temporary intravascular volume increase as a result of volume redistribution from the interstitial space. In an attempt to minimise the effects of variable baseline neurohormonal and haemodynamic status, I excluded any patients who were receiving more that their 10th consecutive dose of ivF. In fact,

almost half of the patients were receiving their 1st-3rd dose, and only three (9.7 %) patients were receiving more than their 7th dose of ivF.

Of the echocardiographic variables, statistically significant reductions were observed in vMVE, TVE, SIVCe, SIVCi, SIVCDe and SIVCDi, in addition to a statistically significant increase in MVDT, suggesting reduction in cardiac preload or intravascular volume following ivF. Maximal changes in tricuspid inflow indices occurred earlier than mitral inflow indices, suggesting that right heart measures may indeed change quicker in response to volume changes compared to left-sided measures due to the right heart being more distensible [Efthimiadis et al., 1999; Areias et al., 1998]. However, the maximal percentage change from baseline for each of these variables was small (Figure 5.1), particularly when the intra-observer variability of each marker is taken into account. Considering the intra-observer variability values established prior to the study (Table 5.2), the maximal percentage change was only marginally above the intra-observer variability for the mitral and tricuspid inflow indices (e.g., for vMVE, the maximal percentage change from baseline was 8.8 % whereas the intra-observer variability was 7.1 %). This suggests that the maximal percentage change from baseline for these biomarkers is unlikely to be greater than their respective within-subject normal variance, which as discussed in Chapter Three, is determined by both the intra-observer variability and within-subject biological variance.

For the IVC indices, the maximal percentage change from baseline in response to ivF was in fact less than the respective intra-observer variability (e.g., for SIVCe, intra-observer variability was 6.7 % whereas maximal percentage change from baseline was 5.1 %), suggesting that the changes in IVC indices did not occur over and above their within-subject

normal variance. It is also unclear as to why there was a statistically significant reduction in vMVE and not MVE, as the strain phase of the Valsalva manoeuvre should in principle, attenuate the mitral inflow response to changes in volume status [Hamilton et al., 1994]. Thus, the statistically significant changes observed in biomarker levels in response to ivF in this cohort of patients are unlikely to represent biologically significant changes. It is therefore reasonable to conclude that the repeated measures assessment of the above echocardiographic variables is unlikely to be clinically useful for tracking volume changes in the current study setting.

On the other hand, the statistically significant reduction in dECW and statistically significant increase in some whole-body, trunk and right lung impedance values following ivF all occurred to a large extent above the respective values for intra-device variability. For example, the maximal percentage reduction for dECW of 2.1 % was much larger than the intra-device variability of 0.22 %. This suggests that the changes in BIA measures might have occurred above their respective within-subject normal variance, although it is not possible to comment at this point on the magnitude of this within-subject normal variance. However, the observed BIA responses were small, as illustrated in Figure 5.5, with maximal changes from baseline of around 10 % at most. This suggests that these changes, despite being statistically significant, are unlikely to be biologically significant. Even if these changes in BIA measures were biologically significant, the small magnitude of change in the BIA measures would make their repeated measures assessment unsuitable for mapping changes in volume status in the current study setting.

Furthermore, whereas the changes in whole-body BIA measures at least appear consistent with the expected reduction in overall ECW following ivF, with statistically significant reduction in dECW and statistically significant increase in Z at current frequencies of 5kHz, 50kHz and 100 kHz (smaller frequency currents representing ECW), the statistically significant changes in only pZ_{50kHz} and $apZ_{50\,kHz}$ and apZ_{200kHz} were less consistent.

Thus, the potential clinical utility of BIA in monitoring patients receiving ivF for the treatment of acute pulmonary oedema demonstrated in a small number of studies which showed much larger percentage change from baseline was not replicated here. Noble et al. [2000] showed that compared to placebo in normal volunteers, ivF resulted in increased urine output, increased mean thoracic impedance by 13.6%, and increased lung impedance by 7.8%. Using a single frequency device, Coodley et al. [1994] showed that after 3 days of diuretic treatment (oral or intravenous), weight reduced by 4.1(0.6) Kg and resistance and reactance increased by 20.8(2.7)% and 22.7(6.1)% respectively. Similarly, Soderberg et al. [2001], using a multi frequency device, showed a reduction of 2.9(1.7) Kg in body weight, of 3.2(2.4) L in TBW, of 2.7(1.6) L in ECW and 0.5(3.2) L in ICW over three days in 12 patients with acute congestion who were being treated with ivF.

The lack of clinically significant response to ivF of the physical biomeasures studied may be explained firstly by a genuine lack of sensitivity of the bioamarkers at tracking volume status. The performance of the echocardiographic variables was severely limited by their large intra-observer variability and small relative changes from baseline, whereas the changes in BIA variables were not as substantial as those seen in other studies. Secondly, the amplitude of overall volume depletion in this cohort in response to ivF may not have been large enough to

induce significant change in biomarkers levels. For example, the large percentage changes in BIA response observed due to reduction in volume status following diuretic treatment in the studies by Coodley et al. [1994] and Soderberg et al. [2001] were only achieved over three days. However, the potential utility of a suitably sensitive biomarker would have been strengthened by the fact that its levels changed significantly in response to smaller volume changes.

Thirdly, the overall volume response to ivF in this heterogeneous cohort of patients may have been null as a result of differing volume response between the subjects studied. Despite small within-subject variability in furosemide responsiveness (unit sodium excreted per unit urinary furosemide), the between-subject patient response to ivF is highly variable [MacFadyen et al., 2004; Cioffi et al., 2003]. Close inspection of the individual patient responses in Figures 5.1, 5.3 and 5.5 confirms not only large variability in individual patient responses to ivF, but also large between-subject variance for individual baseline measures. This may be explained by widely different baseline neurohormonal, renal and haemodynamic status due to different aetiologies and stages of disease, concurrent cardiovascular medication, degree of renal dysfunction, prior dosing with ivF, and volume status, all these factors modulating subsequent response to ivF [Volpe et al., 1992; Anand et al., 1989]. Thus, while some patients may have had a priori expanded interstitial and intravascular volumes with reduction in both compartments and thus reduction in ECW overall following ivF, others may not have had much change in overall ECW due to redistribution of volume from an expanded interstial space into a hypovolaemic intravascular space. Furthermore, patients with renal dysfunction (39 % of the cohort) may exhibit diuretic resistance such that the doses of ivF being

administered during the study may not have been sufficient to have a net effect on overall volume status.

5.6   Limitations

There were a number of limitations to this study. The changes in biomarker levels following ivF were not compared to a gold standard such as blood volume analysis or haemodynamic measures. It is thus difficult to establish the exact volume status at baseline and the volume response to ivF. The use of gold standards would have clarified whether the lack of clinically significant change in biomeasures was due to lack of biomarker sensitivity or lack of significant volume change induced by ivF. Furthermore, surrogate measures of volume status such as urine output, blood pressure and body weight were not documented. These were not performed as the emphasis of this pilot study was to establish the feasibility and to gain familiarity with the set pattern of obtaining physical biomeasurements, such that measuring body weight, blood pressure and urine output may have jeopardised the achievement of these goals due to time constraints.

As mentioned above, the baseline volume status and response to ivF was variable amongst this heterogeneous cohort due to a number of factors. Some of these factors could have been controlled either at the beginning or during the study in order to minimse this between-subject variance. The aetiology of acute cardiogenic pulmonary oedema varied, as did the LVEF and renal function of the patients (29 % of patients had normal LVEF and 39 % of patients had renal impairment). Equally, subjects had variable concomitant medication, such as ACE inhibitors and oral nitrates, which may have compounded the overall fluid or venodilatory response to ivF. The oral fluid and salt intake of patients was also was not controlled.

Nevertheless, these factors are in common with routine clinical practice and this study was thus performed in a very realistic setting.

The regression equations of commercial BIA devices providing estimates of body water composition are validated against radioisotope dilution techniques [Hannan et al., 1995]. However, BIA regression equations are generally validated only in non-obese and euvolaemic population groups [De Lorenzo et al., 1994]. These will be less valid in patients with acute fluid and electrolyte shifts, such as in this study. Raw impedance values should thus be interpreted in preference to the estimates of body water composition.

5.7 Summary

Repeated measures assessment of BIA and echocardiographic variables were both feasible and well tolerated in a potentially unstable group of patients within the time constraints of the study. Intra-observer variability for echocardiographic variables reduced following practice and increased familiarity with the set pattern of repeated measures of physical biomeasures, however, it remained large. The intra-device variability of BIA variables was much smaller compared to echocardiographic variables. No clinically significant change in the physical biomeasures was seen in response to ivF, indicating that their use would be limited as non-invasive tests of volume change in this setting, which is very realistic of normal clinical practice involving a heterogeneous group of patients. However, because the findings may have been compounded by the heterogeneity of the cohort, further assessment of the performance of these biomarkers is warranted.

Chapter Six

Studies in Normal Volunteers

Establishing the Normal Variance of Blood and Physical Biomarkers in Normal Volunteers and their Differential Response to an Acute Load of Oral Water and Intravenous Saline

6.1　　　Introduction

The acute ingestion of oral water in normal volunteers results in a transient reduction in plasma volume within a few minutes of the intervention followed by gradual increase back to baseline values within 30 minutes to an hour. This effect on plasma volume depends on study populations, the rate of water ingestion and the temperature of the water [Jordan et al., 2000; Callegaro et al., 2007; Endo et al., 2001], the mechanism for this response possibly involving simultaneous activation of a pressor response and increased vagal tone. This results in an initial increase in mean arterial blood pressure, which then decreases, causing fluid shift between the intravascular and interstitial spaces. Thus, Endo et al. [2001] demonstrated that 1L of tap water at 25°C ingested over 2 minutes resulted in a decrease in plasma volume within 4 minutes of the start of drinking, followed by an increase in plasma volume to above baseline by 12-15 minutes, monitoring being discontinued after 50 minutes.

In contrast, the acute infusion of normal saline in normal volunteers over approximately 30 minutes increases plasma volume by 9% of volume infused, interstitial space volume by 13% of volume infused, and ECW by 23% of volume infused by the end of the infusion as evidenced by radioisotope studies [Ernest et al., 2001]. Lobo et al. [2009] have also shown that an intravenous normal saline infusion of 1L over 1h results in approximately 68% of the volume infused having moved from the intravascular space into the interstitial space (about 200mL increase in blood volume and approximately 800mL increase in the interstitial space) within 1h of the start of the infusion. Following the 1h measures, changes in blood volume plateaued, whereas interstitial volume reduced gradually to approximately –200mL by the end

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of a 6h monitoring period, these changes were consistent with body weight returning to normal by the end of the experiment.

Thus, acute oral water and intravenous saline loading constituted two different mechanisms by which controlled and consistent volume changes could be induced in normal volunteers. The response patterns of repeated measures of selected biomarkers to these two different interventions could thus be compared to their normal variance patterns, which in turn could be established by performing repeated measures without any intervention. The biomarkers whose response patterns deviate the most from their normal variance pattern would thus potentially be the most sensitive biomarkers to induced volume changes.

6.2   Hypotheses (H1)

I therefore hypothesised that:

- Acute volume changes in normal volunteers following oral water and intravenous saline might induce changes in repeated measures of the selected panel of blood biomarkers and physical biomeasures.
- These changes in repeated measures of biomarker levels might be detectable above the within-subject normal varianceof these biomarkers.
- Normal volunteers will remain *asymptomatic* in response to the differential changes in volume status induced by acute volume loading.

6.3 Methods

6.3.1 Study population

30 normal volunteers were recruited from hospital staff and patients' relatives at the Sandwell and West Birmingham Hospitals. Posters were placed within the hospital compounds, and normal volunteers previously involved with studies at the Centre for Cardiovascular Sciences were also contacted. The volunteers were asymptomatic with no significant past medical history, were on no medication likely to affect fluid homeostasis, and did not abuse alcohol or illicit substances. They had a normal physical examination, including blood pressure, normal routine biochemical and haematological parameters (full blood count, urea and electrolytes, liver function and thyroid function tests, blood glucose and serum calcium). They also had a normal screening echocardiogram (comprehensive study as per local hospital protocol), and negative urine dipstick (Bayer Multistix 10SG, MediSupplies, Dorset).

6.3.2 Volume loading protocol

Volunteers attended the research centre on 3 occasions, at least 5 days apart, at the same time on each occasion, to receive a different intervention each time. They fasted from 21:00 the previous day and abstained from alcohol, smoking and caffeine. They were allowed normal fluid intake (they drank when they felt thirsty) in order to avoid baseline dehydration, and were asked to drink similar amounts prior to attending the study centre on each occasion.

On each occasion, their body weight was recorded on arrival. Their blood pressure and heart rate were measured in triplicate after they rested semi recumbent for 30 minutes. They then

had baseline measurements of echocardiographic and BIA variables using the set pattern of measurement described in Chapter Five. An intravenous cannula was then inserted and baseline blood samples were taken. This was followed by bladder voiding and a urine collection. They were given in random order, an oral water load (Oral Water, 1.5L of mineral water at room temperature, to drink in 20 minutes), an intravenous infusion of normal saline (IV Saline, 1.5L at body temperature over 20 minutes), or no intervention (Control), as illustrated in Figure 6.1.

Subsequent blood samples were withdrawn from the intravenous cannula after discarding an initial 5 mL of blood. Blood samples were repeated at 0.5, 1, 2, 4, 6 and 8 h after the intervention and echocardiographic and BIA measures were repeated at 1, 2 and 4h respectively. Urine volumes were collected throughout the 8-hour period to establish urine volume between 0-4h [Urine volume (4h)], urine volume between 4-8h [Urine volume (8h)], and total urine volume [Urine volume (total), calculated as Urine volume (4h) + Urine volume (8)].

Figure 6. 1. Volume loading protocol for normal volunteers.
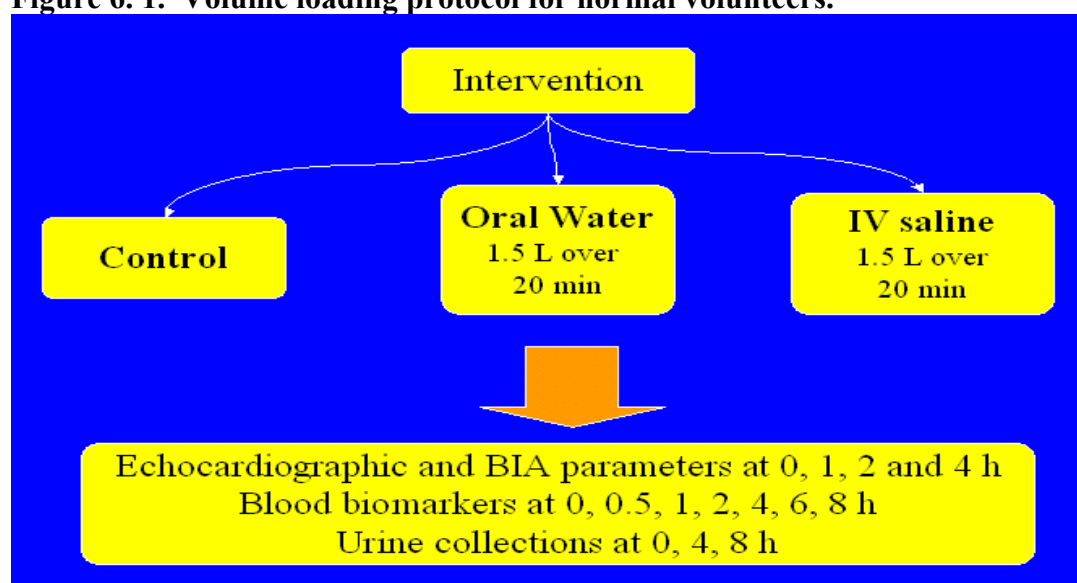
Volunteers relaxed semi-recumbent in between measurements, were given lunch after the 4h measurements, and had a snack after the 6h measurements. Food and snacks were similar for all patients. They continued to abstain from caffeine, alcohol and smoking, and were allowed to ingest 1L of additional fluid at room temperature during the 8h monitoring protocol with meals in addition to their respective volume loading protocol.

6.3.3 Blood biomarkers

Blood samples were collected and stored for Hct, ANP and BNP analyses as described in Chapter Four. IMA and cTn were not studied as their levels in normal volunteers would in theory, be undetectable by available methods of analysis.

6.3.3.1 *Comparison of blood sampling from venesection and intravenous cannula*

In order to ensure that blood collection from the intravenous cannula did not impact on measured values compared to direct venesection, simultaneous blood collection from direct venepuncture and from the intravenous cannula following discarding of an initial 5mL of blood were compared using 4 normal volunteers at random (NV21, NV22, NV24 and NV27). Each sample was analysed three times and the mean value calculated for each normal volunteer (Table 6.1). The mean values of samples collected by venesection and from the intravenous cannula were then compared using paired t-test. There was no significant difference between both groups, p= 0.771. This is in accordance with previous studies [Sliwa at al., 1997; Zlotowski et al., 2001].

Table 6.1. Simultaneous Haematocrit (Hct) values obtained by withdrawal of blood from intravenous cannula and direct venesection.

Patient	Analysis	Hct from intravenous cannula (%)	Mean Hct from intravenous cannula (%)	Hct from Venepuncture (%)	Mean Hct from Venepuncutre (%)
NV 22	1	38.5		38.0	
	2	38.5		37.8	
	3	38.4	38.5	37.8	37.9
NV 23	1	30.8		31.9	
	2	30.8		31.0	
	3	31.0	30.9	31.0	31.3
NV 24	1	34.4		34.9	
	2	34.6		35.5	
	3	34.7	34.6	35.6	35.3
NV 27	1	39.2		38.7	
	2	39.2		39.2	
	3	38.8	39.1	38.7	38.7

Combined means compared using paired t-test.

6.3.4 Physical biomeasures

Echocardiographic variables determined included mitral Doppler indices and IVC indices as described in Chapter Four. The tricuspid inflow traces were again noted to not be as well-defined as the mitral inflow traces, similar to the situation in Chapter Five. As such, they were felt to be inappropriate biomarkers in the study setting as there would be considerable measurement error involved in analysising these traces. I thus chose not to include these in this part of the thesis. BIA measures were determined with electrodes placed distally, proximally, and across the right -lung in the anterior-posterior and the sternum-rib positions.

6.3.5   Statistical analysis

Within-subject normal variance was determined for each biomarker as described in Chapter Three over firstly, the 8h monitoring period on the Control day (within-day CVt_{ws}), and secondly, over the 3 attendances which were at least 5 days apart (between-day CVt_{ws}). Within-day within-subject normal variance was determined for blood biomarkers using the 7 measurements at 0, 0.5, 1, 2, 4, 6 and 8h, and for physical biomeasures using the 4 measurements at 0, 1, 2 and 4h obtained over the 8h monitoring period. Between-day within-subject normal variance was calculated using the 3 baseline values on each attendance day (Time 0h). Within-day and between-day RCV for each biomarker were also calculated as described in Chapter Three. Furthermore, the patterns of biomarker levels on the Control day would constitute the *mean within-subject normal variance* for each biomarker against which biomarker responses to Oral Water and IV Saline would be compared. Between-subject normal variance for each biomarker was determined by using the baseline values from all 30 normal volunteers on the Control day and dividing their cumulative standard deviation by their cumulative mean and multiplying by 100. Analytical variance (CVa) for blood biomarkers and intra-observer and intra-device variability (CVo) for physical biomeasures which were determined in Chapter Five were also tabulated to illustrate their relative contributions to overall variance of the biomarkers.

General linear model repeated measures two-factor within-subject ANOVA was used to compare response patterns of each biomarker to each of the three interventions, and to establish the difference between the main interaction effect of intervention and time (intervention*time), so that for each response curve, measures at each time point, e.g., at 1h

on the Control day, were directly compared to measures at the same time point on the other intervention days. The main effect of time and the main effect of intervention alone were *not* documented. If there was a significant main interaction effect between response patterns for a particular biomarker, Bonferroni post-hoc analysis was used to outline the interventions for which the response curves were significantly different. The Bland-Altman method was used to assess the agreement between the long-axis and short-axis views for the measurement of IVC diameter. All the biomarkers assessed in this study were normally distributed.

Power calculations for the study were based on previous work by Lobo et al. [2010], Heringlake et al. [2004], and Singer et al. [1998]. A sample size of 30 volunteers would give a power of >85% for the detection of significant changes in ANP (116 %), BNP (130%), and Hct (4%) with an alpha value of 5%. No previous studies have investigated the effects of acute volume load on mitral Doppler indices, IVC indices and BIA in normal volunteers receiving acute volume loads, such that data for the latter variables is lacking to estimate power.

6.4   Results

Baseline demographic and clinical variables for the 30 normal volunteers are shown in Table 6.2. All normal volunteers remained haemodynamically stable throughout the study. The mean age was 49 (9) years, and there were almost equal numbers of male and female volunteers.

Table 6.2. Baseline demographic and clinical variables of 30 normal volunteers. Mean (SD), n(%).

Variable	Value
Male (%)	16 (53)
Ethnicity (%)	
Caucasian	17 (57)
Afro Caribbean	7 (23)
Asian	5 (17)
Mixed	1 (3)
Age (years)	49 (9)
Weight (Kg)	76.6 (12.4)
Height (cm)	175.6 (10.0)
Body surface area (m^2)	1.92 (0.20)
Body mass index (Kg/m^2)	24.7 (2.9)
Haemoglobin (g/dl)	13.8 (1.1)
Haematocrit (%)	40.3 (2.9)
Creatinine (mg/dL)	0.88 (0.15)
Systolic BP (mmHg)	128 (10)
Diastolic BP (mmHg)	78 (8)
Heart rate (bpm)	73 (10)

Table 6.3 illustrates the order of interventions as they were received by normal volunteers; there was no significant difference between the order of the interventions, p=0.136.

Table 6.3. Order of interventions for normal volunteers.

	Control	Oral Water	IV saline
First intervention	11	5	14
Second intervention	9	14	7
Third intervention	10	11	9

Comparison using Chi-square.

Normal volunteers drank comparable amounts of fluid (in addition to their Oral Water load on the day of this intervention) on all three occasions. The mean oral intake was 983 (217) mL on the Control day, 940 (211) mL on the Oral Water day and 997 (167) mL on the IV Saline day, p=0.329.

6.4.1 Estimated blood volume

There was a small but statistically significant increase in estimated blood volume on the Control day between the baseline value and all subsequent values, $p<0.0001$. Figure 6.2 shows the estimated blood volume response to the three interventions. There was a significant main interaction effect (intervention*time) for the estimated blood volume curves in response to the three interventions. On post-hoc analysis for the effect of intervention, the estimated blood volume response to IV Saline increased significantly compared to normal variance and Oral Water. There was however, no significant difference in the reponse to Oral Water compared to the Control day.

Figure 6.2. Estimated blood volume response to Control, Oral Water and IV saline.
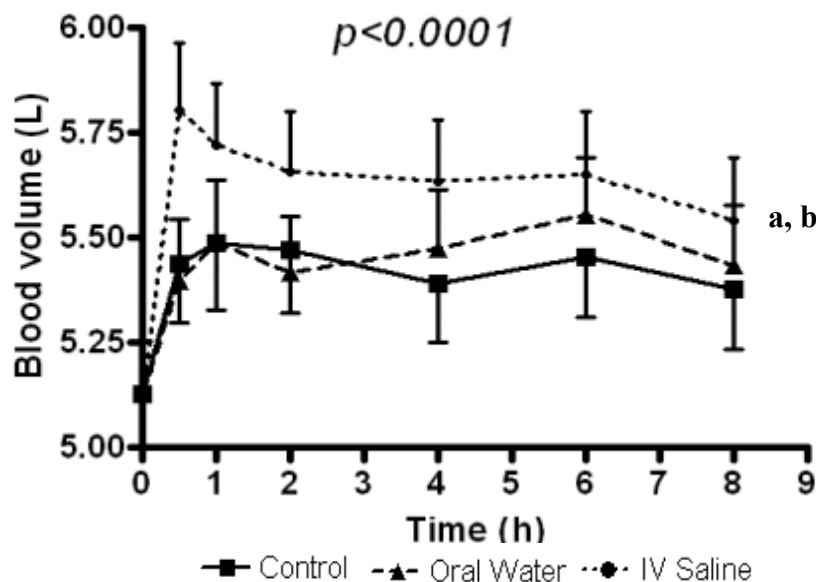
Mean (SE). p value obtained from repeated measures two-factor within-subject ANOVA for main interaction effect of intervention and time.
From Bonferroni post-hoc analysis, significant effect of intervention ($p<0.05$) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

6.4.2   Blood biomarkers

6.4.2.1   Normal variance

All blood biomarker levels at baseline were within normal limits as illustrated by the 0h measures on the Control, Oral Water and IV Saline days in Figure 6.3. Components of their variance are shown in Table 6.4. Hct had the smallest, and BNP the largest between-subject and within-subject total variance.

Table 6.4. Within-day and between-day within-subject total variance (CVt_{ws}) and reference change values (RCV), analytical variation (CVa) and between-subject total variance (CVt_{bs}) for blood biomarkers in normal volunteers.

Biomarker	Within-day CVt_{ws} (%)	Within-day RCV (%)	Between-day CVt_{ws} (%)	Between-day RCV (%)	CVa (%)	CVt_{bs} (%) n=30
ANP	9.9	27.4	12.5	34.6	5.0	44.3
BNP	43.9	121.7	68.4	189.6	11.6	89.1
Hct	3.5	9.7	4.6	12.8	0.6	8.5

ANP: atrial natriuretic peptide; BNP: B-type natriuretic peptide; Hct: haematocrit

On the Control day, a significant drop in Hct was observed between the 0h and all subsequent measures, $p<0.0001$. The maximum change occurred at the 1h measurement, after which the Hct levels reached a plateau. Similarly, ANP values increased significantly over the 8h monitoring period, with all subsequent values being significantly higher than the baseline value, $p<0.0001$. The maximal increase occurred at the 6h measurement. On the other hand, BNP levels were not significantly different from each other over the 8h monitoring period, $p=0.356$.

6.4.2.2   Response patterns to differential volume loading protocols

The response of blood biomarkers to the three different interventions is illustrated in Figure 6.3. There was a significant main interaction effect for the Hct and ANP response curves to

the three interventions. Thus, when compared to its normal variance pattern, the Hct response

pattern decreased with IV Saline, but initially increased following Oral Water before

eventually falling. Post-hoc analysis of intervention effect demonstrated that this differential

Hct response was only significant between IV Saline and Oral Water (p=0.03); there was no

significant difference between the response patterns to Oral Water or IV Saline compared to

normal variance (p=0.716 and p=0.118 respectively).

The ANP response pattern to IV Saline increased compared to its normal variance pattern,

whereas that to Oral Water was similar to its normal variance pattern. Post-hoc analysis of

intervention effect revealed significant difference between the response to IV Saline

compared to those to both Control and Oral Water (p<0.0001 respectively). However, there

was no significant difference between the response to Oral Water compared to normal

variance (p=0.250).

There was no significant main interaction effect for the BNP response patterns to Control,

Oral Water and IV Saline (p=0.084).

Figure 6.3. Blood biomarker response to Control, Oral Water and IV Saline.
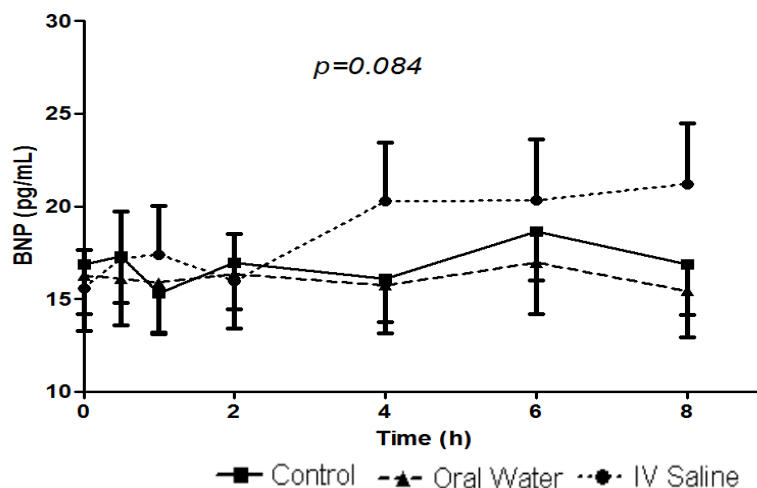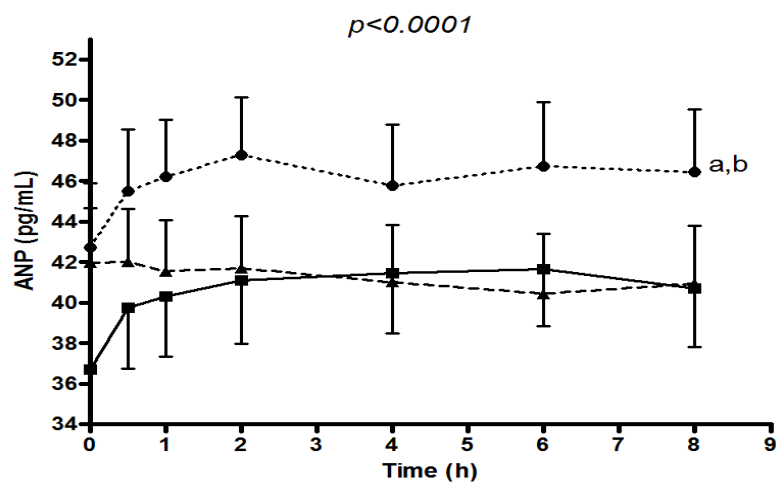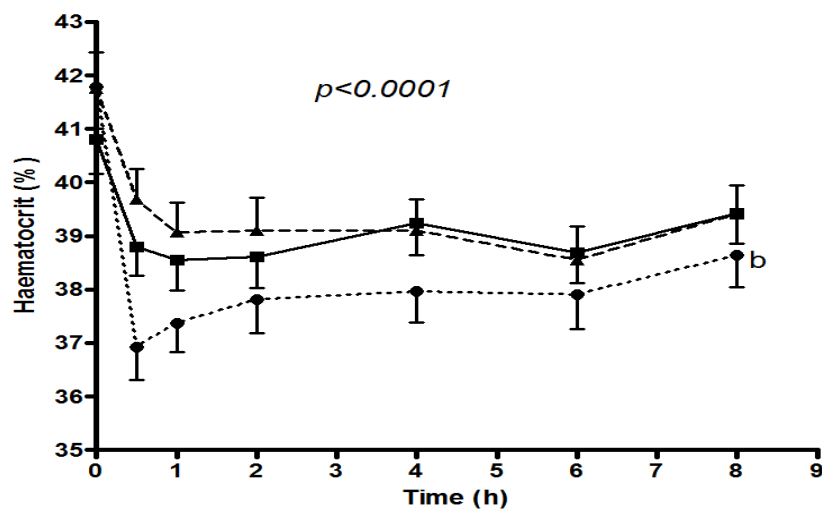
ANP: Atrial natriuretic peptide; BNP: B-type natriuretic peptide. Mean (SE).
p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.
From Bonferroni post-hoc analysis, significant effect of intervention (p<0.05) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

6.4.3 Echocardiographic measures

6.4.3.1 Mitral Doppler indices

6.4.3.1.1 Normal variance

Components of the variance of mitral Doppler indices are shown in Table 6.5; there were non-significant trends in their within-subject total variance patterns on the Control day.

Table 6.5. Within-day and between-day within-subject total variance (CVt$_{ws}$) and reference change values (RCV), intra-observer variability (CVo) and between-subject total variance (CVt$_{bs}$) for mitral Doppler indices in normal volunteers.

Biomarker	Within-day CVt$_{ws}$ (%)	Within-day RCV (%)	Between-day CVt$_{ws}$ (%)	Between-day RCV (%)	CVo (%)	CVt$_{bs}$ (%) n=30
MVE	7.7	21.3	8.5	23.5	3.4	17.1
vMVE	9.6	26.6	9.8	27.1	7.5	21.9
MVDT	14.3	39.6	14.9	41.3	13.9	24.1
vMVDT	17.1	49.0	17.6	48.8	5.87	26.0
MVE/A	9.9	27.4	11.6	32.1	5.1	19.6
vMVE/A	11.7	32.4	9.4	26.0	8.2	22.6
E/Ea(l)	12.5	34.6	10.7	29.6	5.4	26.2
E/Ea(s)	12.7	35.2	8.8	24.4	7.8	24.6

MV: mitral inflow; TV: tricuspid inflow; DT: Deceleration time; E: early peak velocity; A: late peak velocity; v: Valsalva manoeuvre.Ea: tissue Doppler early diastolic mitral annulus velocity; l: lateral wall; s: septal wall.

6.4.3.1.2 Response patterns to differential volume loading protocols

Response patterns of mitral Doppler indices to the three interventions are shown in Figure 6.4. There was significant main interaction effect only for MVE, vMVE, MVE/A, and E/Ea(l) response curves. When compared to their respective within-subject normal variance patterns, response curves of these four biomarkers generally increased markedly following IV Saline, and to a lesser extent, following Oral Water, values peaking at the 1h measurement. Post-hoc analysis of intervention effect revealed significant difference only between MVE and vMVE response to IV Saline compared to both Oral Water and Control ($p < 0.0001$ respectively).

Figure 6.4. Mitral inflow indices response to Control, Oral Water and IV Saline.
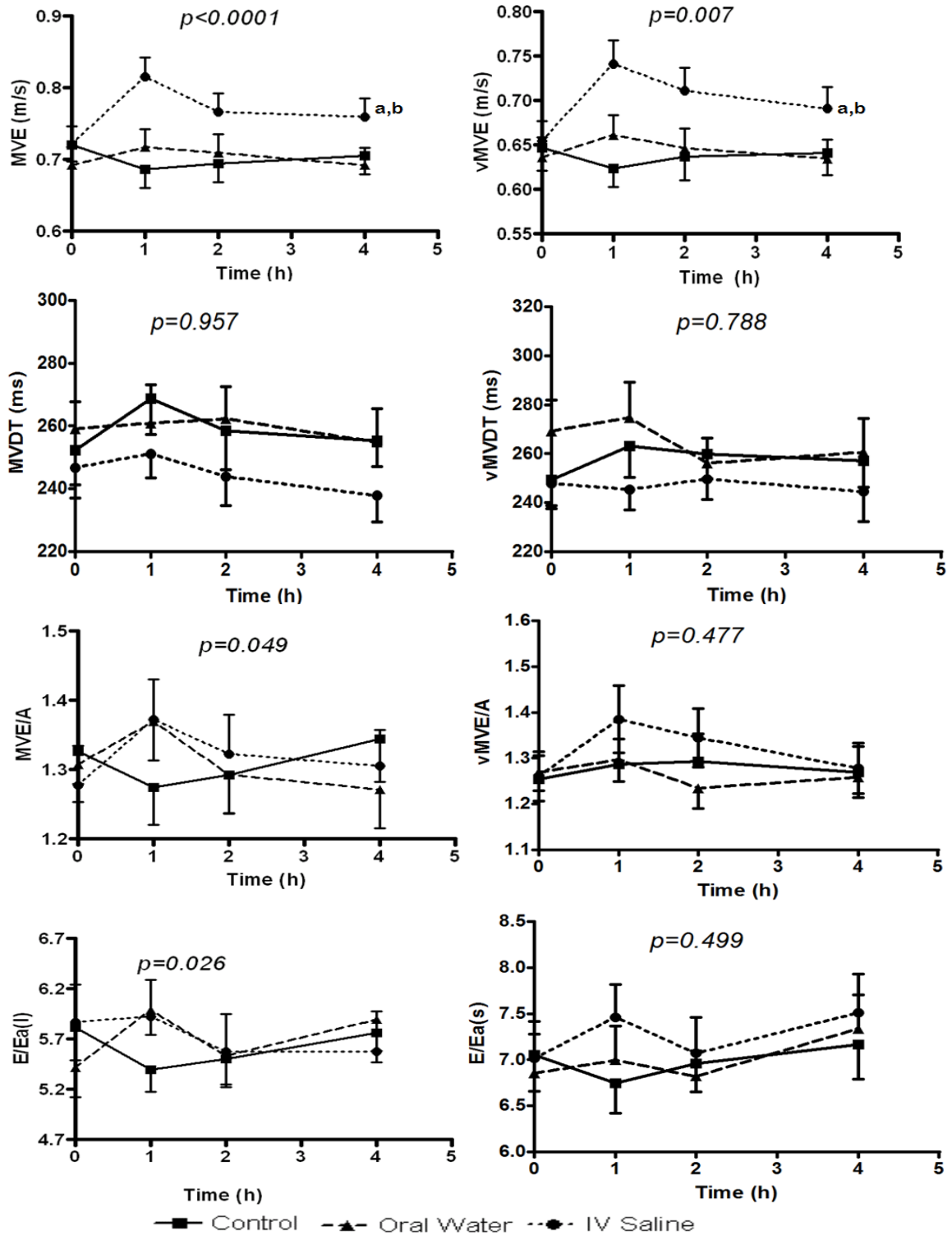
MV: mitral inflow; E: early peak velocity; A: mitral late peak velocity; DT: deceleration time; v: Valsalva manoeuvre; Ea: tissue Doppler early diastolic mitral annulus velocity; l: lateral wall; s: septal wall. Mean (SE). p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.

From Bonferroni post-hoc analysis, significant effect of intervention (p<0.05) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

6.4.3.2　　　　　Inferior vena cava indices

25 (83%) of normal volunteers had long-axis IVC diameter larger than 2cm, and 23 (77%) of normal volunteers had short-axis IVC diameter larger than 2 cm at baseline. The bias (mean difference) between IVC diameters measured in the long-axis and short-axis views was relatively small and clinically acceptable at less than 0.10 cm, as shown in Figure 6.5. The variability of measures using both methods appeared consistent regardless of the vessel diameter.

Figure 6.5. Bland-Altman plot of long-axis and short-axis measures of IVC diameters.
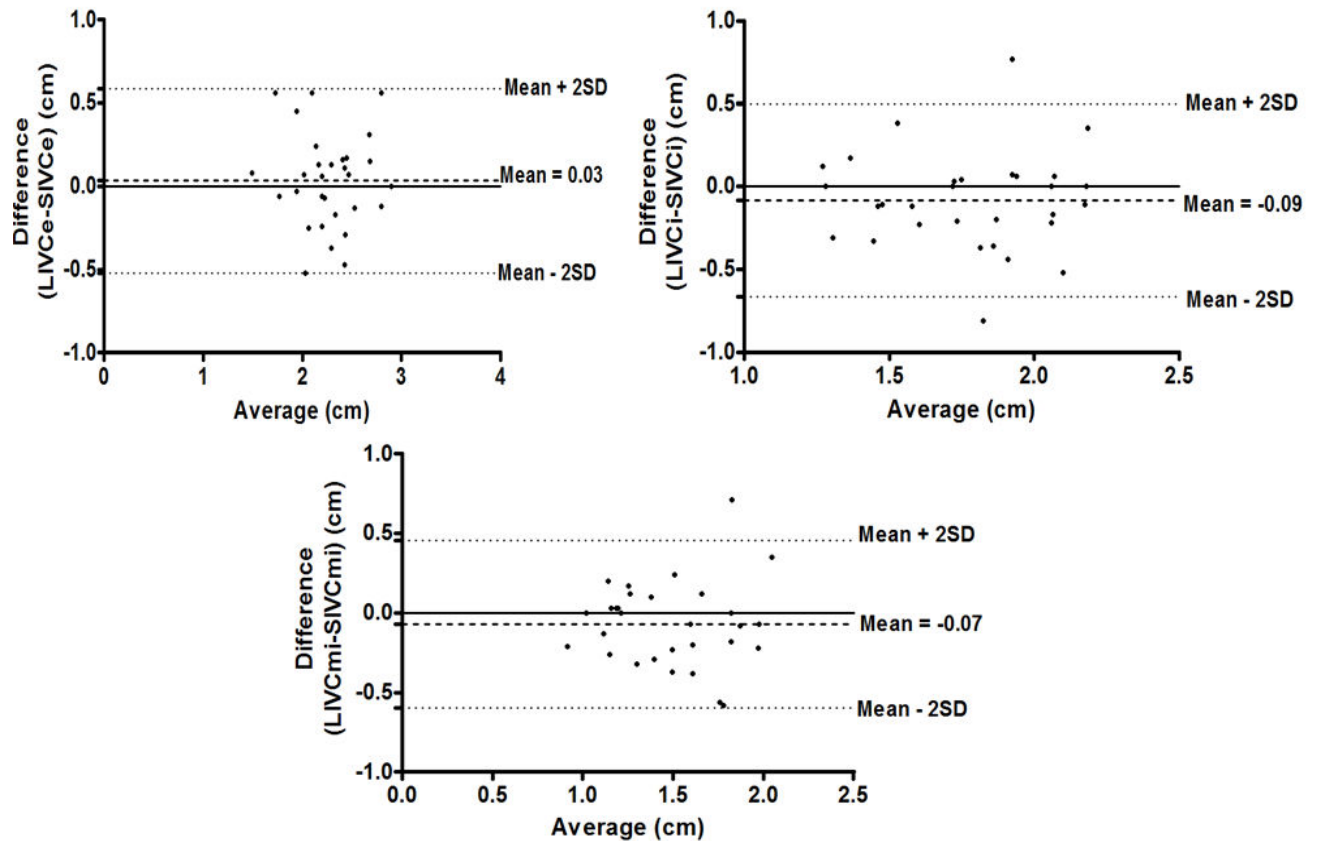
 L: long-axis; S: short-axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration; mean: mean difference between LIVC and SIVC measures; SD: standard deviation of the mean.

The components of the variance of long-axis and short-axis IVC diameters are shown in Table 6.6. There were non-significant trends in normal variance patterns for all IVC indices on the Control day.

Table 6.6. Within-day and between-day within-subject total variance (CVt$_{ws}$) and reference change values (RCV), intra-observer variability (CVo) and between-subject total variance (CVt$_{bs}$) for inferior vena cava indices in normal volunteers.

Biomarker	Within-day CVt$_{ws}$ (%)	Within-day RCV (%)	Between-day CVt$_{ws}$ (%)	Between-day RCV (%)	CVo (%)	CVt$_{bs}$ (%) n=30
LIVCe	8.0	22.2	10.2	28.3	4.2	15.3
LIVCi	9.8	27.1	10.9	31.2	7.8	18.7
LIVCmi	12.3	34.1	13.8	38.2	13.8	23.8
SIVCe	7.7	21.3	8.9	24.7	2.7	16.1
SIVCi	11.2	31.0	11.3	31.3	7.3	17.8
SIVCmi	11.7	32.4	14.6	40.4	5.7	22.8

L: long axis; S: short axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration.

6.4.3.2.2 *Response patterns to differential volume loading protocols*

Response patterns of long-axis and short-axis IVC indices to the three interventions are shown in Figures 6.6 and 6.7 respectively. In general, the response curves for IVCdiameters (IVCe, IVCi, IVCmi and their respective indexed measures) increased following IV Saline when compared to the Control day. This response appeared more marked for short-axis measures compared to long-axis measures, with significant main interaction effect demonstrated for the SIVCe, SIVCmi, SIVCDe and SIVCDmi response patterns. Post-hoc analysis of intervention effect revealed significant difference between these biomarkers' response patterns to IV Saline compared to only Oral Water [SIVCe: p=0.046; SIVCmi: p=0.028; SIVCDe: p=0.040; SIVCDmi: p=0.027). There was also no significant main interaction effect for any of the IVCCI indices.

Figure 6.6. Long-axis inferior vena cava indices response to Control, Oral Water and IV Saline.
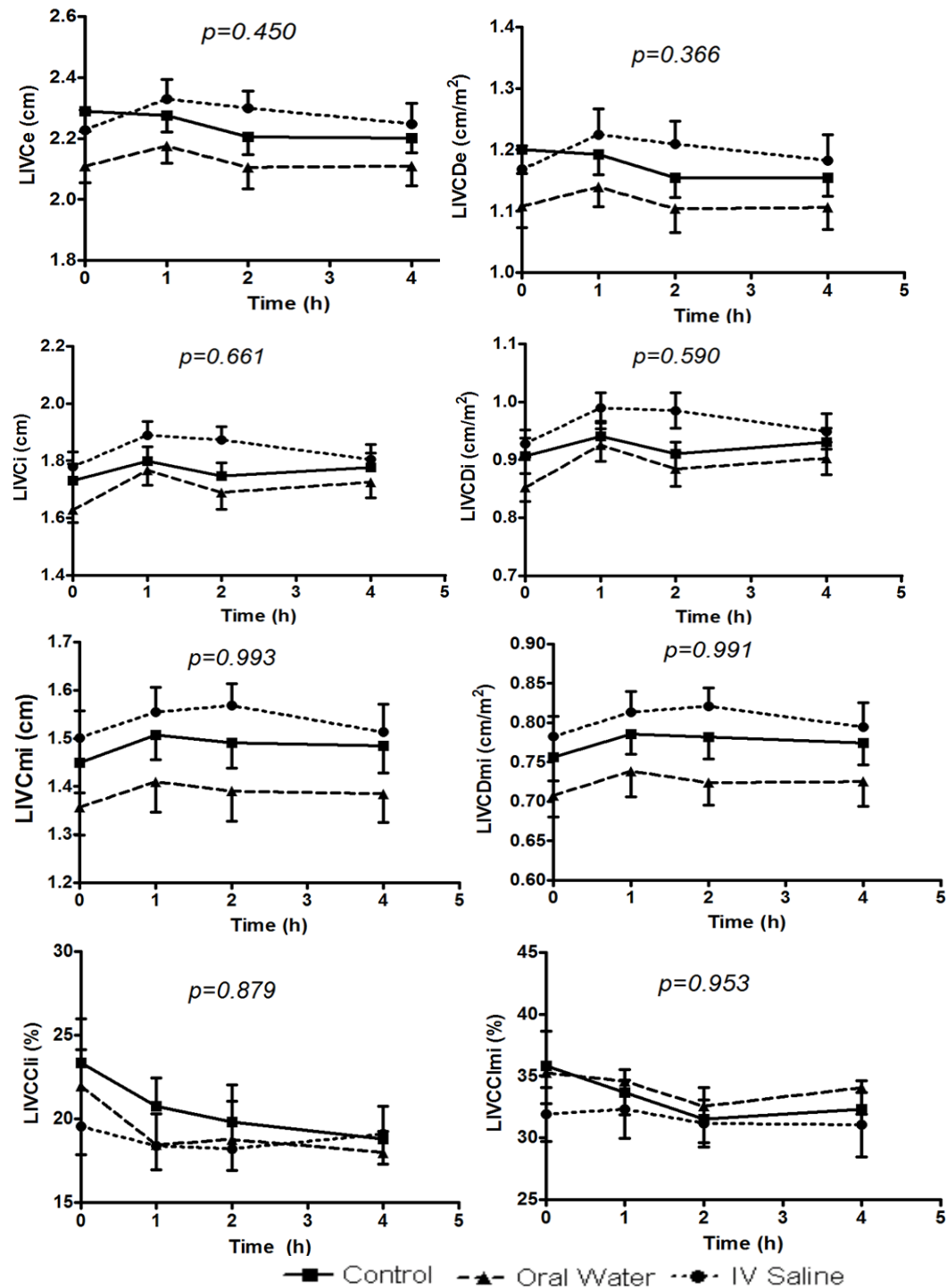
L: long axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration; IVCD: IVC corrected for body surface area; IVCCI: inferior vena cava collapse index.

p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.

From Bonferroni post hoc analysis, significant effect of intervention ($p < 0.05$) between:

[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

Figure 6.7. Short-axis inferior vena cava indices response to Control, Oral Water and IV Saline.
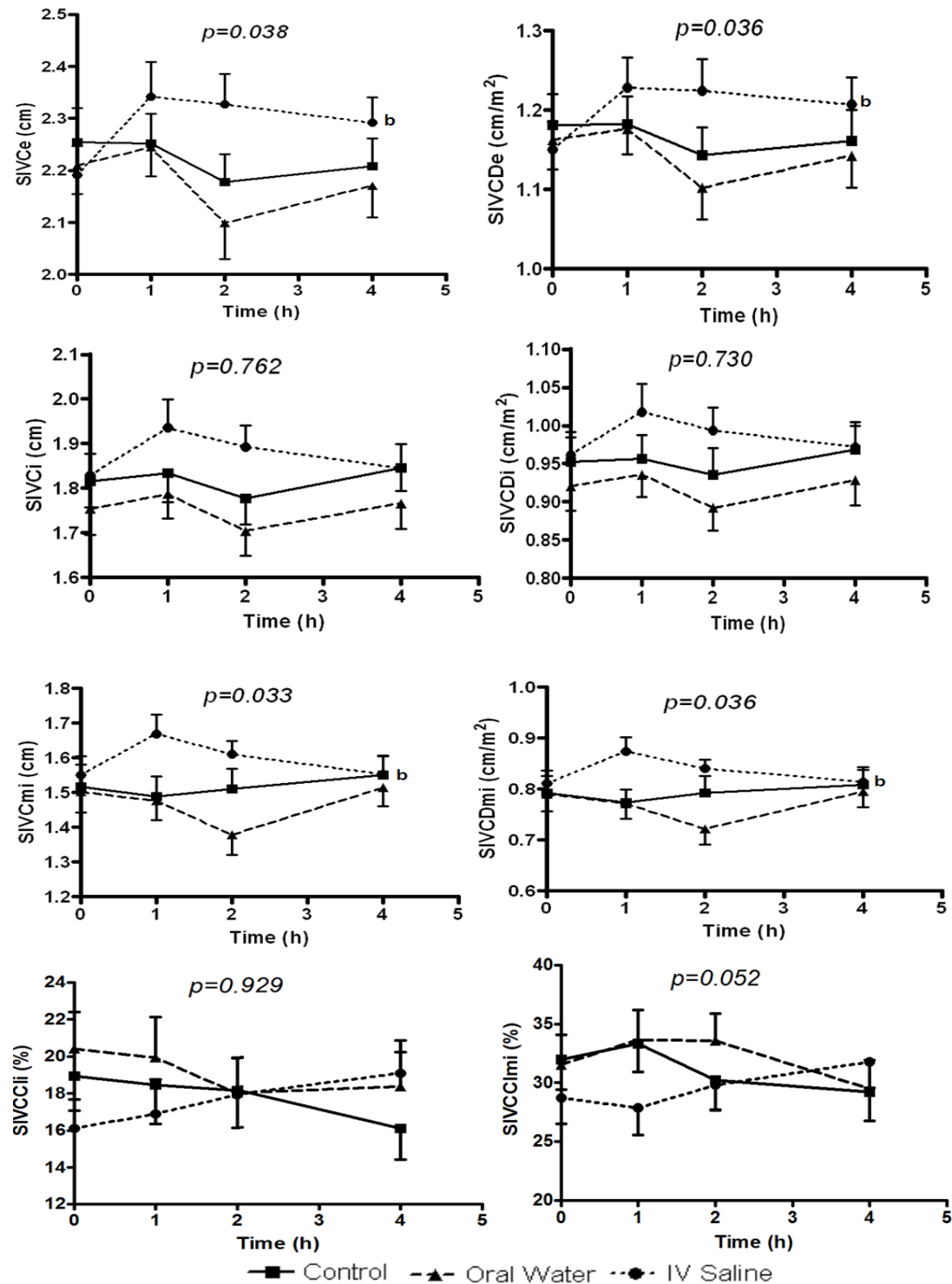
S: short axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration; IVCD: IVC corrected for body surface area; IVCCI: IVC collapsibility index. Mean (SE).

p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.

From Bonferroni post hoc analysis, significant effect of intervention (p<0.05) between:

[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

6.4.4 Bioimpedance measures

6.4.4.1 Normal variance

The components of the total variance of whole-body, trunk and right-lung BIA are shown in Table 6.7. The variance of all BIA parameters was notably smaller when compared to those of blood biomarkers and echocardiographic measures, with variance increasing the smaller the body segment being studied.

There was a general trend for all estimates of body water composition to reduce, and for all impedance values to increase over the monitoring period on the Control day. These trends were statistically non-significant for BIA variables measured with electrodes placed distally. However, most of the trunk and right-lung parameters changed significantly over the monitoring period, most of the significant changes being between baseline values and all subsequent measures ($p<0.005$ for pTBW, pECW, $pZ5_{kHz}$, $pZ50_{kHz}$, pZ_{200kHz}, apZ_{50kHz}, apZ_{200kHz}, rlZ_{5kHz}, rlZ_{50kHz}, rlZ_{100kHz}, rlZ_{200kHz}; p=NS for pZ_{100kHz}, apZ_{5kHz}, apZ_{100kHz}).

Table 6.7. Within-day and between-day within-subject total variance (CVt$_{ws}$) and reference change values (RCV), intra-device variability (CVo) and between- subject total variance (CVt$_{bs}$) for bioimpedance measures in normal volunteers.

Biomarker	Within-day CVt$_{ws}$ (%)	Within-day RCV (%)	Betwen-day CVt$_{ws}$ (%)	Betwen-day RCV (%)	CVo (%)	CVt$_{bs}$ (%) n=30
dTBW	0.9	2.5	2.4	6.6	0.22	10.4
dECW	0.7	1.9	2.2	6.1	0.22	7.8
dICW	0.9	2.5	1.8	5.0	0.36	11.2
dZ$_{5kHz}$	1.2	3.3	2.8	7.8	0.22	14.6
dZ$_{50kHz}$	1.1	3.0	2.6	7.2	0.35	15.5
dZ$_{100kHz}$	1.2	3.3	2.6	7.2	0.51	15.8
dZ$_{200kHz}$	1.3	3.6	2.6	18.7	0.54	16.0
pTBW	1.2	3.3	4.8	13.3	0.63	15.0
pECW	1.7	4.7	4.5	12.5	0.43	12.8
pICW	1.6	7.1	5.1	14.1	0.34	17.8
pZ$_{5kHz}$	3.2	8.9	2.7	7.5	0.74	8.6
pZ$_{50kHz}$	2.9	8.0	2.8	7.8	0.47	9.4
pZ$_{100kHz}$	2.9	8.0	3.0	8.3	0.50	9.9
pZ$_{200kHz}$	2.0	5.5	3.1	8.6	0.50	10.7
apZ$_{5kHz}$	4.6	12.7	6.3	17.5	2.62	18.4
apZ$_{50kHz}$	5.0	13.9	7.2	19.9	2.1	19.6
apZ$_{100kHz}$	5.6	15.5	7.5	20.8	2.4	20.4
apZ$_{200kHz}$	6.2	17.2	7.4	20.5	2.85	21.4
srZ$_{5kHz}$	6.3	17.5	6.3	17.5	0.82	18.2
srZ$_{50kHz}$	5.6	15.5	6.5	18.0	0.95	19.7
srZ$_{100kHz}$	7.2	19.9	6.6	18.3	1.69	20.9
srZ$_{200kHz}$	7.2	19.9	7.0	19.4	1.10	22.5

d: whole-body; p: trunk; ap: antero-posterior electrode position for right-lung BIA; sr: sternum-rib electrode position for right-lung BIA; TBW: total body water; ECW: extracellular water; ICW: intracellular water; Z: impedance; 5-200kHz: current frequency.

6.4.4.2 Response patterns to differential volume loading protocols

Response of whole-body, trunk, and right-lung BIA measuress are illustrated in Figures 6.8, 6.9 and 6.10 respectively. All response patterns for estimates of body water composition determined both distally and proximally increased markedly following IV Saline, compared to the normal variance and Oral Water curves. Response of all distal and proximal impedance

values determined at all four current frequencies reduced markedly following IV Saline compared to normal variance and following Oral Water, with values still much lower than the normal variance curves by the end of the 4h monitoring period.

There was a significant main interaction effect for all whole-body and trunk BIA response patterns. Post-hoc analysis for the effect of intervention showed significant difference mainly between the IV Saline response curves and both the Oral Water and Control curves ($p<0.0001$). There was no significant difference between the Oral Water response patterns compared to normal variance ($p=0.141$).

Response curves for right-lung impedance values determined with both the anterior-posterior and sternum-rib electrode placements at all four current frequencies appeared less sensitive as markers of differential response to Oral Water and IV Saline compared to normal variance. Significant main interaction effect was only found for apZ_{5kHz} and srZ_{50kHz} response patterns on the three separate days, with significant difference between responses to IV Saline compared to Control and following Oral Water on post-hoc analysis.

Figure 6.8. Whole body bioimpedance response to Control, Oral Water and IV Saline.
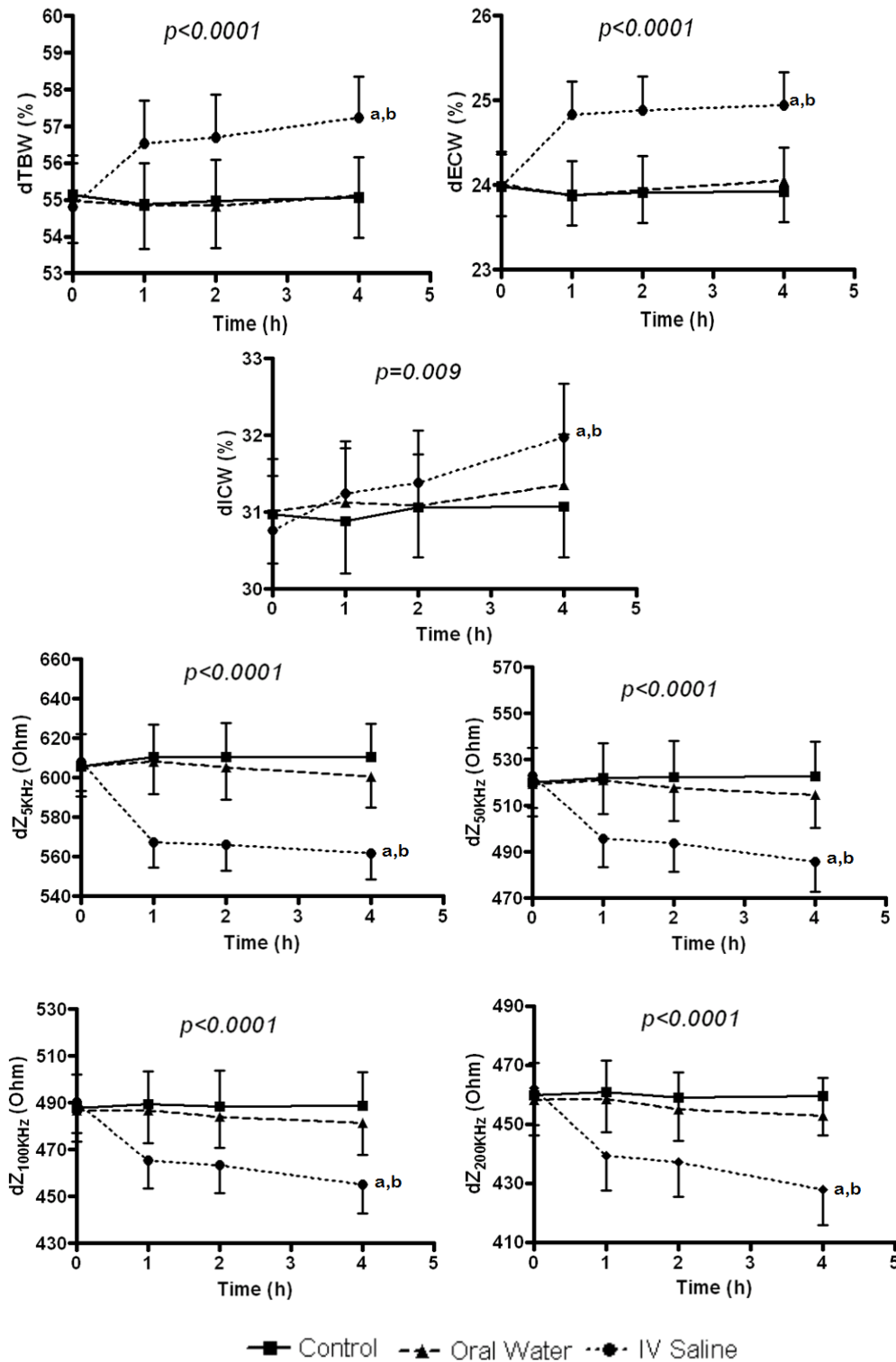
d: whole-body; TBW: total body water; ECW: extracellular water; ICW: intracellular water; Z: impedance; 5-200kHz: current frequency. Mean (SE).

p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.

From Bonferroni post hoc analysis, significant effect of intervention (p<0.05) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

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Figure 6.9. Trunk (proximal) bioimpedance response to Control, Oral Water and IV saline.
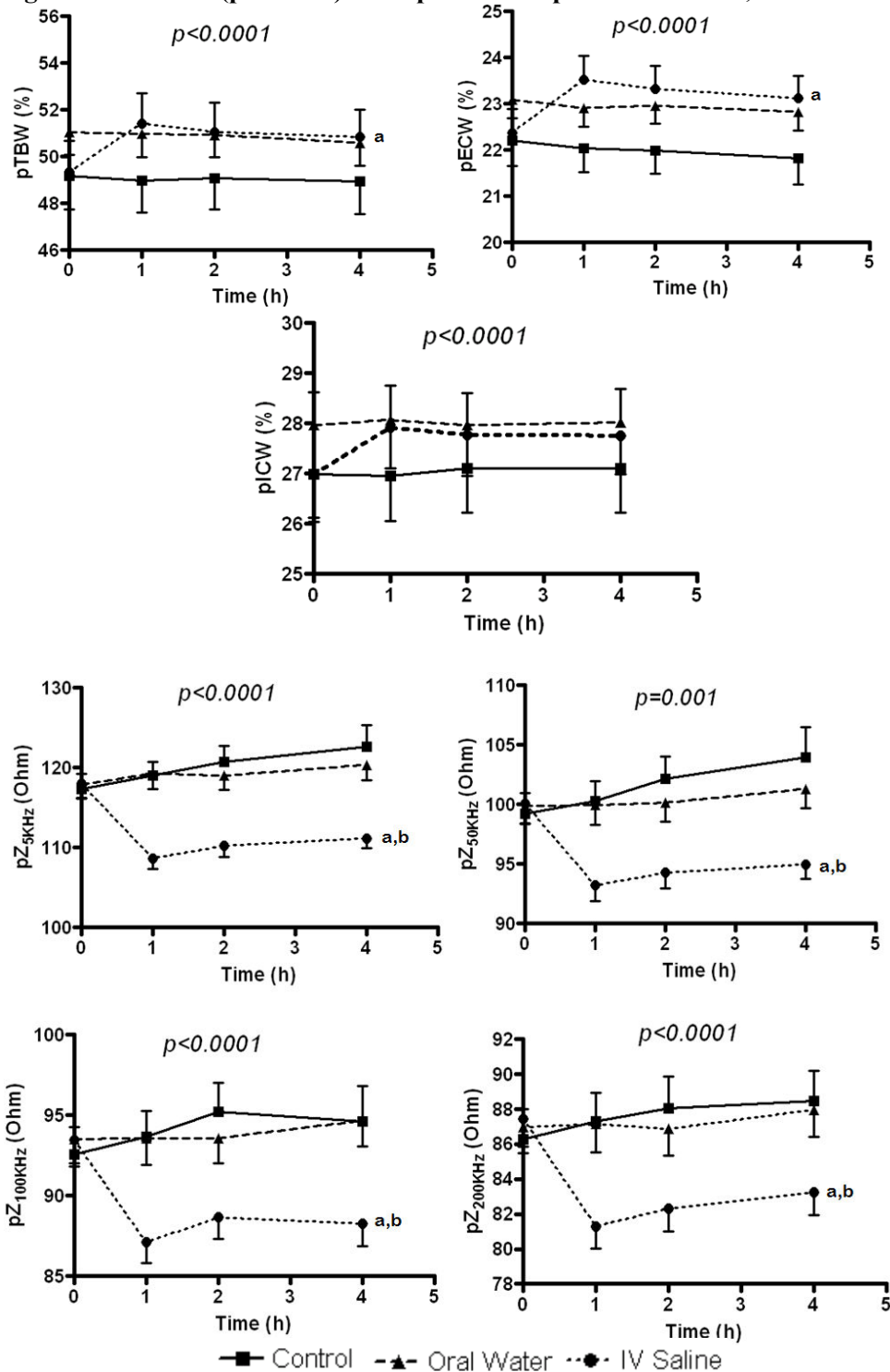
p: trunk; TBW: total body water; ECW: extracellular water; ICW: intracellular water; Z: impedance; 5-200kHz: current frequency. Mean (SE).

p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.

From Bonferroni post hoc analysis, significant effect of intervention ($p < 0.05$) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

Figure 6.10. Right-lung impedance response (with anterior-posterior and sternum-rib electrode placements) to Control, Oral Water and IV Saline.
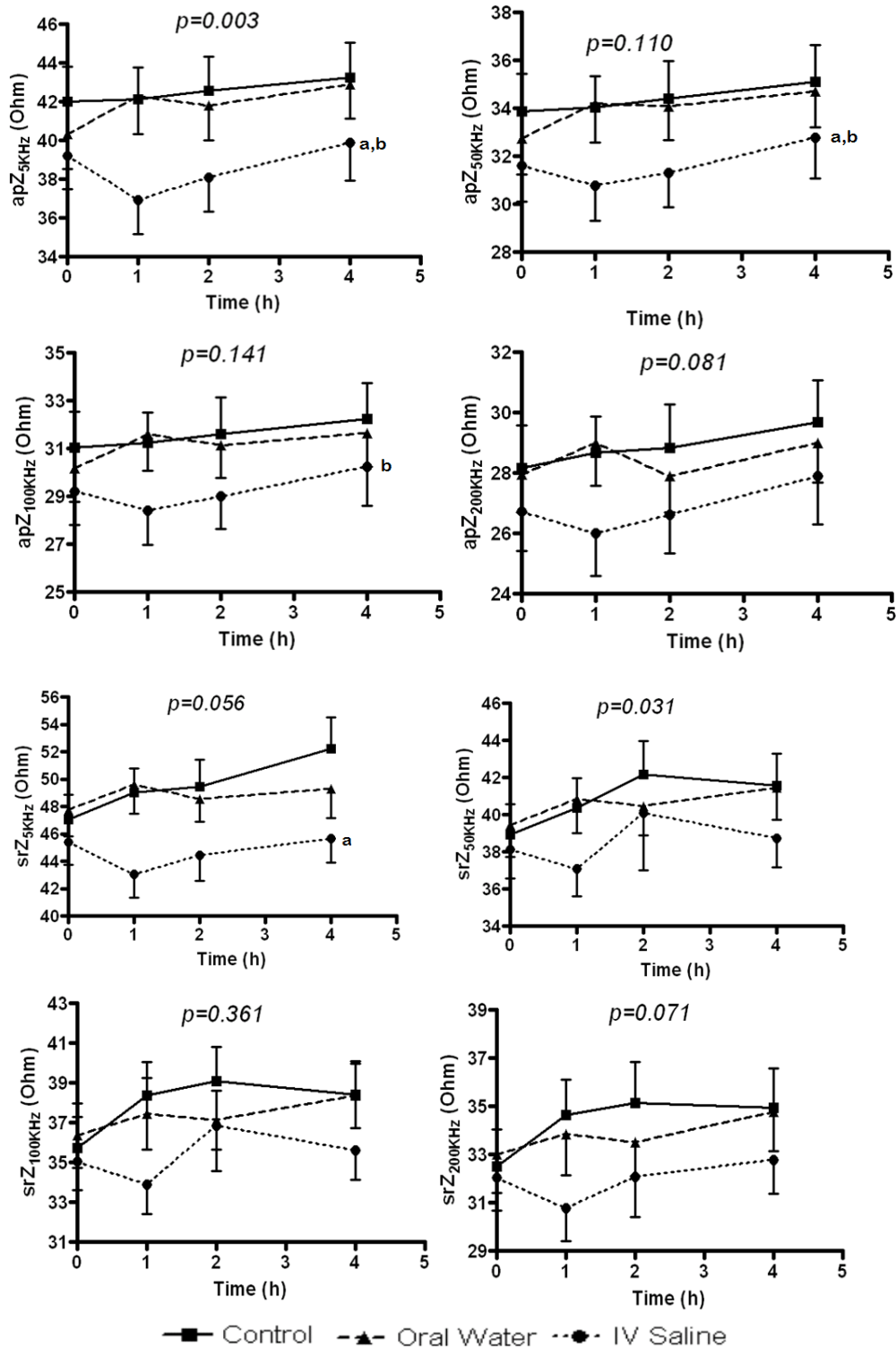
Z: impedance; 5-200kHz: current frequency; ap: anterior-posterior electrode position; sr: sternum-rib electrode position. Mean (SE).
p value obtained from repeated measures two-factor within subject ANOVA for main interaction effect of intervention and time.
From Bonferroni post hoc analysis, significant effect of intervention (p<0.05) between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

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6.4.5 Urine volume response to differential volume loading protocols

Total urine volume rose significantly following both Oral Water and IV Saline compared to the Control day as shown in Table 6.8. An interesting observation was the larger relative increase following Oral Water compared to IV Saline at the 4h measurement.

Table 6.8. Urine volume response to Control, Oral Water and IV Saline.

Urine volume (mL)	Control	Oral Water	IV saline	p
4h	528 (241) [a,c]	1643 (437) [b,c]	934 (363) [a,b]	<0.0001
8h	298 (141) [a,c]	430 (215) [c]	476 (186) [a]	<0.0001
Total	826 (282) [a,c]	2073 (454) [b,c]	1409 (443) [a]	<0.0001

p value obtained from repeated measures one-factor within-subject ANOVA
Significant difference on Bonferroni post-hoc analysis between:
[a] IV Saline and Control   [b] IV saline and Oral Water   [c] Control and Oral Water

6.5 Discussion

6.5.1 Normal variance

Within-subject and between-subject total variance for a number of biomarkers relevant to the pathophysiology of abnormal volume status in LVSD were established in normal volunteers in this Chapter. Within-subject variance was observed to be higher the longer the time period over which sampling was performed (between-day CVt_{ws} larger than within-day CVt_{ws}), and the more times it was measured over a specific period of time. For example, for blood biomarkers over the 8h monitoring period on the Control day, within-subject variance was higher when calculated using 7 measures (at 0, 0.5, 1, 2, 4, 6 and 8h) compared to just using 5 measures (at 0, 2, 4, 6 and 8h). Within-subject variance was smaller compared to between-subject variance, as is the case with most biomarkers. The largest variance (both between-

subject and within-subject) was observed for BNP, and the smallest variance was observed for BIA measures.

Knowledge of the different components of variance of a biomarker enables the clinician to interpret a measured level of the biomarker appropriately in the relevant clinical context. For example, the highest ANP value measured in this group of normal volunteers was 69 pg/mL on the Control day. The between-day within-subject variance of ANP (calculated as 12.5%) implies that ANP levels of up to 78 pg/mL would still be within normal limits for this individual, and if the principle of RCV were applied to this individual (between-day RCV 34.6%), his ANP level would have to be higher than 92 pg/mL before this implies a significant change in clinical status, such as the possible diagnosis of HF, or significant increase in volume status. Both these values are however above the manufacturer-recommended population cut-off value of 70 pg/mL, but are clearly occurring in a healthy, asymptomatic individual, supported by a completely normal physical and echocardiographic examination.

Furthermore, some biomarker levels changed statistically significantly with time on the Control day, such as Hct, ANP, estimated blood volume and right lung impedance. Being aware of this trend in normal variance for these particular biomarkers is important for future studies, where an intervention may be applied which does not have significant biological effect, but where these statistically significant trends in normal variance may erroneously be attributed to that particular intervention.

6.5.1.1 *Blood biomarkers*

The normal variance of Hct and ANP followed a similar pattern, with an initial significant drop for Hct and increase for ANP following the baseline measures compared to all subsequent measures, which eventually plateaued. These patterns have been previously documented. Renbourn [1947] demonstrated a significant drop in Hct levels between 07:00 and subsequent measures up to 02:30, and showed that this variance could be affected by, but not due to, posture, sleep, exertion or food intake. Furthermore, the variance pattern of Hct was similar whether in normal volunteers or in ill-health. Although there are no studies directly involving the normal variance of ANP, Heringlake et al. [2004] showed that NTproANP levels increased significantly from baseline level measured at 09:00 to subsequent values measured over a 10h monitoring period.

Similarly, estimated blood volume, based on changes in Hct values, increased significantly from the baseline value to all other subsequent values, this observation not having been previously documented. In my study, these changes cannot be attributed to postural changes as patients were semi-recumbent throughout the study with the exception of standing to void for urine collections. Furthermore, the collection of blood from the intravenous cannula was shown to produce comparable biomarker values to venesection and thus they *cannot* be attributed to dilutional changes during blood sampling from the cannula. Overall, this normal variance could be attributed to the diurnal secretion of ANP, modulating changes downstream with subsequent increase in blood volume and thus reduction in Hct. However, it may also involve variations in neurogenic tone. A study by Bau et al. [2008] demonstrated brachial artery diameter at 07:00 to be smaller compared to 17:00 and 22:00, suggesting that

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vasodilatation occurs subsequently from 07:00. Blood volume may increase as a result of net movement of volume from the interstial space into the intravascular compartment in response to this vasodilatation.

Despite these initial significant changes in Hct and ANP, their normal variance was relatively small. The within-subject variance of Hct was the smallest of the blood biomarkers, being comparable to that of the BIA variables. This was also consistent with the findings of Thirup et al. [2003] who documented a within-subject variance of 3 % in healthy individuals over a period ranging from 1 day to 2 months.

The observed within-day within-subject variance for ANP of 9.9 % was markedly lower compared to that of 65 % observed by McDowell et al. [2002] in normal volunteers. ANP has a short half-life of 2-5 minutes, and is secreted in bursts, thus potentially explaining the large variability in McDowell's study [Pedersen et al., 1999]. McDowell's observations were also based on samples measured every two minutes over a 90-minute observation period in the morning. As previously discussed, ANP levels in my study increased significantly between the baseline value and all subsequent values, which in turn were not significantly different from each other. Thus, McDowell et al. may have been measuring this increased short-lived variability in the morning. Furthermore, the larger number of repeated measures used to calculate within-subject normal variance (45 measures in total) would also have contributed to their higher estimation of this variance. The variance data generated from this Chapter is, however, more clinically useful as patients will routinely attend for investigations at any time of the day rather than only in the morning.

The observed within-subject variance for BNP in this study was similar to that measured by Wu et al. [2003] in normal volunteers, who published values for within-day within-subject biological variance of 43.6% which they calculated from 4 samples measured on the same day. The calculated within-day within-subject biological variance for my cohort of normal volunteers was 42.7% (calculated as described in Chapter Three).

6.5.1.2   Physical biomeasures

Data on intra- and inter-observer variability is available for a number of echocardiographic variables [Khan et al., 2004; Rijsterborgh et al., 1990], as are data on between-subject variance, mainly generated to establish population reference values. However, data on the variation of echocardiographic measures *within* a subject over a specified period of time is surprisingly *non-existent*. This is thus the first study to establish the within-subject total variance of the echocardiographic measures studied here.

The small within-subject total variance demonstrated for all BIA measures in my study was consistent with findings from a study in paediatric patients which demonstrated the within-subject variance of body water composition and resistance values using a non-invasive BIA device [Andersen et al., 2010]. The within-day within-subject variance was 1.1-2.8%, and the between-day within-subject variance was 2.4-5.7%, variance increasing with reducing age.

6.5.2   **Changes in estimated blood volume to differential volume loading**

The significant overall increase in estimated blood volume observed following IV Saline, with intravascular volume still expanded at the end of the 8h monitoring period is consistent with findings from previous studies, which have in addition demonstrated that the interstitial space had re-equilibrated, and that a proportion of the fluid infused had still not been excreted by the end of similar monitoring periods. [Ernest et al., 2001; Lobo et al., 2009 ; Reid et al., 2003; Lobo et al., 2001].

The absence of any significant change in estimated blood volume observed following Oral Water loading compared to normal variance was in contrast to the initial significant reduction in plasma volume observed by some authors which occurred within minutes of the intervention [Callegaro et al., 2007; Endo et al., 2001]. However, these acute changes in plasma volume were short-lived, normalising within 15-30 minutes. My first biomarker measurements were made 0.5h after the start of Oral Water drinking, it is therefore likely that these initial changes were not recorded in my experiments.

Furthermore, the rate of ingestion of Oral Water in the present study was slower compared to previous studies. Callegaro et al. [2007] instructed their normal volunteers to drink 500mL of mineral water at 22°C in less than 5 minutes; Endo et al. [2001] instructed their volunteers to ingest 1L of tap water at 25°C over 2 minutes, the latter reporting that the same experiment using 500mL of water ingested over the same time period had caused a similar pattern of response but of lesser magnitude.

The mechanisms for the transient activation of the SNS and simultaneous vagal response that result in increased systemic blood pressure and extravasation of fluid into the interstitial space and thus of reduction of plasma volume following the acute ingestion of oral water are still not well understood, but may involve autonomic response to activation of osmoreceptors within the orophaynx, gut or portal system [Andersen et al., 2000]. The afferent response may involve the hypoosmotic property of oral water, as drinking normal saline does not result in similar SNS activation and increased vagal tone compared to oral water ingestion [Brown et al., 2005]. The study by Akasihi et al. [1989] may explain the significantly higher urine volume at 4h observed in my study following Oral Water compared to both Control and IV Saline (Table 6.8). They demonstrated that normal volunteers who drank minimal amounts of water (enough to keep the pharynx moist) over 20 minutes experienced a hypotonic diuresis, whereas those who drank the same amount within seconds did not. Unfortunately, urine osmolality was not measured in my study to explore this.

6.5.3 Changes in blood biomarkers to differential volume loading

As potential biomarkers of intravascular volume, the response pattern of the blood biomarkers studied would be expected to change according to estimated blood volume changes in response to IV Saline and Oral Water. Thus, ANP and BNP response, if suitably sensitive, would be expected to rise, and Hct response to reduce significantly following IV Saline compared to normal variance. While no significant changes were observed between the responses to Oral Water and normal variance as expected [Yamasaki et al., 1998], Hct levels following IV Saline, although showing an overall reduction, did not differ statistically to the normal variance curve. The ANP response curve to IV Saline (Figure 6.3), although showing

a statistically significant difference to both Oral Water and normal variance, does not appear to show changes of different magnitude to the normal variance pattern, the only difference between the two curves being a higher starting point at baseline on the IV Saline day. The lack of significant change of the BNP curve following IV Saline compared to normal variance was expected from previous documented lack to senisitivities to acute volume loading [Lang et al., 1993, Larsen et al., 2006; James et al., 2005, Heringlake et al., 2004]. Thus, none of the three blood biomarkers studied appear to be sensitive enough to map the acute changes in volume status induced in the study setting, and would not be clinically useful for monitoring volume status.

6.5.4 Changes in echocardiographic variables to differential volume loading

6.5.4.1 Mitral Doppler indices

The only mitral inflow indices that appeared sensitive to changes in volume status were MVE and vMVE. As potential markers of cardiac preload and thus of intravascular volume, their responses to both Oral Water and IV Saline mirrored the changes in estimated blood volume, with levels still elevated by the end of the 4h monitoring period following IV Saline. Furthermore, the magnitude of the maximal percentage change from the corresponding measure on the control day was of the order of 18-19 %, which was larger than the maximal percentage change observed for BIA measures following IV Saline compared to normal variance, of the order of 8-10 %.The fact that MVE still changed significantly with the Valsalva manoeuvre, which should attenuate its sensitivity, further supports its potential for tracking volume changes of the magnitude observed in this study. However, the fact that the maximal percentage change from baseline of MVE and vMVE, despite occurring above their

respective within-subject total variance, was less than their respective within-subject RCV suggests that these results need to be interpreted with caution, and their potential clinical application for monitoring of volume status needs further clarification. This is particularly true in the context of the routine care of LVSD patients, in whom much less acute and large changes in volume status might be expected to take place during decompensation.

Although there was a statistically significant main interaction effect for the MVE/A and E/Ea(l) curves, post hoc analysis for the effect of intervention did not show any statistically significant effect of either Oral Water or IV Saline compared to normal variance, suggesting that these markers would not be sensitive enough to track the changes in estimated blood volume induced in my normal volunteers despite their previously good correlations demonstrated with spot measurements of LV filling pressures [Rivas-Gotz et al., 2003]. The lack of sensitivity of MVDTmay be secondary to the large intra-observer variability and within-subject total variance of its measurement.

6.5.4.2 *Inferior vena cava indices*

While several studies have demonstrated fair to good correlation between IVC indices and right atrial pressure or PCWP in various settings, no study so far has evaluated their potential utility at tracking changes in volume status. Despite the apparently higher sensitivity to volume changes of SIVC indices compared to LIVC indices, with statistically significant main interaction effect demonstrated for SIVCe, SIVCmi, SIVCDe and SIVCDmi, there was significant intervention effect only between the Oral Water and IV Saline response curves, and no significant difference of IV Saline response compared to normal variance. This implies

that changes in SIVC indices were of too little magnitude in response to the significant rise in intravascular volume following IV Saline. This could be explained by the lack of capacity of the IVC in my volunteers to distend to a much larger extent as the majority of the volulnteers had IVC diameters on expiration of >2cm at baseline with the IVCCI indices being all <50 %, which in turn could be due to the normal volunteers being well hydrated at baseline. Furthermore, in agreement with Brennan's study [2007], indexing the IVC for body surface area did *not* statistically improve the predictive accuracy of this marker for mapping volume changes in response to differential volume loading.

The changes in IVCCI indices did not appear consistent with the observed changes in estimated blood volume. Thus despite the good correlation between IVCCI indices and invasive haemodynamic indices demonstrated in previous studies [Cheriex et al., 1989], IVCCI indices are not sensitive enough for tracking acute volume changes in the current setting.

6.5.5 Changes in bioimepedance measures to differential volume loading

The largest and most consistent changes in biomarkers in response to acute volume loading were observed with the BIA variables following IV saline, in particular all whole-body measures, and impedance values using trunk measures, with significant difference between IV Saline and both the normal variance and Oral Water response curves. In addition, the maximal percentage change in these markers occurred over and above both their respective within-subject total variance and within-subject RCV. This comparably larger change in BIA response compared to the other biomarkers studied in this section may be explained by the

fact that BIA is indeed measuring ECW. As previously discussed, the infusion of IV Saline

results in an increase not only in blood volume, but also in a comparatively larger initial

increase in interstitial space, such that BIA would reflect the overall increase in both

compartments following IV Saline. This would also be consistent with the larger relative

increase in ECW compared to ICW.The significant concurrent changes observed in ICW may

occur as a result of osmostic processes.

Right-lung measures did not seem particularly sensitive at tracking volume changes to

differential volume loading in this cohort. Thus, although redistribution of volume from the

intravascular space into the interstitial space does occur following IV Saline, this may not

occur significantly within the lungs. It is however, more likely that right-lung BIA measures

are not as sensitive as whole body measures at tracking volume changes, given their relatively

larger within-subject variance.

6.5.6   Differential urine volume response

The more marked urine volume response with Oral Water compared to IV Saline was an

interesting observation, and is not well documented in the literature. An increase in ANP

secondary to atrial stretch [Anderson et al., 1986], and a reduction in renin, aldosterone and

angiotensin II levels in response to IV Saline would both mediate diuresis and natriuresis,

resulting in increased urinary volume [Singer et al., 1998]. However, plasma osmolality does

not change significantly following IV saline and studies have failed to demonstrate a rise in

ADH within 30 minutes of IV saline infusion. This thus results in the increased excretion of

iso-osmotic urine with normal sodium content [Lobo et al., 2001]. As previously discussed, a

proportion of the infused volume is still not excreted by the end of similar monitoring periods to that used in this study, such that blood volume is still expanded. Only 1409 (443) mL had been excreted at the end of the 8h monitoring period compared to the total volume input of 2.5 L (initial 1.5L IV Saline and 1L oral fluids throughout the study).

Oral water drinking, on the other hand, produces rapid reductions in plasma osmolality and plasma sodium, which gradually return back to normal [Kimura et al., 1997]. This in turn reduces ADH levels, resulting in increased diuresis of lower osmolality urine with no significant change in blood volume as demonstrated ealier in my study. Intravenous infusion of 5% dextrose compared to normal saline in normal volunteers results in a 3-fold increase in urine output of much lower osmolality, thus further supporting the hypoosmolar mechanism of diuresis observed [Lobo et al., 2001]. Simultaneous serum and plasma osmolalities as well as urinary sodium would be required to clarify the differential neuroendocrine mechanisms.

6.6   Limitations

The volume loading protocols used in this study are based on previously documented volume changes following acute oral water and IV Saline in normal volunteers. Thus, utility of the blood biomarkers and physical biomeasures studied here are defined on the basis of a presumed acute and sustained *extracellular* volume expansion following IV Saline and no change in intravascular volume following Oral Water at the time points when biomarkers were measured, mapped with some degree of accuracy by the calculated estimated blood volume. For the relative sensitivities of the biomarkers studied to volume changes to be more robust, gold standards such as blood volume analysis or invasive haemodynamic measures

would be required. I also did not perform repeated measures of body weight which would have consisted of a simple means of shedding further light on the changes in volume status observed in this study. Furthermore, repeated measures of blood pressure may have helped to clarify the observed significant changes in the normal variance of ANP and Hct. Simultaneous plasma and urine osmolalities would have contributed to further understanding of the differential urine volume response to IV Saline and Oral Water.

Establishing differential response of physical biomeasures over a longer period (8h) may have been more informative as some markers had not reduced to baseline by 4h. Thus, whereas relative sensitivities have been established to increases in volume status, it is not possible to extrapolate these findings to subsequent reductions in some compartments of volume status.

A large number of biomarkers were studied, which may explain the large proportion of statistically significant results with perhaps not much evidence for biological significance for some biomarker responses. Great care was thus taken in interpreting these results in the light of the potential clinical application of the biomarkers studied for monitoring volume status. Furthermore, the aim of the study was to identify the most potentially useful markers, such that it was important to study a large number of markers in order to maximise the possibility of identifying the most sensitive markers, and thus to optimise the use of the interventions applied.

6.7   Summary

In this series of observations, the normal variance of a panel of blood biomarkers and physical biomeasures of relevance to volume status in LVSD was established in normal volunteers. The response pattern of these biomarkers to Oral Water and IV Saline as a deviation from their normal variance was demonstrated, and is a particular strength of this study, with each volunteer acting as his/her own control. Overall, changes in the levels of the blood biomarkers and echocardiographic measures, with the possible exception of MVE and vMVE, did not appear to be sensitive enough to have clinical utility for monitoring acute volume changes. The most potentially useful markers in this respect were all whole-body BIA measures and trunk impedance measures, whose levels changed consistenly not only over and above their normal variance, but also their respective RCV. These sensitivities need to be tested further in the LVSD population.

Chapter Seven

Studies in Stable Patients with LVSD

Blood and Urine Biomarkers, and Physical Biomeasures of Response to Diuretic-induced Volume Changes in Left Ventricular Systolic Dysfunction

7.1 Introduction: Volume changes in response to diuretic manipulation

Temporary withdrawal of diuretics in selected LVSD patients is well tolerated, and permanent withdrawal is possible in a small proportion of these patients, higher LVEF and lower baseline dose of diuretics being positive predictive factors [Grinstead et al., 1994]. Using an implantable haemodynamic monitor, Braunschweig and colleagues [2002] demonstrated that a temporary 50% reduction of chronic diuretic dose for a week, followed by complete withdrawal of diuretic for another week in 4 stable LVSD patients resulted in increased haemodynamic measures of LV filling pressure. The trend towards increasing cardiac pressures was apparent from the start of diuretic dose reduction, but was more marked after the complete withdrawal of diuretic. Using a combination of blood biomarker (BNP, sCreatinine), haemodynamic and functional (NYHA class, 6-minute walk test) measures, the optimal oral dose of diuretic was determined for each patient. Pressure levels normalised to the initial values within a few days after reinitiation of the optimised oral diuretic dose. Although some patients developed worsening symptoms and signs during the study protocol, none of them needed their diuretic to be reinitiated earlier than planned.

In this third and last series of investigations, careful manipulation of diuretics constituted a means of inducing controlled volume changes in stable LVSD patients on small doses of chronic oral diuretic treatment (up to 80 mg of furosemide daily). Unlike Braunschweig's study, where diuretics were withdrawn for weeks, resulting in the onset of symptoms and signs of volume overload in some patients towards the end of the study period, the temporary withdrawal of diuretics for a few days in stable LVSD patients would provide a means of controlled volume expansion with little likelihood of decompensation. This would be similar

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to the common occurrence of patients being variably non-compliant with their diuretic treatment [MacFadyen et al., 2004]. The administration of ivF after this short phase of diuretic withdrawal, followed by reinitiation of chronic oral diuretic treatment would, in theory, restore volume balance after a few days in these patients who would in principle, remain asymptomatic.

7.2   Hypotheses (H1)

I therefore hypothesised that:

- The temporary withdrawal of chronic oral diuretic dosing over a three-day period will produce an asymptomatic increase in intravascular volume and LV filling pressure in stable LVSD patients. Interstitial space volume is also likely to increase.

- ivF and reinstatement of chronic dose of oral diuretics following this diuretic withdrawal phase will result in an asymptomatic decrease and re-equilibration of intravascular volume, LV filling pressure and interstitial volume.

- These asymptomatic diuretic-induced volume changes will be reliably mapped by repeated measures of a panel of blood and urine biomarkers, and physical biomeasures of volume status.

- Asymptomatic volume expansion in stable patients with LVSD secondary to diuretic withdrawal may give rise to a degree of subclinical myocardial ischaemia as part of the process of cardiac remodelling; this might be reflected by changes in biomarkers of ischaemia.

- A proportion of patients in this cohort of clinically euvolaemic and stable LVSD patients may be intravascularly dehydrated, and diuretic withdrawal may result in

improvement in markers of renal tubular function. Alternatively, diuretic withdrawal may result in increased markers of renal tubular function by an increase in renal venous congestion.

7.3   Methods

7.3.1   Study populatiom

Stable LVSD patients with an LVEF of <40% (by MUGA or Simpson's rule on echocardiography in the last 6 months) and who were on chronic furosemide dosing (40-80mg daily) were recruited. Patients also had to be on clinically optimised LVSD therapy including RAAS blockade, beta-blockers, and spironolactone if indicated. Exclusion criteria included recent hospitalisation for any major illness 3 months prior to enrolment into the study, poor treatment compliance, uncontrolled hypertension or diabetes, clinically significant other illness such as malignancy, unstable stroke disease or neurodegenerative illness, history of ongoing alcohol or substance abuse, and any patient in whom planned diuretic withdrawal would be likely to cause symptomatic decompensation.

Patients were recruited from the HF Services at City and Sandwell Hospitals. The HF services database at Sandwell Hospital was systematically screened for patients enrolled with the services with documented LVEF<40% who were on 40-80mg of furosemide daily. These patients' notes were then assessed to determine whether they would fit the criteria for the study. Unlike at Sandwell Hospital, no such database was available at City Hospital. Thus, all notes held in the HF Services at City Hospital had to be assessed for patients' suitability.

Potential candidates thus identified were then discussed with their respective HF nurse to assess their general suitability further. Selected patients were then contacted and invited to take part in the study. Patients who were interested underwent a (one-hour) screening visit, which constituted of the following:

- Thorough cardiovascular, past medical and drugs history.

- Thorough assessment of HF symptoms.

- Clinical examination to ensure absence of signs of abnormal volume status and clinical instability and to ascertain baseline clinical status, such as blood pressure and heart rate, and the presence of signs such as the longstanding presence of lung crackles not attributed to abnormal volume status in a patient with pulmonary fibrosis.

- Suitability for intravenous cannulation.

- Screening echocardiogram to assess echocardiographic windows and to confirm LVEF by Simpson's biplane formula.

- Screening blood tests including full blood count, urea and electrolytes, eGFR and liver function tests.

- Medication could be adjusted at this stage to optimise LVSD treatment, but was not needed for any of the patients eventually enrolled in the study. If medication had been altered, a further appointment to assess adequate response was planned.

- Explanation of the LVSD protocol and explanation of plan of action in case of decompensation during the protocol. Information leaflets and contact details were provided, including a 24-h mobile phone number to contact me during the 7-day protocol in case of emergencies. All patients' questions and concerns were addressed.

- Gaining written informed consent to participating in the study.

- Booking of week for LVSD protocol for each patient, and transport arrangements made.

7.3.2   Study design

Patients took part in a 7-day diuretic manipulation protocol, as shown in Figure 7.1. On Day 1 they received their usual chronic diuretic dose at 0h (*Baseline*). They then omitted their oral diuretic on Days 2, 3 and 4 (*diuretic withdrawal phase*), *presumed maximal volume expansion* during the protocol being achieved at 0h on Day 4. Following the 0h measure on Day 4, they were given a standard bolus dose of ivF (50mg) in order to start the re-equilibration phase of volume manipulation; this was then followed by reinstatement of their usual diuretic dose from Day 5 onwards to Day 7 (*diuretic resumption phase*). *Re-equilibrium* of volume status was presumed to be achieved at 0h on Day 7.

Patients attended the study centre on Days 1, 2, 3, 4 and 7 at the same time on each occasion (between 07:30 and 08:30) in small groups, with attendances between patients staggered by 30 minutes to enable me to perform timed repeated measures in each individual patient. Blood and urine biomarkers and physical biomeasures were performed at the same time for each individual patient on each day of attendance (0h measures). After the 0h measures, usual oral diuretic was given on Day 1, and a bolus dose of ivF (50 mg) was given on Day 4. Thus, on Days 1 and 4, patients were in addition, monitored over an 8h period with repeated measures of biomarkers at 0.5, 1, 2, 4, 6 and 8h for blood biomarkers, at 1, 2 and 4h for physical biomeasures, and at 4 and 8h for urine biomarkers and urine volume, in a similar procotol to the normal volunteers study.

Figure 7.1. 7-day diuretic manipulation protocol and study design.

	Baseline	Diuretic withdrawal			Diuretic resumption		
Day	1 Tue Baseline at 0h	2 Wed	3 Thurs	4 Fri Presumed maximal volume expansion at 0h	5 Sat	6 Sun	7 Mon Presumed volume re-equilibrium at 0h
Usual dose of oral furosemide	√	×	×	× ivF following 0h measures	√	√	√
Attendance to study centre	√	√	√	√	×	×	√
8-h monitoring*	√	×	×	√	×	×	×
Blood biomarker	√	√	√	√	×	×	√
Echo	√	√	√	√	×	×	√
BIA	√	√	√	√	×	×	√
Urine	√	√	√	√	×	×	√
Body weight	√	√	√	√	×	×	√
VAS	√	√	√	√	√	√	√

*8-h monitoring: Blood biomarkers measured at 0, 0.5, 1, 2, 4, 6 and 8h; urine biomarkers at 0, 4 and 8h; bioimpedance and echocardiographic measures at 0, 1, 2, 4h; lunch after the 4h measures, snack after the 6h measures. Usual oral furosemide on Day 1 after 0h measures, ivF on Day 4 after 0h measures.
Echo: echocardiographic variable; BIA: bioimepedance analysis; VAS: visual analogue score; ivF: intravenous furosemide 50 mg; √: attended/given/measured; ×: not attended/given/measured.

While mainly interested in the longitudinal effect of diuretic withdrawal followed by resumption provided by the 0h measures on Days 1, 2, 3, 4 and 7, the study also provided two further cross-sectional settings for the study of volume status in the form of the 8h monitoring periods on Days 1 and 4. The 8h monitoring period on Day 1 would enable me to establish the within-subject normal variance of the panel of biomarkers, whereas the 8h monitoring period on Day 4 would provide insight into the volume depletion stimulus provided by ivF. Thus, the results will be reported as:

1. Normal variance (8h monitoring on Day 1).

The 8h monitoring period on Day 1 would enable the calculation of within-day within-subject variance for all biomarkers in stable LVSD patients on optimised medical treatment, including their chronic oral diuretic, using the 0, 0.5, 1, 2, 4, 6 and 8h measures for blood biomarkers, the 0, 1, 2 and 4h measures for physical biomeasures, and the 0, 4 and 8h measures for urine biomarkers.

2. Longitudinal response pattern of repeated measures of each biomarker to diuretic-induced volume manipulation over the 7-day protocol.

This would be defined from the analysis of the five 0h biomarker measures on Days 1, 2, 3, 4 and 7, with the Day 1 measure as **baseline** biomarker measure on optimised LVSD treatment, the Day 2, 3 and 4 measures as measures of volume status during the **diuretic withdrawal phase** (Day 4 0h measure being the measure of presumed **maximal volume expansion** during the protocol), and the Day 7 measure reflecting presumed **re-equilibrium** of volume status.

3. Response pattern of repeated measures of biomarkers to ivF at presumed maximal volume expansion (8h monitoring Day 4).

 This would be outlined by the 0, 0.5, 1, 2, 4, 6 and 8h measures for blood biomarkers, the 0, 1, 2 and 4h measures for physical biomeasures, and the 0, 4 and 8h measures for urine biomarkers, ivF being given after the 0h measures. This 8h monitoring period was primarily performed to ensure there were no complications from the bolus dose of ivF. This response pattern could not be compared directly to the normal variance pattern on Day 1 in a similar manner to the normal volunteers study, where biomarker responses to different interventions were directly compared to normal variance. Firstly, the 0h biomarker values on Days 1 and 4 in the LVSD cohort were different, the former occurring during a presumed euvolaemic state, the latter occurring at

presumed maximal volume expansion. Secondly, the dosing and means of administration of furosemide were different, and I did not have anticipated furosemide responsiveness data to enable me to correct for this. This acute response would therefore be interpreted in the context of maximal changes from baseline measures, taking into account their respective normal variance.

Patients starved from 21:00 the prior evening, refrained from alcohol, caffeine and smoking. However, they were allowed normal fluid intake (drank when they were thirsty) to avoid dehydration. On arrival to the study centre on each occasion, their body weight and height were measured. They rested supine for 30 minutes, following which their blood pressure and heart rates were measured in triplicate. This was then followed by bladder voiding, urine collection and the insertion of an intravenous cannula with blood sampling for blood biomarker analyses. BIA and echocardiographic measurements were then performed using a similar pattern to Chapters Five and Six.

On each day of the protocol, in addition to being asked to document specifically symptoms of decompensation (dyspnoea, oedema, chest pain, orthopnoea, paroxysmal nocturnal dyspnoea and perceived reduction in exercise capacity), patients were also asked to rate their sense of general well being on a non-graduated visual analogue score on a scale of 0 to 10 [Sneed et al., 2001].

Patients were reviewed on a daily basis during the diuretic withdrawal phase. They were examined on each occasion for any signs of acute decompensation. To ensure further patient safety, I was available by telephone 24h a day for any emergencies. In case of

decompensation, admission to the acute Cardiology ward was pre-planned for observation and/or treatment.

7.3.3 Blood biomarkers

As outlined in Chapter Four, Hct was measured as a potential biomarker of haemoconcentration or haemodilution; ANP and BNP were measured as the most obvious biomarkers of cardiac stretch or LV filling pressure. Although BNP levels were not expected to change significantly during the 8h monitoring period following either chronic oral or acute intravenous diuresis, significant changes in baseline BNP measures over the 7-day protocol might occur [Braunschweig et al., 2002; Salvatore et al., 2005]. IMA and cTnI were also measured as potential sensitive markers of subclinical myocardial ischaemia which could result from abnormal volume status as part of the remodelling process [Braunwald et al., 2008]. Although cTnT is more specific for myocardial ischaemia compared to cTnI, cTnT levels were undetectable by our assay in 100 samples selected randomly as discussed in Chapter Four. cTnT was therefore not further studied.

7.3.4 Markers of renal tubular function

As outlined in Chapter Four, the renal tubular markers uKIM -1, uNAG, uNGAL and sNGAL were determined as potential measures of subclinical acute renal injury [Vaidya et al., 2008]. All urinary biomarker levels were divided by respective uCreatinine in order to correct for urinary concentration which would undoubtedly be affected by varying furosemide treatment during the study protocol.

7.3.5   Physical biomeasures

Echocardiographic measures obtained included mitral inflow indices and IVC indices as outlined in Chapter Four. BIA measures were obtained with electrodes placed distally, proximally and across the right lung (anterior-posterior and sternum-rib positions).

7.3.6   Statistical analysis

Within-day within-subject variance for each biomarker was determined using measurements over the 8h monitoring period on Day 1, as outlined in Chapter Three. For blood biomarkers, 7 measurements (time points 0, 0.5, 1, 2, 4, 6 and 8h) were used, for urinary tubular markers, 3 measurements (time points 0, 4, 8h) were used, and for physical biomeasures, 4 measurements (0, 1, 2, 4h) were used. Within-day RCV for each biomarker was also calculated as described in Chapter Three. Furthermore, between-subject variance for each biomarker was determined by using the baseline values from all 30 LVSD patients on Day 1 and dividing their cumulative standard deviation by their cumulative mean and multiplying by 100. Analytical variance (CVa) for blood and urine biomarkers, and intra-observer or intra-device variability (CVo) for physical biomeasures calculated in Chapter Five are also documented to illustrate their relative contributions to total variance.

General linear model repeated measures one-factor within-subject ANOVA was used to establish the longitudinal response of parameters to diuretic manipulation over the 7-day protocol using the five 0h measures on Days 1, 2, 3, 4 and 7. Bonferroni post-hoc analysis was performed to outline significant differences in values measured at different time points.

Similar analyses were performed to describe the within-subject normal variance pattern of biomarkers in LVSD patients on Day 1, and their response patterns to acute ivF on Day 4 both over the 8h monitoring period. For illustration purposes, both normal variance curves (Day 1) and response to ivF curves (Day 4) are shown on the same graph. ANP, BNP, cTnI, sCreatinine, uCreatinine, uKIM-1, uNGAL and uNAG were non-normally distributed. Non-normally distributed data was log_{10}-transformed prior to repeated measures ANOVA. The Bland-Altman method was used to assess the agreement between the long-axis and short-axis views for the measurement of IVC diameters.

Power calculations for the study were based on previous work by Braunschweig et al. [2002] and Galve et al. [2005]. A sample size of 30 patients would give a power of > 85 % to detect significant changes in ANP (46 %), BNP (145%), and mitral valve inflow indices (14%) with an alpha value of 5 %. No previous studies have involved studying the effect of chronic diuretic manipulation in LVSD on E/Ea indices, IVC indices, Hct, renal tubular markers, and BIA variables, such that data for the latter variables is lacking to estimate power.

7.4   Results

Baseline demographic and clinical variables of the 30 recruited patients are shown in Tables 7.1 and 7.2 respectively. Patients were mostly male (87%), with mean LVEF of 25(8)%. Two-thirds of patients had ischaemic cardiomyopathy, with the majority being in NYHA Class II. They were all on RAAS blockade. Two-thirds of patients were on 40mg of furosemide daily, the rest were on 80mg of furosemide daily.

All patients remained haemodynamically stable without signs of acute decompensation throughout the 7-day diuretic manipulation protocol. Thus all patients completed the study in accordance with the protocol; no emergency calls were made, and no hospital admissions for acute decompensation were required.

Table 7.1. Baseline demographic variables of LVSD patients. Mean (SD), n (%).

Characteristic	Value
Male, n(%)	26 (87)
Age (years)	70 (7)
Height (m)	1.74 (0.09)
Weight (Kg)	80.2 (19.4)
BMI (Kg/m^2)	26.3 (5.5)
Ethnicity, n(%)	
Caucasian	23 (77)
Afro Caribbean	3 (10)
Asian	4 (13)

Table 7.2. Baseline clinical variables of cohort of 30 LVSD patients. Mean (SD), Median (IQ range), n (%).

Characteristic	Value
Haemoglobin (g/dL)	13.8 (1.8)
Haematocrit (%)	42.3 (4.7)
Sodium (mmol/L)	139 (3)
Urea (mmol/L)	9.5 (4.8)
Serum Creatinine (mg/dL)	1.66 (1.29-2.32)
Estimated glomerular filtration rate (mL/min/1.73 m^2)	55 (16)
Albumin (g/L)	45 (3)
Systolic blood pressure (mmHg)	134 (21)
Diastolic blood pressure (mmHg)	76 (11)
Mean blood pressure (mmHg)	105 (14)
Mean heart rate	64 (14)
Left ventricular ejection fraction (%)	25 (8)
Aetiology of LVSD, n(%)	
Ischaemia	20 (67)
Hypertension	1 (3)
Idiopathic dilated cardiomyopathy	5 (17)
Others	4 (13)
Heart Rhythm, n(%)	
Sinus rhythm	19 (63)
Atrial fibrillation/flutter	11 (37)
NHYA class, n(%)	
I	1 (3)
II	26 (87)
III	3 (10)
Comorbidities, n(%)	
Diabetes	10 (33)
Hypertension	14 (47)
Hypercholesterolaemia	5 (17)
Coronary artery disease	20 (67)
Cerebrovascular/peripheral vascular disease	3 (3)
Lung disease	6 (20)
Smoker	7 (23)
Medication, n(%)	
Beta blockers	23 (77)
ACE inhibitors	20 (67)
Angiotensin II receptor blockers	10 (33)
Aspirin	17 (57)
Warfarin	12 (40)
Lipid lowering	22 (73)
Vasodilators	9 (30)
Dose of daily diuretic	
Furosemide 40 mg	20 (67)
Spironolactone 25 mg	*5*
Spironolactone 50 mg	*1*
Furosemide 80 mg	10 (33)
Spironolactone 25 mg	*3*
Spironolactone 50 mg	*1*

7.4.1 Non-graduated visual analogue scores and body weight

Despite all patients remaining asymptomatic on direct questioning during the 7 days, their visual analogue scores showed a non-significant decrease from Day 1 to Day 6, and an increase on Day 7, as shown in Table 7.3. Body weight showed a non-significant increase from Day 1 to Day 4 before reducing on Day 7.

Table 7.3. Visual analogue scores and body weight of patients throughout 7-day protocol. Mean (SD).

Day	1	2	3	4	5	6	7	p
Visual analogue score	7.43 (1.50)	7.33 (1.86)	7.21 (1.92)	7.07 (1.83)	6.65 (2.23)	6.49 (2.35)	7.17 (1.69)	0.127
Body weight (Kg)	80.2 (19.3)	80.8 (19.2)	81.5 (19.2)	81.8 (19.3)	-	-	81.3 (19.4)	0.149

Visual analogue score=0: Unwell; visual analogue score=10: Well.

7.4.2 Blood biomarkers

At baseline, all patients had elevated ANP levels. However, BNP levels were not particularly elevated in this cohort of patients; only 5(17%) patients had BNP levels >400pg/mL. The majority of patients [18(60%)] had BNP levels 100-400 pg/mL, and 7(23%) had BNP levels <100pg/mL. The majority of patients had elevated cTnI levels [26(87%)], and elevated IMA levels [29(97%)]. Only 1 patient had a Hct <35%.

Normal Variance (0, 0.5, 1, 2, 4, 6 and 8h measures on Day 1)

The components of the variance of blood biomarkers are shown in Table 7.4. Overall,

variance was small for Hct and IMA, moderate for ANP and BNP, and large for cTnI.

Table 7.4. Within-day within subject total variance (CVt_{ws}) and reference change values (RCV), analytical coefficient of variation (CVa) and between-subject variance (CVt_{bs}) of blood biomarkers.

Biomarker	Normal range	Baseline Mean (SD)/ Median (IQR)	CVt_{ws} (%)	RCV (%)	CVa (%)	CVt_{bs} (%)
ANP (pg/mL)	<70	794 (264 – 2543)	10.2	28.3	5.0	185.9
BNP (pg/mL)	<100	155 (96-295)	16.9	46.8	11.6	116.2
cTnI (ng/mL)	<0.004	0.019 (0.009-0.019)	46.6	129.1	3.3	134.5
IMA (KU/L)	<85	126 (28)	5.4	15.0	2.2	22.2
Hct (%)	> 35	42.3 (4.7)	3.3	9.1	0.6	11.1

Hct: haematocrit; ANP: atrial natriuretic peptide; BNP: B-type natriuretic peptide; IMA: ischaemia modified albumin; cTnI: cardiac troponin I.

The mean within-day within-subject variance of each cardiovascular blood biomarker is

illustrated in Figure 7.2 as the curve on Day 1. Hct levels showed a small but statistically

significant drop from 0h compared to all subsequent values, p<0.0001. For ANP and IMA,

there was a small but significant trend towards increasing values with time (both p<0.0001).

There were non-significant changes in BNP (p=0.183) and TnI (p=0.397) values with time.

Figure 7.2. Normal variance of blood biomarkers over 8 hours on chronic oral diuresis (Day 1), and response to acute intravenous diuresis following maximal volume expansion (Day 4).
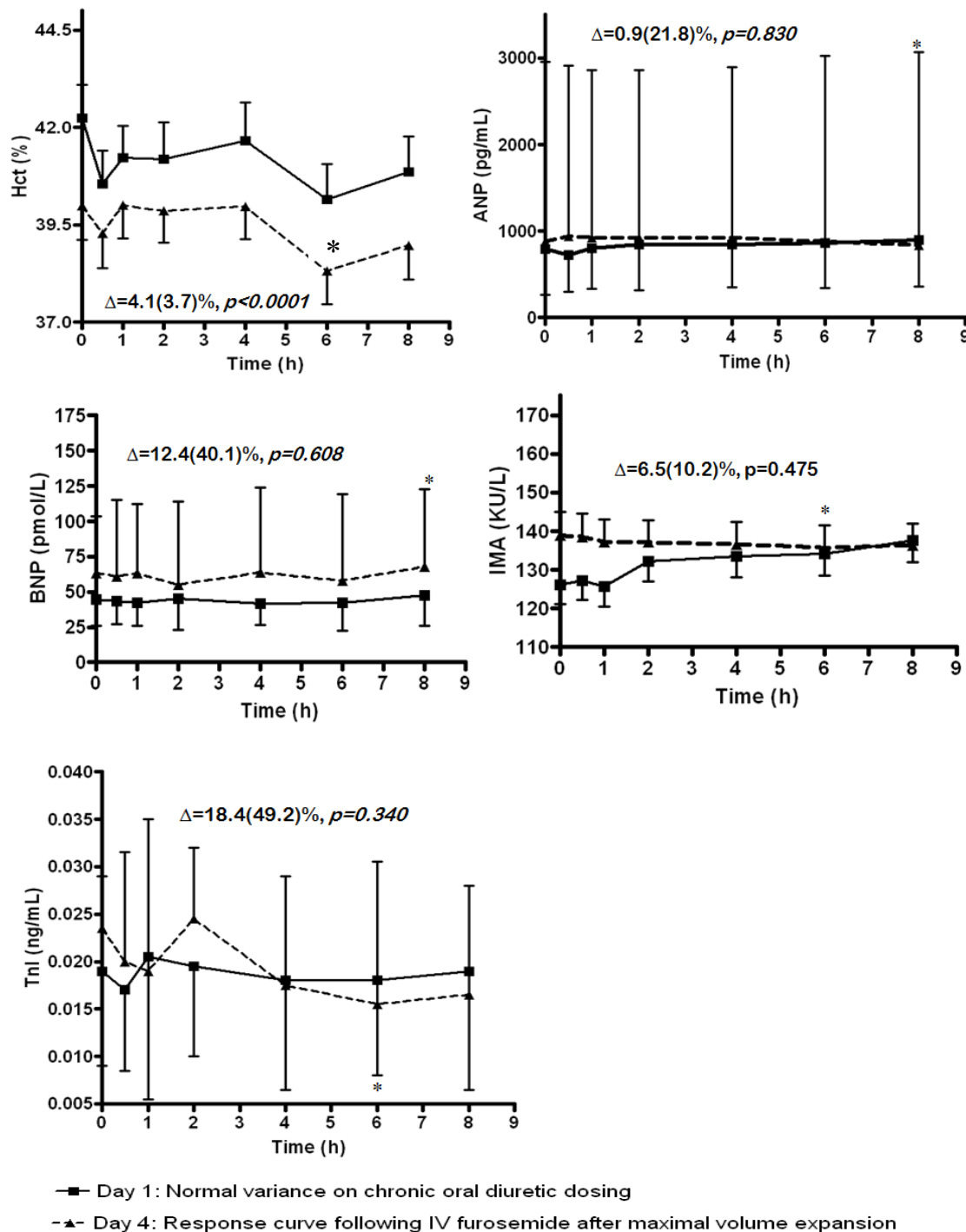
Hct: haematocrit; ANP: atrial natriuretic peptide; BNP: B-type natriuretic peptide; IMA: ischemia modified albumin; cTnI: cardiac troponin I. Mean (SE) for Hct and IMA; median (IQR) for ANP, BNP and TnI. Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4.
Day 4: Δ: maximal percentage change from 0h measure, ∗: time of maximal change from 0h measure. p value obtained from repeated measures one-factor within subject ANOVA.

7.4.2.2 *Longitudinal response to diuretic manipulation over 7-day protocol (0h measures on Days 1, 2, 3, 4 and 7)*

The longitudinal response of blood biomarkers to diuretic withdrawal followed by reinstitution of diuretic treatment is shown in Table 7.5, and blood biomarker responses in individual patients to volume manipulation are illustrated in Figures 7.3 and 7.4. Hct values showed a significant decrease following diuretic withdrawal. However, Hct levels continued to reduce and failed to re-equilibrate to baseline values following diuretic reinstatement, with Hct levels at their lowest on Day 7. Similar significant trends were observed for the ANP and IMA responses, whereby levels showed statistically significant increases following diuretic withdrawal, but levels continued to increase following diuretic reinstatement. BNP levels showed statistically significant increase to a maximum on Day 4 following diuretic withdrawal and reduced to baseline values on Day 7 after diuretic resumption. cTnI levels did not change significantly over the 7-day protocol.

Table 7.5. Longitudinal response of blood biomarkers to 7-day diuretic-induced volume manipulation protocol. Mean (SD), median (IQR).

| Variable | Baseline Day 1(0h) | Diuretic withdrawal | | | Diuretic resumption Day 7 | p |
		Day 2	Day 3	Day 4 (0h)		
Hct (%)	42.3 (4.7)[2,3,4,7]	41.1 (4.9)[1,3,4,7]	40.1 (4.9)[1,2]	40.0 (4.8)[1,2]	39.7 (4.7)[1,2]	**<0.0001**
ANP (pg/mL)	794 (264-2543)[2,3,4,7]	937 (347-2748)[1]	879 (384-2845)[1]	880 (343-2704)[1]	1183 (421-2794)[1]	**0.011[#]**
BNP (pg/mL)	155 (96-295)[2]	142 (63-239)[1,3,4]	150 (92-294)[2]	219 (98-354)[2]	148 (72-365)	**0.001[#]**
IMA (KU/L)	126 (28)[2,3,4,7]	136 (30)[1]	139 (30)[1]	139 (32)[1]	138 (31)[1]	**<0.0001**
cTnI (ng/mL)	0.019 (0.009-0.032)	0.020 (0.010-0.039)	0.017 (0.007-0.027)	0.024 (0.010-0.029)	0.021 (0.008-0.031)	0.323[#]

Hct: haematocrit; ANP: atrial natriuretic peptide; BNP: B-type natriuretic peptide; IMA: ischaemia modified albumin; cTnI: cardiac troponin I.
p values obtained from repeated measures one-factor within-subject ANOVA
#: p values obtained after repeated measures ANOVA of $\log_{10}$-transformed values
Significant difference on Bonferroni post hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

Figure 7.3. Haematocrit and atrial natriuretic peptide response over 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7).
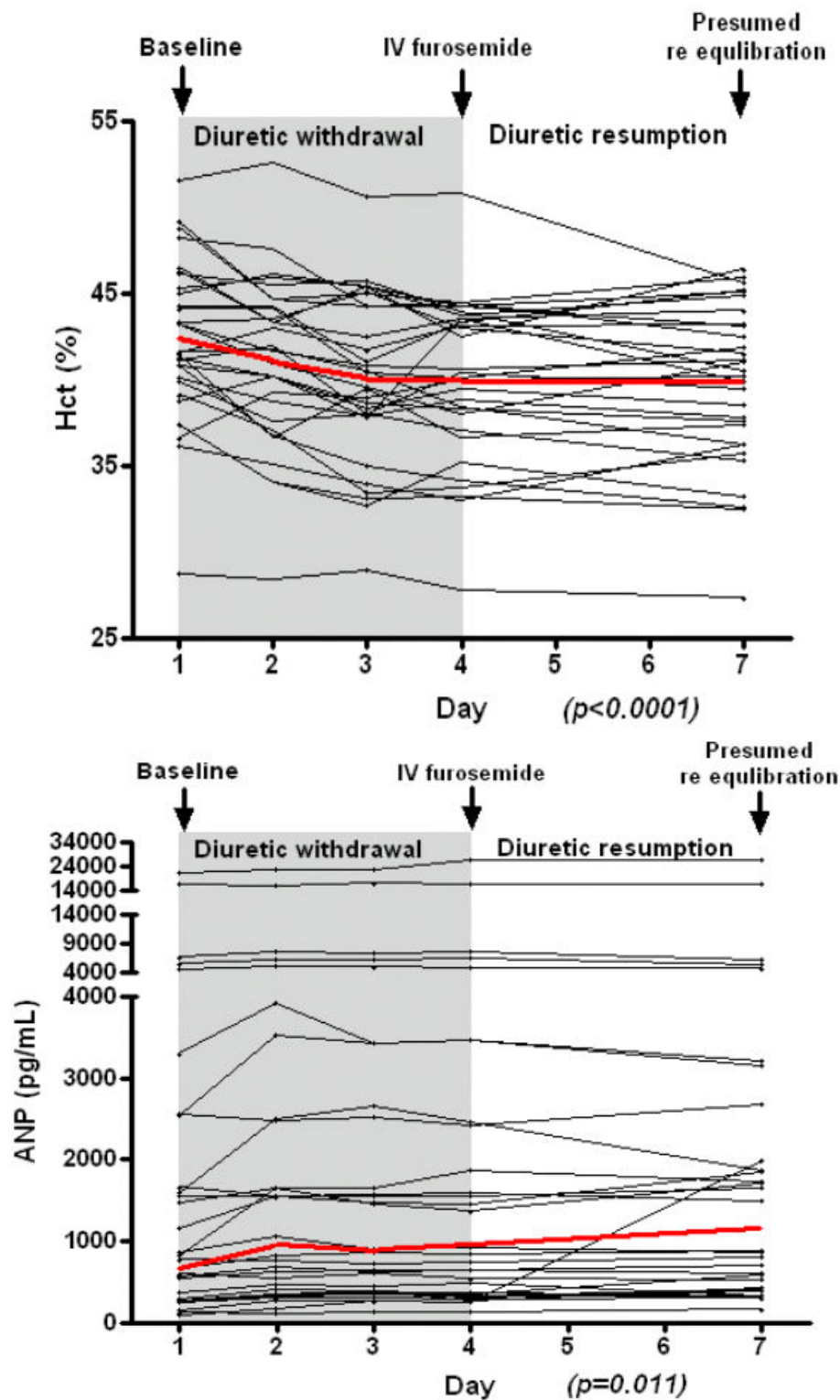
Hct: haematocrit; ANP: atrial natriuretic peptide. Baseline: 0h on Day 1; IV furosemide: 50 mg bolus given after Day 4 0h measures.
Red line joins mean (Hct) and median (ANP) values.

Figure 7.4. B-type natriuretic peptide and ischaemia modified albumin response over 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7).
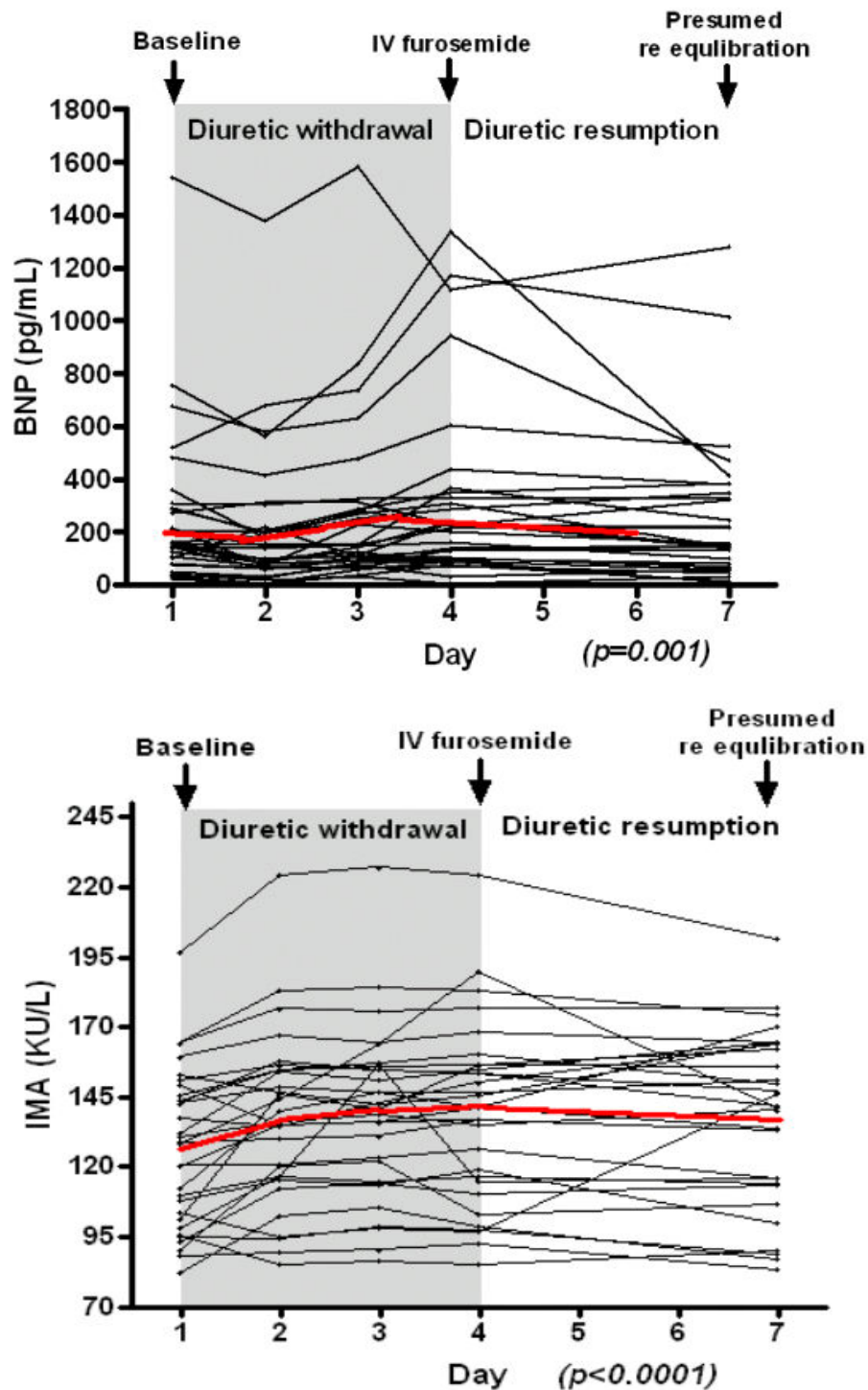
BNP: B-type natiuretic peptide; IMA: ischaemia modified albumin.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus given after Day 4 0h measures.
Red line joins mean (IMA) and median (BNP) values.

7.4.2.3   *Response to acute intravenous diuresis following presumed maximal volume expansion (0, 0.5, 1, 2, 4, 6 and 8h measures on Day 4)*

The response curves of cardiovascular blood biomarkers to ivF on Day 4 at presumed maximal volume expansion are shown as the Day 4 curves in Figure 7.2. Time points of maximal biomarker change from 0h measures are highlighted, and maximal percentage change from 0h is shown for each biomarker.   All maximal changes from 0h occurred at, or after 6h; the maximal percentage changes not exceeding within-subject variance for some biomarkers, or not being markedly higher than the within-subject variance for others. Furthermore ANP, IMA, BNP and TnI levels did not change significantly from baseline in response to ivF.

7.4.3   **Markers of renal tubular function**

Mean eGFR was reduced [55(16) mL/min/1.73 m^2], with all patients having an eGFR below the normal range of >90 mL/min/1.73 m^2. 2(7%) patients had an eGFR <30 mL/min/1.73 m^2, 18(60%) patients had an eGFR 30-60 mL/min/1.73 m^2; 10(33%) patients had eGFR 60-90 mL/min/1.73 m^2. Baseline median sCreatinine was also raised at 1.66 (1.29- 2.32)mg/dL, with 24(80%) patients having a sCreatinine above the normal range of 1.20 mg/dL. Baseline median uKIM-1 levels were elevated at 562(132-1357)ng/gCr, with 22(73%) patients having uKIM-1 levels above the normal range of >200ng/gCr.   Median uNAG levels were 8.5(5.8 – 13.4)U/gCr (normal value <3U/gCr), with 27(90%) patients having uNAG levels >3U/gCr. Median sNGAL levels were also increased at 470(333-601)ng/mL, with all patients having a sNGAL level above the normal range (<20ng/mL). Median uNGAL levels measured 1.4(0.5-

26)µg/gCr (normal <60µg/gCr) were not elevated in the overall study population, with only 3

patients (10%) having elevated baseline levels.

7.4.3.1   *Normal variance (0, 4, and 8h measures on Day 1)*

The components of the variance of markers of renal tubular function are shown in Table 7.6.

The variance of the urine biomarkers was large in general.

Table 7.6. Within-day within-subject total variance (CVt_{ws}) and reference change values (RCV), analytical coefficient of variation (CVa) and between-subject variance (CVt_{bs}) of markers of renal tubular function.

Biomarker	Baseline Mean (SD)/ median (IQR)	CVt_{ws}(%)	RCV (%)	CVa (%)	CVt_{bs} (%)
sCreatinine (mg/dL)	1.66 (1.29-2.32)	24.7	68.5	NA	45.1
uCreatinine (mg/dL)	81.7 (42.0-115.4)	57.8	160.1	<20	56.1
uKIM-1/Cr (ng/gCr)	562 (99-1379)	76.1	210.9	<20	94.3
uNGAL/Cr (µg/gCr)	1.4 (0.5-26.2)	88.8	246.1	<20	235.7
sNGAL (ng/mL)	477 (176)	12.6	34.9	<20	37.0
uNAG/Cr (U/gCr)	8.5 (5.7-14.0)	33.2	92.0	<20	70.2

u: urinary; s: serum; KIM-1: kidney injury molecule-1; NGAL: N-acetyl beta-d-glucosaminidase; NAG: neutrophil gelatinase associated lipocalin; /Cr: corrected for urinary creatinine concentration.NA: not available.

The mean within-day within-subject normal variance for each renal tubular biomarker is

illustrated in Figure 7.5 as the curve on Day 1. There were statistically significant trends

towards a reduction in all four urinary renal tubular markers at the 4h measurement, followed

by an increase in levels at the 8h measurement (uKIM-1: p=0.001; uNGAL: p=0.033; uNAG:

p=0.002; uCreatinine: p<0.0001). There were no significant changes in sNGAL levels

(p=0.163).

Figure 7.5. Normal variance of renal tubular biomarkers on chronic oral diuresis over 8h (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).
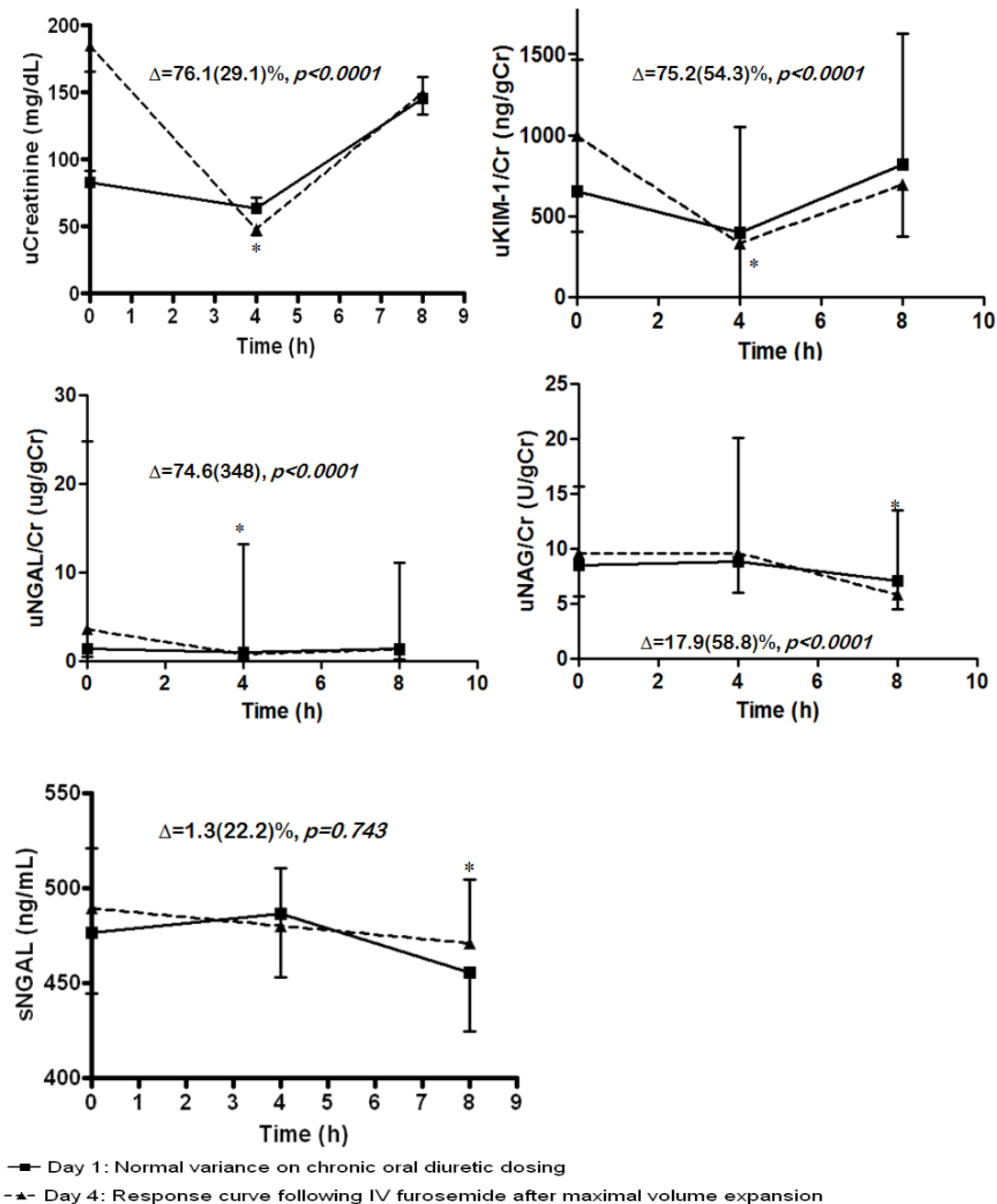
KIM-1: kidney injury molecule-1; NGAL: N-acetyl beta-d-glucosaminidase; NAG: neutrophil gelatinase associated lipocalin; s: serum; u: urinary. Mean (SE) for sNGAL; median (IQR) for uKIM-1, uNGAL, uNAG, uCreatinine.

Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4.

Day 4: Δ: maximal percentage change from 0h measure, $*$: time of maximal change from 0h measure. p value obtained from repeated measures one-factor within-subject ANOVA.

7.4.3.2 Longitudinal response to diuretic manipulation over 7-day protocol (0h measures on Days 1, 2, 3, 4 and 7)

The longitudinal response of renal tubular biomarkers to diuretic withdrawal followed by diuretic resumption is shown in Table 7.7 and Figures 7.6 and 7.7. As a positive control for diuretic manipulation, uCreatinine increased during diuretic withdrawal and reduced to near-baseline values on Day 7. uKIM-1 and uNAG levels showed statistically significant changes during the protocol, with levels increasing during diuretic withdrawal, and reducing to baseline on Day 7 following diuretic resumption. However, post-hoc analysis revealed significant difference for uNAG only between Days 2 and 3. sCreatinine, sNGAL and uNGAL levels showed no significant changes during the protocol.

Table 7. 7 Response of renal tubular markers to 7-day diuretic-induced volume manipulation protocol. Mean (SD), median (IQR).

| Variable | Baseline Day 1(0h) | Diuretic withdrawal | | | Diuretic resumption Day 7 | p |
		Day 2	Day 3	Day 4 (0h)		
uKIM-1/Cr (ng/gCr)	562 (99-1379)[2,3,4]	1025 (587-1944)[1,7]	1115 (579-1732)[1,7]	995 (401-1545)[1]	506 (189-1641)[2,3]	**<0.0001[#]**
uNGAL/Cr (ug/gCr)	1.4 (0.5-26.2)	2.7 (0.5-33.3)	1.6 (0.3-32.4)	3.1 (0.5-24.3)	2.5 (0.5-24.9)	0.723[#]
uNAG/Cr (U/gCr)	8.3 (5.7-14.0)	7.7 (5.6-13.2)[3]	9.9 (6.3-15.2)[2]	9.6 (4.8-15.7)	8.6 (5.4-15.1)	**0.044[#]**
sNGAL (ng/mL)	477 (176)	505 (200)	469 (157)	489 (173)	501 (212)	0.459
uCreatinine (mg/dL)	81.7 (42.0-115.4)[2,3,4]	189.1 (149.9-225.2)[1,7]	181.1 (118.8-227.0)[1,7]	167.9 (102.7-205.8)[1,7]	84.1 (54.6-109.4)[2,3,4]	**<0.0001[#]**
sCreatinine (mg/dL)	1.66 (1.29-2.32)	1.70 (1.31-2.18)	1.52 (1.29-2.13)	1.66 (1.40-2.21)	1.64 (1.31-2.21)	0.994[#]

u: urinary; s: serum; KIM-1: kidney injury molecule-1; NGAL: N-acetyl beta-d-glucosaminidase; NAG: neutrophil gelatinase associated lipocalin; /Cr: normalised for urinary creatinine.
p values obtained from repeated measures one-factor within subject ANOVA.
#: p values obtained after repeated measures ANOVA of $\log_{10}$ transformed values.
Significant difference on Bonferroni post hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 2 and other day
[4]: Day 2 and other day   [7]: Day 2 and other day

Figure 7.6. Response of uKIM-1 and uNGAL to 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7)
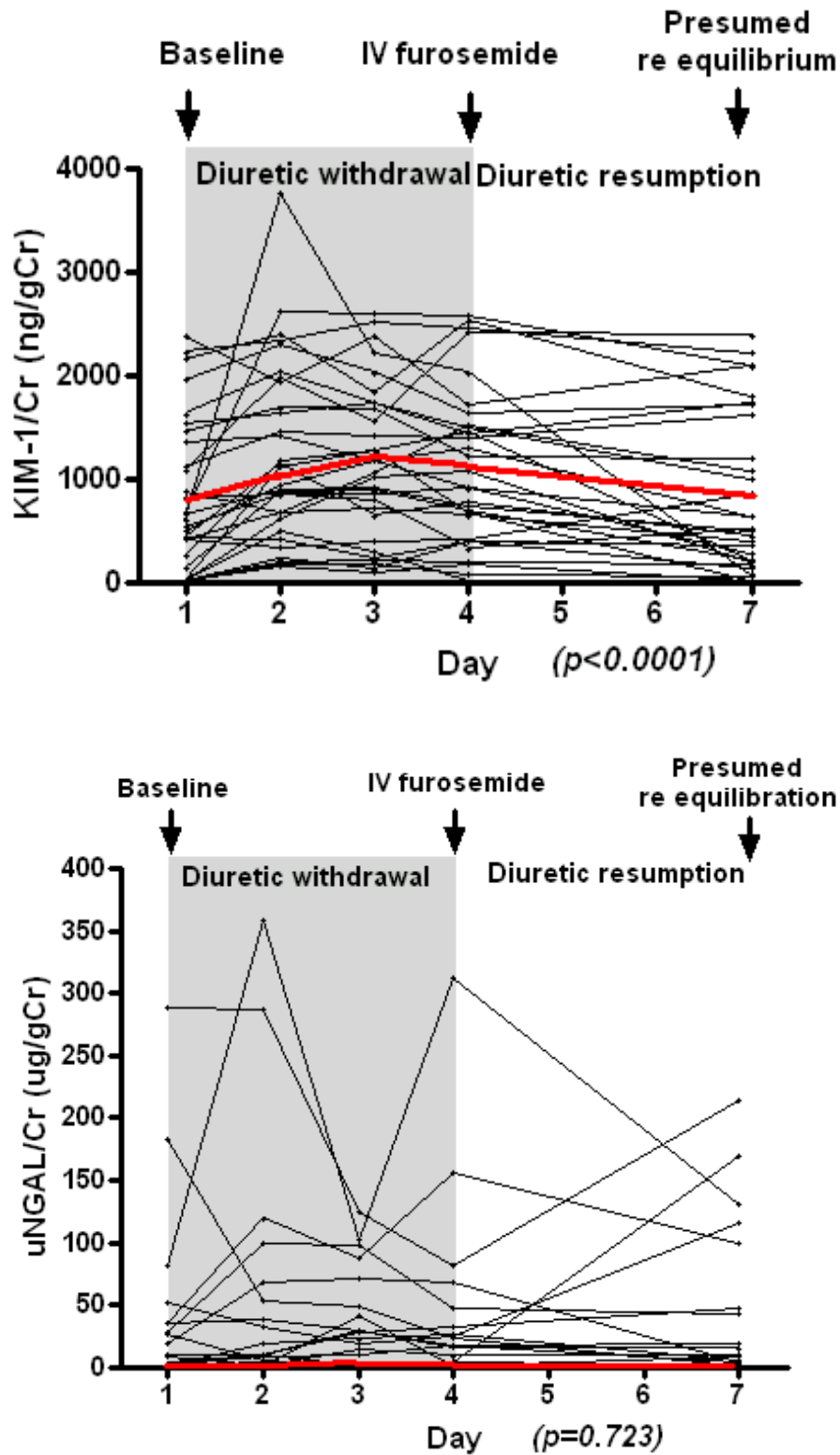
u: urinary; s: serum; KIM-1: kidney injury molecule-1; NGAL: N-acetyl beta-d-glucosaminidase.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.
Red line joins median biomarker values.

Figure 7.7. Response of uNAG and uCreatinine to 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7).
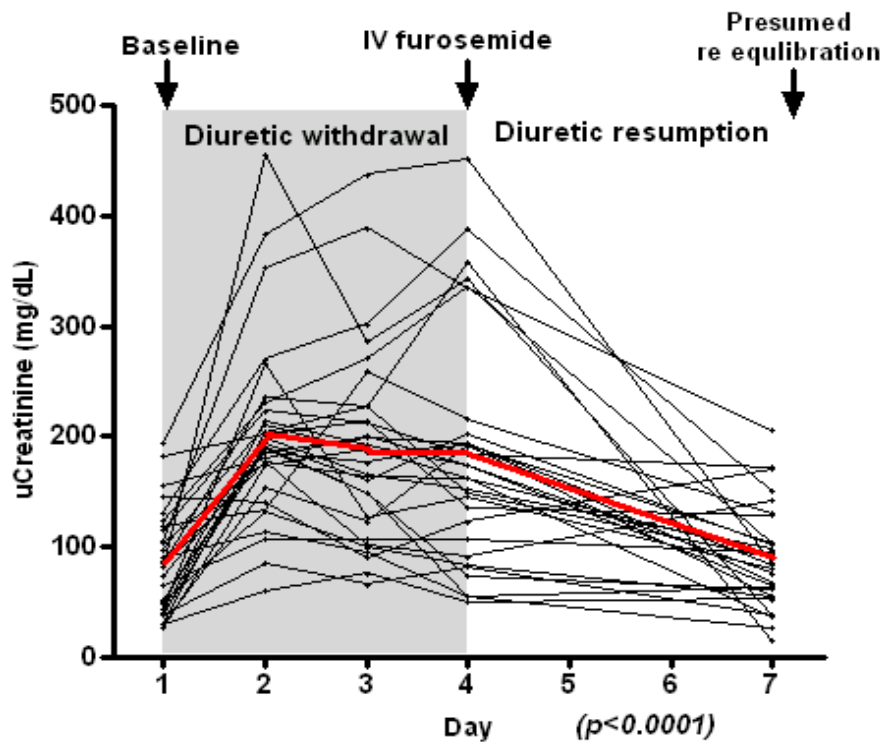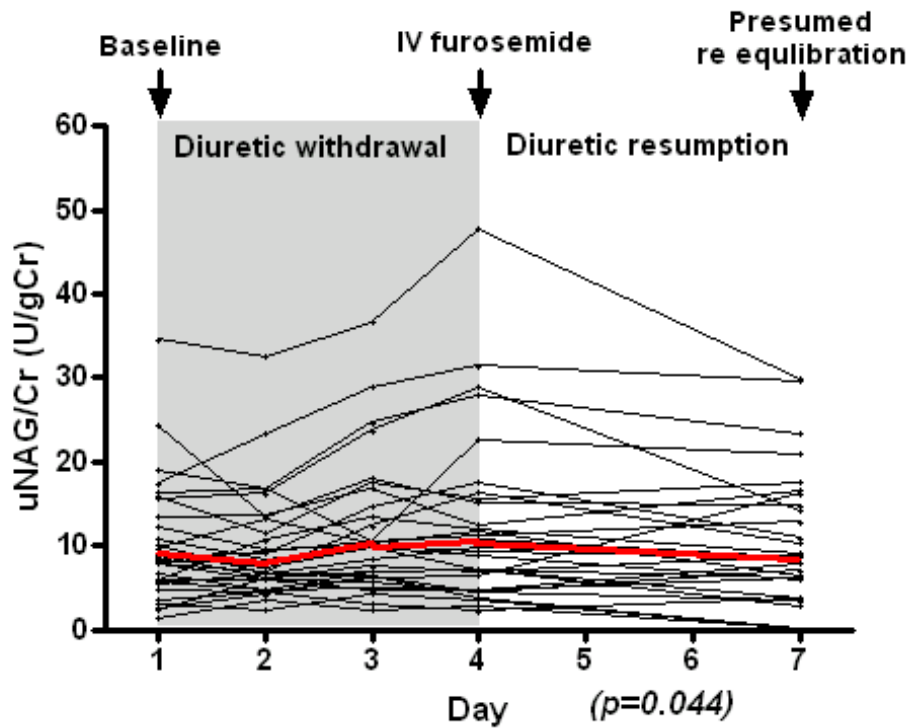
u: urinary; NAG: neutrophil gelatinase associated lipocalin.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.
Red line joins median biomarker values.

7.4.3.3 *Response to acute intravenous diuresis following presumed maximal volume expansion (0, 4 and 8h measures on Day 4)*

The response curves of renal tubular biomarkers to ivF on Day 4 are shown as the Day 4 curves in Figure 7.5. Time points of maximal biomarker change from 0h measures are highlighted, and maximal percentage change from 0h is shown for each biomarker. uCreatinine reduced significantly 4h after ivF, and increased by 8h. All urinary renal tubular markers levels showed a significant trend to a marked reduction in levels at 4h (8h for uNAG), followed by a subsequent increase at 8h. However, the maximal percentage change in these biomarker levels was either marginally above their within-subject normal variance or lower than their respective within-subject normal variance. sNGAL did not change significantly in response to ivF.

7.4.4 Echocardiographic variables

7.4.4.1 *Mitral Doppler indices*

7.4.4.1.1 Normal variance (0, 1, 2 and 4h measures on Day 1)

The components of the variance of mitral Doppler indices are shown in Table 7.8. The variance of these measures overall was moderate, with significant contribution from the intra-observer variability, e.g., for MVDT, CVtws was 15.3 %, whereas CVo was 13.9 %.

Table 7.8. Within-day within-subject total variance (CVt$_{ws}$) and reference change values (RCV), intra-observer variability (CVo) and between-subject variance (CVt$_{bs}$) of mitral Doppler indices.

Biomarker	Baseline Mean (SD) /median (IQR)	CVt$_{ws}$ (%)	RCV (%)	CVo (%)	CVt$_{bs}$ (%)
MVE (m/s)	0.84 (0.24)	10.0	27.7	3.4	28.2
vMVE (m/s)	0.76 (0.21)	11.9	33.0	7.54	27.6
MVDT (ms)	268 (85)	15.3	42.4	13.9	31.6
vMVDT (ms)	266 (78)	17.4	48.2	5.9	29.3
MVE/A	0.85 (0.78-1.65)	12.1	33.5	5.1	77.4
vMVE/A	0.84 (0.77-0.92)	16.9	46.8	8.2	74.4
E/Ea(l)	9.8 (3.6)	14.2	39.3	5.4	37.1
E/Ea(s)	13.7 (5.4)	13.4	37.1	7.8	39.5

MV: mitral valve; E: early peak velocity, v: Valsalva manoeuvre; DT: early deceleration time; A: late peak velocity; Ea: tissue Doppler early diastolic mitral annulus velocity; l: lateral wall; s: septal wall.

The mean within-day within-subject variance for mitral Doppler indices is shown in Figure 7.8 as the curves on Day 1. There were significant trends towards a reduction in MVE and vMVE after the 0h measures over 4h (p<0.0001 respectively). MVDT, vMVDT, MVE/A, vMVE/A, E/Ea(l) and E/Ea(s) values did not change significantly with time on Day 1.

Figure 7.8. Normal variance of mitral Doppler indices on chronic oral diuresis (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).
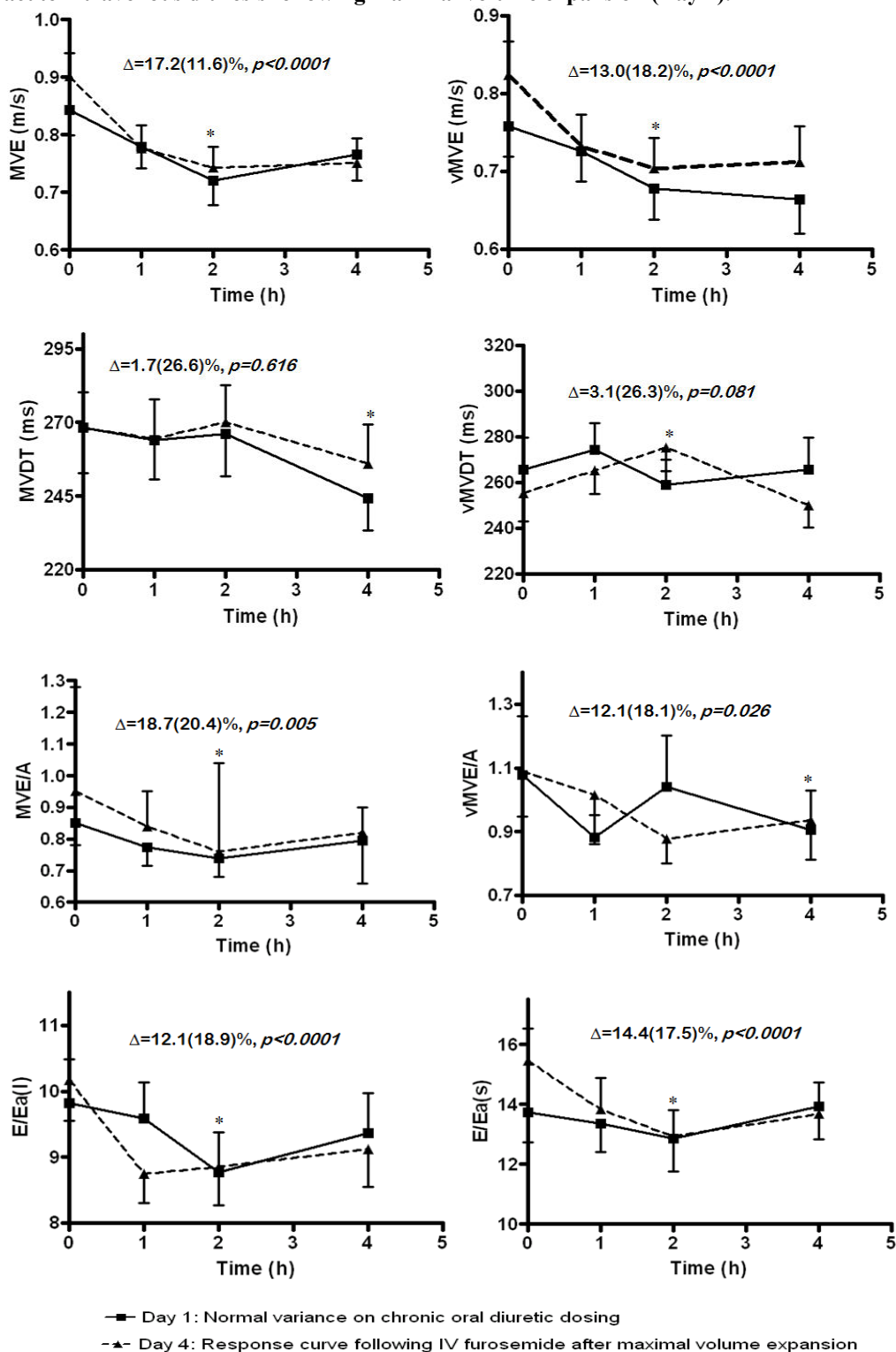
MV: mitral valve; E: early peak velocity, v: Valsalva manoeuvre; DT: early deceleration time; A: late peak velocity; Ea: tissue Doppler early diastolic mitral annulus velocity; l: lateral wall; s: septal wall. Mean (SE). Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4.

Day 4: Δ: maximal percentage change from 0h measure, ∗: time of maximal change from 0h mesure. p value obtained from repeated measures one-factor within-subject ANOVA.

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7.4.4.1.2   Longitudinal response to diuretic manipulation over 7-day protocol (0h measures on Days 1, 2, 3, 4 and 7)

The responses of mitral Doppler indices to diuretic withdrawal followed by diuretic resumption are shown in Table 7.9 and Figure 7.9. MVE, vMVE and MVE/A showed statistically significant increase from baseline to Day 4 following diuretic withdrawal. These biomarker levels then reduced back to baseline values as a result of diuretic resumption. Although E/Ea indices increased from baseline to Day 4 and reduced on diuretic resumption, these changes were statistically non-significant.

Table 7.9. Changes in mitral inflow indices in response to 7-day diuretic manipulation. Mean (SD), median (IQR).

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
MVE (m/s)	0.84 (0.24)[4]	0.82 (0.24)[4]	0.86 (0.21)	0.90 (0.22)[1,2]	0.84 (0.24)	**0.008**
vMVE (m/s)	0.76 (0.21)	0.78 (0.27)	0.76 (0.22)	0.82 (0.24)[7]	0.75 (0.22)[4]	**0.010**
MVDT (ms)	268 (85)	259 (59)	271 (80)	268 (66)	249 (46)	0.454
vMVDT (ms)	266 (78)	249 (67)	269 (69)	255 (67)	239 (57)	0.133
MVE/A	0.85 (0.78-1.65)	0.78 (0.69-0.96)[4]	0.89 (0.74-0.98)	0.95 (0.79-1.28)[2]	0.85 (0.75-0.94)	**0.040**[#]
vMVE/A	0.84 (0.77-0.92)	0.81 (0.67-0.95)	0.83 (0.72-0.98)	0.92 (0.70-1.2)	0.77 (0.64-0.97)	0.425[#]
E/Ea(l)	9.8 (3.6)	10.0 (4.3)	9.6 (3.1)	10.2 (3.3)	9.8 (3.4)	0.741
E/Ea(s)	13.7 (5.4)	13.3 (4.9)	15.3 (9.3)	15.5 (5.8)	14.3 (5.5)	0.232

MV: mitral valve; E: early peak velocity, v: Valsalva manoeuvre; DT: early deceleration time; A: late peak velocity; Ea: tissue Doppler early diastolic mitral annulus velocity; l: lateral wall; s: septal wall.
p values obtained from repeated measures one-factor within-subject ANOVA.
#: p values obtained after repeated measures ANOVA of $\log_{10}$-transformed values.
Significant difference on Bonferroni post-hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

Figure 7. 9. Response of MVE and MVE/A to diuretic-induced volume changes over 7-day protocol (0h measures on Days 1, 2, 3, 4 and 7).
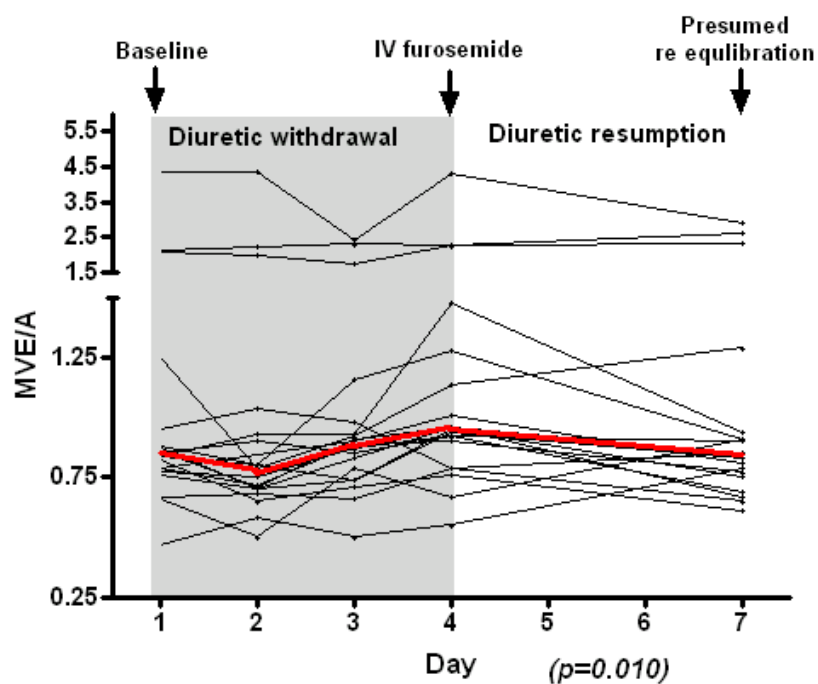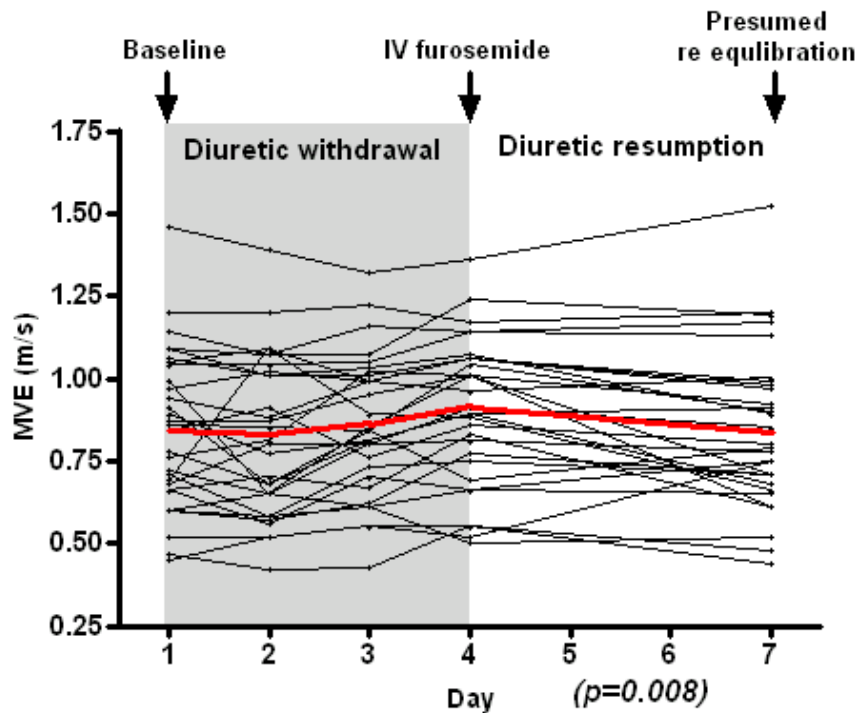
MV: mitral valve; E: early peak velocity; A: late peak velocity.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.
Red line joins mean (MVE) and median (MVE/A) values.

7.4.4.1.3   Response to acute intravenous diuresis following presumed maximal
                volume expansion (0, 1, 2 and 4h measures on Day 4)

The response curves of mitral Doppler indices to ivF on Day 4 are illustrated as the Day 4

curves in Figure 7.8. Time points of maximal biomarker change from 0h measures are

highlighted, and maximal percentage change from 0h is shown for each biomarker.   MVE,

vMVE, MVE/A, vMVE/A, E/Ea(l) and E/Ea(s) showed statistically significant reduction

following intravenous diuresis. However, the maximal percentage changes in biomarker

levels were either smaller than the respective within-subject variance or not markedly larger

than the within-subject variance in other cases.

7.4.4.2   Inferior vena cava indices

IVC views were obtained easily and consistently in all patients in this study. 15 (50 %) of

patients had long-axis IVC diameter on expiration larger than 2 cm, and 18 (60 %) of patients

had short-axis IVC diameter on expiration larger than 2 cm. Bland-Altman plots of the

agreement between the long-axis and short-axis views for the measurement of IVC diameters

are shown in Figure 7.10. The bias (mean difference) between the two methods was small ($<$

0.10 cm for IVCe and IVCi). The variability of measures using both methods appeared

consistent regardless of the vessel diameter.

Figure 7.10. Bland-Altman plots of agreement between long-axis and short-axis measurement of IVC diameters
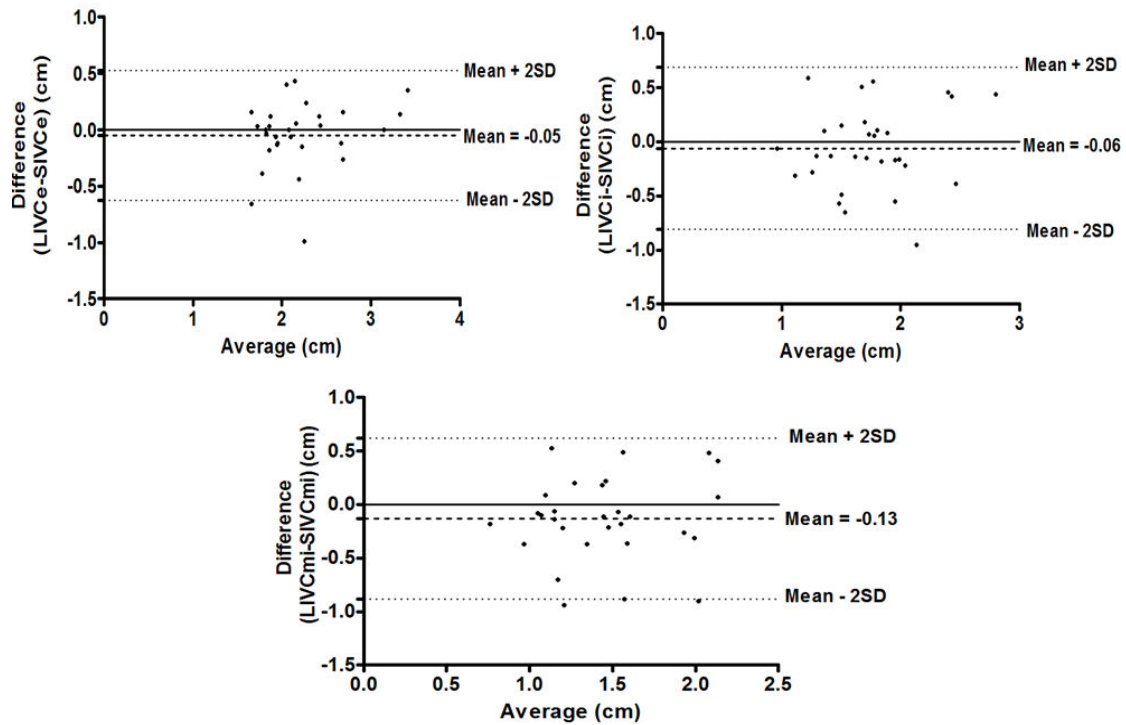
L: long-axis; S: short-axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration; mean: mean difference; SD: standard deviation.

7.4.4.2.1   Normal variance (0, 1, 2 and 4h measures on Day 1)

The components of the variance of IVC indices are shown in Table 7.10. These were similar to the variance of mitral inflow indices.

Table 7.10. Within-day within-subject total variance (CVt$_{ws}$) and reference change values (RCV), intra-observer variability (CVo) and between-subject variance (CVt$_{bs}$) of inferior vena cava indices.

Biomarker	Baseline	CVt$_{ws}$ (%)	RCV (%)	CVo (%)	CVt$_{bs}$ (%)
LIVCe (cm)	2.18 (0.52)	9.7	26.9	4.2	24.1
LIVCi (cm)	1.72 (0.49)	11.6	32.1	7.8	28.4
LIVCmi (cm)	1.38 (0.43)	13.8	38.2	13.8	31.6
SIVCe (cm)	2.23 (0.46)	9.5	26.3	2.7	20.5
SIVCi (cm)	1.78 (0.44)	13.1	36.3	7.3	24.7
SIVCmi (cm)	1.51 (0.40)	15.0	41.6	5.7	26.8

L: long axis; S: short-axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration.

The mean within-day within-subject variance for selected IVC indices is illustrated in Figure 7.11 as the curves on Day 1. There was general trend towards a reduction in all IVC indices over the 4h monitoring period. This trend was significant for LIVCe (p=0.013), LIVCDe (p=0.006), SIVCe (p=0.012), SIVCi (p=0.002) and SIVCDe (p=0.010).

Figure 7.11. Normal variance of selected IVC indices on chronic oral diuresies (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).
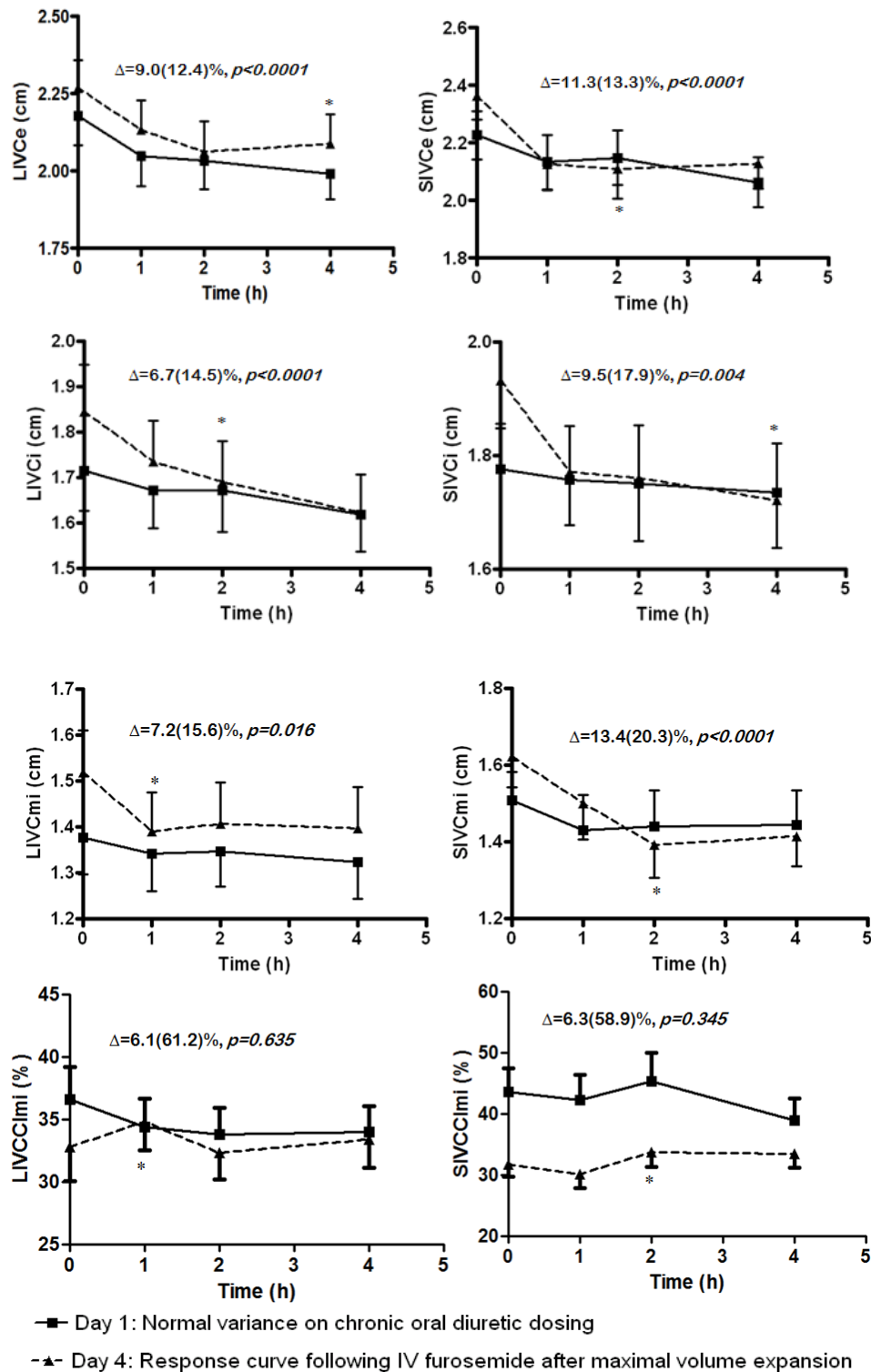
L: long axis; S: short-axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration. Mean (SE).
Oral furosemide given following 0h measures on Day 1, ivF given following 0h measures on Day 4.
Day 4: Δ: maximal percentage change from 0h measures, ∗: time of maximal change from 0h measure. p value obtained from repeated measures one-factor within subject ANOVA.

7.4.4.2.2   Longitudinal response to diuretic manipulation during 7-day protocol
               (0h measures on Days 1, 2, 3, 4 and 7)

The response of IVC indices to diuretic withdrawal followed by reinstitution of diuretic

treatment is shown in Table 7.11 and in Figure 7.12 for LIVC indices, and in Table 7.12 for

SIVC indices. All LIVC and LIVCD indices showed statistically significant increase during

diuretic withdrawal, and reduction following diuretic resumption. However, there was no

statistically significant difference between the time points on post-hoc analysis for these

biomarkers, except for LIVCi and LIVCDi. LIVCCI indices did not change significantly.

Table 7.11. Response of long-axis inferior vena cava indices to 7-day diuretic manipulation. Mean (SD).

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
LIVCe (cm)	2.18 (0.52)	2.11 (0.55)	2.16 (0.53)	2.27 (0.50)	2.09 (0.49)	**0.012**
LIVCi (cm)	1.72 (0.49)	1.69 (0.49)[4]	1.78 (0.47)	1.85 (0.56)[2,7]	1.67 (0.44)[4]	**0.006**
LIVCmi (cm)	1.38 (0.43)	1.37 (0.46)	1.44 (0.45)	1.52 (0.49)	1.38 (0.43)	**0.022**
LIVCDe (cm/m^2)	1.13 (0.25)	1.09 (0.26)	1.11 (0.24)	1.17 (0.25)	1.09 (0.25)	**0.015**
LIVCDi (cm/m^2)	0.89 (0.23)	0.87 (0.23)[4]	0.91 (0.23)	0.95 (0.27)[2,7]	0.86 (0.22)[4]	**0.014**
LIVCDmi (cm/m^2)	0.71 (0.21)	0.70 (0.22)	0.74 (0.23)	0.78 (0.24)	0.71 (0.22)	**0.028**
LIVCCIi (%)	20.9 (14.1)	19.9 (9.4)	17.7 (10.2)	19.0 (13.7)	20.3 (10.1)	0.683
LIVCCImi (%)	36.6 (14.4)	35.5 (11.2)	33.3 (12.8)	32.8 (14.9)	33.8 (13.1)	0.531

L: long axis; IVC: inferior vena cava diameter; IVCD: IVC corrected for body surface area; IVCCI: IVC collapsibility index.
p values obtained from repeated measures one-factor within subject ANOVA.
Significant difference on Bonferroni post-hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

Figure 7.12. Long-axis IVCe and IVCi response to 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7).
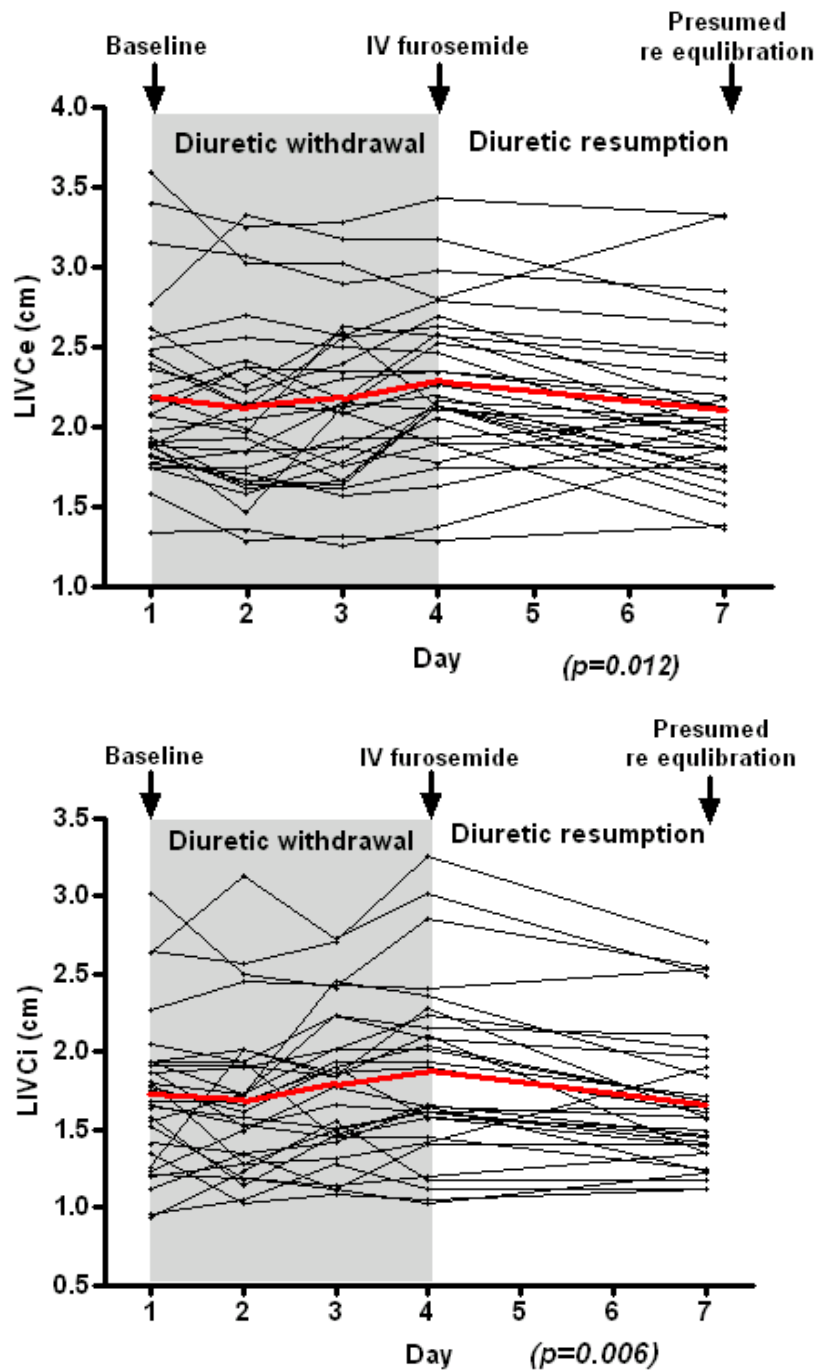
L: long axis; IVC: inferior vena cava diameter; e: expiration; i: inspiration; mi: maximal inspiration.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.
Red line joins mean values.

Similarly, SIVCe, SIVCmi, SIVCDe and SIVCDmi showed statistically signifcant increase from Day 1 to Day 4 and reduced following diuretic resumption (Table 7.12). There was a similar but non-significant trend for SIVCi and SIVCDi. Unlike for LIVCCI indices, both SIVCCI indices showed statistically significant changes during the 7-day protocol. SIVCCmi reduced gradually from baseline to Day 4 following diuretic withdrawal, and increased marginally from Day 4 to Day 7, however, post-hoc analysis did not show any significant difference between these measures. SIVCCmi reduced from baseline to Day 4, but continued to reduce following diuretic resumption.

Table 7.12. Response of short-axis inferior vena cava indices to 7-day diuretic manipulation protocol. Mean (SD).

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
SIVCe (cm)	2.23 (0.46)	2.13 (0.43)[4]	2.20 (0.49)[4]	2.36 (0.45)[2,3,7]	2.17 (0.44)[4]	**0.001**
SIVCi (cm)	1.78 (0.44)	1.80 (0.46)	1.81 (0.46)	1.93 (0.46)	1.81 (0.42)	0.077
SIVCmi (cm)	1.51 (0.40)	1.46 (0.40)[1]	1.48 (0.47)	1.62 (0.44)[1]	1.46 (0.47)	**0.027**
SIVCDe (cm/m^2)	1.15 (0.21)	1.10 (0.19)[4]	1.13 (0.23)[4]	1.21 (0.21)[2,3,7]	1.13 (0.24)[4]	**0.002**
SIVCDi (cm/m^2)	0.92 (0.21)	0.92 (0.22)	0.93 (0.22)	0.99 (0.22)	0.94 (0.24)	0.114
SIVCDmi (cm/m^2)	0.78 (0.19)	0.75 (0.19)[4]	0.76 (0.23)	0.83 (0.20)[2]	0.75 (0.24)	**0.033**
SIVCCIi (%)	20.2 (12.5)	16.4 (7.9)	22.9 (13.7)[7]	18.6 (10.3)	16.1 (11.8)[3]	**0.032**
SIVCCImi(%)	43.6 (21.4)	32.2 (9.5)	41.4 (19.7)	31.7 (11.1)	32.6 (15.0)	**0.001**

S: short axis; IVC: inferior vena cava diameter; IVCD: IVC corrected for body surface area; IVCCI: IVC collapsibility index.
p values obtained from repeated measures one-factor within subject ANOVA.
Significant difference on Bonferroni post-hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

7.4.4.2.3   Response to acute intravenous diuresis following presumed maximal
            volume expansion (0, 1, 2 and 4h measures on Day 4)

The response of IVC indices to ivF on Day 4 is shown in Figure 7.11 as the Day 4 curves for

LIVCe, L IVCi, LIVCmi, LIVCCImi and their corresponding short-axis indices. All LIVC

and SIVC diameters reduced significantly from baseline to the 4h measures, with their

respective LIVCD and SIVCD indices following the same significant trend. However,

maximal percentage change in biomarker levels from 0h was either smaller or marginally

larger than the respective within-subject variance. None of the IVCCI indices changed

significantly following ivF.

7.4.5   Bioimpedance analysis

7.4.5.1   Whole-body BIA

7.4.5.1.1   Normal variance (0, 1, 2 and 4h measures on Day 1)

The components of the variance of whole-body BIA measures are shown in Table 7.13. The

variance of whole body BIA was generally very small.

**Table 7.13. Within-day within-subject total variance (CVt$_{ws}$) and reference change
values (RCV), intra-device variability (CVo) and between-subject variance (CVtb$_s$) of
whole-body bioimpedance measures.**

Biomarker	Baseline	CVt$_{ws}$ (%)	RCV (%)	CVo (%)	CVt$_{bs}$ (%)
dECW (%)	24.3 (2.0)	0.9	2.5	0.2	8.4
dICW (%)	31.8 (3.1)	0.9	2.5	0.4	9.3
dTBW(%)	56.6 (5.5)	1.0	2.8	0.2	9.8
dZ$_{5kHz}$ (Ω)	545 (90)	1.8	5.0	0.5	16.6
dZ$_{50\ kHz}$ (Ω)	482 (84)	1.8	5.0	0.6	17.4
dZ$_{100\ kHz}$ (Ω)	458 (81)	1.8	5.0	0.4	17.6
dZ$_{200kKHz}$ (Ω)	435 (77)	1.9	5.3	0.3	17.6

d: distal; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-200 kHz:
current frequency.

The mean within-day within-subject variance for distal BIA indices is shown in Figure 7.13 as the curves on Day 1. Despite very small increments between all 4 timed measures, there were significant trends towards a reduction in dTBW and dECW from baseline to the 4h measures (p<0.0001 respectively). Similarly, despite small amplitudes of change between the 4 timed measures, all impedance values showed significant trends towards increasing values over the 4h monitoring period (Z_{5kHz}: p<0.0001; Z_{50kHz}: p<0.0001; Z_{100kHz}: p<0.0001; Z_{200kHz}: p=0.001). The dICW normal variance pattern showed a non-significant reducing trend over 4h (p=0.182).

Figure 7.13. Normal variance of whole-body bioimpedance measures on chronic oral diuresis (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).
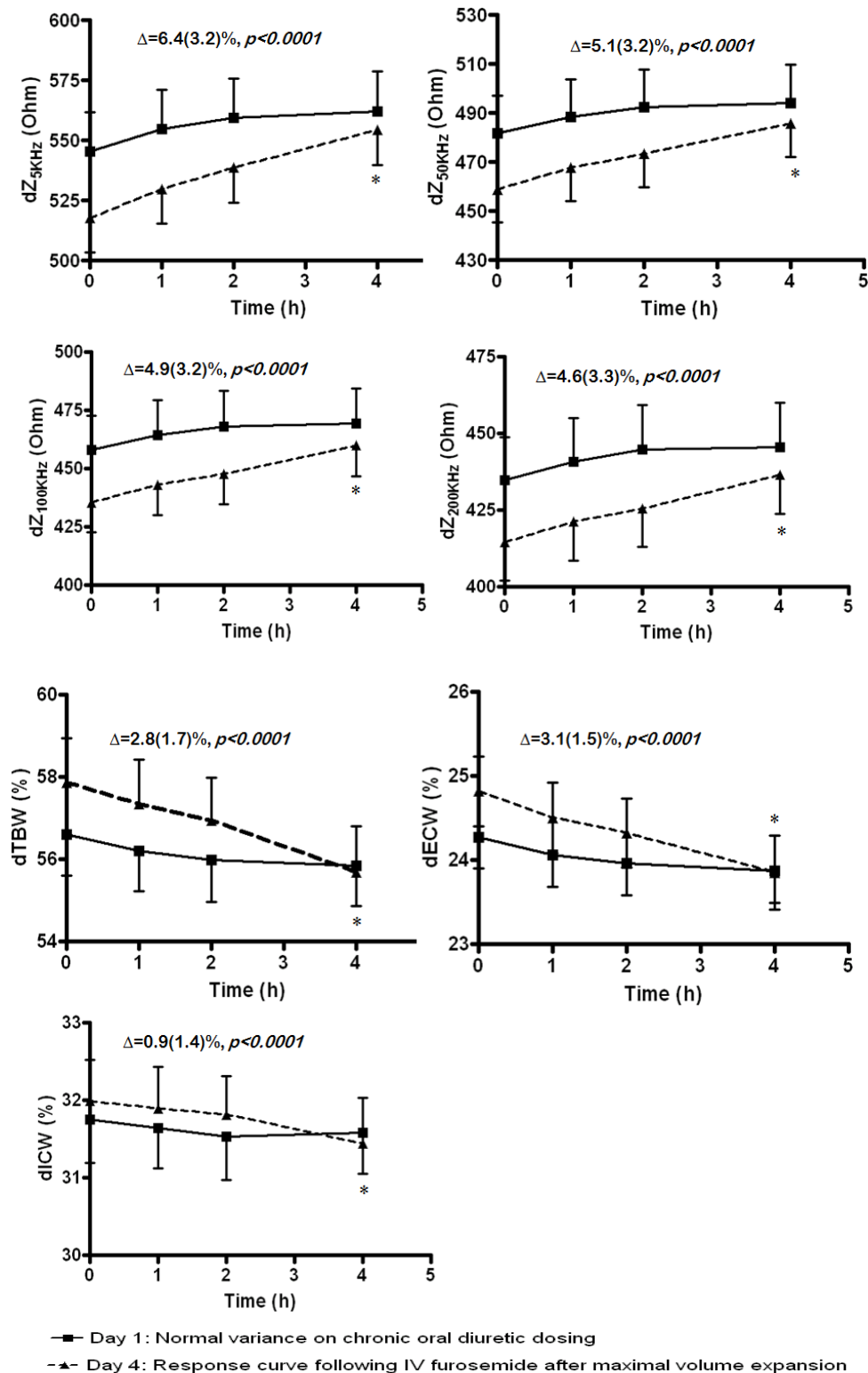
d: distal; Z: impedance; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-200 kHz: current frequency. Mean (SE).
Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4.
Day 4: Δ: maximal percentage change from 0h measure, *:time of maximal change from 0h measure. p value obtained from repeated measures one-factor within subject ANOVA.

7.4.5.1.2   Longitudinal response to diuretic manipulation during 7-day protocol
              (0h measures on Days 1, 2, 3, 4 and 7)

The response of whole-body BIA measures to diuretic withdrawal followed by reinstitution of diuretic treatment is shown in Table 7.14 and for dZ_{5kHz} and dECW in Figure 7.14. Despite all estimates of body water composition showing statistically significant increase from baseline to Day 4 following diuretic withdrawal, and reducing by Day 7, these were by only small increments. All impedance values at all four current frequencies showed statistically significant reduction from baseline to Day 4 and increased following diuretic resumption by Day 7.

Table 7.14. Response of whole-body (distal) BIA measures to 7-day diuretic manipulation protocol. Mean (SD).

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
dECW (%)	$24.3\ (2.1)^{3,4}$	$24.1\ (2.2)^{3,4,7}$	$24.5\ (2.3)^{1,2,4}$	$24.8\ (2.3)^{1,2,3}$	$24.5\ (2.3)^{2}$	**<0.0001**
dICW (%)	$31.7\ (3.1)$	$31.5\ (3.0)^{3,4,7}$	$31.8\ (3.1)^{2}$	$31.9\ (2.9)^{2}$	$31.9\ (2.9)^{2}$	**0.027**
dTBW (%)	$56.4\ (5.6)^{3,4}$	$56.0\ (5.7)^{3,4}$	$56.9\ (5.8)^{1,2,4}$	$57.6\ (6.0)^{1,2,3,7}$	$56.7\ (5.6)^{4}$	**<0.0001**
dZ_{5kHz} (Ω)	$541\ (89)^{2,4}$	$553\ (82)^{1,3,4,7}$	$529\ (77)^{2,4}$	$515\ (77)^{1,2,3,7}$	$531\ (79)^{2,4}$	**<0.0001**
$dZ_{50\ kHz}$ (Ω)	$473\ (79)^{2,4}$	$483\ (76)^{1,3,4,7}$	$463\ (69)^{2,4}$	$451\ (68)^{1,2,3}$	$461\ (64)^{2}$	**<0.0001**
$dZ_{100\ kHz}$ (Ω)	$455\ (80)^{3,4}$	$462\ (76)^{3,4,7}$	$444\ (71)^{1,2,4}$	$434\ (71)^{1,2,3}$	$443\ (71)^{2}$	**<0.0001**
$dZ_{200\ kHz}$ (Ω)	$432\ (76)^{4}$	$439\ (73)^{3,4,7}$	$422\ (68)^{2}$	$413\ (68)^{1,2}$	$421\ (67)^{2}$	**<0.0001**

d: distal; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-200 kHz: current frequency.
p values obtained from repeated measures one-factor within-subject ANOVA.
Significant difference on Bonferroni post-hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

Figure 7.14. Whole-body Z_{5kHz} and ECW response to 7-day diuretic manipulation protocol (0h measures on Days 1, 2, 3, 4 and 7).
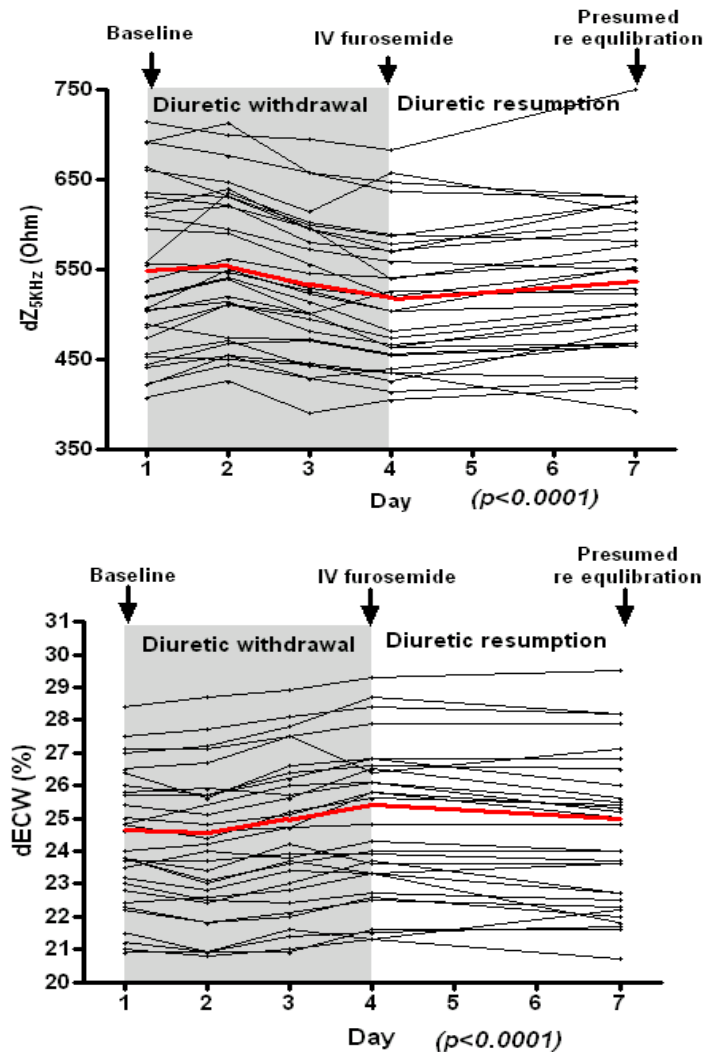
d: distal; ECW: extracellular water; Z: impedance.
Red line joins mean values.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.

7.4.5.1.3   Response to acute intravenous diuresis following presumed maximal volume expansion (0, 1, 2 and 4h measures on Day 4)

The response curves of whole-body BIA measures to ivF on Day 4 are illustrated as the Day 4 curves in Figure 7.1. All impedance curves showed statistically significant increase from 0h to 4h, all estimates of body water composition showed statistically significant decrease after

the administration of ivF. The maximal percentage changes in biomarker values changed above their respective within-subject variance except for dICW.

7.4.5.2 *Proximal BIA measures*

Proximal impedance values were smaller than corresponding distal impedance values, but larger than corresponding right lung impedance values as observed in Chapters Five and Six. Furthermore, estimates of body water percentage were also smaller when measured proximally compared to distally.

7.4.5.2.1 Normal variance (0, 1, 2 and 4h measures on Day 1)

The components of the variance of proximal BIA measures are shown in Table 7.15.

Table 7.15. Within-day within subject total variance (CVt_{ws}) and reference change values (RCV), intra-device variability (CVo) and between-subject variance (CVt_{bs}) of proximal bioimpedance measures.

Biomarker	Baseline	CVt_{ws} (%)	RCV (%)	CVo (%)	CVt_{bs} (%)
pECW (%)	21.9 (2.6)	1.9	5.3	0.4	12.0
pICW (%)	26.7 (3.7)	1.7	4.7	0.5	14.0
pTBW (%)	48.6 (6.2)	1.5	4.2	0.2	12.8
pZ_{5kHz} (Ω)	122 (16)	3.5	9.7	0.7	13.2
$pZ_{50\ kHz}$ (Ω)	107 (15)	3.1	8.6	0.5	13.9
$pZ_{100\ kHz}$ (Ω)	101 (14)	3.3	9.1	0.5	14.1
$pZ_{200\ kHz}$ (Ω)	96 (14)	3.2	8.9	0.5	15.0

p: proximal; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-200 kHz: current frequency.

The mean within-day within-subject variance for proximal BIA indices is shown in Figure 7.15 as the curves on Day 1.There were significant reductions in estimated body water compartments from 0-4h, and significant increases for all impedance values at all four current frequencies (pECW: $p<0.0001$; pICW: $p=0.006$; pTBW: $p<0.0001$; Z_{5kHz}: $p<0.0001$; Z_{50kHz}: $p<0.0001$; Z_{100kHz}: $p<0.0001$; Z_{200kHz}: $p<0.0001$ respectively).

Figure 7.15. Normal variance of proximal BIA measures on chronic oral diuresis (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).

p: proximal; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-200 kHz: current frequency. Mean (SE).

Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4.

Day 4: Δ: maximal percentage change from 0h measure, ∗:time of maximal change from 0h measure. p value obtained from repeated measures one-factor within subject ANOVA.

7.4.5.2.2   Longitudinal response to diuretic manipulation during 7-day protocol
   (0h measures on Days 1, 2, 3, 4 and 7)

The response of proximal BIA measures to diuretic withdrawal followed diuretic resumption

is shown in Table 7.16 and for pZ_{5kHz} and pECW in Figure 7.16. Of the estimated body water

compartments, only pECW showed a statistically significant increase from Day 1 to Day 4

during diuretic withdrawal, with values remaining elevated from baseline on Day 7. All

impedance values showed a statistically significant reduction from Day 1 to Day 4, but did

not change significantly from Day 4 to Day 7 following diuretic resumption.

Table 7.16. Proximal (trunk) BIA responses to 7-day diuretic manipulation. Mean (SD)

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
pECW (%)	22.1 (2.6)[7]	22.0 (2.6)[4,7]	22.2 (2.6)	22.5 (2.8)[2]	22.5 (2.9)[1,2]	**<0.0001**
pICW (%)	26.8 (3.8)	27.0 (3.6)	27.0 (3.4)	26.9 (3.7)	27.0 (3.8)	0.445
pTBW (%)	48.8 (6.3)	49.0 (6.1)	49.3 (5.9)	49.3 (6.3)	49.6 (6.5)	0.071
pZ_{5kHz} (Ω)	121 (15)[4]	121 (12)[3,4,7]	117 (11)[2]	115 (12)[1,2]	117 (13)[2]	**<0.0001**
$pZ_{50\,kHz}$ (Ω)	106 (14)[4]	106 (11)[3,4]	103 (11)[2]	102 (11)[1,2]	103 (12)	**<0.0001**
$pZ_{100\,kHz}$ (Ω)	100 (14)	100 (11)[4]	98 (10)	97 (11)[2]	97 (12)	**0.002**
$pZ_{200\,kHz}$ (Ω)	95 (14)	95 (10)[3,4]	92 (10)[2]	92 (11)[2]	92 (11)	**0.009**

p: proximal; ECW: extracellular water; ICW: intracellular water; TBW: total body water; Z: impedance; 5-
200 kHz: current frequency.
p values obtained from repeated measures one-factor within subject ANOVA
Significant difference on Bonferroni post hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

Figure 7.16. pZ$_{5kHz}$ and pECW response to 7-day diuretic manipulation (0h measures on Days 1, 2, 3, 4 and 7)
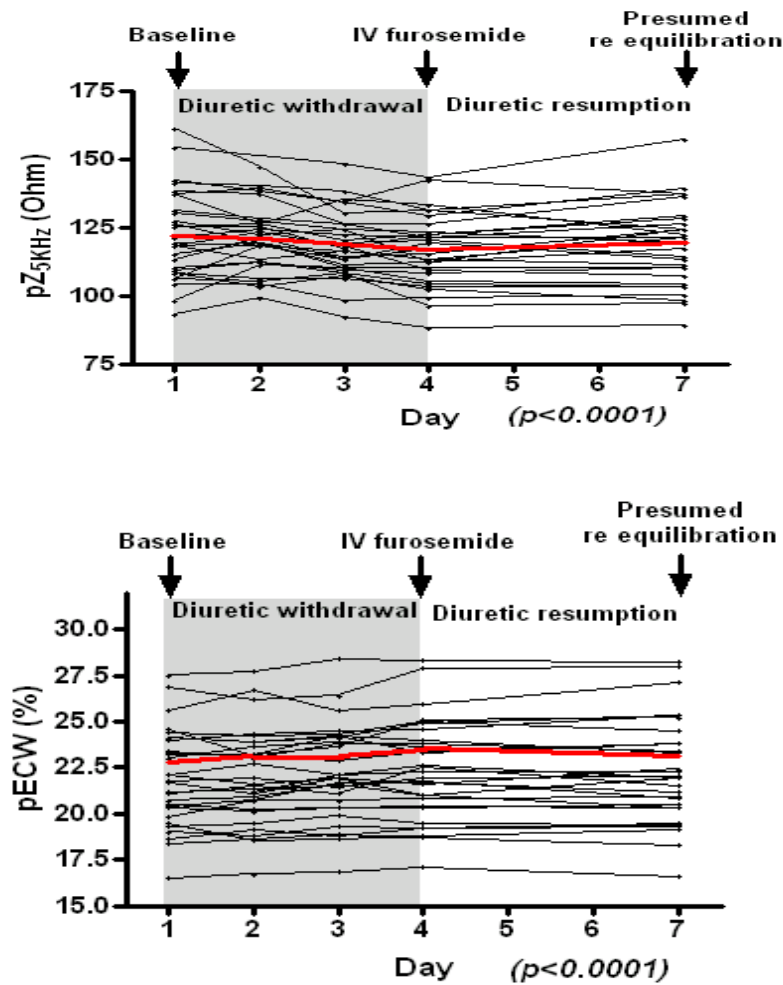
p: proximal; ECW: extracellular water; Z: impedance; 5 kHz: current frequency.
Red line joins mean values.
Baseline: 0h on Day 1; IV furosemide: 50 mg bolus of furosemide given after Day 4 0h measures.

7.4.5.2.3   Response to acute intravenous diuresis following maximal volume
             expansion (0, 1, 2 and 4h measures on Day 4)

The response curves of proximal BIA measures to ivF on Day 4 are illustrated as the Day 4

curves in Figure 7.15. Time points of maximal biomarker change from 0h measures are

highlighted, and maximal percentage change from 0h is shown for each biomarker. All

impedance curves increased significantly from 0-4h, and all estimates of body water

compartments curves decreased significantly over that time. The maximal percentage change in biomarker levels was larger than the respective within-subject variance.

7.4.5.3   *Right-lung BIA*

7.4.5.3.1   Normal variance (0, 1, 2 and 4h measures on Day 1)

The components of the variance of right-lung BIAmeasures are shown in Table 7.17. This was larger than for the corresponding distal and proximal impedance values, but nevertheless still small.
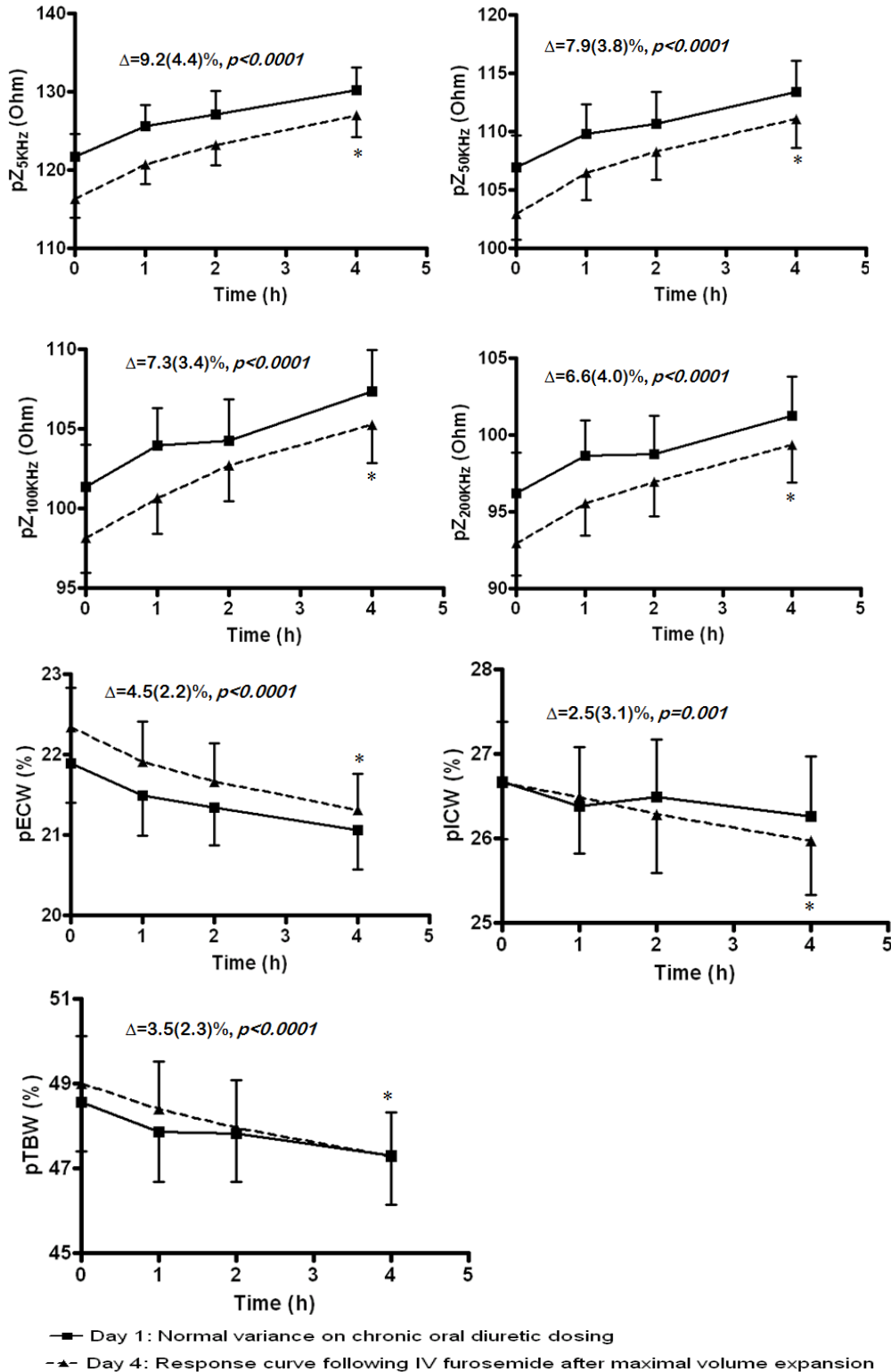
Table 7.17. Within-day within-subject total variance (CVt_{ws}) and reference change values (RCV), intra-device variability (CVo) and between-subject variance (CVt_{bs}) of right-lung bioimpedance measures.

Biomarker	Baseline	CVt_{ws} (%)	RCV (%)	CVo (%)	CVt_{bs} (%)
apZ_{5kHz} (Ω)	53 (9)	4.8	13.3	0.8	16.6
apZ_{50kHz} (Ω)	44 (8)	4.7	13.0	1.0	18.9
apZ_{100kHz} (Ω)	40 (8)	5.0	13.9	1.7	19.9
apZ_{200kHz} (Ω)	37 (8)	5.1	14.1	1.1	21.2
srZ_{5kHz} (Ω)	55 (11)	6.6	18.3	2.6	19.7
srZ_{50kHz} (Ω)	47 (10)	6.8	18.8	2.1	21.7
srZ_{100kHz} (Ω)	44 (10)	6.8	18.8	2.4	23.4
srZ_{200kHz} (Ω)	41 (10)	7.2	19.9	2.9	24.0

ap: anterior-posterior electrode position; sr: sternum-rib electrode position; Z: impedance; 5-200kHz: current frequency.

The mean within-day within-subject variance of right-lung BIA measures derived from both electrode positions is shown in Figure 7.17 as the curves on Day 1. All impedance patterns from both right-lung electrode placements increased significantly from baseline to the 4h measures (all p<0.0001).

Figure 7.17. Normal variance of right lung BIA measures on chronic oral diuresis (Day 1) and response to acute intravenous diuresis following maximal volume expansion (Day 4).
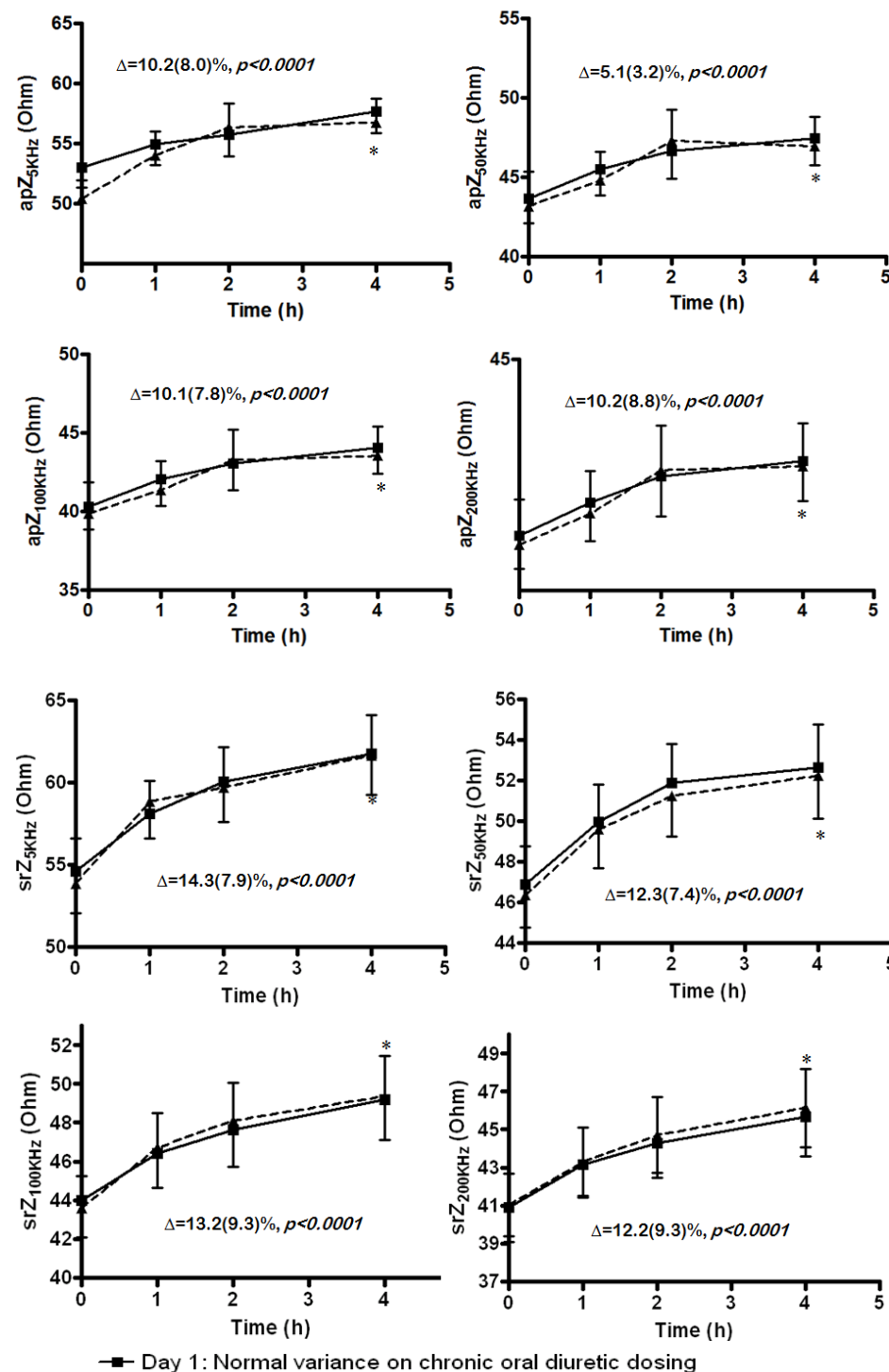
sr: sternum-rib; ap: anterior-posterior electrode positions; Z: impedance; 5-200 kHz: current frequency. Mean (SE). Oral furosemide given following 0h measures on Day 1; ivF given following 0h measures on Day 4. *Day 4*: Δ: maximal percentage change from 0h measure, *:time of maximal change from 0h measure. p value obtained from repeated measures one-factor within subject ANOVA.

7.4.5.3.2   Longitudinal response to diuretic manipulation during 7-day protocol
               (0h measures Days 1, 2, 3, 4 and 7)

The response of right-lung BIA measures to diuretic withdrawal followed by reinstitution of diuretic treatment is shown in Table 7.18. Only apZ_{5kHz} changed statistically significantly, reducing from baseline to Day 4 before increasing again to baseline values following diuretic resumption (p=0.017). However, post-hoc analysis did not reveal significant differences between any time points.

Table 7.18. Right-lung BIA responses to 7-day diuretic manipulation. Mean (SD).

Variable	Baseline Day 1(0h)	Diuretic withdrawal			Diuretic resumption Day 7	p
		Day 2	Day 3	Day 4(0h)		
apZ_{5kHz} (Ω)	53 (9)	54 (10)	53 (9)	50 (8)	54 (11)	**0.017**
apZ_{50kHz} (Ω)	43 (8)	44 (10)	44 (9)	43 (12)	44 (10)	0.579
apZ_{100kHz} (Ω)	40 (8)	41 (10)	40 (9)	39 (11)	41(10)	0.517
apZ_{200kHz} (Ω)	37 (8)	38 (10)	37 (9)	37 (11)	38 (10)	0.558
srZ_{5kHz} (Ω)	54 (11)	58 (12)[2]	56 (12)	54 (10)[2]	55 (11)	0.072
srZ_{50kHz} (Ω)	47 (10)	48 (10)	48 (13)	46 (9)	47 (11)	0.497
srZ_{100kHz} (Ω)	44 (11)	45 (10)	43 (8)	43 (9)	43 (10)	0.267
srZ_{200kHz} (Ω)	41 (10)	42 (10)	42 (14)	41 (9)	40 (10)	0.809

ap: anterior-posterior electrode placement; sr: sternum-rib electrode placement; Z: impedance; 5-200 kHz: current frequency.
p values obtained from repeated measures one-factor within-subject ANOVA.
Significant difference on Bonferroni post-hoc analysis between:
[1]: Day 1 and other day   [2]: Day 2 and other day   [3]: Day 3 and other day
[4]: Day 4 and other day   [7]: Day 7 and other day

7.4.5.3.3   Response to acute intravenous diuresis following maximal volume
            expansion (0, 1, 2 and 4h measures on Day 4)

The response curves of right-lung impedance measures to ivF on Day 4 are illustrated as the

Day 4 curves in Figure 7.17. Time points of maximal biomarker change from 0h measures are

highlighted, and maximal percentage change from 0h is shown for each biomarker. All

impedance curves showed statistically significant increase from 0-4h.The maximal percentage

change in biomarker levels from the 0h measures all occurred above the respective within-

subject normal variance.

7.4.6   Urine volumes and oral intake on Day 1 and Day 4 (0, 4 and 8h measures)

Table 7.19 illustrates the urine volumes and oral intake during the 8h monitoring periods on

Day 1 and Day 4. Patients were allowed comparable amounts of fluid as they would normally

drink, and oral intake was no different on both days. There was a significantly larger total

urine volume on Day 4 compared to Day 1, secondary to the significantly larger diuresis at the

4h measures following ivF.

Table 7.19. Urine output and oral intake during 8h monitoring on Day 1 and Day 4.

Urine volume (mL)	Day 1	Day 4	p value
4 h	878 (416)	1461 (577)	<0.0001
8 h	215 (128)	242 (141)	0.395
Total	1093 (465)	1703 (607)	<0.0001
Oral intake (mL)	653 (264)	742 (347)	0.145

Comparison using t-test.

7.5 Discussion

7.5.1 Normal variance (Day 1 8h monitoring)

7.5.1.1 Blood biomarkers

Of the panel of blood biomarkers studied, normal variance in LVSD has only been established for ANP and BNP so far [Bruins et al., 2004; Wu et al., 2003; McDowell et al., 2002; Araújo et al. 2006]. Consequently, this is the first study to establish the within-subject and between-subject normal variance of Hct, cTnI and IMA in stable LVSD patients. I observed the smallest within-subject variance for Hct, and the largest within-subject variance for cTnI. Furthermore, the within-subject variance of Hct and ANP was similar to that observed in my normal volunteers cohort (Chapter Six), the within-subject variance of IMA and cTnI was larger compared to that of normal volunteers [Wu et al., 2009; Govender et al., 2008], and the within-subject variance of BNP was lower compared to normal volunteers. Overall, the between-subject variance for cardiovascular blood biomarkers appeared much larger in LVSD compared to normal volunteers.

The within-subject total variance of ANP in my cohort of LVSD patients (10.2 %) was in contrast to that observed in the HF cohort in the study by McDowell et al. [2002] of 51 %, but similar to that observed in my cohort of normal volunteers (9.9 %). As discussed in Chapter Six, this discrepancy may be due to the high variability of ANP levels in the morning and the much higher frequency of measurements made by McDowell et al. who measured ANP levels every 2 minutes over 90 minutes in the morning. The findings from my study are more clinically relevant as routine tests in patients will potentially take place at any time of the day.

The observed initial significant drop in Hct levels and increase in ANP levels from the baseline measure showed a similar but more blunted trend compared to that observed in the normal variance curves for the normal volunteers. The fact that Hct levels fall as ANP levels rise again suggests that blood volume is modulated by changes in ANP, resulting in the subsequent changes in Hct levels. The blunted trend may be secondary to reduced capacity for large increases in ANP secretion in view of the already increased levels in all patients in the LVSD cohort [Kjaer et al., 2005].

For BNP, I demonstrated comparable within-day within-subject variance (16.9%) to the studies by Bruins et al. [2004] and O'Hanlon et al. [2007], where the within-subject variance obtained from 2-hourly and one-hourly measures respectively in LVSD ranged between 12 to 15%. The reduced within-subject variance observed for BNP compared to that observed in normal volunteers (44 %) has previously been reported by Wu et al., 2003 and may be secondary to the reduced capacity of myocytes to further release BNP from an already elevated baseline.

Only one study so far has established the normal variance of IMA in healthy individuals [Govender et al., 2008]. IMA levels were measured weekly over a 5-week period.   Between-week within-subject variance was 3% and between-subject variance was 7%. Using a high-sensitivity assay, Wu et al. [2009] reported on the normal variance of cTnI in normal volunteers where blood was taken hourly over a 4h period and on alternate weeks over an 8-week period. Within-day within-subject variance was 9.7% and between-subject variance was 57%. Between-week within-subject variance was 14% and between-subject variance was 63%. The increased within-subject variance observed in my LVSD cohort for both these

biomarkers may be due to the ongoing process of myocardial remodelling or other pathophysiological processes involved in LVSD.

The increased between-subject variance observed in this cohort of LVSD patients for all blood biomarkers compared to normal volunteers could be explained by the presence of differing degrees of haemodilution or haemoconcentrition secondary to diuretic therapy, differing baseline volume status and neurohormomal activation, or concurrent illness.

7.5.1.2   Biomarkers of renal tubular function

The use of renal tubular markers at predicting acute kidney injury is still at a pre-clinical stage. As is often the case, the normal variance of biomarkers and how this impacts on their routine clinical application, tends to be studied after clinical utility has been established for the biomarker, such that normal variance for the renal tubular markers studied in this thesis is as yet unknown. I observed for the first time, very high within-subject variance (33-89%) and between-subject variance (37-235%) for all urinary renal tubular markers in stable LVSD patients on optimal medical treatment, perhaps reflecting their sensitivity to chronic oral furosemide. While elevated uNGAL levels have previously been documented in LVSD [Damman et al., 2007], the median uNGAL at baseline in my cohort was within normal limits with only 3 patients having elevated baseline levels. uNGAL for both studies were collected and analysed in a similar way, the analyses being performed in the same location by the same team in Groningen, such that significant analytical variability is unlikely. Despite similar LVEF and use of RAAS blockade, Damman's population of LVSD patients were younger [59 (11) years], had more severe HF symptoms (93% had NYHA class III/IV) and only 70% were

on diuretics. This suggests that uNGAL levels may only be elevated in patients with more severe HF who are possibly undertreated. The fact that both uKIM-1 and uNAG levels were elevated at baseline in the majority of my patients (these values being observed for the first time in a cohort of stable LVSD patients) suggests that levels of these latter biomarkers, contrary to uNGAL, may be more likely to be elevated in lesser severities of HF.

7.5.1.3 Echocardiographic measures

The within-subject and between-subject variance of the panel of echocardiographic variables studied in this thesis has not been established to date, although Schirmer et al. [2000] determined population reference values corrected for gender and age for MVE, MVA, MVE/A, MVDT. The variance of echocardiograpic variables was moderate and comparable to that of some blood biomarkers, with the intra-observer variability being an important contributor to total variance. The variance of IVC indices was least when measured in expiration and most when measured in maximal inspiration, likely due to easier measurement of the vessel at its maximal diameter. This suggests that IVC measured in expiration should be used preferentially to the other IVC indices. This is further supported by the observation that the bias in the measurement of IVC diameters was smallest for IVCe (-0.05 cm).

7.5.1.4 Bioimpedance measures

BIA measures had the smallest variance of the panel of biomarkers. This variance was smallest for whole-body measures and highest for right-lung measures, consistent with the findings from the normal volunteers study in Chapter Six. and with previous studies [Steiner

et al., 2002; Ng et al., 1993; Verhoeve et al., 1998; Jewkes et al., 1991]. Thus, in clinical practice, it would be preferable to measure whole-body BIA in preference to BIA across smaller body segments.

7.5.2 **Longitudinal response to diuretic manipulation (0h measures on Days 1, 2, 3, 4 and 7)**

7.5.2.1 *Visual analogue score and body weight*

Visual analogue scores are an accurate means of measuring patients' perceived general health and feeling of well being in a number of chronic conditions [Sneed et al., 2001; Cowley et al., 1986]. The use of a non-graduated visual analogue score constituted a simple means of determining whether despite an asymptomatic increase in volume status as evidenced by no change reported on direct questionning regarding dyspnoea, ankle oedema, tiredness, etc., there might be a subconscious deterioration in my volunteers' general feeling of well being. Interestingly, visual analogue scores reduced non-significantly from Day 1 to Day 6, and increased from Day 6 to Day 7, perhaps reflecting the patients' anxiety during the diuretic manipulation protocol due to the potential risks of decompensation and the psychological and emotional stress of attending the study centre for timed measures. Patients may have felt relieved on the last day of the protocol when they were reassured that they had remained stable during the protocol. Nevertheless, the non-significant change observed implies that the changes in volume status induced during the protocol were indeed subclinical.

The non-significant changes in body weight during this study are not surprising. Although body weight has been shown to increase up to 7-11 days prior to the onset of decompensation [Chaudhry et al., 2007], and the absence of an increase in body weight (2Kg or 2% increase in

body weight over 48-72 h) has high specificity for excluding clinical deterioration, weight gain has poor sensitivity at predicting clinical deterioration. This is because weight gain can also occur as a result of increases in non-fluid body mass, but also because fluid retention may occur without a significant increase in body weight due to the simultaneous loss of lean mass secondary to cardiac cachexia. Furthermore, decompenstation can result from volume redistribution rather than volume overload. It is also possible that the magnitude of volume changes in my LVSD patients was too small to be reflected in significant changes in body weight. The mean weight increased by a maximum of 1.6 (0.49) Kg which was achieved on Day 4. This is lower than the increase in weight observed in Braunschweig's study [2002], which ranged from 2.5-4.0 Kg, which was associated with increased intracardiac pressures.

7.5.2.2   *Blood biomarkers*

Of the blood biomarkers studied, only BNP levels seemed to track presumed volume changes with some accuracy in response to diuretic manipulation over the 7-day protocol, with levels increasing significantly to peak at presumed maximal volume expansion on Day 4, and reducing again to baseline value on Day 7. However, the percentage increase in BNP levels from Day 1 to Day 4 was 41 %, which is above its within-subject normal variance (17 %), but lower than its within-subject RCV (47 %). The lack of significant change from within-subject variance and RCV may also explain why Lewin et al. [2005] failed to show the utility of *serial* BNP monitoring for predicting clinical deterioration in a group of stable LVSD patients on an outpatient basis. They measured BNP at recruitment into their study, and on decompensation, and used the difference between these values to determine the optimal cut-off value for their receiver operating curve, only achieving a sensitivity and specificity of 47%

and 77% respectively for a BNP increase of >100 pg/mL. Baseline BNP levels were much higher in their cohort of patients that decompensated clinically [781 (898) pg/mL], such that an increment of 100 pg/mL does not exceed the within-subject normal variance of BNP. Thus, depite previous studies suggesting that BNP may reflect medium to long-term haemodynamic changes [Disomma et al., 2008; Paterna et al., 2005; Metra et al., 2007], my findings suggest that the relatively small amplitude of change in BNP levels may not be sensitive enough to track the subclinical volume changes induced over the 7-day protocol, particularly when considered in the context of LVSD patients with higher baseline BNP levels.

ANP levels, like BNP levels, increased following diuretic withdrawal, however, they continued to increase following diuretic resumption. Similar trends were observed in the Hct and IMA response curves, whereby the Hct levels continued to reduce, and the IMA levels continued to rise following diuretic resumption potentially suggesting that plasma volume continued to increase following resumption of diuretic treatment [Yamasaki et al., 1998; Lobo et al., 2010]. While this may a priori seem unlikely due to the significant diuresis observed on Day 4 after the bolus dose of ivF, and the subsequent resumption of oral diuretic, an increase in intravascular volume may have resulted from redistribution of volume from the interstitial space, with a net overall reduction in ECW. The release of ANP in response to atrial and ventricular stretch is quicker than the release of BNP in response to ventricular stretch in LVSD following different volume loading protocols [Gabrielsen et al., 2000; Larsen et al., 2006]. However, the secretion of ANP and BNP in response to volume depletion may be inherently different, with the ANP response being slower in this instance; this has not been previously studied.

While the increase in IMA following diuretic withdrawal supports my hypothesis that some degree of ischaemic stress is induced by volume expansion/furosemide withdrawal, possibly secondary to the remodelling process, the reason for the continuing increase following diuretic resumption is unclear. It might suggest ongoing subclinical cardiac ischaemia as part of ongoing LV remodelling despite resumption of diuretics, but could also be linked to the same volume modulating mechanisms involved in the ANP and Hct response. IMA levels do correlate with echocardiographic estimates of LV filling pressure (E/Ea) in stable end stage renal failure [Sharma et al., 2005], and correlate inversely to LVEF in LVSD [Franceschi et al., 2009], however, they have not been linked to invasive haemodynamic measures. The nature of the response of IMA thus needs to be clarified in further studies. cTnI as expected did not change significantly with presumed volume changes, reflecting understandably its poor sensitivity to small volume changes.

7.5.2.3 *Renal tubular biomarkers*

The effects of diuretic manipulation on markers of renal tubular function were studied as they are more sensitive than conventional measures of renal function such as sCreatinine; their levels increase within hours of acute kidney injury [Vaidya et al., 2008]. Withdrawal of diuretics resulted in a significant increase in uCreatinine, and reinitiation of diuretic resulted in return of uCreatinine to baseline values. The percentage increase from baseline on Day 4 was 106 %, which is above the within-subject total variance of uCreatine (58 %), but less that its within-subject RCV (160 %). Thus, despite this very large increment in uCreatinine levels, uCreatinine may be limited as a potential marker of volume change, in the same respect as BNP. The statistically significant increase of uKIM-1 and uNAG corrected for urine

concentration during diuretic withdrawal followed by return to baseline values of the biomarkers after re-initiation of oral diuretics was relatively smaller compared to the changes seen in uCreatinine, with the least change observed for uNAG. Furthermore, the maximal percentage changes in both these biomarkers during diuretic manipulation occurred over their respective within-subject variance, but much lower than their respective within-subject RCV. This will limit their potential clinical application for volume status monitoring.

However, the observed response of these renal biomarkers to diuretic manipulation was of considerable interest from a pathophysiological point of view. The response of uKIM-1 and uNAG, which are mostly markers of hypoxic renal tubular injury, suggest that contrary to the hypothesis that furosemide may contribute to renal impairment in LVSD as a result of dehydration, it may in fact, be beneficial to renal tubular function. This would potentially explain why the addition of hydorcholorothiazide to angiotensin-receptor blocker treatment resulted in more pronounced reduction in both u KIM-1 and uNAG concentrations in normal volunteers in a study by Waanders et al. [2009].

A number of potential mechanisms may explain these observed changes in renal tubular markers following diuretic manipulation. In LVSD, reduced renal blood flow is the main determinant of renal function [Damman et al., 2007], however, venous congestion also plays a role in worsening renal function, especially in the presence of already reduced renal perfusion [Mullens et al., 2009; Damman et al., 2009]. Diuretics may thus have a protective renal effect by their effects on volume modulation, whether by reducing venous congestion and/or improving renal blood flow. Unfortunately, I did not have surrogate measures of renal blood flow in my panel of non-invasive biomarkers to shed more light on the potential mechanisms

of these changes. While an inverse correlation has been observed between right atrial pressure and renal blood flow in LVSD [Kos et al., 1998], the effect of blood volume on renal blood flow in LVSD has not been documented.

Furthermore, the linkage of diuretics to deleterious renal effects and worsening of outcome [Dries et al., 2000; Domanski et al., 2006] and the association of increasing doses of diuretics to worsening renal function [Eshaghian et al., 2006], may merely represent the fact that higher risk patients with worse renal function require higher doses of loop diuretics to treat abnormal volume status rather than representing adverse treatment effect of loop diuretics through dehydration and neurohormonal activation . The diuretic response to furosemide depends on its urinary concentration (mainly by tubular secretion), the time of delivery to its site of action in the ascending loop of Henle (dependent on cardiac output and renal blood flow) and the dynamics of the response at the loop of Henle (determined by RAAS activation) [Ho et al., 2010]. Thus, in LVSD patients with reduced renal function, a combination of reduced proximal tubular secretion due to underlying renal dysfunction, reduced furosemide delivery due to reduced renal blood flow and increased neurohormonal activation my all contribute to the need for higher doses of furosemide to achieve adequate diuresis.

However, the apparent beneficial renal effects of diuretic withdrawal may occur independently/in conjuction with other mechanisms not involving volume modulation, such that the observed changes in uCreatinine, uKIM-1 and uNAG may have been secondary to the direct renal effects of furosemide therapy.Oxygen tensions in the medulla are comparatively low, with only 10% of renal blood flow going to the inner medulla. The high metabolic demands of the loop of Henle and relatively low Hct levels in the medulla mean that the

medulla is more prone to ischaemia with reduced renal blood flow such as in LVSD [Evans et al., 2008]. Furosemide may thus improve medullary oxygen tensions by reducing the Na-K-Cl_2 activity and by increasing prostaglandins [Gerber et al., 1980].

The lack of response of sCreatinine to diuretic manipulation over 7 days is not surprising as its changes in response to renal injury lag behind changes in markers of renal tubular function by at least 12h (Mishra et al., 2005). The reasons for the lack of response of sNGAL and uNGAL however, are unclear. NGAL levels are known to be elevated in a number of pathophysiological conditions other than simply tubular dysfunction [Schmidt-Ott et al., 2007]. Furthermore, while both uKIM-1 and uNAG are markers that represent proximal tubular injury, uNGAL levels are more dependent of production of NGAL in the distal tubule following renal injury, with only a small contribution to urinary levels from sNGAL filtered through the glomerulus [Schmidt-Ott et al., 2007]. Thus, changes in diuretic treatment may primarily affect proximal tubular function as opposed to the distal tubule.

7.5.2.4 *Echocardiographic variables*

7.5.2.4.1 Mitral Doppler indices

Despite significant statistical changes in MVE, vMVE and MVE/A in response to diuretic manipulation, the amplitude of the changes their measures were very small, with the maximal percentage changes observed being lower than their respective within-subject variance, let alone their respective RCV. Thus, unlike their response in the study in normal volunteers, where MVE and vMVE levels changed above their respective within-subject variance in

response to IV Saline, they do not appear to be sensitive markers of the smaller changes in volume status in the stable LVSD cohort.

I also observed no significant change in E/Ea indices over the 7-day protocol. As previously discussed, E/Ea indices have varying correlations to mean PCWP in a wide variety of cardiac conditions [Mansencal et al., 2004; Mansencal et al., 2007; Nagueh et al., 1997]; good correlation with spot haemodynamic measures clearly not translating into good performance of these biomarkers in tracking volume changes. Furthermore, E/Ea indices are less reliable at predicting LV filling pressure in the presence of basal regional motion wall abnormalities, especially at the septum [Lim et al., 2009]. Two-thirds of my cohort of LVSD patients had ischaemic cardiomyopathy, thus with a large likelihood of having significant regional wall abnormalities. In addition, Mullens et al. [2009] showed no correlation between E/Ea indices and PCWP in a group of advanced decompensated LVSD patients with similar mean LVEF [28 (8) %] to that in my cohort of LVSD patients. Importantly however, some of the patients in their study had biventricular pacemakers and/or significant mitral regurgitation which may have confounded their findings.

7.5.2.4.2   Inferior vena cava indices

Despite statistically significant changes in some LIVC and SIVC indices with increase in values following diuretic withdrawal and decrease in values following diuretic resumption, post-hoc analysis showed little difference between individual time points, and the amplitude of the changes in measures was very small. All maximal percentage changes occurred below the within-subject total variance for each respective biomarker.This may be explained by the

fact that a large proportion (>50 %) of the patients had IVC diameters >2cm at baseline, thus limiting the amount of dilatation in response to volume expansion. In agreement with the study by Brennan et al. [2007], indexing the indices for body surface area did not improve their sensitivity. Furthermore, the changes in the IVCCI indices were inconsistent with biologically significant change, for example, SIVCCI indices reduced consistently during diuretic manipulation.

7.5.2.5   *Bioimpedance analysis*

Similarly to the echocardiographic variables, none of the BIA measures appeared to have potential clinical application for the monitoring of volume status in the setting of this study. Despite all body water compartments increasing following diuretic withdrawal and reducing following diuretic resumption, statistically significant changes were only seen with whole-body TBW, ECW and ICW, and trunk ECW. The amplitude of these changes however, similar to that of all impedance values, was very small despite the maximal percentage change occurring over and above the within-subject total variance as well as the within-subject RCV for all these measures.

7.5.3   Response to acute intravenous diuresis following presumed maximal volume expansion (Day 4 8h monitoring)

Patients were studied on Day 4 after their 0h measures in addition over 8h mainly to ensure there were no complications following a bolus dose of ivF. Biomarker responses were documented over this time period in response to acute intravenous diuresis. This response could not be directly compared to the normal variance pattern of the biomarkers on Day 1 for

reasons outlined earlier in this Chapter. A significant diuresis occurred as a result of ivF administration, as evidenced by a significantly larger urine volume at 4h compared to Day 1 [(1461 (577)mL versus 878 (416)mL, $p<0.0001$], suggesting significant volume depletion in response to ivF.

All the biomarkers studied were inadequate for mapping this level of change in volume status. Overall, their levels did not change significantly from the 0h measures, and their maximal percentage change from 0h was inferior to their within-day within-subject variance determined on Day 1. The mitral Doppler indices, IVC indices, and urine biomarkers of tubular function showed statistically significant change from the 0h measures, however, their maximal percentage change were in most cases, inferior to both their within-day within-subject variance and RCV. The BIA measures showed statistically significant change following ivF and their maximal percentage change was superior to their corresponding within-subject variance, and in some cases, their RCV as well. However, the relative changes were too small to suggest that these biomarkers would be suitable for monitoring of acute volume changes in this cohort.

7.6   Limitations

There are a number of limitations to this section of my study. Patients were not standardised in terms of their salt and water intake during the study protocol, such that their baseline hydration states may have varied considerably. While the significant changes in uCreatinine and non-significant trends of body weight change over the diuretic manipulation protocol suggest subclinical increase in volume status during diuretic withdrawal and reduction in

volume status during diuretic reinitiation, it is difficult to gauge the amplitude of this volume change, and thus to determine whether the observed lack of sensitivity of the biomarkers was genuine or whether the volume stimuli were too small. However, the volume stimuli used in this study are very realistic of a routine clinical setting; diuretic manipulation and/or non-adherence is a real clinical phenomenon.The lack of gold standard measures of volume status thus limits the ability of this study to make any definite conclusions regarding the sensitivity of the biomarkers studied at tracking volume changes. The use of blood volume analysis, haemodynamic measures, as well as isotopic determination of renal blood, would have also enabled the determination of the exact changes occurring in specific volume compartments, and would have clarified, for example, the ANP, Hct and IMA responses, as well as the reason for the changes in renal tubular markers.Unfortunately, the use of central lines or pulmonary artery catheters was not feasible, nor was the use of radioactive material to perform blood volume analysis within the setting of the study.

In the absence of gold standard measures of volume status, surrogate measures could have been used. However, the equation used for estimation of blood volume in the normal volunteers study was only validated in normal volunteers, and cannot be applied to LVSD patients undergoing dynamic fluid shifts and who were on concomitant vasoactive medication. Blood pressure and heart rate were also not measured. Furthermore, the longitudinal response of biomarkers to diuretic manipulation over the 7-day protocol could have been compared directly to the within-subject normal variance of respective biomarkers on their usual medication over a similar period in a similar way to the normal volunteers study. This would enable further insight into the sensitivities of each bioamarker, with the most sensitive deviating the most from their normal variance. However, establishing this

normal variance pattern over 7 days would involve doubling the study time, which was not practically feasible in the frailer LVSD cohort.

The significant changes seen in uKIM-1 and uNAG corrected for urinary concentration in response to diuretic manipulation may in addition have been due to changes in uCreatinine. Normalising the renal biomarker levels to uCreatinine should in theory correct for differences in urinary flow rate as a result of diuretic manipulation, on the assumption that uCreatinine is constant. However, the rate of creatinine excretion by the kidneys may also have differed in response to diuretic manipulation as a result of changes in GFR. This possible effect on uCreatinine concentration is likely to have been small. However, timed urine collections to allow the calculation of both uCreatinine concentration and renal biomarker levels over 24 hours may have been more appropriate [Waikar SS et al., 2010]. Unfortunately, this was not feasible within the study setting.

A large number of statistically significant changes in biomarker levels was observed, some of which may have resulted from the large number of statistical comparisons made rather than due to physiologically significant changes in response to the volume manipulation protocols used. This has been recognised, such that despite this observation, I concluded that none of the biomarkers studied appeared to have potential clinical utility for monitoring volume changes in this study setting. However, the aim of this study was to identify the most potentially useful markers of volume change, such that it was important to study a large number of markers in order to optimise the interventions applied in this population of LVSD patients.

7.7   Summary

In this study, I demonstrated, in some cases for the first time, the normal variance of blood and urine biomarkers and physical biomeasures in stable LVSD patients on optimised medical therapy. The largest variance was observed for the urine renal tubular markers and smallest variance for BIA measures. None of the biomarkers studied appeared to have potential clinical utility for the monitoring of the subtle changes in volume status induced in this cohort of patients, whether over the medium term or acutely in response to ivF. Their utility in this context seems to be limited in some instances by large within-subject variance, or by very small incremental changes in response to the volume changes induced. I also demonstrated, for the first time, an increase in some renal tubular markers as a result of diuretic withdrawal, suggesting potential renal benefit of diuretic treatment.

Chapter Eight

Summary, Suggestions for Future Studies and Conclusion

8.1   Summary

The main aim of this thesis was to establish the feasibility and potential clinical utility of repeated measures assessment of non-invasive biomarkers in tracking change in volume status. This was particularly in the context of patients with LVSD, where abnormal volume homeostasis prevails, and abnormal volume status, whether present overtly or subclinically, is linked to symptoms and poor prognosis. The overall clinical goal driving this programme of work is the need to prevent acute decompensation, and for different LVSD therapies to be optimised on an individual basis, tailored to the baseline volume status of each individual, thus achieving an equilibrium between optimal symptom control and volume status with minimal detrimental neurohormonal activation and detrimental effects on the kidneys.

Extensive review of the literature identified potential non-invasive means of volume assessment. A panel of these non-invasive biomarkers was selected on the basis of their availability and use in current clinical practice, their ease of applicability and reproducibility by a variety of health care professionals and patients alike, as opposed to biomarkers which were still at an experimental stage. I also explored interventions which would result in volume changes in a number of conditions, and applied these to different volunteer groups in order to test the comparative performance of the panel of blood biomarkers and physical biomeasures to volume changes. Literature review also revealed that, despite the wide clinical and investigational application of some of these biomarkers to guide diagnosis, prognosis, and in some cases to guide treatment in LVSD, there was little or no documentation of their normal variance. In cases where the normal variance was established, measured values of biomarkers were not always interpreted in the context of this variance, and in some studies, variance

determined in one group of volunteers (e.g., normal volunteers) was applied to interpret measured biomarker values in a different group of volunteers (e.g., stable cardiac patients).

I observed smallest variance for BIA measures, moderate variance for echocardiographic variables, and largest variance for urinary renal tubular markers. Variance for blood biomarkers was small (Hct and IMA), moderate (ANP and BNP) or large (cTnI). I demonstrated that measurement variance in the form of intra-observer variability, was the main contributor to the total variance of echocardiographic measures. This measurement variance could be reduced by devising a systematic way to perform the measurements and with practice and increased familiarity with the set pattern of performing the measurements. This has implications for the training of staff employed to perform physical biomeasures, in particular echocardiographic measures, for monitoring purposes. I demonstrated reduced within-subject variance for some biomarkers in LVSD patients compared to normal volunteers (BNP), and increased within-subject variance in LVSD compared to normal volunteers for others (IMA, cTnI, echocardiographic and BIA variables). Overall, between-subject variance was larger in LVSD compared to normal volunteers for all biomarkers. These observations have implications for the interpretation of these biomarkers, whose use in clinical practice is already widely established. Thus, clinicians need to be aware of these issues when using biomarker levels to diagnose disease in unselected groups of patients or in monitoring disease progression or response to treatment in select groups of patients.

Establishing the within-subject variance of biomarkers was crucial for the interpretation of the sensitivity of biomarker responses to volume manipulations within this programme of work. However, the clinical application of this concept is not well-tested. Clearly, a random

measurement of a biomarker in a clinically stable patient which falls outside population

reference values but within the within-subject normal variance for that individual should be

reassuring. However, it is unclear whether every biomarker measure that falls outside this

within-subject variance indicates clinical instability, and how much deviation from the within-

subject variance would be clinically significant. The clinical applicability of RCV appears

limited in that respect, these being merely laboratory-generated percentages suggesting

statistically significant differences between serial measures in an attempt to aid clinical

decision-making. It is however, unclear whether they actually predict

decompensation/worsening of disease in practice.

Furthermore, I observed that within-subject or between-subject variance tends to increase the

longer the time frame over which it is measured. In addition, the within-subject variance

increases the more times it is measured over a specific period of time. Assuming clinical

practice evolved to incorporate the widespread use of within-subject normal variance for the

interpretation of biomarker levels within an individual patient, it is unclear how this within-

subject normal variance would be calculated in practice. How many measures of the

biomarker would be needed and over how long a period of time they should be collected is

not known, in addition to how often the biomarker level would need to be repeated to enable

satisfactory monitoring of disease status. These factors will undoubtedly be limited by the

resources available. The impact on the patient of increased frequency of measurents also

needs to be carefully assessed.

The key to applying repeated measures of biomarkers to predict clinical decompensation

could lie in the *persistent or sustained* deviation of biomarker measures from their within-

subject normal variance on repeated measurements. Studies do show that persistently elevated body weight, invasively-measured haemodynamic variables, or fluid index measured from implantable devices in clinically stable patients precede decompensation. It is as yet unclear whether intensification of treatment at the point of this *asymptomatic sustained deviation* from normal variance results in better outcomes.

The panel of biomarkers studied in the three experiments showed, in general, little sensitivity at tracking the volume changes induced by the interventions used, except for whole-body BIA and trunk impedance in the normal volunteers in response to IV Saline. While this may be partly due to a failure of the protocols to induce large enough overall amplitudes of change in volume status, especially in the heterogeneous group of patients with decompensated heart failure, and to some extent in the patients with stable LVSD, the findings are more likely to relate to poor performance of the biomarkers as markers of volume change. In fact, the settings applied in these latter two experiments are very realistic of routine clinical situations, and the emphasis of the thesis was to identify biomarkers which would be sensitive enough to track even small amplitudes of volume change in order to detect sublclinical deterioration. Thus, the sensitivity of biomarkers that changed significantly would have been in theory, strenghthened.

I undertook to study a large number of potential candidate biomarkers for the accurate mapping of volume change in a variety of settings in order to optimise the chances of identifying a suitably sensitive marker. The large number of statistical comparisons performed is thus likely to inevitably give rise to a number of statistically significant results at random. This may explain why, despite a large number of statistically significant changes in some

biomarkers, only few response patterns were consistent with potential clinical significance, and thus utility.

To decide whether those biomarkers whose levels showed statistically significant trends of response in the various settings would display clinically useful characteristics, I first of all examined the post-hoc analysis, which did not reveal any significant differences between the different time points or interventions in some instances, e.g., IVC indices, thus unlikely to suggest biologically significant biomarker response. Secondly, I examined the magnitude of the biomarker changes in response to volume change in the context of their respective within-subject total variance and within-subject RCV. Changes in biomarker levels that occurred below the respective within-subject variance clearly demonstrated lack of sensitivity for tracking volume changes, e.g., MVE in the LVSD cohort. Some changes in biomarker levels occurred above the within-subject variance but below the within-subject RCV, again potentially suggesting limited clinical applicability. In theory, changes in biomarker levels over and above both the within-subject variance and the within-subject RCV should imply potential clinical utility for tracking volume changes, e.g., BIA measures in the patients with stable LVSD. However, the amplitude of the changes in BIA measures was generally very small (around or less than 5%) such that the changes were either inconsistent with physiological significance (e.g., right lung BIA in the LVSD cohort) or, even if consistent with physiological significance (e.g., whole-body $Z5_{kHz}$ in the LVSD cohort), the small degree of change would limit their applicability as markers of volume status.

The limitations of blood biomarkers as non-invasive markers of volume change include their lack of sensitivity, the large within-subject variance for some biomarkers, the discomfort of

venepuncture, and the latency of the availability of their results which is entirely dependent on local laboratory processes.

For urine biomarkers, it is still unclear whether the changes observed are secondary to the small volume changes induced in the study in stable LVSD patients or whether these are due to volume-independent effects of diuretic manipulation. In any case, their extremely large variance will limit their application as markers of volume change. Furthermore, their results are not immediately available as is the case for blood biomarkers. However, the fact that uKIM-1 and uNAG increased following diuretic withdrawal and reduced following diuretic resumption in the LVSD patients suggests potential beneficial renal effects of furomide in LVSD, possibly involving the proximal tubule.

The use of echocardiographic measures for monitoring volume status is attractive as these measures are freely available as part of a routine study, and the use of hand held echoarcardiography could be easily disseminated to be used by a number of health care personel for the targeted measurement of some of the easily and consistently imaged variables. However, the potential for echocardiogaphic variables as markers of volume change is severely hampered by operator experience (as I demonstrated by in Chapter Five), their relatively large intra- and inter-observer variability and patients'echogeneicity. MVE indices were the potentially most promising echocardiographic markers of volume change due to the sensitivity shown during volume loading with IV Saline in the normal volunteers even following the Valsalva manoeuvre. However, these significant changes in MVE indices were induced following the relatively larger volume change in response to IV Saline in normal volunteers, which if induced in stable LVSD patients for example, might have resulted in

acute decompensation, thus defeating the purpose of using these markers to prevent decompensation.

BIA was the least invasive, time consuming and operator-dependent of all biomarkers studied, with the least variance of its measurements and with results being immediately available. However, the amplitude of change in their levels was too small to imply clinical utility in mapping volume change, except in the setting of the large volume changes induced by IV Saline in the normal volunteers study, which as previously discussed, if induced in LVSD patients, may already have resulted in decompensation. The main issue with bioimpedance, however, seems to lie in its lack of specificity for volume change; significant changes in bioimpedance can occur due to other physiological or pathological processes, or simply for no apparent reason. Catanzariti et al. [2009] showed that of 362 OptiVol alerts registered over a 12-month period in a cohort of stable HF patients, only 175 resulted in a clinical event, of which only 145 were due to worsening of HF (40 % of alerts), the remaining were due to for example, sepsis, respiratory pathologies, or arrhythmias. Moreover, 120 (33 %) alerts were unexplained. Thus while most whole body and trunk BIA measures changed statistically significantly in response to volume manipulation in all three study settings, these changes may not have specifically indicated volume change.

8.2   Suggestions for future studies

Study limitations have been discussed following each stage of investigation. In view of these limitations, future studies are needed to clarify and further investigate the hypotheses tested while addressing the limitations encountered during this programme of work.

The main limitation highlighted in all three sectional studies was the lack of gold standard measures of different volume compartments against which to compare biomarker responses to different volume manipulation protocols. Future studies should ideally employ radioisotope analysis for the determination of not only different body water compartments, but also to determine isotopic renal blood flow in an attempt to elucidate the mechanisms for increased markers of tubular function secondary to diuretic withdrawal. Furthermore, invasive haemodynamic measures should be considered to not only provide accurate measures of LV filling pressure, cardiac output and systemic vascular resistance but also renal venous pressure.

The control of volume homeostasis in LVSD involves a number of pathophysiological mechanisms as discussed in Chapter One. Some of these mechanisms occur upstream and thus modulate volume change, such as the processes involved in neurohormonal activation. These biomarkers would in theory, predict change in volume, rather than simply track subclinical or clinical change in volume status. It is thus forseeable that biomarkers depicting each of these different processes, whether upstream or downstream, can be used to map volume status longitudinally in individual patients. A multi-marker strategy of non-invasive repeated measures assessment of these biomarkers could become clinically applicable, offering a graded measure of impending decompensation and potentially mapping body water compartments, invasive haemodynamic measures or measures of baseline neurohormonal activation and renal function/injury. Considered together with clinical assessment, they would allow an integrated approach to the individual patient such that therapy can be tailored to each individual patient, whether in the subclinical or symptomatic phase of abnormal volume

status. Furthermore, it is forseeable that biomarker measurement technology will evolve to enable finger-prick testing of blood biomarkers, or rapid measurement of markers of renal function by spot urine testing, thus enabling even quicker and less intrusive testing, as well as telemonitoring of repeated measures as is already the case with invasive impedance measures from implantable cardiac devices.

Given the general lack of sensitivity of the biomarkers studied in this thesis for mapping changes in volume status, these being chosen primarily due to their availalility in current clinical practice, the clinical applicability of other potential biomarkers must be investigated, which are possibly still at an investigational stage. Future studies will also need to demonstrate how much deviation from normal variance would imply a change in clinical status, and whether intensification of treatment based purely on changes in biomarker levels has a significant impact on outcome. Furthermore, the practical and financial implications of monitoring biomarkers on a large scale in the context of an increasing HF population need to be established. Once suitably sensitive non-invasive biomarkers of volume change have been identified and validated against gold standards, algorithms for a multi-marker strategy need to be developed and tested for beneficial effects on morbidity and mortality.

8.3   Conclusion

In conclusion, the repeated measures assessment of the biomarkers studied in this thesis appeared, in general, limited in terms of its clinical applicability for tracking subclinical volume changes, and would thus have limited potential for application in the monitoring of volume status in LVSD. This is despite established good correlations of their spot

measurements with isolated measures of volume status, thus illustrating that good sensitivity of a biomarker for monitoring purposes is discerned primarily by its capacity for rapid and clinically appreciable changes in response to clinical changes, and not necessarily by good correlation with static measures of change in clinical status. Thus, this thesis has highlighted some important principles underlying the clinical application of repeated measures assessment for monitoring of clinical status , in particular volume status, with an important strength of the study involving performing repeated measures assessment of biomarkers within individuals to various volume stimuli such that each volunteer acted as his/her own control.

Chapter Nine

References

Abraham WT, Fisher WG, Smith AL, Delurgio DB, Leon AR, Loh E, Kocovic DZ,Packer M, Clavell AL, Hayes DL, Ellestad M, Trupp RJ, Underwood J, Pickering F,Truex C, McAtee P, Messenger J; MIRACLE Study Group. Multicenter InSync Randomized Clinical Evaluation. Cardiac resynchronization in chronic heart failure. N Engl J Med 2002; 346(24):1845-53.

Adams KF Jr, Fonarow GC, Emerman CL, LeJemtel TH, Costanzo MR, Abraham WT, Berkowitz RL, Galvao M, Horton DP. Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). Am Heart J 2005; 149(2): 209-16.

Adamson PB, Magalski A, Braunschweig F, Böhm M, Reynolds D, Steinhaus D, Luby A, Linde C, Ryden L, Cremers B, Takle T, Bennett T. Ongoing right ventricular hemodynamics in heart failure: clinical value of measurements derived from an implantable monitoring system. J Am Coll Cardiol 2003; 41(4): 565-71.

Adlbrecht C, Kommata S, Hülsmann M, Szekeres T, Bieglmayer C, Strunk G, Karanikas G, Berger R, Mörtl D, Kletter K, Maurer G, Lang IM, Pacher R. Chronic heart failure leads to an expanded plasma volume and pseudoanaemia, but does not lead to a reduction in the body's red cell volume. Eur Heart J 2008; 29(19): 2343-50.

Agostoni PG, Marenzi GC, Pepi M, Doria E, Salvioni A, Perego G, Lauri G, Giraldi F, Grazi S, Guazzi MD. Isolated ultrafiltration in moderate congestive heart failure. J Am Coll Cardiol 1993; 21(2):424-31.

Akaishi T, Shingai T, Miyaoka Y, Homma S. Hypotonic diuresis following oropharyngeal stimulation with water in humans. Neurosci Lett 1989; 107(1-3): 70-4.

Albert SN, Hirsch EF, Economopoulos B, Albert CA. Triple-tracer technique for measuring red-blood-cell, plasma and extracellular-fluid volume. J Nucl Med 1968; 9(1): 19-23.

Almeida FA, Suzuki M, Maack T. Atrial natriuretic factor increases haematocrit and decreases the plasma volume in nephrectomised rats. Life Sci 1986; 39: 1193-99.

Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined -a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction.J Am Coll Cardiol 2000;36(3):959-9.

Alonsozana LG, Chrostenson RH. The case for cardiac troponin T: marker for effective risk stratification of patients with acute cardiac ischaemia. Clin Chem 1996; 42: 803-08.

Alvelos M, Ferreira A, Bettencourt P, Serrao P, Pestana M, Cerqueira-Gomes M, Soares-ds-Silva P. The effect of dietary sodium restriction on neurohormonal activity and renal dopaminergic response in patients with heart failure. Eur J Heart Fail 2004; 6: 593-99.

Ammann P, Pfisterer M, Fehr T, Rickli H. Raised cardiac troponins. BMJ 2004; 328(7447): 1028-29.

Anand IS, Ferrari R, Kalra GS, Wahi PS, Poole-Wilson PA, Harris P. Oedema of cardiac origin: studies of body water and sodium, renal function, haemodynamic indices, and plasma hormones in untreated congestive cardiac failure. Circulation 1989; 80: 299-305.

Andersen LJ,Jensen TUS,Bestle MH,Bie P. Gastrointestinal osmoreceptors and renal sodium excretion in humans. Am J Physiol Regulatory Integrative Comp Physiol 2000; 278: R287-94.

Andersen TB, Jødal L, Arveschoug A, Eskild-Jensen A, Frøkiær J, Brøchner-Mortensen J. Precision and within- and between-day variation of bioimpedance parameters in children aged 2-14 years. Clin Nutr. 2010 [Epub ahead of print].

Anderson JV, Donckier J, McKenna WJ, Bloom SR. The plasma release of atrial natiuretic peptide in man. Clin Sci (Colch.) 1986; 71: 151-55.

Androne AS, Hryniewicz K, Hudaihed A, Mancini D, Lamanca J, Katz SD. Relation of unrecognized hypervolemia in chronic heart failure to clinical status,hemodynamics, and patient outcomes. Am J Cardiol 2004; 93(10):1254-59.

Androne AS, Katz SD, Lund L, LaManca J, Hudaihed A, Hryniewicz K, Mancini DM. Hemodilution is common in patients with advanced heart failure. Circulation 2003; 107 (2): 226-29.

Araújo JP, Azevedo A, Lourenço P, Rocha-Gonçalves F, Ferreira A, Bettencourt P. Intraindividual variation of amino-terminal pro-B-type natriuretic peptide levels in patients with stable heart failure. Am J Cardiol 2006; 98(9): 1248-50.

Areias JC, Matias A, Montenegro N. Venous return and right ventricular diastolic function in ARED flow fetuses. J Perinat Med 1998; 26 (3): 157-67.

Arteaga RB, Hreybe H, Patel D, Landolfo C. Derivation and validation of a diagnostic model for the evaluation of left ventricular filling pressures and diastolic function using mitral annulus tissue Doppler imaging. Am Heart J 2008; 155(5): 924-29.

Auricchio A, Stellbrink C, Block M, Sack S, Vogt J, Bakker P, Klein H, Kramer A, Ding J, Salo R, Tockman B, Pochet T, Spinelli J. Effect of pacing chamber and atrioventricular delay on acute systolic function of paced patients with congestive heart failure. The Pacing Therapies for Congestive Heart Failure Study Group. Circulation 199; 99(23): 2993-3001.

Balga I, Solenthaler M, Furlan M. Should whole-body red cell mass be measured or calculated? Blood Cells Mol Dis 2000; 26(1): 25-31.

Barclay JL, Kruszewski K, Croal BL, Cuthbertson BH, Oh JK, Hillis GS. Relation of left atrial volume to B-type natriuretic peptide levels in patients with stable chronic heart failure. Am J Cardiol 2006; 98 (1): 98-101.

Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. Characterisation of the Co2+ and Ni 2+ binding amino-acid residues of the N-terminus of human albumin: an insight into the mechanism of a new assay for myocardial ischemia. Eur J Biochem 2001; 268:42-47.

Bartel T, Muller S, Borges AC. Doppler-derived diastolic indices in dilated cardiomyopathy: a haemodynamic evaluation relating per- and afteload parameters to flow velocity. Can J Cardiol 1996; 12 (10): 953-58.

Basit M, Bakshi N, Hashem M, Allebban Z, Lawson N, Rosman HS, Maciejko JJ. The effect of freezing and long-term storage on the stability of cardiac troponin T. Am J Clin Pathol 2007; 128 (1): 164-67.

Basnight MA, Gonzalez MS, Kershenovich SC, Apleton CP. Pulmonary venous flow velocity: relation to haemodynamics, mitral flow velocity and left atrial volume and ejection fraction. J Am Soc Echocardiogr 1991; 4: 457-558.

Bau PFD, Bau CHD, Naujorks AA, Rosito GA, Fuchs FD. Diurnal variation of vascular diameter and reactivity in healthy young men. Braz J Med Biol Res. 2008; 41 (6): 500-503.

Bauersachs J, Fraccarollo D, Ertl G, Gretz N, Wehling M, Christ M. Striking increase in natriuresis by low-dose spironolactone in congestive heart failure only in combination with ACE inhibition. Circulation 2000; 102: 2325-28.

Beetham R, Monk C, Keating L, Benger JR, Kendall J. Effects of storage at –20 °C on ischaemia modified albumin results. Ann Clin Biochem 2006; 43 (6): 500-02.

Belenky A, Smith A, Zhang B, Lin S, Despres N, Wu AH, Bluestein BI. The effect of class-specific protease inhibitors on the stabilisation of B-type natriuretic peptide in human plasma. Clin Chim Acta 2004; 340: 163-72.

Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure-definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004; 8: R204-12.

Ben Driss A, Tabet JY, Meurin P, Weber H, Renaud N, Grosdemouge A, Bourmayan C. Role of B-type natriuretic peptide and echocardiographic indices in predicting the development of acute heart failure following beta-blocker uptitration in chronic heart failure patients with left ventricular systolic dysfunction. Int J Cardiol 2007; 115 (2): 257-58.

Ben Driss A, Tabet JY, Meurin P, Weber H, Renaud N, Grosdemouge A, Bourmayan C. [BNP and echocardiography can predict the occurrence of acute cardiac decompensation during titration of bisoprolol in chronic systolic heart failure]. Arch Mal Coeur Vaiss 2007; 100 (11): 934-40.

Berger R, Moertl D, Peter S, Ahmadi R, Huelsmann M, Yamuti S, Wagner B, Pacher R. N-terminal pro-B-type natriuretic peptide-guided, intensive patient management in addition to multidisciplinary care in chronic heart failure a 3-arm, prospective, randomized pilot study. J Am Coll Cardiol 2010; 55 (7): 645-53.

Beshai JF, Grimm RA, Nagueh SF, Baker JH 2nd, Beau SL, Greenberg SM, Pires LA, Tchou PJ; RethinQ Study Investigators. Cardiac-resynchronization therapy in heart failure with narrow QRS complexes. N Engl J Med 2007; 357 (24): 2461-71.

Bhagavan NV, Lai EM, Rios PA, Yang J, Ortega-Lopez AM, Shinoda H, Honda SA, Rios CN, Sugiyama CE, Ha CE. Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischaemia and myocardial infarction. Clin Chem 2003; 49: 581-85.

Binanay C, Califf RM, Hasselblad V, O'Connor CM, Shah MR, Sopko G, Stevenson LW, Francis GS, Leier CV, Miller LW. Evaluation study of congestive heart failure and pulmonary artery catheterization effectiveness: the ESCAPE trial. JAMA 2005; 294 (13):1625-33.

Biolo A, Fisch M, Balog J, Chao T, Schulze PC, Ooi H, Siwik D, Colucci WS. Episodes of acute heart failure syndrome are associated with increased levels of troponin and extracellular matrix markers. Circ Heart Fail 2010; 3(1): 44-50.

Biomarkers Definitions Working Group. Biomarkers and surrogate end-points: preferred definitions and conceptual framework. Clini Pharmacol Ther 2001; 69: 89-95.

Blair JE, Brennan JM, Goonewardena SN, Shah D, Vasaiwala S, Spencer KT. Usefulness of hand-carried ultrasound to predict elevated left ventricular filling pressure. Am J Cardiol 2009; 103 (2): 246-47

Blehar DJ, Dickman E, Gaspari R. Identification of congestive heart failure via respiratory variation of inferior vena cava diameter. Am J Emerg Med 2009; 27(1): 71-75.

Boccanelli A, Liberatore SM, Prati PL. Captopril versus frusemide in moderate heart failure. Lancet 1986; 2 (8519): 1331.

Bodor GS, Porter S, Landt Y, Ladenson JH. Development of monoclonal antibodies for an assay of cardiac troponin I and preliminary results in suspected cases of myocardial infarction. Clin Chem 1992; 38: 2203-14.

Boerrigter G, Burnett JC Jr. Recent advances in natriuretic peptides in congestive heart failure. Expert Opin Investig Drugs 2004; 13: 643-52.

Bonfils PK, Damgaard M, Taskiran M, Goetze JP, Norsk P, Gadsbøll N. Impact of diuretic treatment and sodium intake on plasma volume in patients with compensated systolic heart failure. Eur J Heart Fail 2010; 12 (9): 995-1001.

Bourge RC, Abraham WT, Adamson PB, Aaron MF, Aranda JM Jr, Magalski A, Zile MR, Smith AL, Smart FW, O'Shaughnessy MA, Jessup ML, Sparks B, Naftel DL, Stevenson LW; COMPASS-HF Study Group. Randomized controlled trial of an implantable continuous hemodynamic monitor in patients with advanced heart failure: the COMPASS-HF study. J Am Coll Cardiol 2008; 51 (11): 1073-79.

Boyle A, Sobotka PA. Redefining the therapeutic objective in decompensated heart failure: haemoconcentration as a surrogate for plasma refill rate. J Cardiac Fail 2006; 12(4): 247-49.

Brater DC. Pharmacokinetics of loop diuretics in congestive heart failure. Br Heart J1994; 72 (Suppl.): S40-43.

Brater DC. Resistance to diuretics: emphasis on a pharmacological perspective.Drugs. 1981; 22 (6): 477-94.

Braunschweig F, Fahrleitner-Pammer A, Mangiavacchi M, Ghio S, Fotuhi P, Hoppe UC, Linde C. Correlation between serial measurements of N-terminal pro brain natriuretic peptide and ambulatory cardiac filling pressures in outpatients with chronic heart failure. Eur J Heart Fail 2006; 8 (8): 797-803

Braunschweig F, Ford I, Conraads V, Cowie MR, Jondeau G, Kautzner J, Lunati M, Munoz Aguilera R, Man Yu C, Marijianowski M, Borggrefe M, van Veldhuisen DJ. Can monitoring of intrathoracic impedance reduce morbidity and mortality in patients with chronic heart failure? Rationale and design of the Diagnostic Outcome Trial in Heart Failure (DOT-HF). Eur J Heart Fail 2008; 10 (9): 907-16.

Braunschweig F, Kjellström B, Söderhäll M, Clyne N, Linde C. Dynamic changes in right ventricular pressures during haemodialysis recorded with an implantable haemodynamic monitor. Nephrol Dial Transplant 2006; 21 (1): 176-83.

Braunschweig F, Linde C, Eriksson MJ, Hofman-Bang C, Rydén L. Continuous haemodynamic monitoring during withdrawal of diuretics in patients with congestive heart failure. Eur Heart J 2002; 23 (1): 59-69.

Braunwald E. Biomarkers in heart failure. N Engl J Med 2008; 358: 2148-59.

Braunwald E, Zipes DP, Libby P, Bonow R, editors. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine (7th Edition). Philadelphia: Saunders; 2004.

Brennan JM, Blair JE, Goonewardena S, Ronan A, Shah D, Vasaiwala S, Kirkpatrick JN, Spencer KT. Reappraisal of the use of inferior vena cava for estimating right atrial pressure. J Am Soc Echocardiogr 2007; 20: 857-61.

Brezis M, Agmon Y, Epstein FH. Determinants of intrarenal oxygenation. Effects of diuretics. Am J Physiol 1994; 267 (6 Pt 2): F1059-62.

Bristow MR, Saxon LA, Boehmer J, Krueger S, Kass DA, De Marco T, Carson P, DiCarlo L, DeMets D, White BG, DeVries DW, Feldman AM; Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) Investigators. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. N Engl J Med 2004; 20; 350 (21): 2140-50.

Bristow MR. β-Adrenergic receptor blockade in chronic heart failure. Circulation 2000; 101: 558-69.

Brock H, Gabriel C, Bibl D, Necek S. Monitoring intravascular volumes for postoperative volume therapy. Eur J Anaesthesiol 2002; 19 (4): 288-94.

Brown CM,Barberini L, Dulloo AG, Montani JP. Cardiovascular responses to water drinking: does osmolality play a role? Am J Physiol Regul Comp Physiol 2005; 289: R1687-92.

Brown MA, Mitar DA, Whitworth JA. Measurement of plasma volume in pregnancy. Clin Sci 1992; 83: 29-34.

Bruins S, Fokkema MR, Romer JWP, Dejongste MJL, van der Dijs FPL, can den Ouweland JMW, Muskiet FAJ. High intraindividual variation of B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with stable chronic heart failure. Clin Chem 2004; 50 (11): 1052-2058.

Butman SM, Ewy GA, Standen JR, Kern KB, Hahn E. Bedside cardiovascular examination in patients with severe chronic heart failure: importance of rest or inducible jugular venous distension. J Am Coll Cardiol1993; 22 (4): 968-74.

Callegaro GC, Moraes RS, Negrao CE, Trombetta IC, Rondon MU, Teixeira MS, Silva SC, Ferlin EL, Krieger EM, Ribeiro JP. Acute water ingestion increases arterial blood pressure in hypertensive and normotensive subjects. J Hum Hypertens 2007; 21: 564-70.

Capolaghi B, Charbonnier B, Dumontet M, Hennache B, Henninot J, Laperche T, Lavoinne A, Lefèvre G, Morin C, Pateron D. Prescription, assay and interpretation of cardiac troponins tests: guidelines from SFBC-CNBC troponin working group Ann Biol Clin (Paris) 2005; 63 (3): 245-61.

Capomonella S, Febo O, Caporotondi A, Guazzotti G, Gnemmi M, Rossi A, Pinna G, Maestri R, Cobelli F. Non-invasive estimation of right atrial pressure by combined Doppler echocardiographic measurements of the inferior vena cava in patients with congestive heart failure. Italian Heart Journal 2000; 1(10): 684-90.

The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. Lancet.1999 ; 353 (9146): 9-13.

The Carvedilol Prospective Randomised Cummulative Survival (COPERNICUS) Study Group. Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med 2001; 344: 1651-58.

Catanzariti D, Lunati M, Landolina M, Zanotto G, Lonardi G, Iacopino S, Oliva F, Perego GB, Varbaro A, Denaro A, Valsecchi S, Vergara G; Italian Clinical Service Optivol-CRT Group. Monitoring intrathoracic impedance with an implantable defibrillator reduces hospitalizations in patients with heart failure. Pacing Clin Electrophysiol. 2009; 32(3):363-70.

Chakko S, Woska D, Martinez H, de Marchena E, Futterman L, Kessler KM, Myerberg RJ. Clinical, radiographic, and hemodynamic correlations in chronic congestive heart failure: conflicting results may lead to inappropriate care. Am J Med 1991; 90 (3): 353-59.

Charach G, Rabinovich P, Grosskopf I, Weintraub M. Transthoracic monitoring of the impedance of the right lung in patients with cardiogenic pulmonary edema. Crit Care Med 2001; 29 (6):1137-44.

Chaudhry SI, Phillips CO, Stewart SS, Riegel B, Mattera JA, Jerant AF, Krumholz HM. Telemonitoring for patients with chronic heart failure: a systematic review. J Card Fail 2007; 13 (1): 56-62.

Chaudhry SI, Wang Y, Concato J, Gill TM, Krumholz HM. Patterns of weightchange preceding hospitalization for heart failure. Circulation 2007; 116 (14): 1549-54.

Cheng CK, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol 2004; 10 (1): 42-53.

Chen HH. Heart failure: a state of brain natriuretic peptide deficiency or resistance or both! J Am Coll Cardiol. 2007; 49 (10): 1089-91.

Chen BH, Fong JF, Chiang CH. Effect of different anticoagulant, underfilling of blood sample and storage stability on selected haemogram. Kaoshing J Med Sci 1999; 15(2): 87-93.

Chen YN, Wei JR, Zeng LJ, Wu MY. Monitoring of cardiac troponin I in patients with acute heart failure. Ann Clin Biochem 1999; 36 (Pt 4): 433-37.

Cheriex Ec, Luenissen KML, Janssen JHA, Mooy JMV, van Hooff JP. Echography of the inferior vena cava is a simple and reliable tool for estimation of 'dry weight' in haemodialysis patients. Nephrology Dialysis Transplantation 1989; 4: 563-68.

Chew SL, Lins RL, Daelemans R, Nuyts GD, De Broe ME. Urinary enzymes in acute renal failure. Nephrol Dial Transplant 1993; 8 (6): 507-11

Chomsky DB, Lang CC, Rayos G, Wilson JR. Treatment of subclinical fluid retention in patients with symptomatic heart failure: effect on exercise performance. J Heart Lung Transplant 1997; 16(8): 846-53.

Christenson RH, Duh SH, Sanhai WR, Wu AH, Holtman V, Painter P, Branham E, Apple FS, Murakami M, Morris DL. Characteristics of an albumin cobalt bidning test for assessment of acute coronary syndrome patients: a multicentre study. Clin Chem 2001; 47: 464-70

Chuang PP, Wilson RF, Homans DC, Stone K, Bergman T, Bennett TD, Kubo SH. Measurement of pulmonary artery diastolic pressure from a right ventricular pressure transducer in patients with heart failure. J Card Fail 1996; 2 (1): 41-46.

Cioffi G, Stefenelli C, Tarantini L, Opasich C. Haemodynamic response to intensive unloading therapy (furosemide and nitroprusside) in patients >70 years of age with left ventricular systolic dysfunction and decompensated heart failure. Am J Cardiol 2003; 92 (9): 1050-56.

Cleland JG, Daubert JC, Erdmann E, Freemantle N, Gras D, Kappenberger L, Tavazzi L. Longer-term effects of cardiac resynchronization therapy on mortality in heart failure [the Cardiac Resynchronization-Heart Failure (CARE-HF) trial extension phase]. Eur Heart J 2006; 27 (16): 1928-32.

Cleland JG, Louis AA, Rigby AS, Janssens U, Balk AH; TEN-HMS Investigators. Noninvasive home telemonitoring for patients with heart failure at high risk of recurrent admission and death: the Trans-European Network-Home-Care Management System (TEN-HMS) study. J Am Coll Cardiol 2005; 45 (10): 1654-64.

Cleland JG, Swedberg K, Follath F, Komajda M, Cohen-Solal A, Aguilar JC, Dietz R, Gavazzi A, Hobbs R, Korewicki J, Madeira HC, Moiseyev VS, Preda I, van Gilst WH, Widimsky J, Freemantle N, Eastaugh J, Mason J. The EuroHeart Failure survey programme--a survey on the quality of care among patients with heart failure in Europe. Part 1: patient characteristics and diagnosis. Eur Heart J 2003; 24 (5): 442-63.

Cleland JG, Ward S, Dutka D, Habib F, Impallomeni M, Morton IJ. Stability of plasma concentrations of N and C terminal atrial natriuretic peptides at room temperature. Heart 1996; 75(4):410-13.

Clerico A, Del Ry S, Giannesi D. Measurement of cardiac natriuretic hormones (atrial natriuretic peptide, brain natriuretic peptide, and related peptides) in clinical practice: the need for a new generation of immunoassay methods. Clin Chem 2004; 46: 1529-34.

Cohn JN, Franciosa JA. Vasodilator therapy of cardiac failure. N Engl J Med 1977:297:27-31, 254-58.

Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, Smith R, Dunkman WB, Loeb H, Wong M, Bhat G, Goldman S, Fletcher RD, Doherty J, Hughes CV, Carson P, Cintron G, Shabetai R, Haakenson C. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. N Engl J Med 1991; 325:303-10.

Cohn JN, Tognoni G; Valsartan Heart Failure Trial Investigators. For the Valsartan Heart Failure Trial Investigators. A randomised trial of the angiotensin-receptor blocker valsartan in chronic heart failure. N Engl J Med 2001; 345: 1667-1675.

Cohn JN, Ferrari R, Sharpe N. Cardiac remodelling: concepts and clinical implications: a consensus paper from an international forum on cardiac remodelling, on behalf of an International Forum on Cardiac Remodelling. J Am Coll Cardiol 2000; 35: 569-82.

Collinson PO, Gaze DC, Bainbridge K, Morris F, Morris B, Price A, Goodacre S. Utility of admission cardiac troponin and "Ischemia Modified Albumin" measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. Emerg Med J 2006; 23 (4): 256-61.

Consuegra-Sanchez L, Bouzas-Mosquera A, Sinha MK, Collinson PO, Gaze DC, Kaski JC. Ischemia-modified albumin predicts short-term outcome and 1-year mortality in patients attending the emergency department for acute ischemic chest pain. Heart Vessels 2008;23(3):174-80

Conway J, Lauwers P. Haemodynamic and hypotensive effects of long term therapy with chlorothiazide. Circulation 1960; 21: 21-27.

Coodley EL, Segal JL, Smith DH, Neutel JM. Bioelectrical impedance analysis as an assessment of diuresis in congestive heart failure. Ann Pharmacother 1995; 29 (11): 1091-95.

Corboy JC, Walker RJ, Simmonds MB, Wilkins GT, Richards AM, Espiner EA. Plasma natriuretic peptides and cardiac volume during acute changes in intravascular volume in haemodialysis patients. Clin Sci (Lond) 1994; 87 (6): 679-84.

Costanzo MR. Ultrafiltration in the management of heart failure. Curr Opin Crit Care 2008; 14(5):524-30.

Costanzo MR, Guglin ME, Saltzberg MT, Jessup ML, Bart BA, Teerlink JR, Jaski BE, Fang JC, Feller ED, Haas GJ, Anderson AS, Schollmeyer MP, Sobotka PA; UNLOAD Trial Investigators. Ultrafiltration versus intravenous diuretics for patients hospitalized for acute decompensated heart failure. J Am Coll Cardiol 2007; 49(6):675-83.

Cotter G, Dittrich HC, Weatherley BD, Bloomfield DM, O'Connor CM, Metra M, Massie BM. The PROTECT pilot study: a randomized, placebo-controlled, dose-finding study of the adenosine A1 receptor antagonist rolofylline in patients with acute heart failure and renal impairment.Card Fail 2008;14(8):631-40.

Cotter G, Metra M, Milo-Cotter O, Dittrich HC, Gheorghiade M. Fluid overload in acute heart failure--re-distribution and other mechanisms beyond fluid accumulation. Eur J Heart Fail 2008;10(2):165-69.

Cowley AJ, Stainer K, Wynne RD, Rowley JM, Hampton JR. Symptomatic assessment of patients with heart failure: double-blind comparison of increasing doses of diuretics and captopril in moderate heart failure. Lancet 1986; 2(8510):770-72.

Cruden NL, Witherow FN, Webb DJ, Fox KA, Newby D. Bradykinin contributes to the systemic haemodynamic effects of chronic angiotensin-converting enzyme inhibition in patients with heart failure. Arterioscler Thomb Vasc Biol 2004; 24: (6): 1043-48.

Damman K, Navis G, Smilde TD, Voors AA, van der Bij W, van Veldhuisen DJ,Hillege HL. Decreased cardiac output, venous congestion and the association with renal impairment in patients with cardiac dysfunction. Eur J Heart Fail 2007; 9(9):872-78.

Damman K, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Urinary neutrophil gelatinase associated lipocalin (NGAL), a marker of tubular damage, is increased in patients with chronic heart failure. Eur J Heart Fail 2008;10(10):997-1000.

Damman K,van Deursen VM, Navis G,Voors AA, van Veldhuisen DJ, Hillege HL. Increased central venous pressure is associated with impaired renal function and mortality in a broad spectrum of patients with cardiovascular disease. J Am Coll Cardiol 2009;53(7):582-88.

Das SR, Dries DL, Drazner MH, Yancy CW, Chae CU. Relation of lower hematocrit to progression from asymptomatic left ventricular dysfunction to symptomatic heart failure. Am J Cardiol 2005; 96(6):827-31.

Davidov M, Kakaviatos N., Finnerty FA Jr. Intravenous administration of furosemide in heart failure. JAMA 1967; 200: 824-29.

Davis ME, Richards AM, Nicholls MG, Yandle TG, Frampton CM, Troughton RW. Introduction of metoprolol increases plasma B-type cardiac natriuretic peptides in mild, stable heart failure. Circulation 2006;113(7):977-85

De Lorenzo A, Deurenberg P, Andreoli A, Sasso GF, Palestinin M, Docimo R. Multifrequency impedance in the assessment of body water losses during dialysis. Ren Physiol Biochem 1994; 17: 326-32.

De Marco T, Daly PA,Liu M, Kayser S, Parmley WW, Chatterjee K.Enalaprilat, a new parenteral angiotensin-converting enzyme inhibitor: rapid changes in systemic and coronary haemodynamics and humoral profile in chronic heart failure. J Am Coll Cardiol 1987; 9(5): 1131-38.

Dickstein K, Chang P, Willenheimer R, Haunso S, Remes J, Hall C, Kjekshus J. Comparison of the effects of lorsartan and enalapril on clinical status and exercise performance in patients with moderate or severe chronic heart failure. J Am Coll Cardiol 1995; 26 (2): 438-45.

Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, Strömberg A, van Veldhuisen DJ, Atar D, Hoes AW, Keren A, Mebazaa A, Nieminen M, Priori SG, Swedberg K; ESC Committee for Practice Guidelines, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008. Eur Heart J 2008; 29(19): 2388-442.

Di Donato M, Mori F, Barletta G, Dabizzi RP, Fantini F. Effect of dextran infusion on left atrial size in normal subjects. Cardiology 1982; 69: 257-64.

Dikshit K, Vyden JK, Forrester JS, Chatterjee K, Prakash R, Swan HJ. Renal and extrarenal haemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. N Engl J Med 1973; 288: 1087-90.

Disomma S, Magrini L, Pittoni V, Marino R, Peacock WF, Maisel A. Usefulness of serial assessment of natriuretic peptides in the emergency department for patients with acute decompensated heart failure. Congest Heart Fail 2008;14(4 Suppl 1): 21-24.

Dokainish H, Zoghbi WA, Lakkis NM, Al-Bakshy F, Dhir M, Quinones MA, Sherif F, Nagueh SF. Optimal non invasive assessment of left ventricular filling pressures. Circulation 2004; 109: 2432-39.

Dokainish H, Zoghbi WA, Lakkis NM, Ambriz E, Patel R, Quinones MA, Nagueh SF. Incremental predictive power of B-type natriuretic peptide and tissue Doppler echocardiography in the prognosis of patients with congestive heart failure. J Am Coll Cardiol 2005; 45(8):1223-26.

Domanski M, Norman J, Pitt B, Haigney M, Hanlon S, Peyster E. Diuretic use, progressive heart failure, and death in patients in the studies of left ventricular dysfunction (SOLVD). J Am Coll Cardiol 2003: 42: 705-08.

Domanski M, Tian X, Haigney M, Pitt B. Diuretic use, progressive heart failure, and death in patients in the DIG study. J Card Fail 2006;12(5):327-32.

Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Samimi-Fard S, Kaski JC. Relation of ischemia-modified albumin levels and left ventricular systolic function in patients with ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention. Clin Chim Acta 2008; 388(1-2):196-99.

Drazner MH, Hamilton MA, Fonarow G, Creaser J, Flavell C, Stevenson LW. Relationship between right and left-sided filling pressures in 1000 patients with advanced heart failure. J Heart Lung Transplant 1999; 18(11):1126-32.

Drazner MH, Rame JE, Dries DL. Third heart sound and elevated jugular venous pressure as markers of the subsequent development of heart failure in patients with asymptomatic left ventricular dysfunction. Am J Med 2003; 114(6): 431-37.

Drazner MH, Rame JE, Stevenson LW, Dries DL. Prognostic importance of elevated jugular venous pressure and a third heart sound in patients with heart failure. New Engl J Med 2001; 345:574-81.

Dries DL, Exner DV, Domanski MJ, Greenberg B, Stevenson LW. The prognostic implications of renal insufficiency in asymptomatic and symptomatic patients with left ventricular systolic dysfunction. J Am Coll Cardiol 2000; 35(3): 681-89.

Drighil A, Madias JE, Mathewson JW, El Mosalami H, El Badaoui N, Ramdani B, Bennis A. Haemodialysis: effects of acute decrease in preload on tissue Doppler imaging indices of systolic and diastolic function of the left and right ventricles. Eur J Echocardiogr 2008; 9(4):530-35.

Dupuy AM, Terrier N, Dubois M, Boularan AM, Cristol JP. Heparin plasma sampling as an alternative to EDTA for BNP determination on the Access-Beckman Coulter-effect of storage at –20 degrees C. Clin Lab 2006; 52(7-8): 393-97.

Efthimiadis GK, Parharidis GE, Gemitzis KD, Nouskas IG, Karvounis HI, Styliadis IK, Louridas GE. Doppler echocardiographic evaluation of right ventricular diastolic function in isolated valvular aortic stenosis. J Heart Valve Dis 1999; 8 (3):261-69.

Ellis K, Dreiscach AW, Lertora JL. Plasma elimination of cardiac troponin I in end stage renal disease. South Med J 2001;94(10): 993-96.

Ellison DH. Diuretic therapy and resistance in congestive heart failure. Cardiology 2001; 96: 132-43.

Endo Y, Torii R, Yamazaki F, Sagawa S, Yamauchi K, Tsutsui Y, Morikawa T, Shiraki

K. Water drinking causes a biphasic change in blood composition in humans. Pflugers Arch. 2001; 442(3):362-68.

England JM, Walford DM, Waters DA. Reassessment of the reliability of the haematocrit. Br J Haematol 1972; 23 (2): 247-56.

Ennezat PV, Ennezat CA, Vijayaraman P, Lachmann J, Asseman P, Cohen-Solal A,Sonnenblick EH, LeJemtel TH. Dissociation between improvement in left ventricular performance and functional class in patients with chronic heart failure. J Cardiovasc Pharmacol 2005; 46(3): 262-68.

Ernest D, Belzberg AS, Dodek PM. Distribution of normal saline and 5% albumin infusions in cardiac surgical patients. Crit Care Med 2001; 29 (12): 2299-302.

Eshaghian S, Horwich TB, Fonarow GC. Relation of loop diuretic dose to mortality in advanced heart failure. Am J Cardiol 2006; 97(12): 1759-64.

Evans RG, Gardiner BS, Smith DW, O'Connor PM. Intrarenal oxygenation: unique challenges and the biophysical basis of homeostasis. Am J Physiol Renal Physiol. 2008; 295 (5): F1259-70.

Feigenbaum MS, Welsch MA, Mitchell M, Vincent K, Braith RW, Pepine CJ. Contracted plasma and bood volume in chronic heart failure. J Am Coll Cardiol 2000; 35: 51-55.

Felker GM, Adams KF Jr, Gattis WA, O'Connor CM. Anemia as a risk factor and therapeutic target in heart failure. J Am Coll Cardiol 2004; 44(5): 959-66.

Felker GM, Cuculich PS, Gheorghiade M. The Valsalva manoeuvre: a bedside 'biomarker' for heart failure. Am J Med 2006; 119: 117-22.

Fellahi JL, Caille V, Charron C, Deschamps-Berger PH, Vieillard-Baron A. Noninvasive assessment of cardiac index in healthy volunteers: a comparison between thoracic impedance cardiography and Doppler echocardiography. Anesth Analg 2009;108(5):1553-59.

Fennessy PA, Campbell JH, Campbell GR. Perindopril inhibits both the development of atherosclerosis in the cholesterol-fed rabbit and lipoprotein binding to smooth muscle cells in culture. Atherosclerosis 1994; 106 (1): 29-41.

Ferguson JL, Beckett GJ, Stoddart M, Walker SW, Fox KA. Biochemical markers of myocyte injury in heart failure. Heart 2004; 90: 1110-13.

Ferguson JL, Beckett GJ, Stoddart M, Walker SW, Fox KA. Myocardial infarction redefined: the new ACC/ESC definition, based on cardiac troponin, increases the apparent incidence of infarction. Heart 2002; 88: 343-47.

Flather MD, Shibata MC, Coats AJ, Van Veldhuisen DJ, Parkhomenko A, Borbola J,Cohen-Solal A, Dumitrascu D, Ferrari R, Lechat P, Soler-Soler J, Tavazzi L, Spinarova L, Toman J, Böhm M, Anker SD, Thompson SG, Poole-Wilson PA. Randomized trial to determine the

effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). Eur Heart J 2005; 26 (3):215-25.

Fokkema MR, Herrmann Z, Muskiet FA, Moecks J. Reference change values for brain natriuretic peptides revisited. Clin Chem 2006; 52 (8): 1602-03.

Fonarow GC, Heywood JT, Heidenreich PA, Lopatin M, Yancy CW. Temporal trends in clinical characteristics, treatments, and outcomes for heart failure hospitalizations, 2002 to 2004: findings from Acute Decompensated Heart Failure National Registry (ADHERE).Am Heart J 2007;153(6):1021-8.

Fouad FM, Tarazi RC, Bravo EL, Hart NJ, Castle LW, Salcedo EE. Long term control of congestive heart failure with captopril. Am J Cardiol 1982; 49: 1489-96.

Franceschi F, Deharo JC, Giorgi R, By Y, Monserrat C, Condo J, Ibrahim Z, Saadjian A, Guieu R. Peripheral plasma adenosine release in patients with chronic heart failure. Heart 2009; 95 (8): 651-55.

Franciosa JA, Dunkman WB, Leddy CL. Hemodynamic effects of vasodilators and long-term response in heart failure. J Am Coll Cardiol 1984; 3(6): 1521-30.

Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH,Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation 1990; 82(5):1724-29.

Francis GS, McDonald K, Chu C, Cohn JN. Pathophysiologic aspects of end-stage heart failure. Am J Cardiol 1995; 75: 11A-16A.

Francis GS, Siegel RM, Goldsmith SR, Olivari MT, Levine TB, Cohn JN.Acute vasoconstrictor response to intravenous furosemide in patients with chronic congestive heart failure. Activation of the neurohormonal axis. Ann Intern Med 1985; 103: 1-6.

Frantz RP, Olson LJ, Grill D, Moualla SK, Nelson SM, Nobrega TP, Hanna RD, Backes RJ, Mookadam F, Heublein D, Bailey KR, Burnett JC. Carvedilol therapy is associated with a sustained decline in brain natriuretic peptide levels in patients with congestive heart failure. Am Heart J 2005; 149 (3): 541-47.

Fraser CG. Biological variation: From Principles to Practice. Washington, DC: ACC Press, 2001.

Fraser CG. Inherent biological variation and reference values. Clin Chem Lab Med 2004; 42: 758-64.

Fraser CG. Reference change values: the way forward in monitoring. Ann Clin Biochem 2009; 46 (Pt3): 264-65.

Gabriel RS, Kerr AJ, Sharma V, Zeng IS, Stewart RA. B-type natriuretic peptide and left ventricular dysfunction on exercise echocardiography in patients with chronic aortic regurgitation. Heart 2008; 94 (7): 897-902.

Gabrielsen A, Brie P, Holstein-Rathlou NH, Christensen NJ, Warberg J, Dige-Petersen H, Frandsen E, Galatius S, Pump B, Sorensen VB, Norsk P. Neuroendocrine and renal effects of intravascular volume expansion in compensated heart failure. Am J Physiol Regul Integr Comp Physiol 2001; 281: R459-67.

Garrido IP, Roy D, Calviño R, Vazquez-Rodriguez JM, Aldama G, Cosin-Sales J, Quiles J, Gaze DC, Kaski JC. Comparison of ischaemia modified albumin levels in patients undergoing percutaneous coronary intervention for unstable angina pectoris with versus without coronary collaterals. Am J Cardiol 2004; 93: 88-90.

Gelman S. Venous function and central venous pressure: a physiologic story. Anesthesiology 2008;108(4):735-48.

Gentile F, Mantero A, Lippolis A, Ornaghi M, Azzollini M, Barbier P, Beretta L, Casazza F, Corno R, Faletra F, Giagnoni E, Gualtierotti C, Lombroso S,Mattioli R, Morabito A, Pepi M, Todd S, Pezzano A. Pulmonary venous flow velocity patterns in 143 normal subjects aged 20 to 80 years old. An echo 2D colour Doppler cooperative study. Eur Heart J 1997;18(1):148-64.

Gerhardt W, Nordin G, Herbert AK, Burzell BL, Isaksson A, Gustavsson E, Haglund S, Müller-Bardorff M, Katus HA. Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: lower recoveries in early than in late phases of myocardial injury. Clin. Chem 2000; 46(6 Pt 1): 817-21.

Gerber JG, Nies AS. Furosemide-induced vasodilation: importance of the state of hydration and filtration. Kidney Int 1980; 18(4):454-59.

Gheorghiade M, Abraham WT, Albert NM, Gattis Stough W, Greenberg BH, O'Connor CM, She L, Yancy CW, Young J, Fonarow GC. Relationship between admission serum sodium concentration and clinical outcomes in patients hospitalized for heart failure: an analysis from the OPTIMIZE-HF registry. Eur Heart J 2007; 28(8):980-88.

Gheorghiade M, Gattis Stough W, Adams KF Jr, Jaffe AS, Hasselblad V, O'Connor CM. The Pilot Randomised study of nesiritide versus dobutamine in heart failure (PRESERVD-HF). Am J Cardiol 2005; 96 (6A):18G-24G.

Gheorghiade M, Zannad F, Sopko G, Klein L, Piña IL, Konstam MA, Massie BM, Roland E, Targum S, Collins SP, Filippatos G, Tavazzi L. Acute heart failure syndromes: current state and framework for future research. Circulation. 2005; 112 (25): 3958-68.

Gidenne S, Ceppa F, Fontan E, Perrier F, Burnat P. Analytical performance of the albumin cobalt binding (ACB) test on the Cobas MIRA plus analyzer. Clin Chem Lab Med 2004; 4: 455-61.

Givertz MM, Slawsky MT, Moraes DL, McIntyre KM, Colucci WS. Noninvasive determination of pulmonary artery wedge pressure in patients with chronic heart failure. Am J Cardiol 200; 87 (10): 1213-5; A7.

Goldberg LR, Piette JD, Walsh MN, Frank TA, Jaski BE, Smith AL, Rodriguez R,Mancini DM, Hopton LA, Orav EJ, Loh E. Randomized trial of a daily electronic home monitoring system in patients with advanced heart failure: the Weight Monitoring in Heart Failure (WHARF) trial. Am Heart J 2003; 146 (4): 705-12.

Goonewardena SN, Gemignani A, Ronan A, Vasaiwala S, Blair J, Brennan JM, Shah DP, Spencer KT. Comparison of hand-carried ultrasound assessment of the inferior vena cava and N-terminal pro-brain natriuretic peptide for predicting readmission after hospitalization for acute decompensated heart failure. JACC Cardiovasc Imaging. 2008;1 (5): 595-601.

Gordon EP,Schnittger I,Fitzgerald PJ, Williams P, Popp RL.Reproducibility of left ventricular volumes by two-dimensional echocardiography. J Am Coll Cardiol 1983;2(3):506-13.

Gottlieb SS, Brater DC, Thomas I, Havranek E, Bourge R, Goldman S, Dyer F, Gomez M, Bennett D, Ticho B, Beckman E, Abraham WT. BG9719 (CVT-124), an A1 adenosine receptor antagonist, protects against the decline in renal function observed with diuretic therapy. Circulation 2002; 105 (11): 1348-53.

Gottlieb SS, Dickstein K, Fleck E, Kostis J, Levine TB, LeJemtel T, DeKock M. Haemodynamic and neurohormonal effects of angiotensin II antagonist losartan in patients with congestive heart failure. Circulation 1993; 88: 1602-09.

Gottlieb SS, Skettino SL, Wolff A, Beckman E, Fisher ML, Freudenberger R, Gladwell T, Marshall J, Cines M, Bennett D, Liittschwager EB. Effects of BG9719 (CVT-124), an A1-adenosine receptor antagonist, and furosemide on glomerular filtration rate and natriuresis in patients with congestive heart failure. J Am Coll Cardiol 2000; 35 (1): 56-59.

Govender R, De Greef J, Delport R, Becker PJ, Vermaak WJ. Biological variation of ischaemia-modified albumin in healthy subjects. Cardiovasc J Afr. 2008;19(3):141-44.

Gower WR Jr, Chiou S, Skolnick KA, Vesely DL. Molecular forms of circulating atrial natriuretic peptides in human plasma and their metabolites. Peptides 1994; 15: 861-67.

Grinstead WC, Francis MJ, Marks GF, Tawa CB, Zoghbi WA, Young JB. Discontinuation of chronic diuretic therapy in stable congestive heart failure secondary to coronary artery disease or to idiopathic dilated cardiomyopathy. Am J Cardiol 1994; 73(12):881-86.

Gruson D, Rousseau MF, de Coninck V, Ahn SA, Ketelslegers JM. Influence of sampling and storage conditions on B-type natriuretic peptide immunoreactivity ofr 3 automated assays. Clin Chem 2006; 52(4): 766-67.

Gulati GL, Hyland LJ, Kocher W, Schwarting R. Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature. Arch Pathol Lab Med 2002; 126 (3): 336-42.

Gunduz A, Turkmen S, Turedi S, Mentese A, Yulug E, Ulusoy H, Karahan SC, Topbas M. Time-dependent variations in ischemia-modified albumin levels in mesenteric ischemia. Acad Emerg Med 2009; 16(6): 539-43.

Gupta S, Neyses L. Diuretic usage in heart failure: a continuing conundrum in 2005. Eur Heart J 2005; 26 (7): 644-49.

Guven S, Alver A, Mentese A, Ilhan FC, Calapoglu M, Unsal MA. The novel ischemia marker 'ischemia-modified albumin' is increased in normal pregnancies. Acta Obstet Gynecol Scand 2009; 88 (4): 479-82.

Guyton AC, Hall JE. Textbook of Medical Physiology (11th Edition). Philadelphia: Saunders; 2000.

Hakligör A, Kösem A, Seneş M, Yücel D. Effect of albumin concentration and serum matrix on ischemia-modified albumin. Clin Biochem. 2010; 43 (3): 345-48.

Hamaad A, Lip GYH, MacFadyen RJ. Acute coronary syndromes presenting solely with heart failure symptoms: are they under recognised? Eur J Heart Fail 2004; 6(6): 683-86.

Hamilton WF, Woodbury RA, Haper HT. Arterial cerebrospinal and venous pressures in man during coughing and strain. Am J Physiol 1994; 14: 42-50.

Han WK, Alinani A, Wu CL, Michaelson D, Loda M, McGovern FJ, Thadhani R, Bonventre JV. Human kidney injury molecule-1 is a tissue and urinary tumor marker of renal cell carcinoma. J Am Soc Nephrol 2005; 16 (4): 1126-34.

Hannan WJ, Cowen SJ, Plester CE, Fearon KC, deBeau A. Comparison of bio-impedance spectroscopy and multi-frequency bio-impedance analysis for the assessment of extracellular and total body water in surgical patients. Clin Sci (Lond) 1995; 89(6): 651-58.

Harada K, Shiota T, Takahashi Y, Suzuki T, Tamura M, Takada G. Right ventricular diastolic filling in the first day of life. Tohoku J Exp Med 1994; 172 (3): 227-35.

Harris EK, Brown SS. Temporal changes in the concentration of serum constituents in healthy men. Distributions of within-person variances and their relevance to the interpretation of differences between successive measurements. Ann Clin Biochem 1979; 16: 169-76.

Heilbrunn SM, Shah P, Bristow MR, Valantine HA, Ginsburg R, Fowler MB. Increased beta-receptor density and improved haemodynamic response to catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. Circulation 1989; 79: 483-90.

Henning RJ, Weil MH. Effect of afterload reduction on plasma volume during acute heart failure. Am J Cardiol 1978; 42 (5): 823-27.

Hensen J, Abraham WT, Durr J, Schrier RW. Aldosterone in congestive heart failure: analysis of determinants and role in sodium retention. Am J Nephrol 1991; 3: 721-29.

Heringlake M, Heide C, Bahlmann L, Eichler W, Pagel H, Schmucker P, Wergeland R, Armbruster FP, Klaus S. Effects of tilting and volume loading on plasma levels and urinary excretion of relaxin, NT-pro-ANP, and NT-pro-BNP in male volunteers. J Appl Physiol 2004; 97 (1): 173-79.

Herrlin B, Nyquist O, Sylven C. Induction of a reduction in haemoglobin by enalapril in stable, moderate heart failure: a double blind study. Br Heart J 1991; 66: 199-205.

Heywood JT, Fonarow GC, Costanzo MR, Mathur VS, Wigneswaran JR, Wynne J. High prevalence of renal dysfunction and its impact on outcome in 118,465 patients hospitalized with acute decompensated heart failure: a report from the ADHERE database. J Card Fail 2007; 13(6):422-30.

Hillege HL, Girbes AR, de Kam PJ, Boomsma F, de Zeeuw D, Charlesworth A, Hampton JR, van Veldhuisen DJ. Renal function, neurohormonal activation, and survival in patients with chronic heart failure. Circulation 2000; 102 (2): 203-10.

Hillis GS, Ujino K, Mulvagh SL, Hagen ME, Oh JK. Echocardiographic indices of increased left ventricular filling pressure and dilation after acute myocardial infarction. J Am Soc Echocardiogr 2006; 19 (4): 450-56.

Hirata T, Wolfe SB, Popp RL, Helman CH, Feigenbaun H. Estimation of left atrial size using ultrasound. Am Heart J 1969; 78: 43-52.

Ho KM, Power BM. Benefits and risks of furosemide in acute kidney injury. Anaesthesia. 2010; 65(3):283-93.

Hogenhuis J, Voors AA, Jaarsma T, Hillege HL, Hoes AW, van Veldhuisen DJ. Low prevalence of B-type natriuretic peptide levels < 100 pg/mL in patients with heart failure at hospital discharge. Am Heart J 2006; 151 (5): 1012.e1-5.

Holst M, Strömberg A, Lindholm M, Willenheimer R. Liberal versus restricted fluid prescription in stabilised patients with chronic heart failure: result of a randomised cross-over study of the effects on health-related quality of life, physical capacity, thirst and morbidity. Scand Cardiovasc J 2008 ; 42 (5): 316-22.

Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. Circulation 2003 108 (7): 833-38.

Hurrell DG, Nishimura RA, Ilstrup DM, Appleton CP. Utility of preload alteration in assessment of left ventricular filling pressure by Doppler echocardiography: a simultaneous catheterization and Doppler echocardiographic study. J Am Coll Cardiol 1997;30(2):459-67.

Iwanaga Y, Nishi I, Furuichi S, Noguchi T, Sase K, Kihara Y, Goto Y, Nonogi H. B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure: comparison between systolic and diastolic heart failure.J Am Coll Cardiol 2006;47(4):742-48.

International Committee for Standardisation in Haematology: Recommended methods for measurements of red-cell and plasma volume. J Nucl Med 1980; 21: 793-800.

James KB, McCarthy PM, Jaalouk S, Bravo EL, Betkowski A, Thomas JD, Nakatani S, Fouad-Tarazi FM. Plasma volume and its regulatory factors in congestive heart failure after implantation of long-term left ventricular assist devices. Circulation 1996; 93 (8):1515-19.

James KB, Troughton RW, Feldschuh J, soltis D, Thomas D, Fouad-Tarazi F. Blood volume and brain natriuretic peptide in congestive heart failure: a pilot study. Am Heart J 2005; 150 (5): 984.

Januzzi JL Jr, Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, Tung R, Cameron R, Nagurney JT, Chae CU, Lloyd-Jones DM, Brown DF, Foran-Melanson S, Sluss PM, Lee-Lewandrowski E, Lewandrowski KB. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005; 95(8):948-54.

Jentzer JC, DeWald TA, Hernandez AF. Combination of loop diuretics with thiazide-type diuretics in heart failure. J Am Coll Cardiol 2010; 56 (19):1527-34

Jewkes C, Sear JW, Verhoeff F, Sanders DJ, Foëx P. Non-invasive measurement of cardiac output by thoracic electrical bioimpedance: a study of reproducibility and comparison with thermodilution. Br J Anaesth 1991; 67 (6): 788-94.

Jordan J, Shannon JR, Black BK, Ali Y, Farley M, Costa F, Diedrich A, Robertson RM, Biaggioni I, Robertson D. The pressor response to water drinking in humans: a sympathetic reflex? Circulation 2000; 101: 504-09.

Jourdain P, Jondeau G, Funck F, Gueffet P, Le Helloco A, Donal E, Aupetit JF, Aumont MC, Galinier M, Eicher JC, Cohen-Solal A, Juillière Y. Plasma brain natriuretic peptide-guided therapy to improve outcome in heart failure: the STARS-BNP Multicenter Study. J Am Coll Cardiol 200; 49(16):1733-39.

Jugdutt BI. Nitrates in myocardial infarction. Cardiovasc Drugs Ther 1994; 8: 635-46

Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, Duarte MM, Moresco RN. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. Clin Biochem 2010; 43(4-5): 450-54.

Kalra PR, Anagnostopoulos C, Bolger AP, Coats AJ, Anker SD. The regulation and measurement of plasma volume in heart failure. J Am Coll Cardiol 2002;39(12):1901-08.

Kamath SA, Drazner MH, Tasissa G, Rogers JG, Stevenson LW, Yancy CW. Correlation of impedance cardiography with invasive hemodynamic measurements in patients with advanced heart failure: the BioImpedance CardioGraphy (BIG) substudy of the Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness (ESCAPE) Trial. Am Heart J 2009; 158 (2): 217-23.

Kato M,Kinugawa T, Omodani H, Osaki S, Ahmmed GU, Ogino K, Hisatome I, Miyakoda H, Thames M. Responses of plasma norepinephrine and renin-angiotensin-aldosterone system to dynamic exercise in patients with congestive heart failure. J Car Fail 1996; 2 (2): 103-09.

Kelley WE, Januzzi JL, Christenson RH. Increases in cardiac troponin in conditions other than acute coronary syndrome and heart failure. Clin Chem 2009; 55 (12): 2098-2112.

Khan S, Bess RL, Rosman HS, Nordstrom CK, Cohen GI, Gardin JM. Which echocardiographic Doppler left ventricular diastolic function measurements are most feasible in the echocardiographic laboratory? Am J Cardiol 2004; 94: 1099-1101.

Kim WJ, Laterza OF, Hock KG, Pierson-Perry JF, Kaminski DM, Mesguich M,Braconnier F, Zimmermann R, Zaninotto M, Plebani M, Hanna A, Cembrowski GS, Scott MG. Performance of a revised cardiac troponin method that minimizes interferences from heterophilic antibodies. Clin Chem 2002; 48 (7):1028-34.

Kimura T, Yamamoto T, Ohta M, Ota K, Shoji M, Funyu T, Mori T, Sahata T, Omata K, Abe K. Impaired vasopressin suppression and enhanced atrial natriuetic hormone release following an acute water load in primary aldosteronism. Eur J Endocrinol 1997; 137: 154-61.

Kinet JP, Soyeur D, Balland N, Saint-Remy M, Collignon P, Godon JP. Hemodynamic study of hypotension during hemodialysis. Kidney Int 1982; 21 (6): 868-76.

Kjaer A, Appel J, Hildebrandt P, Petersen CL. Basal and exercise-induced neurodendocrine activation in paitents with heart failure and in normal subjects. Eur J Heart Fail 2005; 6:29-39.

Kjellström B, Igel D, Abraham J, Bennett T, Bourge R. Trans-telephonic monitoring of continuous haemodynamic measurements in heart failure patients. J Telemed Telecare 2005; 11(5): 240-44.

Kos T, Pacher R, Wimmer A, Bojic A, Hülsmann M, Frey B, Mayer G, Yilmaz N, Skvarilova L, Spinar J, Vitovec J, Toman J, Woloszcuk W, Stanek B. Relationship between kidney function, hemodynamic variables and circulating big endothelin levels in patients with severe refractory heart failure. Wien Klin Wochenschr 1998; 11(3): 89-95.

Kosiborod M, Curtis JP, Wang Y, Smith GL, Masoudi FA, Foody JM, Havranek EP,Krumholz HM. Anemia and outcomes in patients with heart failure: a study from the National Heart Care Project. Arch Intern Med 2005; 165 (19): 2237-44.

Kroll MH, Srisawasdi P. The clearance of BNP modeled using the NT-proBNP-BNP relationship. Biosystems 2007; 88 (1-2): 147-55.

Krum H, Carson P, Farsang C, Maggioni AP, Glazer RD, Aknay N, Chiang YT, Cohn JN. Effect of valsartan added to background ACE inhibitor therapy in patients with heart failure: results from Val-HeFT. Eur J Heart Fail 2004; 6(7):937-45.

Kuecherer HF, Muhiudeen IA, Kusumoto FM, Lee E, Moulinier LE, Cahalan MK,

Schiller NB. Estimation of mean left atrial pressure from transesophageal pulsed Doppler echocardiography of pulmonary venous flow. Circulation 1990; 82(4):1127-39.

Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, Heitmann BL, Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C; Composition of the ESPEN Working Group. Bioelectrical impedance analysis--part I: review of principles and methods. Clin Nutr 2004; 23 (5): 1226-43.

Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise J, Solomon S, Spencer KT, St John Sutton M,Stewart W; American Society of Echocardiography's Nomenclature and Standards Committee; Task Force on Chamber Quantification; American College of Cardiology Echocardiography Committee; American Heart Association; European Association of Echocardiography, European Society of Cardiology. Recommendations for chamber quantification. Eur J Echocardiogr 2006;7(2):79-108.

Lang CC, Choy AM,Turner K,Tobin R,Coutie W, truther AD.The effect of intravenous saline loading on plasma levels of brain natriuretic peptide in man. J Hypertens 1993;11(7): 737-41.

Larsen AI, Dickstein K, Ahmadi NS, Aarsland T, Kvaløy JT, Hall C. The effect of altering haemodynamics on the plasma concentrations of natriuretic peptides in heart failure. Eur J Heart Fail 2006; 8(6): 628-33.

Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, Angelici L, Barlera S, Parrinello G, Maggioni AP, Tognoni G, Cohn JN; Val-HeFT Investigators. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation 2007; 116 (11): 1242-49.

Latini R, Masson S, Anand I, Salio M, Hester A, Judd D, Barlera S, Maggioni AP, Tognoni G, Cohn JN; Val-HeFT Investigators. The comparative prognostic value of plasma neurohormones at baseline in patients with heart failure enrolled in Val-HeFT. Eur Heart J 2004; 25 (4): 292-99.

La Vecchia L, Mezzena G, Ometto R, Finocchi G, Bedogni F, Soffiati G, Vincenzi M. Detectable serum troponin I in patients with heart failure of nonmyocardial ischemic origin. Am J Cardiol 1997; 80 (1): 88-90.

Leclercq C, Walker S, Linde C, Clementy J, Marshall AJ, Ritter P, Djiane P, Mabo P, Levy T, Gadler F, Bailleul C, Daubert JC. Comparative effects of permanent biventricular and right-univentricular pacing in heart failure patients with chronic atrial fibrillation. Eur Heart J 2002; 23(22):1780-87.

Lefevre GF. Analytical performance of the ACB test for ishaemia on the Konealb 20. Clin Chem 2003; 49 (Suppl 6): A 33.

Lewin J, Ledwidge M, O'Loughlin C, McNally C, McDonald K. Clinical deterioration in established heart failure: what is the value of BNP and weight gain in aiding diagnosis? Eur J Heart Fail 2005; 7 (6): 953-37.

Lewis SM, Bain BJ, Bates I, Editors. Dacie and Lewis Practical Haematology (9th Edition). London: Churchill Livingstone; 2001.

Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007; 18 (3): 904-12.

Light DB, Schweibert EM, Karlson KH, Stanton BA. Atrial natriuretic peptide inhibits a cation channel in renal inner medullary collecting duct cells. Science 1989; 243: 383-85.

Lim HS, Kang SJ, Choi JH, Ahn SG, Choi BJ, Choi SY, Yoon MH, Hwang GS, Tahk SJ, Shin JH. Is E/E' reliable in patients with regional wall motion abnormalities to estimate left ventricular filling pressure? Int J Cardiovasc Imaging 2009; 25 (1): 33-39.

Little WC, Barr WK, Crawford MH. Altered effect of the Valsalva manoeuvre on left ventricular volume in patients with cardiomyopathy. Circulation 1985; 71(2): 227-33.

Lobo DN, Stanga Z, Aloysius MM, Wicks C, Nunes QM, Ingram KL, Risch L, Allison SP. Effect of volume loading with 1 liter intravenous infusions of 0.9% saline, 4% succinylated gelatine (Gelofusine) and 6% hydroxyethyl starch (Voluven) on blood volume and endocrine responses: a randomized, three-way crossover study in healthy volunteers. Crit Care Med 2010; 38(2):464-70.

Lobo DN, Stanga Z, Simpson JA, Anderson JA, Rowlands BJ, Allison SP. Dilution and redistribution effects of rapid 2-litre infusions of 0.9% (w/v) saline and 5% (w/v) dextrose on haematological parameters and serum biochemistry in normal subjects: a double-blind crossover study. Clin Sci (Lond) 2001; 101 (2): 173-79.

Logeart D,Saudubray C,Beyne P,Thabut G,Ennezat PV,Chavelas C,Zanker C,Bouvier E,Solal AC. Comparative value of Doppler echocardiography and B-type natriuretic peptide assay in the etiologic diagnosis of acute dyspnea. J Am Coll Cardiol 2002;40(10):1794-800.

Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, Asjo B, Malamba S, Downing R. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol 2004; 11(1): 29-34.

MacFadyen RJ. Intraindividual temporal variance of biomarkers and the definition of individualised prognosis in cardiovascular patients. J Hum Hypertens 2006; 20: 383-86.

MacFadyen RJ, Barr CS, Struthers AD. Aldosterone blockade reduces vascular collagen turnover, improves heart rate variability and reduces early morning rise in heart rate in heart failure patients. Cardiovas Res 1997; 35(1): 30-34.

MacFadyen RJ, Gorski JC, Brater DC, Struthers AD. Furosemide responsiveness, non-adherence and resistance during the chronic treatment of heart failure: a longitudinal study. Br J Clin Pharmacol 2003; 57(5): 622-31.

MacFadyen RJ, Lee A Morton, JJ Pringle, SD Struthers AD. How often are angiotensin II and aldosterone concentration raised during chronic ACE inhibitor treatment in cardiac failure. Heart 1999; 82(1): 57-61.

Maeda K, Tsutamoto T, Wada A, Hisanga T, Kinoshita M. Plasma brain natriuretic peptide as a biochemical marker of high left ventricular end-diastolic pressure in patients with symptomatic left ventricular dysfunction. Am Heart J 1998; 135: 825-32.

Magalski A, Adamson P, Gadler F, Böehm M, Steinhaus D, Reynolds D, Vlach K, Linde C, Cremers B, Sparks B, Bennett T. Continuous ambulatory right heart pressure measurements with an implantable hemodynamic monitor: a multicenter,12-month follow-up study of patients with chronic heart failure. J Card Fail 2002; 8 (2): 63-70.

Mair J, Falkensammer G, Poelzl G, Hammerer-Lercher A, Griesmacher A, Pachinger O. B-type natriuretic peptide (BNP) is more sensitive to rapid hemodynamic changes in acute heart failure than N-terminal proBNP. Clin Chim Acta 2007; 379 (1-2): 163-66.

Maisel A, Hollander JE, Guss D, McCullough P, Nowak R, Green G, Saltzberg M, Ellison SR, Bhalla MA, Bhalla V, Clopton P, Jesse R. Primary results of the Rapid Emergency Department Heart Failure Outpatient Trial (REDHOT). A multicenter study of B-type natriuretic peptide levels, emergency department decision making, and outcomes in patients presenting with shortness of breath. J Am Coll Cardiol 2004; 44 (6): 1328-33.

Maisel AS, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AH, Clopton P, Steg PG, Westheim A, Knudsen CW, Perez A, Kazanegra R, Herrmann HC, McCullough PA; Breathing Not Properly Multinational Study Investigators. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002; 347 (3): 161-67.

Maisel A, Mueller C, Adams K Jr, Anker SD, Aspromonte N, Cleland JG, Cohen-Solal A, Dahlstrom U, DeMaria A, Di Somma S, Filippatos GS, Fonarow GC, Jourdain P, Komajda M, Liu PP, McDonagh T, McDonald K, Mebazaa A, Nieminen MS, Peacock WF, Tubaro M, Valle R, Vanderhyden M, Yancy CW, Zannad F, Braunwald E. State of the art: using natriuretic peptide levels in clinical practice. Eur J Heart Fail 2008; 10 (9): 824-39.

Malino T. Novel risk markers and clinical practice. N Engl J Med 2003; 349: 1587-89.

Mansencal N, d'Allones LR, Beauchet A, Fabre S, Digne F, Farcot JC, Joseph T, Dubourg O. Reliability of echocardiography for haemodynamic assessment of end-stage heart failure. Am J Cardiol 2007; 100: 998-1001.

Marcus GM, Michaels AD, De Marco T, McCulloch CE, Chatterjee K. Usefulness of the third heart sound in predicting an elevated level of B-type natriuretic peptide. Am J Cardiol 2004; 93 (10): 1312-13.

Marenzi G, Grazi M, Susini G, Lauri G, Assanelli E, Guazzi M, Agostoni P. Intra- and extravascular volumes in congestive heart failure and their redistribution following extracorporeal ultrafiltration. Cardiologia 1998; 43 (11): 1193-200.

Marenzi G, Lauri G, Grazi M, Assanelli E, Campodonico J, Agostoni P. Circulatory response to fluid overload removal by extracorporeal ultrafiltration in refractory congestive heart failure. J Am Coll Cardiol 2001; 38 (4): 963-68.

Marin-Grez M, Fleming JT, Steinhausen M. Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. Nature 1986; 324: 473-74.

Massie BM, O'Connor CM, Metra M, Ponikowski P, Teerlink JR, Cotter G, Weatherley BD, Cleland JG, Givertz MM, Voors A, DeLucca P, Mansoor GA, Salerno CM, Bloomfield DM, Dittrich HC; PROTECT Investigators and Committees. Rolofylline, an adenosine A1-receptor antagonist, in acute heart failure. N Engl J Med 2010; 363 (15): 1419-28.

Matsumori A, Kawai C, Yamada T, Ohkusa T, Morishima S, Tamaki N, Watanabe Y, Yonekura Y, Endo K, Konishi J. Mechanism and significance of myocardial uptake of antimyosin antibody in myocarditis and cardiomyopathy: clinical and experimental studies. Clincial Immunology and Immunopathology 1993; 68: 215-219.

Matsusaka T, Ichikawa I. Biological functions of angiotensin and its receptors. Annu Rev Physiol 1997; 59: 395-412.

McDonagh TA,Gardner RS,Chong KS,Dargie HJ.Can we use B-type natriuretic peptides to monitor patients with heart failure?Biomark Med 2007;1(3):349-53.

McDonagh TA, Holmer S, Raymond I, Luchner A, Hildebrant P, Dargie HJ. NT-proBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. Eur J Heart Fail 2004; 6 (3): 269-73.

McDowell G, Maguire S, Shaw C, Nicholls DP, Hall C. Variability of NT-proANP and C-ANP. Eur J Clin Invest 2002; 32 (8): 545-48.

McIntyre KM, Vita JA, Lambrew CT, Freeman J, Loscalzo J. A noninvasive method of predicting pulmonary-capillary wedge pressure. N Engl J Med 1992 ; 327 (24): 1715-20.

McMurray JJ, Ostergren J, Swedberg K, Granger CB, Held P, Michelson EL,Olofsson B, Yusuf S, Pfeffer MA. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function taking angiotensin-converting-enzyme inhibitors: the CHARM-Added trial. Lancet 2003; 362(9386):767-71.

McMurray JJ, Teerlink JR, Cotter G, Bourge RC, Cleland JG, Jondeau G, Krum H, Metra M, O'Connor CM, Parker JD, Torre-Amione G, van Veldhuisen DJ, Lewsey J,Frey A, Rainisio M, Kobrin I. Effects of tezosentan on symptoms and clinical outcomes in patients with acute heart failure: the VERITAS randomized controlled trials. JAMA 2007; 298 (17): 2009-19.

MERIT-HF study group. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CRXL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 1999; 353: 2001-07.

Metra M, Nodari S, Parrinello G, Specchia C, Brentana L, Rocca P, Fracassi F, Bordonali T, Milani P, Danesi R, Verzura G, Chiari E, Dei Cas L. The role of plasma biomarkers in acute heart failure. Serial changes and independent prognostic value of NT-proBNP and cardiac troponin-T. Eur J Heart Fail 2007; 9 (8): 776-86.

Metra M, Nodari S, Parrinello G, Bordonali T, Bugatti S, Danesi R, Fontanella B, Lombardi C, Milani P, Verzura G, Cotter G, Dittrich H, Massie BM, Dei Cas L. Worsening renal function in patients hospitalised for acute heart failure: clinical implications and prognostic significance. Eur J Heart Fail 2008; 10 (2): 188-95.

Metra M, Teerlink JR, Voors AA, Felker GM, Milo-Cotter O, Weatherley B,Dittrich H, Cotter G. Vasodilators in the treatment of acute heart failure: what we know, what we don't. Heart Fail Rev 2009; 14 (4): 299-307.

Michalsen A, Konig G, Thimme W. Preventable causative factors leading to hospital admission with decompensated heart failure. Heart 1998; 80: 437-41.

Miller WL, Hartman KA, Burritt MF, Grill DE, Rodeheffer RJ, Burnett JC Jr, Jaffe AS. Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time. Circulation 2007; 116 (3): 249-57.

Miller WL, Hartman KA, Burritt MF, Grill DE, Jaffe AS. Profiles of serial changes in cardiac troponin T concentrations and outcome in ambulatory patients with chronic heart failure. J Am Coll Cardiol. 2009 Oct 27;54(18):1715-21.

Milliez P, Thomas O, Haggui A, Schurando P, Squara P, Cohen-Solal A, Mebazaa A, Leenhardt A. Cardiac resynchronisation as a rescue therapy in patients with catecholamine-dependent overt heart failure: results from a short and mid-term study. Eur J Heart Fail 2008; 10 (3): 291-97.

Milzman D,Napoli A,Hogan C,Zlidenny A,Janchar T.Thoracic impedance vs chest radiograph to diagnose acute pulmonary edema in the ED. Am J Emerg Med 2009; 27(7):770-5.

Mishra J, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery.Lancet 2005;365(9466):1231-38.

Moe GW, Canepa-Anson R, Howard RJ, Armstrong PW. Sequential changes in atrial pressures, dimensions, and plasma atrial natiuretic factor concentrations during volume loading in haemodynamically normal human subjects. J Lab Clin Med 1991; 117(6): 528-34.

Moe GW, Howlett J, Januzzi JL, Zowall H. N-terminal pro-B-type natriuretic peptide testing improves the management of patients with suspected acute heart failure: primary results of the

Canadian prospective randomized multicenter IMPROVE-CHF study. Circulation 2007; 115 (24): 3103-10.

Moreno FLL, Hagan A, Holmen JR, Pyror A, Strickland RD, Castle cH. Evaluation of size and duynamics of the inferior vena cava as an index of right-sided cardiac function. Am J Cardiol 1984; 53: 579-85.

Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. Circulation 2007; 115 (8): 949-52.

Moss AJ, Hall WJ, Cannom DS, Klein H, Brown MW, Daubert JP, Estes NA 3rd, Foster E, Greenberg H, Higgins SL, Pfeffer MA, Solomon SD, Wilber D, Zareba W. Cardiac-resynchronization therapy for the prevention of heart-failure events. N Engl J Med 2009; 361 (14): 1329-38.

Mueller T, Gegenhuber A, Dieplinger B. Long-term stability of endogenous B-type natiuretic peptide (BNP) and amino terminal proBNP (NT-proBNP) in frozen plasma samples. Clin Chem Lab Med 2004; 42: 942-44.

Mueller C, Laule-Kilian K, Schindler C, Klima T, Frana B, Rodriguez D, Scholer A, Christ M, Perruchoud AP. Cost-effectiveness of B-type natriuretic peptide testing in patients with acute dyspnea. Arch Intern Med 2006; 166 (10): 1081-87.

Mueller C, Scholer A, Laule-Kilian K, Martina B, Schindler C, Buser P, Pfisterer M, Perruchoud AP. Use of B-type natriuretic peptide in the evaluation and management of acute dyspnea. N Engl J Med 2004; 350 (7): 647-54.

Mullens W, Abrahams Z, Francis GS, Sokos G, Taylor DO, Starling RC, Young JB, Tang WH. Importance of venous congestion for worsening of renal function in advanced decompensated heart failure. J Am Coll Cardiol 2009; 53 (7): 589-96.

Mullens W, Abrahams Z, Francis GS, Taylor DO, Starling RC, Tang WH. Prompt reduction in intra-abdominal pressure following large-volume mechanical fluid removal improves renal insufficiency in refractory decompensated heart failure. J Card Fail 2008; 14 (6): 508-14.

Mullens W, Abrahams Z, Skouri HN, Francis GS, Taylor DO, Starling RC, Paganini E, Tang WH. Elevated intra-abdominal pressure in acute decompensated heart failure: a potential contributor to worsening renal function? J Am Coll Cardiol 2008; 51 (3): 300-06.

Mullens W, Borowski AG, Curtin RJ, Thomas JD, Tang WH. Tissue Doppler imaging in the estimation of intracardiac filling pressure in decompensated patients with advanced systolic heart failure. Circulation 2009; 119 (1): 62-70.

Muscari A, Berzigotti A, Bianchi G, Giannoni C, Ligabue A, Magalotti D, Sbano D, Zacchini A, Zoli M. Non-cardiac determinants of NT-proBNP levels in the elderly: relevance of haematocrit and hepatic steatosis. Eur J Heart Fail 2006; 8 (5): 468-76.

NACB Writing Group, Wu AH, Jaffe AS, Apple FS, Jesse RL, Francis GL, Morrow DA, Newby LK, Ravkilde J, Tang WH, Christenson RH, Cannon CP, Storrow AB. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: use of cardiac troponin and B-type natriuretic peptide or N-terminal proB-type natriuretic peptide for etiologies other than acute coronary syndromes and heart failure. Clin Chem 2007; 53 (12): 2086-96.

Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962; 51: 224-32.

Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quinones MA. Doppler tissue imaging: a nonivasive technique for evaluation of left ventricular relaxation and estimation of filling pressure. J Am Coll Cardiol 1997; 30 (6): 1527-33.

Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. J Am Soc Nephrol 2006; 17 (1): 17-25

Nath J, Vacek JL, Heidenreich PA. A dilated inferior vena cava is a marker of poor survival. Am Heart J 2006; 151 (3): 730-35.

Natori H, Tamaki S, Kira S. Ultrasonographic evaluation of ventilatory effect on inferior vena caval configuration. American Review of Respiratroy Disease 1979; 120 (2): 421-27.

Nelson GI, Silke B, Ahuja RC, Hussain M, Taylor SH. Haemodynamic advantages of isosorbide dinitrate over furosemide in acute heart failure following myocardial infarction. Lancet 1983; 1(8327): 730-33.

Neuberg GW,Kukin ML,Penn J,Medina N,Yushak M,Packer M.Haemodynamic effects of renin inhibition by enalkiren in chronic congestive heart failure. Am J Cardiol 1991;67:63-66.

Neuberg GW, Miller AB, O'Connor CM, Belkin RN, Carson PE, Cropp AB, Frid DJ, Nye RG, Pressler ML, Wertheimer JH, Packer M; PRAISE Investigators. Prospective Randomized Amlodipine Survival Evaluation. Diuretic resistance predicts mortality in patients with advanced heart failure. Am Heart J 2002; 144: 31-38.

Ng HW, Coleman N, Walley TJ, Mostafa SM, Breckenridge AM. Reproducibility and comparison of cardiac output measurement by transthoracic bioimpedance and thermodilution methods in critically ill patients. Clin Intensive Care 1993; 4 (5): 217-21.

Ng Kam Chuen MJNK, MacFadyen RJ. Variable aetiologies contributing to the anaemia of systolic heart failure are important to individual patient management. Eur Heart J 2007; 28 (2): 143-45.

Nieminen MS, Brutsaert D, Dickstein K, Drexler H, Follath F, Harjola VP,Hochadel M, Komajda M, Lassus J, Lopez-Sendon JL, Ponikowski P, Tavazzi L. EuroHeart Failure Survey II (EHFS II): a survey on hospitalized acute heart failure patients: description of population. Eur Heart J 2006; 27 (22): 2725-36.

Nishimura RA, Appleton CP, Redfield MM,Ilstrup DM,Holmes DR Jr,Tajik AJ. Noninvasive Doppler echocardiographic evaluation of left ventricular filling pressures in patients with cardiomyopathies: a simultaneous Doppler echocardiographic and cardiac catherterisation study. J Am Coll Cardiol 1996; 28 (5): 1226-33.

Noble TJ, Harris ND, Morice AH, Milnes P, Brown BH. Diuretic induced change in lung water assessed by electrical impedance tomography. Physiol Meas 2000; 21 (1): 155-63.

Noble TJ,Morice AH,Channer KS,Milnes P,Harris ND,Brown BH. Monitoring patients with left ventricular failure by electrical impedance tomography.Eur J Heart Fail 1999; (4):379-84.

Nohria A, Hasselblad V, Stebbins A, Pauly DF, Fonarow GC, Shah M, Yancy CW, Califf RM, Stevenson LW, Hill JA. Cardiorenal interactions: insights from the ESCAPE trial. J Am Coll Cardiol 2008; 51 (13): 1268-74.

Ogawa T, Linz W, Stevenson M, et al. Evidence for load-dependent and load-independent determinants of cardiac natriuretic peptide production. Circulation 1996, 93: 2059-67

O'Hanlon R, O'Shea P, Ledwidge M, O'Loughlin C, Lange S, Conlon C, Phelan D, Cunningham S, McDonald K. The biologic variability of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in stable heart failure. J Cardiac Fail 2007; 13: 50-55.

Ohlsson A, Bennett T, Nordlander R, Rydén J, Astrom J, Rydén L. Monitoring of pulmonary arterial diastolic pressure through a right ventricular pressure transducer. J Card Fail 1995;1:161– 8.

Omar F, van der Watt GF, Pillay TS. Reference change values: how useful are they? J Clin Pathol 2008; 61 (4): 426-27

O'Neill JO, Bott-Silverman CE, McRae AT 3rd, Troughton RW, Ng K, Starling RC,Young JB. B-type natriuretic peptide levels are not a surrogate marker for invasive hemodynamics during management of patients with severe heart failure.Am Heart J 2005; 149(2):363-69.

O'Neill JO, Bott-Silverman CE, McRae AT 3rd, Troughton RW, Ng K, Starling RC, Young JB. B-type natriuretic peptide levels are not a surrogate marker for invasive hemodynamics during management of patients with severe heart failure.Am Heart J 2005; 149 (2): 363-69.

Oohashi S, Endoh H. Does central venous pressure or pulmonary capillary wedge pressure frefelct the status of circualtiong blood volume in patients after extended oesophagectomy. J Anaesth 2005; 19(1): 21-25.

Packer M. The role of vasodilator therapy in the treatment of severe chronic heart failure. Drugs 1986; 32 (Suppl 5): 13-26.

Packer M, Abraham WT, Mehra MR, Yancy CW, Lawless CE, Mitchell JE, Smart FW, Bijou R, O'Connor CM, Massie BM, Pina IL, Greenberg BH, Young JB, Fishbein DP, Hauptman PJ, Bourge RC, Strobeck JE, Murali S, Schocken D, Teerlink JR, Levy WC, Trupp RJ, Silver MA. Utility of impedance cardiography for the identification of short-term risk of clinical

decompensation in stable patients with chronic heart failure. J Am Coll Cardiol 2006; 47 (11):2245-52.

Parisi AF, Harrington JJ, Askenazi J, Pratt RC, McIntyre KM. Echocardiographic evaluation of the Valsalva Maneuver in healthy subjects and patients with and without heart failure. Circulation 1976; 54 (6): 921-27.

Parmley WW.Pathophysiology of congestive heart failure.Clin Cardiol 1992;15 Suppl :I5-12.

Paterna S, Di Pasquale P, Parrinello G, Fornaciari E, Di Gaudio F, Fasullo S, Giammanco M, Sarullo FM, Licata G. Changes in brain natriuretic peptide levels and bioelectrical impedance measurements after treatment with high-dose furosemide and hypertonic saline solution versus high-dose furosemide alone in refractory congestive heart failure: a double-blind study. J Am Coll Cardiol 2005; 45 (12): 1997-2003.

Paterna S, Gaspare P, Fasullo S, Sarullos FM, Di Pasquale P. Normal-sodium diet compared with low-sodium diet in compensated congestive heart failure: is sodium an old enemy or a new friend? Clin Sci 2008; 114: 221-30.

Paterna S, Parrinello G, Cannizarro S, Fasullo S, Torres D, Sarullo FM, Di Pasquale P. Medium term effects of different dosage of diuretic, sodium, and fluid administration on neurohormal and clinical outcome in patients with recently compensated heart failure. Am J Cardiol 2009; 103 (1): 93-102.

Peacock WF, Albert NM, Kies P, White RD, Emerman CL. Bioimpedance monitoring: better than chest x-ray for predicting abnormal pulmonary fluid? Congest Heart Fail 2000; 6(2):86-89.

Peacock WF, Costanzo MR, De Marco T, Lopatin M, Wynne J, Mills RM, Emerman CL. Impact of intravenous loop diuretics on outcomes of patients hospitalized with acute decompensated heart failure: insights from the ADHERE registry. Cardiology 2009;113(1):12-9.

Peacock WF, De Marco T, Fonarow GC, Diercks D, Wynne J, Apple FS, Wu AH; ADHERE Investigators. Cardiac troponin and outcome in acute heart failure. N Engl J Med 2008; 358 (20): 2117-26.

Pedersen EB, Pedersen HB, Jensen KT. Pulsatile secretion of atrial natriuretic peptide and brain natriuretic peptide ain healthy humans. Clin Sci 1999; 97: 201-06.

Pfeffer MA, Frohlich ED. Improvements in clinical outcomes with the use of angiotensin-converting enzyme inhibitors: cross-fertilisation between clinical and basic investigation. Am J Physiol Heart Cir Physiol 2006; 291: H2021-25.

Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Køber L, Maggioni AP, Solomon SD, Swedberg K, Van de Werf F, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM. Valsartan, captopril or both in myocardial infarction complicated by heart failure, left ventricular dysfunction or both. N Engl J Med 2003; 349: 1893-1906.

Pfeffer JM, Pfeffer MA, Braunwald E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. Circ Res 1985; 57: 84-95.

Pfisterer M, Buser P, Rickli H, Gutmann M, Erne P, Rickenbacher P, Vuillomenet A, Jeker U, Dubach P, Beer H, Yoon SI, Suter T, Osterhues HH, Schieber MM, Hilti P, Schindler R, Brunner-La Rocca HP. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. JAMA 2009; 301 (4): 383-92.

Pickkers P, Dormans TP, Russel FG, Hughes AD, Thien T, Schaper N, Smits P. Direct vascular effects of furosemide in humans. Circulation 1997; 96 (6): 1847-52.

Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003; 348(14):1309-21.

Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999; 341(10):709-17.

Pozzoli M. Non-invasive evaluation of the haemodynamic profile in patients with heart failure: estimation of left atrial pressure. Ital Heart J Suppl 2000; 1(10): 1326-33.

Pritchett AM, Jacobsen SJ, Mahoney DW, Rodcheffer RJ, Bailey KR, Redfield MM. Left atrial volume as an index of left atrial size: a population based study. J Am Coll Cardiol 2003; 41: 1036-43.

Publication Committee for the VMAC Investigators (Vasodilatation in the Management of Acute CHF). Intravenous nesiritide vs nitroglycerin for treatment of decompensated congestive heart failure: a randomized controlled trial. JAMA 2002; 287 (12): 1531-40.

Quiles J, Roy D, Gaze D, Garrido IP, Avanzas P, Sinha M, Kaski JC. Relation of ischaemia modified albumin levels following elective angioplasty for stable angina pectoris to duration of ballon-induced myocardial ischaemia. Am J Cardiol 2003; 92: 322-24.

Quinones Ma, Otto CM, Stoddard M, Waggoner A, Zoghbi WA. Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. Recommendations for quantification of Doppler echocardiography: A report from the Doppler Quantification Task Force of the Nomenclature and Standards Committee of the American Society of Echocardiography. J Am Soc Echocardiog 2002; 15: 167-84.

Reid F, Lobo DN, Williams RN, Rowlands BJ, Allison SP. (Ab)normal saline and physiological Hartmann's solution: a randomized double-blind crossover study. Clin Sci (Lond) 2003; 104 (1): 17-24.

Rein AJ, Lewis N, Forst L, Gotsman MS, Lewis BS. Echocardiography of the inferior vena cava in healthy subjects and in patients with cardiac disease. Isr J Med Sci 1982;18(5):581-85.

Reis FJ, Fernandes AM, Bitencourt AG, Neves FB, Kuwano AY, França VH, Macedo CR, Cruz CG, Sahade V, Aras Júnior R. Prevalence of anemia and renal insufficiency in non-hospitalized patients with heart failure. Arq Bras Cardiol 2009; 93(3):268-74.

Remme WJ. Congestive heart failure-pathophysiology and medical treatment. J Cardiovasc Pharmacol 1986; 8 Suppl 1: S36-52.

Renbourn ET. Variation, diurnal and over longer periods of time, in blood haemoglobin, haematocrit, plasma protein, erythrocyte sedimentation rate, and blood chloride. J Hyg (Lond). 1947; 45 (4): 455-467.

RESOLVD Study. Comparison of candesartan, enalapril, and their combination in congestive heart failure. Randomised Evaluation of Strategies for Left Ventricular Dysfunction. Circulation 1999; 100: 1056-64.

Reynolds DW, Bartelt N, Taepke R, Bennett TD. Measurement of pulmonary artery diastolic pressure from the right ventricle. J Am Coll Cardiol 1995; 25: 1176–82.

Richardson A, Scriven AJ, Poole-Wilson AP, Balyliss J, Parameshwar J, Sutton GC. Double-blind comparison of captopril alone against frusemide plus amiloride in mild heart failure. Lancet 1987; 2 (8561): 709-11.

Ricós C, Iglesias N, García-Lario JV, Simón M, Cava F, Hernández A, Perich C, Minchinela J, Alvarez V, Doménech MV, Jiménez CV, Biosca C, Tena R. Within-subject biological variation in disease: collated data and clinical consequences. Ann Clin Biochem 2007;44(Pt 4):343-52.

Rijsterborgh H, Mayala A, Forster T, Vletter W, van der Borden B, Sutherland GR, Roelandt J. The reproducibility of continuous wave Doppler measurements in the assessment of mitral stenosis or mitral prosthetic function: the relative contributions of heart rate, respiration, observer variability and their clinical relevance. Eur Heart J 1990; 11 (7): 592-600.

Rivas-Gotz C, Manolios M, Thohan V, Nagueh SF. Impact of left ventricular ejection fraction on estimation of left ventricular filling pressures using tissue Doppler and flow propagation velocity. Am J Cardiol 2003; 91 (6): 780-84.

Robertson D, Stevens RM, Friesinger GC, Oates JA. The effect of the Valsalva manoeuvre on echocardiographic dimensions in man. Circulation 1977; 55(4): 296-62.

Rodeheffer RJ, Miller WL, Burnett Jr JC. Atrial natriuretic factor release is augmented by leg elevation in human subjects with congestive heart failure. J Am Coll Cardiol 1991; 17: 316a.

Rohde LE, Furian T, Campos C, Biolo A, Rabelo E, Foppa M, Clausell N. Implications of the hemodynamic optimization approach guided by right heart catheterization in patients with severe heart failure. Arq Bras Cardiol 2002; 78 (3): 261-66.

Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. J Am Coll Cardiol 2008; 52 (19): 1527-39.

Rossi J, Bayram M, Udelson JE, Lloyd-Jones D, Adams KF, Oconnor CM, Stough WG, Ouyang J, Shin DD, Orlandi C, Gheorghiade M. Improvement in hyponatremia during hospitalization for worsening heart failure is associated with improved outcomes: insights from the Acute and Chronic Therapeutic Impact of a Vasopressin Antagonist in Chronic Heart Failure (ACTIV in CHF) trial. Acute Card Care. 2007; 9 (2): 82-86.

Rossvoll O, Hatle LK. Pulmonary venous folw velocities recorded by tranthoracic Doppler ultrasound: relation to left ventricular diastoclic pressures. J Am Coll Cardiol 1993; 21: 1687-96.

Russell SD, Selaru P, Pyne DA, Ghazzi MM, Massey KD, Pressler M, Serikoff A,Coats AJ. Rationale for use of an exercise end point and design for the ADVANCE (A Dose evaluation of a Vasopressin ANtagonist in CHF patients undergoing Exercise) trial. Am Heart J 2003; 145 (1): 179-86.

Sakka SG, Bredle DL, Reinhart K, Meier-Hellmann A. Comparison between intrathoracic blood volume and cardiac filling pressures in the early phase of hemodynamic instability of patients with sepsis or septic shock. J Crit Care1999; 14 (2): 78-83.

Salvatore P, Di Pasquale P, Prrinello G, Fornaciara E, Di Gaudio F, Fasullo S, Giammanco M, Sarullo F, Licata G. Changes in brain natriuretic peptide levels and bioelectrical impedance measurements after treatment with high dose furosemide and hypertonic saline solution versus high dose furosemide alone in refractory congestive heart failure. J Am Coll Cardiol 2005; 45: 1997-2003.

Sato Y, Yamada T, Taniguchi R, Nagai K, Makiyama T, Okada H, Kataoka K, Ito H,Matsumori A, Sasayama S, Takatsu Y. Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. Circulation 2001; 103 (3): 369-74.

Sasaki T, Kubo T, Miyamoto T, Komamura K, Hionda K, Miyatake K. Clinical significance of measureing inferior vena cava dimension in patients with acute exacerbation of chronic heart failure. J Cardiol 2001; 37(6): 309-13.

Sbarouni E, Georgiadou P, Kremastinos DT, Voudris V. Ischemia modified albumin: is this marker of ischemia ready for prime time use? Hellenic J Cardiol. 2008; 49 (4): 260-66.

Sbarouni E, Georgiadou P, Koutelou M, Sklavainas I, Panagiotakos D, Voudris V.Ischaemia-modified albumin in dilated cardiomyopathy. Ann Clin Biochem 2009; 46(Pt 3): 241-43.

Sbarouni E, Georgiadou P, Manginas A, Panagiotakos D, Karavolias GK, Voudris V.Ischaemia-modified albumin in pulmonary hypertension.Biomarkers 2010; 15 (3): 238-42.

Schabelman S, Schiller NB, Silverman NH, Ports TA. Left atrial volume estimation by two-dimensional echocardiography. Cathet Cardiovasc Diagn 1981; 7: 165-78.

Schirmer H, Lunde P, Rasmussen K. Mitral flow derived Doppler indices of left ventricular diastolic function in a general population; the Tromso study. Eur Heart J 2000;21(16):1376-86.

Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, Barasch J. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18(2):407-13.

Schrier RW. Body fluid volume regulation in health and disease: a unifying hypothesis. Ann Intern Med 1990; 113 (2): 155-59.

Schrier RW, Abraham WT. Hormones and haemodynamics in heart failure. N Eng J Med 1999; 341 (8): 577-85.

Schroeder KL, Sallustio JE, Ross EA. Continuous haematocrit monitoring during intradialytic hypotension: precipitous decline in plasma refill rates. Nephrol Dial Transplant 2004; 19(3): 652-56.

Schuster CJ, Weil MH, Besso J, Carpio M, Henning RJ.Blood volume following diuresis induced by furosemide. Am J Med 1984; 76: 585-92.

Shah MR, Hasselblad V, Tasissa G, Christenson RH, Binanay C, O'Connor CM, Ohman EM, Stevenson LW, Califf RM. Rapid assay brain natriuretic peptide and troponin I in patients hospitalized with decompensated heart failure (from the Evaluation Study of Congestive Heart Failure and Pulmonary Artery Catheterization Effectiveness Trial). Am J Cardiol 2007; 100 (9): 1427-33.

Sharma GV, Woods PA, Lambrew CT, Berg CM, Pietro DA, Rocco TP, Welt FW, Sacchetti P, McIntyre KM. Evaluation of a noninvasive system for determining left ventricular filling pressure. Arch Intern Med 2002; 162(18):2084-88.

Sharma R, Gaze DC, Pellerin D, Mehta RL, Gregson H, Streather CP, Collinson PO, Brecker SJ. Ischaemia modified albumin predicts mortality in ESRD. Am J Kidney Dis 2006; 47(3): 493-502.

Sharpe N, Murphy J, Smith H, Hannan S. Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. Lancet 1988; 1(8580):255-59.

Shimizu H, Aono K, Masuta K, Asada H, Misaki A, Teraoka H. Stability of brain natriuretic peptide BNP in human blood samples. Clin Chim Acta 1999; 285: 169-72.

Shochat M, Charach G, Meyler S, Meisel S, Weintraub M, Mengeritsky G, Mosseri M, Rabinovich P. Prediction of cardiogenic pulmonary edema onset by monitoring right lung impedance.Intensive Care Med 2006; 32 (8): 1214-21.

Shyu KG, Liou JY, Wang BW, Fang WJ, Chang H. Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in pressure-overloaded rat heart. J Biomed Sci 2005; 12 (2): 409-20.

Silver MA, Cianci P, Brennan S, Longeran-Thomas H, Ahmad F. Evaluation of impedance cardiography as an alternative to pulmonary artery catheterization in critically ill patients. Congest Heart Fail 2004; 10(2 Suppl 2): 17-21.

Silverberg DS, Wexler D, Blum M, Keren G, Sheps D, Leibovitch E, Brosh D, Laniado S, Schwartz D, Yachnin T, Shapira I, Gavish D, Baruch R, Koifman B, Kaplan C, Steinbruch S, Iaina A. The use of subcutaneous erythropoietin and intravenous iron for the treatment of the anemia of severe, resistant congestive heart failure improves cardiac and renal function and functional cardiac class, and markedly reduces hospitalizations. J Am Coll Cardiol. 2000; 35(7):1737-44.

Simon R, Desebbe O, Hénaine R, Bastien O, Lehot JJ, Cannesson M. Comparison of ICG thoracic bioimpedance cardiac output monitoring system in patients undergoing cardiac surgery with pulmonary artery cardiac output measurements] Ann Fr Anesth Reanim 2009 ; 28(6): 537-41.

Singer DRJ, Markandu ND, Buckley MG, Miller MA, Sagnella GA, MacGregor GA. Contrasting endocrine responses to acute oral compared with intravenous sodium loading in normal humans. Am J Physiol 1998; 274: F111-19.

Sinha MK, Gaze D, Tippins JR, Collinson PO, Kaski DC. Ischaemia modified albumin is a sensitive marker of myocardial ischaemia after percutaneous coronary intervention. Circulation 2003; 107: 2403-05.

Sinha MK, Roy D, Gaze DC, Collinson PO, Kaski JC. Role of ischaemia modified albumin, a new biochemical marker of myocardial ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J 2004; 21: 29-34.

Sliwa CM Jr. A comparative study of haematocrits drawn from a standard venepuncture and those drawn from a saline lock device. J Emerg Nurs 1997; 23 (3): 228-31.

Smilde TD, Damman K, van der Harst P, Navis G, Daan Westenbrink B, Voors AA, Boomsma F, van Veldhuisen DJ, Hillege HL. Differential associations between renal function and "modifiable" risk factors in patients with chronic heart failure. Clin Res Cardiol 2009; 98(2):121-29

Smith MW, Espiner EA. Yandle TG, Charles CJ, Richards AM. Delayed metabolism of human brain natriuretic peptide reflects resistance to neutral endopeptidase. J Endocrinol 2000; 167: 239-46.

Smith GL, Lichtman JH, Bracken MB, Shlipak MG, Phillips CO, DiCapua P, Krumholz HM. Renal impairment and outcomes in heart failure: systematic review and meta-analysis. J Am Coll Cardiol 2006;47 (10): 1987-96.

Smith RF, Johnson G, Ziesche S, Bhat G, Blankenship K, Cohn JN. Functional capacity in heart failure. Comparison of methods for assessment and their relation to other indexes of heart failure. The V-HeFT VA Cooperative Studies Group. Circulation 1993; 87 (6 Suppl): VI88-93.

Smye SW, Sutcliffe J, Pitt E. A comparison of four commercial systems used to measure whole-body electrical impedance. Physiol Meas 1993; 14: 473-78.

Sneed NV, Paul S, Michel Y, Vanbakel A, Hendrix G. Evaluation of 3 quality of life measurement tools in patients with chronic heart failure. Heart Lung 2001; 30 (5): 332-40.

Söderberg M, Hahn RG, Cederholm T. Bioelectric impedance analysis of acute body water changes in congestive heart failure. Scand J Clin Lab Invest 2001; 61 (2): 89-94.

Sodolski T, Kutarski A. Impedance cardiography: A valuable method of evaluating haemodynamic parameters. Cardiol J 2007; 14 (2): 115-26.

Sokoll LJ, Baum H, Collinson PO, Gurr E, Haass M, Luthe H, Morton JJ, Nowatzke W, Zingler C. Performance evaluation of Elecsys proBNP in a multicentre study (Abstract). Clin Chem 2002; 48: A91.

Speth M, Seibold K, Katz N. Interaction between heparin and cardiac troponin T and troponin I from patients after coronary bypass surgery. Clin Biochem 2002; 35 (5): 355-62.

Stahl C, Beierlein W, Walker T, Straub A, Nagy Z, Knubben K, Greiner TO, Lippert M, Czygan G, Paule S, Schweika O, Kühlkamp V. Intracardiac impedance monitors hemodynamic deterioration in a chronic heart failure pig model. J Cardiovasc Electrophysiol 2007; 18 (9): 985-90.

Steimle AE, Stevenson LW, Chelimsky-Fallick C, Fonarow GC, Hamilton MA, Moriguchi JD, Kartashov A, Tillisch JH. Sustained hemodynamic efficacy of therapy tailored to reduce filling pressures in survivors with advanced heart failure. Circulation 1997; 96 (4): 1165-72.

Steiner MC, Barton RL, Singh SJ, Morgan MD. Bedside methods versus dual energy X-ray absorptiometry for body composition measurement in COPD.Eur Respir J 2002;19(4):626-31.

Stevenson LW, Perloff JK. The limited reliability of physical signs for estimating haemodynamics in chronic heart failure. JAMA 1989; 261: 884-88.

Stockand JD, Sansom SC. Regulation of filtration rate by glomerular mesangial cells in health and diabetic renal disease. Am J Kidney Dis 1997; 29: 971-81.

Strauss MB, Davies RK, Rosenbaum JD, Rossmeisl EC. "Water diuresis" produced during recumbency by intravenous infusion of isotonic saline solution. J Clin Invest1951;30:862-68.

Swärd K, Valsson F, Sellgren J, Ricksten SE. Differential effects of human atrial natriuretic peptide and furosemide on glomerular filtration rate and renal oxygen consumption in humans. Intensive Care Med 2005; 31 (1): 79-85.

Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. CONSENSUS Trial Study Group. Circulation 1990; 82 (5): 1730-36.

Szatajzel J, Ruedin P, Monin C, Stoermann C, Leski M, Rutishauser W, Lerch R. Effect of altered loading conditions during haemodialysis on left ventricular filling pattern. Eur H J 1993; 14: 655-61.

Szatalawicz VL, Arnold PE, Chaimovitz C, Bichet D, Berl T, Schrier RW. Radioimmunoassay of plasma argernine vasopressin in hypnoatremic patients with congestive heart failure. N Engl J Med 1981; 305: 263-66.

Tabata T, Thomas JD, Klein AL. Pulmonary venous flow by Doppler echocardiography: revisited 12 years later. J Am Coll Cardiol 2003; 41: 1243-50.

Tang WH, Girod JP, Lee MJ, Starling RC, Young JB, Van Lente F, Francis GS. Plasma B-type natriuretic peptide levels in ambulatory patients with established chronic symptomatic systolic heart failure. Circulation 2003; 108 (24): 2964-66.

Tanino Y, Shite J, Paredes OL, Shinke T, Ogasawara D, Sawada T, Kawamori H, Miyoshi N, Kato H, Yoshino N, Hirata K. Whole body bioimpedance monitoring for outpatient chronic heart failure follow up. Circ J 2009;73(6):1074-79.

Tarazi RC, Dustan HP, Frohlich ED. Long term thiazide therapy in essential hypertension: evidence for persistant alteration in plasma volume and renin activity. Circulation 1970; 41: 709-17.

Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R Jr., Ferdinand K,Taylor M, Adams K, Sabolinski M, Worcel M, Cohn JN. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. N Engl J Med 2004; 351: 2049–57.

Thirup P.Haematocrit: within-subject and seasonal variation. Sports Med 2003;33(3): 231-43.

Trachtenberg BH, Hare JM. Biomarkers of oxidative stress in heart failure. Heart Fail Clin 2009; 5 (4): 561-77.

Travers B, O'Loughlin C, Murphy NT, Ryder M, Conlon C, Lewidge M, McDonald K. Fluid restriction in the management of decompensated heart failure: no impact on time to clinical stability. J Card Fail 2007; 13 (2): 128-32.

Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet 2000; 355 (9210): 1126-30.

Tsuyuki RT, McKelvie RS, Arnold JM, Avezum A Jr, Barretto AC, Carvalho AC, Isaac DL, Kitching AD, Piegas LS, Teo KK, Yusuf S. Acute precipitants of congestive heart failure exacerbations. Arch Intern Med 200; 161(19):2337-42.

Turedi S, Patan T, Gunduz A, Mentese A, Tekinbas C, Topbas M, Karahan SC, Yulug E, Turkmen S, Ucar U. Ischemia-modified albumin in the diagnosis of pulmonary embolism: an experimental study. Am J Emerg Med 2009; 27(6): 635-40.

Udelson JE, Orlandi C, Ouyang J, Krasa H, Zimmer CA, Frivold G, Haught WH, Meymandi S, Macarie C, Raef D, Wedge P, Konstam MA, Gheorghiade M. Acute hemodynamic effects of tolvaptan, a vasopressin V2 receptor blocker, in patients with symptomatic heart failure and systolic dysfunction: an international, multicenter, randomized, placebo-controlled trial. J Am Coll Cardiol 2008; 52 (19): 1540-45.

Uretsky BF, Shaver JA, Liang CS, Amin D, Shah PK, Levine TB, Walinsky P, LeJemtel T, Linnemeier T, Rush JE. Modulation of haemodynamic effects with a convertinf enzyme inhibitor: acute haemodynamic dose-dependent relationship of a new angiotensin converting enzyme inhibitor, lisinopril, with observations on long-term clinical, functional, and biochemical responses. Am Heart J 1988; 116 (2): 480-88.

Uretsky BF, Verbalis JG, Murali S, Betschart AR, Kolesar JA, Reddy PS. Control of atrial natriuretic peptide secretion in patients with severe congestive heart failure. J Clin Endocrinol Metab. 1990; 71(1): 146-51.

Vaidya VS, Ferguson MA, Bonventre J. Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol 2008; 48: 463-93.

Vaidya VS, Waikar SS, Ferguson MA, Collings FB, Sunderland K, Gioules C, Bradwin G, Matsouaka R, Betensky RA, Curhan GC, Bonventre JV. Urinary Biomarkers for Sensitive and Specific Detection of Acute Kidney Injury in Humans. Clin Transl Sci 2008;1(3):200-08.

Valeri CR, Dennis RC, Ragno G, Macgregor H, Menzoian JO, Khuri SF. Limitations of the haematocrit level to assess the need for red blood cell transfusion in hypovolaemic anaemic patients. Tranfusion 2006; 46 (3): 365-71.

van der Merwe DE, Henley R, Lane G, Field R, Frenneaux M, Dunstan F, McDowell I. Effect of different sample types and stability after blood collection on N-terminal pro-B-type natriuretic peptide as measured with Roche Elecsys system. Clin Chem 2004; 50: 779-80.

Var-Or D, Lau E, Winkler JV. Novel assay for cobalt-albumin binding and its potential as a marker of myocardial ischaemia-a preliminary report. J Emerg Med 2000; 19: 311-15.

Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. Circulation 2006; 113: 2335-62.

Veall N, Edmonds CJ, Hesp R, Smith T. Methods and problems in the measurement of body water and electrolyte pools with radionuclide tracers. Contrib Nephrol 1980; 21: 10-14.

Verhoeve PE, Cadwell CA, Tsadok S. Reproducibility of nonivasive bioimpedance measurements of cardiac function. J Card Fail 1998; 4 (suppl.): S3 (abstract).

Verma SP, Silke B, Hussain M, Nelson GI, Reynolds GW, Richmond A, Taylor SH.First-line treatment of left ventricular failure complicating acute myocardial infarction: a randomised evaluation of immediate effects of diuretic,venodilator, arteriodilator, and positive inotropic drugs on left ventricularfunction. J Cardiovasc Pharmacol 1987; 10 (1): 38-46.

Volpe M, Tritto C, De Luca N, Mele AF, Lembo G, Rubattu S, Romano M, De Campora P, Enea I, Ricciardelli B, et al. Failure of atrial natriuretic factor to increase with saline load in patients with dilated cardiomyopathy and mild heart failure. J Clin Invest 1991; 88 (5): 1481-19.

Volpe M, Rao MAE, Cuocolo A, Russo R, Nappi A, Mele AF, Enea I, Trimarco B, Condorelli M. Radionuclide monitoring of cardiac adaptations to volume loading in patients with dilated cardiomyopathy and mild heart failure. Circulation 1995; 92: 2511-18.

Volpe M, Tritto C, DeLuca N, Rubattu S, Mele AF, Lembo G, Enea I, deCampora P, Rendina V, Romano M. Angiotensin converting enzyme inhibition restores cardiac and hormonal responses to volume overload in patients with dilated cardiomyopathy and mild heart failure. Circulation 1992; 86 (6): 1800-09.

Waanders F, Vaidya VS, van Goor H, Leuvenink H, Damman K, Hamming I, Bonventre JV, Vogt L, Navis G. Effect of renin-angiotensin-aldosterone system inhibition, dietary sodium restriction, and/or diuretics on urinary kidney injury molecule 1 excretion in nondiabetic proteinuric kidney disease: a post hoc analysis of a randomized controlled trial. Am J Kidney Dis 2009; 53 (1): 16-25.

Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN, Lee HT. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006; 105 (3): 485-91.

Waikar SS, Sabbisetti WS, Bonventre JV. Normalization of urinary biomarkers to creatinine during changes in glomerular filtration rate. Kidney Int. 2010; 78(5):486-94.

Webel AR, Frazier SK, Moser DK, Lennie TA. Daily variability in dyspnea, edema and body weight in heart failure patients. Eur J Cardiovasc Nurs. 2007; 6 (1): 60-65.

Weilenmann D, Rickli H, Follath F, Kiowski W, Brunner-La Rocca HP. Noninvasive evaluation of pulmonary capillary wedge pressure by BP response to the Valsalva maneuver. Chest 2002; 122 (1): 140-45.

Westhuyzen J, Endre ZH, Reece G, Reith DM, Saltissi D, Morgan TJ. Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. Nephrol Dial Transplant 2003; 18 (3): 543-51.

Wilson JR, Rayos G, Yeoh TK, Gothard P, Bak K. Dissociation between exertional symptoms and circulatory function in patients with heart failure. Circulation 1995; 92 (1): 47-53.

Wingender W, Neurser D, Weber H, Morich FJ, Horstmann R, Kuhlmann J. Increase in plasma atrial natriuretic factor and right atrial area during endogenous and exogenous volume loading in healthy volunteers: effect on plasma renin activity, aldosterone and antidiuretic hormone. J Hypertens Suppl 1988; 6 (4): S314-16.

Wright SP, Walsh H, Ingley KM, Muncaster SA, Gamble GD, Pearl A, Whalley GA, Sharpe N, Doughty RN. Uptake of self-management strategies in a heart failure management programme. Eur J Heart Fail 2003; 5 (3): 371-80.

Wu AH, Lu AQ, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high specificity assay: implications for clinical practice. Clin Chem 2009; 55 (1): 508.

Wu AH, Smith A, Rame E, Wians F, Minard F, Giuliani I, Larue C. Analytical assay characterization for 1-108 Pro-B-type natriuretic peptide on the BioPlex 2200 analyzer. Clin Chim Acta 2009; 408 (1-2): 143-44.

Wu AHB, Smith A, Wieczorek S, Mather JF, Duncan B, White M, McGill C, Katten D, Heller G. Biological variation for N-terminal pro- and B-type natriuretic peptides and implications for therapeutic monitoring of patients with congestive heart failure. Am J Cardiol 2003; 92: 628-31.

Wu CC, Lin YP, Yu WC, Lee WS, Hsu TL, Ding PY, Chen CH. The assessment of fluid status in haemodialysis patients: usefulness of the Doppler echocardiographic parameters. Nephrol Dial Transplant 2004;19(3): 644-51.

Yamamoto T, Yamamoto Y. Electrical properties of the epidermal stratum corneum. Med Biol Eng 1976; 14: 151-58.

Yamamoto Y, Yamamoto T. Characteristics of skin admittance for dry electrodes and measurement of skin moisturisation. Med Biol Eng Comp 1986; 24: 71-77.

Yamasaki Y, Nishiuchi T, Kojima A, Saito H, Saito S. Effects of an oral water loading and intravenous administration of isotonic glucose, hypertonic saline, mannitol and furosemide on the release of atrial natriuretic peptide in man.Acta Endocrinol (Copenh) 1998;119(2):269-76.

Yan RT, White M, Yan AT, Yusuf S, Rouleau JL, Maggioni AP, Hall C, Latini R, Afzal R, Floras J, Masson S, McKelvie RS. Usefulness of temporal changes in neurohormones as markers of ventricular remodeling and prognosis in patients with left ventricular systolic dysfunction and heart failure receiving either candesartan or enalapril or both. Am J Cardiol 2005; 96 (5): 698-704.

Yasue H, Yoshimura M, Sumida E, et al. Localisation and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and in patients with heart failure. Circulation 1994; 90: 195-203.

Yeo KT, Wu AH, Apple FS, Kroll MH, Christenson RH, Lewandrowski KB, Sedor FA, Butch AW. Multicentre evaluation of the Rcone NT-proBNP assay and comparison to the Biosite Traige BNP assay. Clin Chim Acta 2003; 338: 107-15.

Yoshimura M. Mizuno Y, Harada E, et al. Interaction on metabolic clearance between A-type and B-type natiuretic peptides in patients with heart failure. Metabolism 2000; 49: 1228-33.

Yoshimura M, Yasue H, Okumura K, Ogawa H, Jougasaki M, Mukoyama M, Nakao K, Imura H. Different secretion patterns of atrial natiuretic peptide and brian natriuretic peptide in patients with congestive cardiac failure. Circulation 1993; 87 (2): 464-69.

Ypenburg C, Bax JJ, van der Wall EE, Schalij MJ, van Erven L. Intrathoracic impedance monitoring to predict decompensated heart failure. Am J Cardiol 2007; 99(4): 554-57.

Yu CM, Wang L, Chau E, Chan RH, Kong SL, Tang MO, Christensen J, Stadler RW, Lau CP. Intrathoracic impedance monitoring in patients with heart failure: correlation with fluid status and feasibility of early warning preceding hospitalization. Circulation 2005;112(6):841-48.

Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the randomized aldactone evaluation study (RALES). Circulation 2000; 102 (22): 2700-06.

Zapico-Muñiz E, Santaló-Bel M, Mercé-Muntañola J, Montiel JA, Martínez-Rubio A, Ordóñez-Llanos J. Ischemia-modified albumin during skeletal muscle ischemia. Clin Chem 2004; 50 (6): 1063-65.

Zema MJ, Masters AP, Margouleff D. Dyspnea: the heart or the lungs? Differentiation using the bedside Valsalva maneuver. Chest 1984; 85: 59-64.

Zema MJ, Restivo B, Sos T, Sniderman KW, Kline S. Left ventricular dysfunction--bedside Valsalva manoeuvre. Br Heart J. 1980; 44 (5): 560-69.

Zhang R, Zhao J, Mandveno A, Potter JD. Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. Cir Res 1995; 76: 1028-35.

Zile MR, Bennett TD, St John Sutton M, Cho YK, Adamson PB, Aaron MF, Aranda JM Jr, Abraham WT, Smart FW, Stevenson LW, Kueffer FJ, Bourge RC. Transition from chronic compensated to acute decompensated heart failure: pathophysiological insights obtained from continuous monitoring of intracardiac pressures. Circulation 2008;118 (14): 1433-41.

Zlotowski SJ, Kupas DF, Wood GC. Comparison of laboratory values obtained by means of routine venipuncture versus peripheral intravenous catheter after a normal saline solution bolus. Ann Emerg Med. 2001; 38 (5): 497-504.