N-terminal pro Brain Natriuretic Peptide as predictor of outcome in scleroderma renal crisis

C.B. Chighizola[1,2], F. Pregnolato[2], P.L. Meroni[1,2], C.P. Denton[3], V.H. Ong[3]

[1]*Department of Clinical Sciences and Community Health, University of Milan, Italy;*
[2]*Experimental Laboratory of Immunological and Rheumatologic Researches, IRCCS Istituto Auxologico Italiano, Milan, Italy;*
[3]*Centre for Rheumatology and Connective Tissue Diseases, Royal Free Hospital, London, UK.*

Cecilia B. Chighizola, MD, PhD
Francesca Pregnolato, MStat, MSc
Pier Luigi Meroni, MD
Christopher P. Denton, PhD, FRCP
Voon H. Ong, PhD, FRCP

Please address correspondence to:
Voon H. Ong,
Centre for Rheumatology and Connective Tissue Diseases,
Royal Free Hospital,
Rowland Hill Street,
London NW3 2PF, United Kingdom.
E-mail: v.ong@ucl.ac.uk

Received on October 11, 2015; accepted in revised form on January 4, 2016.

Clin Exp Rheumatol 2016; 34 (Suppl. 100): S122-S128.

Key words: systemic sclerosis, scleroderma renal crisis, N-TproBNP, outcome, dialysis

Funding: we gratefully acknowledge funding support for this work including a grant from EULAR Orphan Diseases Programme.

Competing interests: none declared.

ABSTRACT

Objective. *Although in scleroderma renal crisis (SRC) outcome has improved to a great extent with the introduction of ACE inhibitors, there remains significant mortality and morbidity with frequent requirement for renal replacement therapy. Therefore, novel biomarkers to identify patients at high risk of poor outcome would be valuable. The aim of this study was to assess the role of the N terminal fragment of pro Brain Natriuretic Peptide (N-TproBNP) as predictor of outcome in SRC.*

Methods. *20 subjects with confirmed SRC were retrospectively enrolled. Clinical data, full blood count, creatinine, eGFR and N-TproBNP at presentation were collected.*

Results. *Patients requiring renal replacement therapy presented significantly higher levels of N-TproBNP and creatinine ($p>0.01$), lower eGFR ($p<0.01$) and haemoglobin levels ($p=0.01$) and shorter disease duration ($p<0.01$) compared to those who did not require dialysis. Whereas all the candidate variables significantly predicted renal outcome in univariate models, N-TproBNP was the only variable to hold significance in predicting renal outcome in a Firth's multivariate logistic regression model ($p=0.05$, OR 7.6). ROC curve of N-TproBNP to identify patients requiring renal replacement therapy provided a sensitivity of 88.9%, with a specificity of 81.8% at a cut-off value of 360 pmol/L (95% CI 0.84-1.00, area under the curve 0.94). In our cohort, this provided a positive predictive value of 80% and a negative predictive value of 90%.*

Conclusion. *N-TproBNP peptide may be a useful biomarker in risk-stratification of renal outcome in SRC, selectively identifying patients likely to require renal replacement therapy.*

Introduction

First described in 1863 by Auspitz, scleroderma renal crisis (SRC) is a life-threatening complication of systemic sclerosis (SSc) (1). It is characterised by an abrupt onset of systemic hypertension with progressive renal failure (2, 3). SRC presents in 10-20% of patients with diffuse cutaneous SSc (dcSSc), much less commonly in the limited subset (lcSSc). It occurs early in disease course, almost invariably within the first five years from diagnosis; up to 25% of patients are diagnosed with SSc at SRC onset (3, 4). Medium-high dose corticosteroids have been linked to SRC, with 60% of patients having received steroids prior to presentation (5). Although there is variability between reports in the incidence of both SRC and anti-RNA polymerase III antibodies (anti-RNAP III), recent studies suggest that 30-59% of SRC patients harbour anti-RNAP III (3, 6). Several additional risk-factors have been identified as predictors of SRC in SSc population: rapidly progressive skin thickening, joint contractures, new-onset anaemia, recent cardiac events such as pericardial effusion and congestive heart failure (7, 8). The introduction of angiotensin converting enzyme inhibitors (ACEIs) has dramatically improved survival at 1 year from 15 to 76% (5, 9). However, prognosis remains poor, with a mortality rate at 5 years between 30 and 40% (3, 10, 11). Penn *et al*. showed that haemodialysis was instituted in 2/3 of patients presenting with SRC; approximately half of subjects on renal replacement therapy subsequently discontinued dialysis (3). Median time to dialysis discontinuation was 11 months, the potential for renal recovery without long-term renal replacement therapy was low beyond 24 months (3). To date, few authors have assessed the role of outcome

predictors in SRC patients: most studies have focused on demographic and clinical variables associated with poor renal outcome; some investigators focused on the predictive role of pathologic features at renal biopsy, with conflicting results (3, 12). However, a non-invasive surrogate biomarker that predicted long-term outcome in SRC has not yet been proposed. Given the high mortality and morbidity SRC still heralds, the identification of such prognostic tool would be valuable. This may facilitate stratification of patients at higher risk of poor outcome that may warrant more aggressive monitoring and treatment. Brain Natriuretic Peptide (BNP) is a neurohormone released from cardiomyocytes in response to pressure overload. BNP opposes the effects of renin-angiotensin-aldosterone system, promoting vasodilatation and natriuresis and increasing glomerular filtration rate (GFR). It is synthesised as a 108-amino acid prohormone, proBNP, then cleaved into a biologically active peptide and an inactive fragment, the 76-residue N-TproBNP (13). BNP is rapidly metabolised while N-TproBNP has a longer half-life resulting in a higher circulating level than BNP. Consequently, N-TproBNP assay is preferred as diagnostic and prognostic tool in cardiac failure and pulmonary arterial hypertension (PAH) (14-16). Since N-TproBNP levels are elevated even in patients with end-stage renal disease receiving renal replacement therapy (17, 18) and its long-term prognostic value is independent of cardiac mass and ejection fraction (19), N-TproBNP may act as biomarker in SRC. Therefore, the aim of this work was to retrospectively assess the role of N-TproBNP together with several clinical and biochemical parameters as a candidate predictor of renal outcome in a cohort of 20 SRC patients.

Patients and methods

Patients

All patients included in this study fulfilled ACR preliminary classification criteria for SSc (20) and were recruited from a single UK centre. Patients were categorised into lcSSc and dcSSc according to LeRoy (21). SRC was defined as a new-onset systemic hypertension >150/85 mmHg with a decrease in estimated GFR (eGFR) >30%. Patients with normotensive SRC were also included; these patients demonstrated increased blood pressure (BP, even though not attaining standard hypertensive levels), with reduction in renal function measured by serum creatinine as described for hypertensive SRC (22). Demographic data were retrospectively collected through review of records; several clinical data were recorded: disease subset, modified Rodnan skin score (mRSS), heart involvement, PAH, autoantibody specificities (antibodies against centromere (ACA), topoisomerase I (ATA), U_3RNP (anti-U_3RNP), anti-RNAP III). Additional parameters such as left ventricular ejection fraction (LVEF), systolic and diastolic BP (sBP and dBP) were recorded at the time of SRC.

Blood sampling and assay

Serum biochemistry including full blood count and creatinine was assessed at the time of SRC presentation. eGFR was computed using the modification of diet in renal disease equation. All patients had N-TproBNP levels tested at the time of SRC presentation; twelve subjects had a repeat N-TproBNP at six-month follow-up. Serum N-TproBNP levels were measured with Roche Modular Analytics E-170. Normal N-TproBNP levels were less than 20 pmol/L.

Statistical analysis

Renal outcome was defined upon dialysis requirement ("dialysis" and "no dialysis" group). Given the number of variables included in this study, principal components analysis (PCA) was applied to ascertain multivariate relationships by clustering groups of variables into components that accounted a common effect. A correlation matrix approach was used as these variables bore different measure units and magnitude orders. N-TproBNP levels were considered after log-transformation in order to achieve normality. The screeplot of eigenvalues and Kaiser criterion were used to determine the components to retain. Absolute and relative contributions were estimated and a scatter plot was drawn. Mann-Whitney test was used to compare candidate variables in two subgroups of patients identified upon renal outcome and disease subset. Fisher's exact test was performed to investigate the distribution of renal outcome between disease subsets and genders. Kruskal-Wallis test was used to compare clinical and biochemical variables between groups with specific autoantibody profile (anti-RNAP III, ATA and anti-U_3RNP).

Associations between variables (N-TproBNP, creatinine, sBP and dBP, LVEF, haemoglobin (Hb), disease duration and mRSS) were determined by Spearman's coefficient (r). Linear univariate regression analyses were performed to investigate the relationship between continuous variables and renal outcome. Quantile classification method was used to initially identify cut-off points for creatinine, eGFR, N-TproBNP, Hb and disease duration; the optimal cut-offs were then set based upon receiver operating characteristic (ROC) curves.

To overcome the quasi-complete separation phenomenon, we applied Firth's penalised maximum likelihood. This procedure has been proven to significantly reduce the small sample bias of maximum likelihood estimates. Firth's penalisation logistic univariate regression analyses were performed to investigate the relationship between dichotomic variables and renal outcome. A Firth's penalisation multivariate logistic regression model was also built to predict renal outcome inserting as dichotomic variables N-TproBNP, creatinine, Hb and disease duration with a step-wise approach. eGFR was not considered because a parsimony model was adopted to explain most variability.

The likelihood ratio test (LRT) was used to compare the saturated model comprising four variables (N-TproBNP, creatinine, Hb and disease duration) against a model considering N-TproBNP only; the most restrictive model is considered the nested "null" while the general model provides the alternative hypothesis: when $p<0.05$, the more restrictive model should be rejected.

The ROC curve was drawn to assess the diagnostic value of N-TproBNP

in patients who required dialysis compared to those who did not.
Serial levels of N-TproBNP were compared using Wilcoxon matched-pair signed rank sum test. Due to the limited sample size and multiple nonparametric variables, continuous variables were expressed as median values (25°-75° percentile).
Statistical analysis was performed using SAS 9.1.5.

Results

Twenty scleroderma patients with a diagnosis of SRC were enrolled in this study. Fourteen patients (70%) were female, 17 subjects (85%) had been diagnosed with dcSSc. Only one patient presented with normotensive SRC. None of the subjects had received steroids at a dosage >7.5 mg daily prior to SRC onset. All patients were treated with ACEIs. Eleven patients (55%) did not require a renal replacement therapy ("no dialysis" group), while in 9 subjects (45%) dialysis was necessary ("dialysis group"). In the latter group, 5 patients recovered sufficient renal function to discontinue dialysis, while 4 remained dialysis-dependent. Anti-RNAP III was the most common autoantibody specificity (9 subjects, 45%), anti-U_3RNP antibodies were demonstrated in 5 patients (25%). All patients had a systolic pulmonary artery pressure below 30 mmHg and a LVEF above 50% on echocardiogram.
Clinical and biochemical variables of patients are presented in Table I. One patient in the "non dialysis" group deceased during follow-up because of squamous-cell lung carcinoma. No other fatal events were observed.

Principal components analysis

PCA identified two main components, accounting for 70.5% of the total variance. The two components were retained with the following eigenvalues: component I 3.57, component II 1.37. The factor maps from the PCA including the most relevant study variables are presented in Figure 1. Component I included renal function tests such as creatinine and eGFR, N-TproBNP, disease duration and Hb. Component II comprised age and LVEF.

Table I. Clinical characteristics of patients enrolled in this study and N-TproBNP levels.

	No dialysis group (n=11)	Dialysis group (n=9)	Total cohort (n=20)	*p*
Disease duration (months)	12 (8.5-35)	6 (1-7)	9 (6-13)	0.006
Disease subsets	9 dcSSc/ 2 lcSSc	8 dcSSc/ 1 lcSSc	17 dcSSc/ 3 lcSSc	1.000
mRSS	23 (18-24)	20 (15-23)	22 (16-24)	0.491
LVEF (%)	60 (58-66)	60 (55-62)	60 (58-62)	0.209
Systolic BP (mm Hg)	205 (170-227)	214 (165-225)	209 (169-227)	0.916
Diastolic BP (mm Hg)	110 (98-120)	115 (110-130)	110 (106-120)	0.375
Creatinine (μmol/L)	145 (129.5-250.5)	442 (297-676)	289 (143-441)	0.003
eGFR (mL/min/1.73m^2)	37 (19.5-50)	15 (15-15)	15 (15-39)	0.003
Hb (g/dL)	11.7 (10.5-12.8)	9.3 (9-11.6)	11 (9-12)	0.015
N-TproBNP (pmol/L)	119 (63.5-194.5)	2052 (1494-4139)	305.5 (110-1987)	0.001

All variables are expressed as median (25° percentile – 75° percentile). dcSSc: diffuse cutaneous systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; LVEF: left ventricular ejection fraction; BP: Blood Pressure; eGFR: estimated glomerular filtration rate; Hb: haemoglobin.

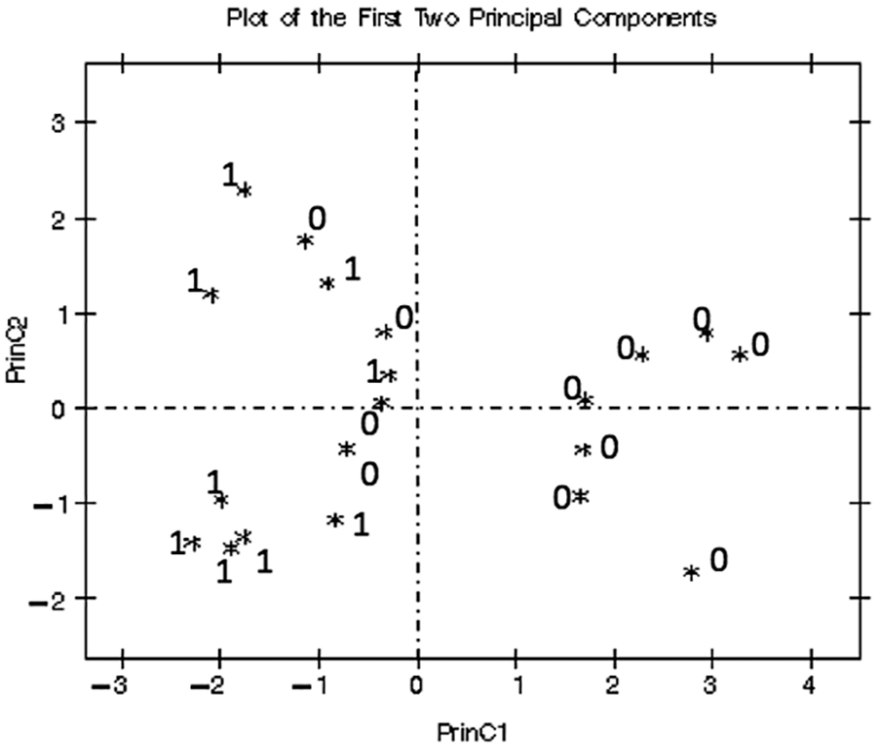

Fig. 1. Scatter plot of Principal Component Analysis (PrinC) with patients stratified based on renal outcome (0= no dialysis; 1 = dialysis).
Subjects in the left portion of the graph (PrinC1<0) are characterised by medium–high levels of both N-TproBNP and creatinine, low disease duration, haemoglobin (Hb) and estimated glomerular filtration rate (eGFR). 75% (9/12) of these patients presented a poor renal outcome. Conversely, patients in the second group clustered in the right portion of the graph (PrinC1>0), presenting lower N-TproBNP and creatinine, a longer disease duration, higher eGFR values and normal Hb levels.
Levels of N-TproBNP and creatinine were defined high when above the cut-off values of 360 pmol/L and 259 µmol/L respectively. Levels of eGFR, Hb and disease duration were defined low when below the following cut-off values: 15 ml/min/1.73m^2, 11.7 mg/dl and 8 months respectively.
Cut-off values were initially identified using the quantile classification method; the optimal cut-offs were then set based upon receiver operating characteristic (ROC) curves.

Component I was the most prominent, accounting for 51% of the total variance. It correlated positively with disease duration (r=0.70; p<0.01), eGFR (r=0.94; p<0.01) and Hb (r=0.81; p<0.01) but inversely with N-TproBNP (r=-0.81; p<0.01) and creatinine (r=-0.80; p<0.01). Component II accounted

for 19.5% of the total variance. Component II correlated positively with age (r=0.88; $p<0.01$) and negatively with LVEF (r=-0.63; $p<0.01$).

Association of renal outcomes and clinical and biochemical variables

Patients requiring renal replacement therapy presented higher creatinine and N-TproBNP values (U=10.00 and U=6.00 respectively, $p<0.01$; Fig. 2) but lower eGFR (U=12.00, $p<0.01$), shorter disease duration and lower Hb levels compared to those not requiring dialysis (U=13.50 and U=17.50 respectively, $p<0.01$). No difference in renal outcome was observed based on disease subset. Nine of the 14 female patients had a worse renal outcome, while none of the males required dialysis ($p=0.01$). No further analysis was performed for autoantibody subtypes due to small sample size. The two subgroups were similar for age at SRC onset, systolic and diastolic BP, LVEF and mRSS (Table I).

Identification of candidate clinical and biochemical variables predictive of renal outcome

The above analyses prompted us to consider five candidate variables potentially predictive of renal outcome in SRC: N-TproBNP, creatinine, eGFR, Hb and disease duration.

Candidate predictor variables

Female patients presented significantly higher N-TproBNP and lower Hb than males (U=8.00, $p<0.01$ and U=15.50, $p=0.04$), no differences were noted for the remaining three variables. No significant difference in creatinine, eGFR, N-TproBNP, disease duration and Hb was observed for disease subset or autoantibody subtypes.

Correlation between candidate predictors of renal outcome in SRC and other clinical and biochemical variables

As expected, significant correlations emerged between all the five variables identified as candidate predictors of renal outcome. A positive correlation was observed between N-TproBNP and creatinine (r=0.62, $p<0.01$), a negative correlation was reported between N-TproBNP and eGFR, Hb, disease duration and age (r=-0.74, r=-0.70, r=-0.66, r=-0.10, $p<0.01$). Noteworthy, the correlation between N-TproBNP levels and LVEF did not quite meet statistical significance (r=-0.46, $p=0.07$); similarly, N-TproBNP did not correlate with sBP or dBP.

Positive correlations emerged between eGFR and Hb as well as disease duration, and between Hb and disease duration; a negative correlation was reported between creatinine and eGFR, Hb and disease duration.

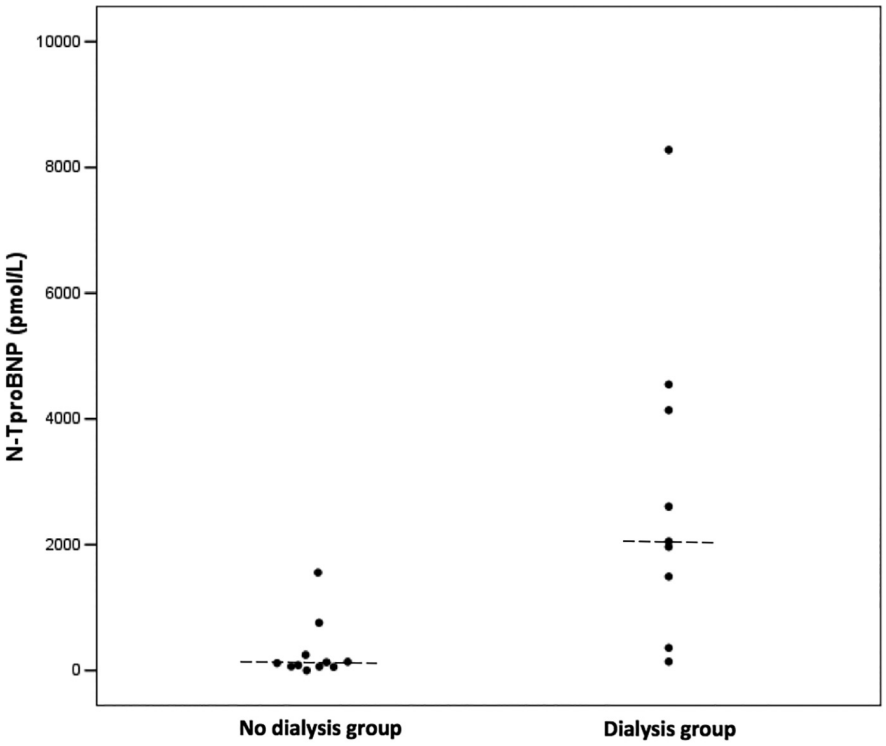

Fig. 2. Dot-plot of N-TproBNP in patients with scleroderma renal crisis based on renal outcome. Dotted lines indicate median values. Median values of N-TproBNP in 'no dialysis' group =119 pmol/L, in 'dialysis' group =2052 pmol/L (U=6.00, $p<0.01$).

Univariate linear regression analyses

As shown in Table II, N-TproBNP, creatinine and Hb significantly predicted the requirement of dialysis ($p=0.02$, $p=0.02$ and $p=0.03$ respectively). Conversely, eGFR and disease duration were not significantly associated with renal outcome.

Identification of cut-off values

The following threshold values were identified: N-TproBNP 360 pmol/L, creatinine 259 μmol/L, eGFR 15 ml/min/1.73m^2, disease duration 8 months and Hb 11.7 mg/dl.

Firth's univariate logistic regression analyses

At logistic regression analysis (Table III), a N-TproBNP value >360 pmol/L conferred an odds ratio (OR) of 21.5 for dialysis requirement ($p<0.01$). Both creatinine above the threshold value of 259 μmol/L and eGFR at the cut-off value of 15 mL/min/1.73m^2 significantly predicted the renal outcome ($p<0.01$), with an OR for dialysis requirement of 13.7. Disease duration above 8 months significantly predicted renal outcome, the OR was 7.3 ($p=0.03$). In contrast, Hb above the threshold value of 11.7 mg/dl was not predictive of dialysis requirement.

Firth's multivariate logistic regression analysis

Multivariate logistic regression model identified N-TproBNP as the only variable to reach statistical significance in predicting renal outcome, with a more accurate CI for OR estimate ($p=0.05$, OR 7.6; Table IV).

Likelihood ratio test

LRT analysis showed that N-TproBNP exerted a strong impact on the fitness of the model (LRT=9.76, $p<0.01$);

conversely, the variables creatinine, Hb and disease duration had little influence on the fitness of the saturated model (LRT=1.22, *p*=NS). However, considering the important biological role of these three variables, we believe that the saturated model offers a more reliable adjusted estimates of the OR of N-TproBNP to predict poor renal outcome compared to the model considering N-TproBNP only (OR 7.6 *vs*. 21.5).

Sensitivity, specificity and predictive values of N-TproBNP

Based on the ROC curve of N-TproBNP that identified patients likely to require renal replacement therapy, a sensitivity of 88.9% was achieved at a cut-off N-TproBNP level of 360 pmol/L, with a specificity of 81.8% (95% CI 0.84-1.00, area under the curve 0.94). In our SSc cohort, 45% of patients presenting with SRC required dialysis, leading to a negative predictive value (NPV) of 90% and a positive predictive value (PPV) of 80%. Considering the lowest estimates of the rate of patients requiring renal replacement therapy (31%) (23), the PPV would be 67% with a NPV of 76%.

N-TproBNP levels at six month follow-up

Twelve patients (60%) had their N-TproBNP levels repeated during a six-month follow up after SRC. There was a statistically significant reduction in N-TproBNP values among patients who did not require dialysis and those who discontinued it (*p*=0.01); the reduction in N-TproBNP levels observed in the "permanent dialysis" group did not achieve statistical significance.

Table II. Univariate regression analyses with continuous predictor variables.

Variable	β	χ^2 Wald	*p*	OR	95% CI	AIC
N-TproBNP	<0.01	5.03	0.02	1.00	1.00-1.00	18.54
Creatinine	0.01	5.45	0.02	1.01	1.00-1.02	19.86
eGFR	-0.51	0.93	0.34	0.60	0.21-1.70	16.68
Hb	-1.0	4.64	0.03	0.37	0.15-0.91	23.03
Disease duration	-0.33	3.53	0.06	0.72	0.51-1.01	21.01

p: *p*-value; OR: odds ratio; CI: confidence interval; AIC: Akaike's information criterion.

Table III. Firth's univariate regression analyses with dichotomic predictor variables.

Variable	β	χ^2	*p*	OR	95% CI	-2LogL
N-TproBNP	3.07	9.76	<0.01	21.5	2.90-294.88	15.95
Creatinine	2.62	7.37	<0.01	13.7	1.98-171.60	18.35
eGFR	2.6	7.37	<0.01	13.7	1.98-171.60	18.35
Hb	1.57	2.52	0.12	4.8	0.70-55.91	23.37
Disease duration	1.99	4.76	0.03	7.3	1.21-58.76	20.95

Cut-offs: N-TproBNP 360 pmol/L; creatinine 259 μmol/L; eGFR 15 ml/min/1.73m^2; Hb 11.7 mg/dL; disease duration: 8 months. *p*: *p*-value; OR: odds ratio; CI: confidence interval; AIC: Akaike's information criterion.

Table IV. Firth's logistic regression analysis model comprising N-TproBNP and creatinine and the two nested models.

Variable	β	χ^2	*p*	OR	95% IC	-2LogL
N-TproBNP	2.03	3.83	0.05	7.6	1.11-115.86	
Creatinine	1.37	1.21	0.27	3.9	0.31-63.38	
Disease duration	0.93	0.64	0.42	2.5	0.23-29.83	
Hb	0.21	0.01	0.90	1.2	0.02-44.96	
						12.48
N-TproBNP	2.28	4.30	0.04	9.7	1.13-152.44	
Creatinine	1.6	1.69	0.19	4.9	0.43-80.99	
Disease duration	1.06	0.77	0.38	2.9	0.25-38.83	
						13.52
N-TproBNP	2.49	5.11	0.02	12.12	1.38-189.53	
Creatinine	2.00	2.91	0.09	7.40	0.74-124.98	
						13.65

p: *p*-value; OR: odds ratio; CI: confidence interval; AIC: Akaike's information criterion.

Discussion

There is a major clinical need for serum or plasma biomarkers, beyond routine measures of kidney function, that may predict outcome or help with clinical management of SRC. In this study, we retrospectively identified 5 candidate biomarkers of renal outcome assessing their prognostic role in a cohort of 20 SRC patients. Overall, our data suggest that in this setting N-TproBNP is the most reliable predictor of renal outcome. Indeed, the high sensitivity and specificity in predicting the requirement of renal replacement therapy provided by N-TproBNP at a cut-off value of 360 pmol/L may confer important clinical implications. Considering also the good NPV and the strong OR for renal replacement therapy, N-TproBNP could be helpful in the risk-stratification of SRC patients at presentation, complementing renal function tests. Our data also suggest that N-TproBNP is even more reliable than kidney function tests in predicting renal outcome among SRC patients. Indeed, N-TproBNP levels above the threshold value of 360 pmol/L conferred a higher OR for dialysis than creatinine above 259 μmol/L and eGFR lower than 15 mL/min/1.73m^2 (21.5 *vs*. 13.7). In addition, N-TproBNP but not creatinine significantly predicted renal outcome in a Firth's multivariate regression model. In addition, LRT clearly showed that N-TproBNP contributed to the significance of the model to a stronger extent compared to the other candidate variables. Interestingly, high serum creatinine at presentation has been reported to confer poor outcome, either early death or requirement for permanent dialysis (3, 7, 9); however, serum creatinine greater than 270 μmol/L did not significantly predict outcome in multivariate regression analysis (9).

Significant renal impairment (creatinine >150 μmol/L) has been demon-

strated to affect N-TproBNP levels (24); in particular, as N-TproBNP displays a lower extrarenal excretion than BNP, renal dysfunction influences N-TproBNP levels to a greater extent than BNP (25). Thus, the role of N-TproBNP as cardiac biomarker in patients with renal insufficiency has been much debated: some authors have suggested that in renal insufficiency N-TproBNP elevations were merely reflective of reduced clearance. However, several investigators have demonstrated that N-TproBNP levels are not spurious even in renal failure setting (24, 26): in heart failure patients, N-TproBNP levels at presentation were a powerful predictor of 60-day mortality regardless of renal function (25). These observations prompted us to hypothesise that N-TproBNP in SRC may represent a marker of overall haemodynamic status. Endothelial dysfunction of the renal small arteries and arterioles is regarded as the initial pathogenic trigger in SRC; it causes an impaired glomerular perfusion, thus inducing hyper-renaemia and subsequent angiotensin-II induced hypertension. Consequently, higher N-TproBNP levels may reflect a stronger activation of renin-angiotensin axis, in turn responsible for an increased production of natriuretic peptides by cardiomyocytes.

Besides renal function tests and N-TproBNP, we identified two other variables as candidate predictors of renal outcome in SRC, namely disease duration and Hb. In this regard, in our study population patients with lower disease duration presented a worse renal outcome, with a strong inverse correlation between N-TproBNP levels and disease duration. These data might thus suggest that patients presenting with SRC earlier on disease course are at higher risk of requiring dialysis. Indeed, in most studies conducted among scleroderma patients, N-TproBNP levels were not correlated with disease duration (27, 28), with an Italian group even observing a positive relationship between disease duration and N-TproBNP (29).

A recent onset of anaemia is not only a well-described precipitating factor for SRC but also a frequent finding in patients with acute renal failure. Accordingly, in our cohort a significant association between Hb and N-TproBNP emerged, with patients requiring dialysis presenting lower Hb levels. Creatinine, Hb and disease duration were not associated with renal outcome in our multivariate model. However, considering the biological importance of the above-cited variables, we believe the saturated model should be considered, with a net OR for N-TproBNP to predict renal outcome of 7.6.

We also assessed the role of demographic confounders potentially affecting the prediction of renal outcome, such as age, gender, BP, and LVEF. In contrast to previous studies reporting older age as an independent predictor of dialysis-free survival (9, 11, 30), in our cohort the age of patients requiring renal replacement therapy was similar to that of subjects not on dialysis. We observed a significant inverse correlation between N-TproBNP and age, even if N-TproBNP levels are described to increase with aging (31). Similarly, past studies identified male sex to be associated with poor renal outcome (7, 9). Even though men displayed lower N-TproBNP levels compared to women, likely because none of the male subjects required dialysis, it is rather unlikely that in this setting gender could display a confounding effect as any difference in N-TproBNP has ever been reported between female and male SSc patients (27, 28). Previous studies conducted in heart failure population have provided conflicting results, with some authors describing similar N-TproBNP levels (13, 32) and others higher N-TproBNP either among male (33) or female subjects (26, 34).

Interestingly, we did not report any variance in low BP and LVEF between SRC patients requiring and those not requiring renal replacement. Conversely, several investigators have reported that patients requiring dialysis displayed a lower BP at presentation (3, 12, 35). Possibly, our finding might be ascribed to the fact that only one patient presented with normotensive SRC. In addition, as previously described by Penn *et al*. (3), we could not report any significant difference in LVEF between the two groups of patients stratified to renal outcome, in contrast to other reports of heart failure as risk-factor for poor outcome in SRC (9). Accordingly, we couldn't demonstrate a significant correlation between N-TproBNP and LVEF, in agreement with a recent study showing that N-TproBNP levels, despite maintaining a prognostic role in patients presenting with acute renal failure, are not closely related to LVEF (36).

As a whole, our data suggest that N-TproBNP provides a reliable surrogate biomarker to identify SRC patients at higher risk of poor renal outcome, independently from cardiorenal function and other demographic, clinical and biochemical variables.

Noteworthy, a significant reduction in serial N-TproBNP levels among patients with better renal outcome was observed, suggesting N-TproBNP could be a useful tool in the follow-up of SRC patients. Changes in N-TproBNP levels may reflect the response to ACEIs: N-TproBNP may allow monitoring treatment response facilitating therapeutic management post-acute crisis. Indeed, several drugs including ACEIs may strongly affect N-TproBNP levels; patients with chronic heart failure in whom treatment was adjusted based on N-TproBNP levels had an improved outcome (32).

Several important issues may limit interpretation of this study. The retrospective design is acknowledged as a weakness; the study cohort was relatively small. The limited sample size may be responsible of the quasi-complete separation phenomenon and the wide range of CI for OR estimates of N-TproBNP in predicting renal outcome, with a borderline statistical significance. These critical issue were, at least partially, overcome thanks to Firth's penalised maximum likelihood, which led to a more accurate estimate of the relative risk provided by N-TproBNP.

In conclusion, our data suggest that N-TproBNP could selectively identify patients at highest risk of poor renal outcome at SRC onset. This may potentially allow more intensive treatment or earlier use of renal replacement therapy to improve outcome, although this would require a prospective study.

As the assay is increasingly available as routine laboratory test, N-TproBNP may provide a practical prognostic biomarker in patients presenting with SRC. We believe that, considering SSc is a relatively rare disease and SRC an uncommon complication with high mortality, this study presents important relevance in clinical practice. N-TproBNP may add to current armamentarium in the management of this life-threatening aspect of SSc, even though future studies are required to prospectively evaluate its utility as outcome predictor in this setting.

References

1. TRAUB YM, SHAPIRO AP, RODNAN GP *et al*.: Hypertension and renal failure (scleroderma renal crisis) in progressive systemic sclerosis. Review of a 25-year experience with 68 cases. *Medicine* (Baltimore) 1983; 62: 335-52.
2. PENN H, DENTON CP: Diagnosis, management and prevention of scleroderma renal crisis. *Curr Opin Rheumatol* 2008; 20: 692-6.
3. PENN H, HOWIE AJ, KINGDON EJ *et al*.: Scleroderma renal crisis: patient characteristics and long-term outcomes. *Q J Med* 2007; 100: 485-94.
4. BARSOTTI S, STAGNARO C, DELLA ROSSA A: Systemic sclerosis: a critical digest of the recent literature. *Clin Exp Rheumatol* 2015; 33: S3-14.
5. STEEN VD, MESDGER TA: Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. *Arthritis Rheum* 1998; 41: 1613-9.
6. MEYER O, DE CHAISEMARTIN L, NICAISE-ROLAND P *et al*.: Anti-RNA polymerase III antibody prevalence and associated clinical manifestations in a large series of French patients with systemic sclerosis: a cross-sectional study. *J Rheumatol* 2010; 37: 125-30.
7. STEEN VD, MEDSGER TA: Long-term outcomes of scleroderma renal crisis. *Ann Intern Med* 2000; 133: 600-3.
8. DEMARCO PJ, WEISMAN MH, SEIBOLD JR *et al*.: Predictors and outcomes of scleroderma renal crisis. The high-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis trial. *Arthritis Rheum* 2002; 46: 2983-9.
9. STEEN VD, CONSTANTINO JP, SHAPIRO AP, MESDGER TA: Outcome of renal crisis in systemic sclerosis: relation to availability of angiotensin converting enzyme (ACE) inhibitors. *Ann Intern Med* 1990; 113: 352-7.
10. TEIXEIRA L, MAHR A, BEREZNE A, NOËL LH, GUILLEVIN L, MOUTHON L: Scleroderma renal crisis, still a life-threatening complication. *Ann N Y Acad Sci* 2007; 1108: 249-58.
11. DENTON CP, BLACK CM: Scleroderma – clinical and pathological advances. *Best Pract Res Clin Rheumatol* 2004; 18: 271-90.
12. BATAL I, DOMSIC RT, SHAFER A *et al*.: Renal biopsy findings predicting outcome in scleroderma renal crisis. *Hum Pathol* 2009; 40: 332-40.
13. HALL C: Essential biochemistry and physiology of (NT-pro)BNP. *Eur J Heart Fail* 2004; 257-60.
14. WILLIAMS MH, HANDLER CE, AKRAM R *et al*.: Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. *Eur Heart J* 2006; 27: 1485-94.
15. IBRAHIM N, JANUZZI JL: The potential role of natriuretic peptides and other biomarkers in heart failure diagnosis, prognosis and management. *Expert Rev Cardiovasc Ther* 2015; 21: 1-14.
16. GIORDANO N, MONTELLA A, CORALLO C *et al*.: Pulmonary hypertension: a correct diagnosis for a suitable therapy in scleroderma patients. *Clin Exp Rheumatol* 2015; 33: S182-9.
17. ZOCCALI C, MALLAMACI F, BENEDETTO FA *et al*.: Cardiac natriuretic peptides are related to left ventricular mass and function and predict mortality in dialysis patients. *J Am Soc Nephrol* 2001; 12: 1508-15.
18. WANG AY, LAM CW, YU CM *et al*.: N-terminal pro-brain natriuretic peptide: an independent risk predictor of cardiovascular congestion, mortality, and adverse cardiovascular outcomes in chronic peritoneal dialysis patients. *J Am Soc Nephrol* 2007; 18: 321-30.
19. MADSEN LH, LADEFOGED S, CORELL P, SCHOU M, HILDEBRANDT PR, ATAR D: N-terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis. *Kidney Int* 2007; 71: 548-54.
20. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-47.
21. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1998; 15: 202-5.
22. STEEN V, MAYES M, MERKEL P: Assessment of kidney involvement. *Clin Exp Rheumatol* 2003; 21: 29-31.
23. WALKER JG, AHERN MJ, SMITH MD *et al*.: Scleroderma renal crisis: poor outcome despite aggressive anti-hypertensive treatment. *Intern Med J* 2003; 216-20.
24. VAN KIMMENADE RR, JANUZZI JL, BAGGISH AL *et al*.: Amino-terminal pro-brain natriuretic peptide, renal function, and outcomes in acute heart failure: redefining the cardiorenal interaction? *J Am Coll Cardiol* 2006; 48: 1621-7.
25. DAS SR, ABDULLAH SM, LEONARD D *et al*.: Association between renal function and circulating levels of natriuretic peptides (from the Dallas Heart study). *Am J Cardiol* 2008; 102: 1394-8.
26. TAKAMI Y, HORIO T, IWASHIMA Y *et al*.: Diagnostic and prognostic value of plasma brain natriuretic peptide in non-dialysis dependent CRF. *Am J Kidney Dis* 2004; 44: 420-8.
27. CHOI HJ, SHIN YK, LEE HJ *et al*.: The clinical significance of serum N-terminal pro-brain natriuretic peptide in systemic sclerosis. *Clin Rheumatol* 2008; 27: 437-42.
28. ORAVEC RM, BREDEMEIER M, LAURINO CC *et al*.: NTproBNP levels in systemic sclerosis: association with clinical and laboratory abnormalities. *Clinical Biochem* 2010; 43: 745-9.
29. SCHIOPPO T, ARTUSI C, CIAVARELLA T *et al*.: N-TproBNP as biomarker in systemic sclerosis. *Clin Rev Allergy Immunol* 2012; 43: 292-301.
30. TEXEIRA L, MOUTHON L, MAHR A *et al*.: Mortality and risk factors of scleroderma renal crisis: a French retrospective study of 50 patients. *Ann Rheum Dis* 2008; 67: 110-6.
31. REDFIELD MM, RODEHEFFER RJ, JACOBSEN SJ, MAHONEY DW, BAILEY KR, BURNETT JC JR: Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Card* 2002; 40: 976-82.
32. JANUZZI JL: The role of natriuretic peptide testing in guiding chronic heart failure management: review of available data and recommendations for use. *Arch Cardiovascular Dis* 2012; 105: 40-50.
33. OLOFSSON M, BOMAN K: Usefulness of natriuretic peptides in primary health care: an exploratory study in elderly patients. *Scand J Prim Health Care* 2010; 28: 29-35.
34. 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: 269-73.
35. GUILLEVIN L, BEREZNE A, SEROR R *et al*.: Scleroderma renal crisis: a retrospective multicentre study on 91 patients and 427 controls. *Rheumatology* (Oxford) 2012; 51: 460-7.
36. BALIK M, JABOR A, OTAHAL M, WALDAUF P, PAVLISOVA M: Impact of acute renal failure on the relationship between N-Terminal pro-B type natriuretic peptide and hemodynamic parameters. *Blood Purif* 2011; 31: 47-54.