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Effect of haemodialysis and residual renal function on serum levels of galectin-3, B-type natriuretic peptides and cardiac troponin T

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Running title: Cardiac biomarkers in haemodialysis patients

Word count:

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nep.13179

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Abstract: 248 words Text (including abstract and references): 3,634 words

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ABSTRACT:

Aim: Levels of plasma markers of myocardial fibrosis (galectin-3), stretch (B-type natriuretic peptide (BNP)) and injury (troponin T (TnT)) may be affected by haemodialysis and residual renal function (RRF) in addition to cardiac pathology. We aimed to determine the association of RRF, urine output and haemodialysis itself on clinically important cardiac biomarkers in haemodialysis patients.

Methods: Adult haemodialysis patients underwent venesection pre- and post-haemodialysis followed by echocardiography and inter-dialytic urine collection to calculate RRF (mL/minute/1.73m^2) and urine output (mL/day). Galectin-3, BNP-32, NT-ProBNP and high-sensitivity TnT were measured and levels compared across tertiles of echocardiographic parameters, RRF and urine output using the non-parametric test for trend across ordered groups.

Results: Twenty-three patients (17 male) with mean age 67.7±13.8 years and median (interquartile range, IQR) dialysis duration 13.6 (9.8-19.1) months participated. Galectin-3 was substantially lower following haemodialysis: 55ng/mL (47-70) versus 23ng/mL (19-27, $p<0.001$), but other biomarkers changed little. By increasing tertile of RRF, post-dialysis galectin-3 was 32.6ng/mL (23.7-36.6), 21.9ng/mL (19.0-23.2) and 19.0ng/mL (16.9-21.0, $p=0.001$) and NT-ProBNP was 10,192ng/L (2,303-21,504), 2,037ng/L (1,224-10,795) and 1,481ng/L (172-2,890, $p=0.016$). Changes were less marked with BNP-32 and hs-TnT. Results were similar for daily urine volume, but left ventricular ejection fraction, left ventricular mass index and E:A ratio were not associated with biomarker concentrations.

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Conclusion: Plasma concentration of galectin-3 is reduced by the haemodialysis procedure. Lower RRF and urine volume are strongly associated with higher levels of galectin-3 and NT-Pro-BNP. These associations are important to the clinical interpretation of these biomarker levels in haemodialysis patients.

Key Words: B-type natriuretic peptide; galectin-3; haemodialysis; residual kidney function; cardiac troponin T

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Introduction

People with end-stage kidney disease (ESKD) have a high prevalence of left ventricular hypertrophy (LVH) and dilated cardiomyopathy[1] and studies with cardiac magnetic resonance imaging have demonstrated the presence of myocardial fibrosis in both a focal or diffuse pattern [2]. The factors that lead to LVH and myocardial fibrosis are complex and varied but include ESKD-related pathways such as intravascular volume expansion, vascular calcification with stiff vessels, anaemia, hormonal activation (sympathetic nervous system and renin-angiotensin-aldosterone system activation), as well as intracellular pathways such as signals leading to apoptosis and activation of the mammalian target of rapamycin[3]. Plasma biochemical markers have been shown to predict clinical outcomes and may be useful tools to both identify patients with left ventricular abnormalities and to potentially guide treatment[4]. However, interpretation of cardiac biochemical markers is made difficult because their levels may be affected by cardiac disease, reduced kidney function and other factors[5].

Galectin-3 is a novel plasma marker of cardiac fibrosis that is associated with incident heart failure and increased mortality in a community cohort[6], but is associated with fibrosis in other organs, including the kidney[7]. In a report combining patients admitted for coronary angiograpy with varying levels of kidney function in one study with haemodialysis patients with diabetes in a randomised controlled trial, levels of galectin-3 increased as glomerular filtration rate (eGFR) declined, and higher galectin-3 levels were associated with cardiovascular events[8]. The AHA/ACC

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clinical practice guideline recommends considering measuring galectin-3 for additive risk stratification in heart failure[9], whilst also recommending measurement of B-type natriuretic peptide (BNP), an established biochemical marker of myocardial stretch for diagnosis and prognostication in heart failure[9]. B-type natriuretic peptide can be measured as the active 32 amino acid hormone, BNP-32, or the inactive 76 amino acid N-terminal fragment, NT-ProBNP. B-type natriuretic peptides are elevated in patients with ESKD[10] and are strongly associated with LVH[11], and single and serial measurements are predictors of adverse outcomes[12, 13]. The plasma concentrations of cardiac troponins I and T are frequently elevated in haemodialysis patients due to cardiac factors and/or dialysis-related factors[14, 15]. Regardless of the reason, elevated cardiac troponin in haemodialysis patients is associated with adverse outcomes[16, 17]. We aimed to determine how measures of cardiac structure and function assessed by echocardiography and measures of residual kidney function were associated with levels of these biochemical markers of cardiac disease in haemodialysis patients.

Materials and Methods

Patients

Adult patients who had received thrice weekly haemodialysis for more than 3 months in satellite units associated with a single institution (Austin Health) who had no current symptoms of cardiovascular disease such as chest pain or dyspnoea were eligible for inclusion. Exclusion criteria included commencement of dialysis more than 2 years prior to recruitment in order to avoid heterogeneity of dialysis duration,

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inability to have an echocardiogram immediately following haemodialysis (due to need for ambulance transport or considered too unwell to manage study procedures), and inability to provide written informed consent. The Austin Health Human Research Ethics Committee approved the study (H2008/03162), which complied with the Statement on Human Experimentation by the National Health and Medical Research Council of Australia; all participants provided written informed consent.

Procedures

Participants underwent venesection pre- and post- hemodialysis followed by an echocardiogram, and then commenced an inter-dialytic urine collection. In order to ensure volume state was as consistent as possible, this was commenced on a mid-week dialysis day. Blood samples were centrifuged and aliquots of serum and plasma stored at -80^{o}C until analysis. Demographic and dialysis data were collected. The timed inter-dialytic collection of urine was performed to determine urine output and residual renal function. The daily urine volume (mL/day) was calculated from the timed collection. The residual renal function (RRF) for hemodialysis patients was calculated according to the formula recommended by the European Best Practice Guidelines for Hemodialysis (Part 1)[18]:

RRF (ml/min/1.73m^2) = 0.5 x (Cl_{Cr} + Cl_{Urea}) x (1.73/BSA), where:

Cl_{Cr} = Creatinine clearance (ml/min) = (U_{Cr} xV)/{0.5 x (Cr_1 + Cr_2) x Time}, and

Cl_{Urea} = Urea clearance (ml/min) = (U_{ur} xV)/{0.5 x (Ur_1 + Ur_2) x Time}.

BSA is body surface area by the Dubois formula; U_{Cr}=urinary creatinine in timed collection, Cr_1= plasma creatinine post dialysis on day urine collection starts, Cr_2=

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plasma creatinine pre-dialysis on day urine collection ends (all in μmol/L); U_{Ur}=urinary urea in timed collection, Ur_1= plasma urea post dialysis on day urine collection starts, Ur_2= plasma urea pre-dialysis on day urine collection ends (all in mmol/L); V=volume of urine in mL; Time=time of collection in minutes.

Laboratory measurements

Galectin-3 was measured by chemiluminescent microparticle immunoassay on an automated platform (Abbott ARCHITECT, Abbott Laboratories, Abbott Park, IL, USA). This assay has an imprecision of less than 10% within the range of galectin-3 of 4.0-114.0ng/mL. B-type natriuretic peptide (BNP-32) was measured by chemiluminescent microparticle immunoassay using an automated platform (Abbott ARCHITECT, Abbott Laboratories, Abbott Park, IL, USA). NT-ProBNP was measured by an electrochemiluminescence immunoassay (proBNP II; Roche Diagnostics, Indianapolis, IN) on an E170 analyzer (Roche Diagnostics). Cardiac troponin T (cTnT) was measured by electrochemiluminescence immunoassay (Troponin T hs; Roche Diagnostics, Indianapolis, IN) using an Elecsys E170 analyzer (Roche Diagnostics). The upper reference limit (99^{th} percentile) is 14ng/L and across the range of values measured, this assay has an imprecision<10%.

Echocardiography

A transthoracic echocardiogram was performed after the mid-week dialysis to ensure participants were at their dry weight. The examination was performed in the left lateral decubitus position using standard parasternal and apical views according to previously published methods [19] to determine left ventricular ejection fraction

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(LVEF), the E:A ratio as a measure of diastolic dysfunction and left ventricular mass indexed for body surface area (LVMI). Measurement of early (E) and late (A) diastolic peak filling velocities enabled calculation of the E:A ratio. LVMI was calculated from measurements of the left ventricular end diastolic diameter (LVEDD), intraventricular septal thickness (IVST) and posterior wall thickness (PWT) according to the formula of Devereux[20]:

$LVMI\ (g/m^2) = 0.8 \times (1.04 \times [(IVST+LVID+PWT)^3 - LVEDD^3]) + 0.6/BSA$

Statistical methods

Measured variables demonstrated skewed or non-normal distributions that were not correctable by transformation and thus limited our ability to use linear regression. Results for these are presented as median (interquartile range). The associations of biomarkers were assessed by dividing measures of residual kidney function and cardiac structure and function into tertiles and comparing biomarker values in these tertiles. The non-parametric test for trend[21] was used to test whether there was a trend for change in biomarker across ordered groups (tertiles) of measured residual kidney function (or cardiac function), and the non-parametric sign test used to determine whether the median of the differences between pre- and post-hemodialysis measurements was different to zero. Analyses were performed using Stata version 11.2 (Satacorp, College Station, Texas).

Results

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Of 70 patients receiving hemodialysis who were screened for participation, 44 were eligible and 23 participated and underwent study procedures (**Figure 1**). The mean age was 67.7±13.8 years, most were male with a short dialysis vintage based on inclusion criteria. Participants mostly received 4 hours of hemodialysis thrice weekly using predominantly high-flux dialysis membranes. Thirteen patients had diabetes and 11 had co-morbid cardiovascular disease (**Table 1**).

Plasma levels of galectin-3 were approximately 50% lower after hemodialysis whereas the other biomarkers changed minimally (**Figure 2;** data in **Supplementary Table S1**). Regression of the change in galectin-3 against the change in volume state either as total weight removed (kg) or weight removed as a percentage of dry weight (%) demonstrated no association of change in galectin-3 with ultrafiltration volume (p=0.64 and p=0.82, respectively).

Because the distributions of biomarker levels were not normal, these were compared across tertiles of kidney and cardiac function measures instead of correlation with continuous variables. Unless specified, results presented are for samples taken after the hemodialysis session.

Measures of Residual Kidney Function

The median (IQR) RRF was 2.8mL/min/1.73m^2 (0.6-7.1). The group was divided into the following tertiles for comparison of biomarker levels: 0-0.67mL/min/1.73m^2 (n=8), 0.68-4.09 mL/min/1.73m^2 (n=8) and e4.10 mL/min/1.73m^2 (n=7). Levels of all four biomarkers were greatest in patients with the lowest RRF (**Figure 3**) and this trend was statistically significant for galectin-3, BNP-32 and NT-ProBNP (**Table 2**).

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Analysis of pre-hemodialysis samples yielded similar results (**Supplementary Table 2**), though changes in BNP-32 were not statistically significant. The median (IQR) measured daily urine volume was 391mL/day (79-616) and the group was divided into the following tertiles: 0-126mL/day (n=7), 127-536mL/day (n=8) and ≥536mL/day (n=8). Participants with the lowest urine output had higher levels of all four biomarkers (**Supplementary Figure 1**), and this trend was statistically significant for each biomarker (**Table 2**). From pre-hemodialysis samples, this trend was statistically significant for BNP-32 and NT-ProBNP only (**Supplementary Table 2**). Anuric participants (urine volume<100mL/day, n=6) had higher median galectin-3 than participants with greater urine volume in post-dialysis samples: 33.6ng/mL (27.0-36.6) versus 20.9ng/mL (18.5-23.1), p=0.002.

Measures of cardiac structure and function

The median (IQR) LVEF was 62% (52-68), suggesting well-preserved ventricular function in this group. Only five participants had LVEF<50%. Tertiles of LVEF were <57% (n=6), 57-64% (n=7) and ≥65% (n=6). Although participants with the lower LVEF had higher levels of all four biomarkers (**Figure 4**), these associations were not statistically significant (**Table 2**). After exclusion of the outlier with a very high galectin-3 in Tertile 3, the association remained non-significant (p=0.09). Inclusion of the missing LVEF measures (n=4) obtained by visual estimation by an experienced reader (PMS) did not substantially alter these findings (data not shown). The median (IQR) LVMI was 124g/m^2 (83-168). Tertiles of LVMI were <110g/m^2 (n=7), 110-135g/m^2 (n=7) and ≥136g/m^2 (n=7) and no association of biomarkers with

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LVMI could be demonstrated (**Table 2**). The median (IQR) E:A ratio was 0.78 (0.62-0.99). Tertiles of E:A ratio were <0.66 (n=7), 0.67-0.92 (n=7) and e 0.93 (n=7). Levels of all four measured biomarkers did not differ significantly across E:A ratio tertiles (**Table 2**). Results for all three cardiac measurements were similar for pre-hemodialysis levels (**Supplementary Table 2**).

Discussion

In this cohort of stable patients receiving chronic hemodialysis in an outpatient setting, galectin-3 was most significantly reduced by the hemodialysis procedure. Whether samples were collected pre- or post-hemodialysis, levels of galectin-3 and NT-ProBNP were highest in people with the lowest RRF or smallest urine output. For BNP-32, this association was not statistically significant in pre-hemodialysis samples. Associations with hsTnT and RRF or urine output were variable. No association could be demonstrated with LVEF, LVMI or the E:A ratio.

Although biomarker levels were lower for all four biomarkers, the change in galectin-3 as a result of dialysis was proportionally the greatest. This marked reduction in plasma levels before and after a completed hemodialysis session with predominantly high-flux dialyzers has not been previously demonstrated. One study that analysed samples taken from the lines either side of a low-flux polysulfone dialyzer 30 minutes into a hemodialysis session in 16 patients did demonstrate a fall in median galectin-3 from 49.7ng/mL (33.2-60.4) before the dialyzer to 34.3ng/mL (27.6-47.3) in blood filtered by the dialyzer[22]. In general, lowering of cardiac biomarkers such as BNP and

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the troponins appears more likely if high-flux membranes are used[23], but high-sensitivity troponin assays have only recently been studied[24]. Galectin-3, at 32-35kDa[25] is similar in size to the troponins and larger than BNP[23], suggesting that it should be more difficult to remove with dialysis. Despite this, levels of galectin-3, but not the other markers, are about 50% lower after dialysis compared to before; this should be considered in interpreting levels or comparing values between studies. Higher levels of all four of these biomarkers have been demonstrated as kidney function declines, with highest levels in people receiving dialysis. However, formal assessment of RRF in people receiving dialysis is not often reported. Where it has been reported, in the Implantable Cardioverter Defibrillators in Dialysis patients (ICD2) Trial, RRF correlated negatively with hs-TnT[15]. Levels of other biomarkers were not reported in this study. We also assessed residual kidney function as daily urine volume, a more practical method in practice. Patients on haemodialysis with a greater urine output tend to have better control of extracellular fluid volume. The strong negative association for both forms of BNP with daily urine output may thus be influenced by volume state as well as kidney function, as myocardial stretch is the main stimulus for release of BNP. In separate studies, both NT-ProBNP and cTnT were higher in dialysis patients assessed as hypervolaemic by a number of clinical and imaging assessments[26], and NT-ProBNP was independently associated with bioimpedance measurements of overhydration[27]. We did not perform bioimpedance measurements and our sample size did not allow for multivariate analysis to tease this out further.

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We demonstrated no significant associations of any of the four measured biomarkers with echocardiographic measures of left ventricular structure and function in this study. This is in contrast to other larger studies in hemodialysis patients in which galectin-3 correlated strongly with LVEF and LVMI[28], as did BNP-32[29], NT-ProBNP [30, 31] and hs-TnT[32]. This may be due to insufficient sample size, the well preserved ejection fraction of our cohort, and the relatively short dialysis vintage. An association of biomarkers with LVEF may be demonstrated with a larger sample including participants with a lower range of LVEF values. Even with too few participants to detect associations with cardiac structure, it is noteworthy that significant associations of a number of biomarkers with RRF and daily urine volume were demonstrated

Conclusions

Thus, levels of the novel biomarker galectin-3 are substantially lower in samples collected post-hemodialysis, and residual kidney function influences the levels of this biomarker and also NT-ProBNP. Timing of measurement in relation to hemodialysis is thus particularly important for interpreting and comparing levels of galectin-3 in studies of patients receiving dialysis. Similarly, consideration of a patient's residual kidney function, by formal assessment of RRF or measurement of urine volume, is critical to the interpretation of these biomarkers in the clinic and should also be incorporated into essential future studies.

Acknowledgements

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This work was supported by a Don and Lorraine Jacquot Fellowship for 2008, and an Austin Medical Research Foundation grant (2008). Abbott Diagnostics (Australia) provided the galectin-3 assay kits that were used in this study. The authors declare that no conflict of interests exists.

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FIGURE LEGENDS

Fig 1 Recruitment of participants to the study.

Fig 2 Levels of (A) Galectin-3, (B) BNP-32, (C) NT-ProBNP and (D) hsTnT before (Pre-HD) and after (Post-HD) hemodialysis. Each line represents an individual participant.

Fig 3 Levels of (A) Galectin-3, (B) BNP-32, (C) NT-ProBNP and (D) hsTnT by tertile of residual renal function. The boxes span the 25th and 75th percentile values with a horizontal line showing the median value. The whiskers show values nearest 1.5 times the interquartile range from the 25th and 75th percentile values, and the dots the values outside of these ranges.

Fig 4 Levels of (A) Galectin-3, (B) BNP-32, (C) NT-ProBNP and (D) hsTnT by tertile of LVEF. The boxes span the 25th and 75th percentile values with a horizontal line showing the median value. The whiskers show values nearest 1.5 times the interquartile range from the 25th and 75th percentile values, and the dots the values outside of these ranges.

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TABLE

Table 1. Baseline characteristics of participants.

Baseline characteristics	**Participants (n=23)**
Demographics	
Age	67.7±13.8
Male	17
Diabetes	13
Cardiovascular disease*	11
Coronary artery disease	8
Stroke	7
Peripheral vascular disease	2
Dialysis variables	
Dialysis vintage (months)	13.9±6.2
Dialysis hours/week	12.3±0.9
High-flux dialyser	21
Anuria (<100mL/day)	6
Urine volume (mL)	391 (79-616)
Ultrafiltration (% dry weight)	2.2±1.2
Urea reduction ratio (%)	77±6
Pre-dialysis blood pressure (mmHg)	145±16/72±11
Post-dialysis blood pressure (mmHg)	132±16/69±8

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Echocardiography	
Ejection fraction (%, n=19)	62 (52-68)
Left ventricular mass index (g/m^2, n=20)	124 (83-168)
E:A ratio (n=22)	0.78 (0.62-0.98)

*Some patients had more than one type of cardiovascular disease

Results are either n, mean±standard deviation or median (interquartile range)

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Table 2. Post-dialysis biomarker concentrations by tertiles of measured parameters (see text for values defining tertiles of each parameter)

	Tertile 1	**Tertile 2**	**Tertile 3**	**P***
Residual GFR				
Galectin-3 (ng/mL)	32.6 (23.7-36.6)	21.9 (19.0-23.2)	19.0 (16.9-21.0)	0.001
BNP-32 (ng/L)	408 (250-594)	195 (162-374)	122 (35-347)	0.04
NT-ProBNP (ng/L)	10,192 (2,303-21,504)	2,037 (1,224-10,795)	1,481 (172-2,890)	0.016
cTnT (ng/L)	63 (48-90)	22 (20-38)	36 (18-63)	0.051
Daily urine volume				
Galectin-3 (ng/mL)	32.6 (23.2-36.6)	23.0 (18.1-23.6)	20.2 (18.0-21.0)	0.001
BNP-32 (ng/L)	408 (290-594)	175 (102-337)	137 (67-276)	0.009
NT-ProBNP (ng/L)	10,192 (3,336-21,504)	2,037 (1,224-10,795	1,315 (534-2,186)	0.003
cTnT (ng/L)	63 (44-90)	22 (20-63)	29 (20-51)	0.034
LVEF				
Galectin-3	31.2 (22.1-34.5)	23.1 (22.8-23.7)	20.6 (20.0-23.2)	0.14

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(ng/mL)				
BNP-32 (ng/L)	425 (239-707)	163 (44-391)	262 (151-347)	0.31
NT-ProBNP (ng/L)	10.795 (1,774-20,921)	1,875 (1,224-2,831)	2,156 (1,207-3,841)	0.13
cTnT (ng/L)	44 (20-87)	37 (20-56)	33 (22-38)	0.67
LVMI				
Galectin-3 (ng/mL)	22.8 (20.0-23.7)	23.2 (20.9-23.5)	22.1 (16.9-29.7)	0.75
BNP-32 (ng/L)	163 (44-473)	204 (151-319)	347 (186-2,964)	0.25
NT-ProBNP (ng/L)	2,037 (896-2,895)	1,422 (1,207-13,890)	6,843 (1,774-30,370)	0.13
cTnT (ng/L)	44 (19-56)	30 (22-43)	53 (20-93)	0.61
E:A Ratio				
Galectin-3 (ng/mL)	23.1 (22.8-34.5)	20.9 (19.0-23.7)	23.2 (16.6-29.7)	0.34
BNP-32 (ng/L)	204 (160-425)	151 (44-473)	319 (186-2,964)	0.46
NT-ProBNP (ng/L)	2,037 (1,224-6,494)	1,713 (896-2,895)	7,318 (1,774-30,370)	0.31
cTnT (ng/L)	38 (20-44)	36 (22-82)	67 (30-93)	0.14

*Non-parametric test for trend of biomarker values across ordered groups (tertiles)

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