Lack of activation of renal functional reserve predicts the risk of significant renal involvement in systemic sclerosis

Riccardo Livi,[1] Serena Guiducci,[2] Federico Perfetto,[1] Gabriele Ciuti,[1] Elisa Grifoni,[1] Letizia Conforti,[2] Felice Galluccio,[2] Alberto Moggi Pignone,[1] Marco Matucci Cerinic[2]

[1]Department of Internal Medicine, University of Florence, Florence, Italy
[2]Department of Medicine and Biomedicine, Division of Rheumatology AOU Careggi, University of Florence, Florence, Italy

Correspondence to
Riccardo Livi, Department of Internal Medicine, University of Florence, Viale Pieraccini 18, I-50139 Firenze, Italy; rlivi@unifi.it

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ABSTRACT

Objective To evaluate if defective activation of renal functional reserve (RFR) in systemic sclerosis (SSc) without clinical signs of renal involvement predicts the risk of developing clinically relevant renal damage.

Methods Twenty-eight normotensive SSc patients with normal renal function and no urinary abnormalities were submitted to an intravenous amino acid load to activate RFR. Nineteen patients (six with diffuse cutaneous SSc (dcSSc)) had an RFR activation defect, while nine (two with dcSSc) showed normal RFR. All patients were followed up for 5 years, with periodic evaluation of renal function, urinary protein excretion and systemic blood pressure (BP).

Results At admission, patients with normal RFR had lower BP than those with abnormal RFR; no age, disease duration or creatinine clearance (CCr) differences were found. Five years later, patients with abnormal RFR showed, with respect to basal values, a significantly higher CCr reduction than patients with normal RFR (mean percent decrease 15.4 ± 9.5 vs 2.6 ± 3.8, $p<0.001$). Among patients with abnormal RFR, 13 (68.4%) showed a CCr reduction of ≥2 ml/min/year, with a final CCr of ≤70 ml/min in eight cases; two patients developed microalbuminuria and 10 grade 1 or 2 systemic hypertension. Significant CCr reduction rates were found in eight patients with high BP and in five patients who remained normotensive. No patient with normal RFR had proteinuria or high BP during follow-up.

Conclusions Lack of RFR activation is an early sign of renal involvement in SSc, and is a harbinger of an increased risk of developing renal insufficiency and systemic hypertension.

INTRODUCTION

Scleroderma renal crisis (SRC) is one of the most acute and dramatic events in systemic sclerosis (SSc). It occurs more often in the diffuse (14–20% of cases) than the limited form of the disease (2% of cases).[1–3] Although the prognosis of SRC greatly improved after the introduction of angiotensin converting enzyme (ACE) inhibitors,[4] it remains a significant cause of mortality and end stage renal failure requiring dialysis.[2 5]

However, pathological studies in SSc have also shown a high prevalence of silent renal changes, mainly vascular lesions, in patients who have never experienced an SRC.[6–8] This insidious form of chronic renal involvement often goes unrecognised when kidney function is evaluated by the usual clinical methods.[6] Renal vascular damage, however, is evident when renal blood flow is explored by angiography, clearance studies or radioisotopic methods[9 10] or when renal circulation is investigated by Doppler ultrasonography.[11]

In SSc, the early recognition of kidney damage is of paramount importance because it may significantly worsen disease prognosis.[12 13]

It has been shown that, in the absence of any clinical signs or symptoms of renal involvement, SSc patients may already have a defect in the activation of renal functional reserve (RFR).[14] RFR is a measure of the ability of the healthy kidney to increase glomerular filtration rate (GFR) in response to an oral protein or intravenous amino acid load, and depends on the activation of preglomerular vasodilating mechanisms.[15 16] In SSc, the lack of activation of RFR reveals an imbalance between vasoconstricting and vasodilating mediators, mainly favouring vasoconstriction. This functional derangement of vascular tone control in SSc has also been demonstrated in skin,[17 18] lung[19] and coronary heart disease.[20]

The aim of this study was to evaluate in asymptomatic SSc patients if defective activation of RFR is an early predictor of the risk of developing clinically relevant renal damage.

PATIENTS AND METHODS

Thirty SSc patients (26 females, 4 males; 22 cases of limited cutaneous SSc (lcSSc) and eight cases of diffuse cutaneous SSc (dcSSc))[21] gave informed consent and were admitted to the study. No patient had ever experienced an SRC, and all had normal renal function (creatinine clearance (CCr) >70 ml/min), 24 h urinary protein excretion <150 mg, normal urinary sediment and normal systemic blood pressure (BP) (systolic BP <140 and diastolic BP <90 mm Hg at repeated measurements). No patient had diabetes. Seven patients had high serum cholesterol levels (range 248–278 mg/dl). One patient was a current smoker and four were ex-smokers. Two patients had Sjogren syndrome and one had autoimmune thyroiditis.

Assessment of internal organ involvement included oesophageal manometry, pulmonary function tests and carbon monoxide transfer factor, high resolution CT of the lungs, and Doppler ultrasonography of the heart and renal arteries.

All patients were submitted to an intravenous amino acid load test for the evaluation of RFR. The detailed protocol of this test has been previously described.[14] Briefly, CCr was measured over two 60 min periods before and after the infusion of a standardised amino acid solution (Freamine III,

8.5% solution, 4.16 ml/min for 2 h; Baxter, Milan, Italy). In each patient, RFR was expressed as the percentage CCr increase after the amino acid load with respect to basal values. On the basis of our previous experience, RFR was defined as normal if the amino acid load elicited a CCr increase of 10% or more.[14]

Ten patients had a normal renal response to the amino acid challenge (normal RFR), showing a CCr increase of 25.4±17.9%; the remaining 20 patients showed no CCr increase or even a CCr reduction (CCr change –7.9±9.9%) after the amino acid load (abnormal RFR).

All patients entered a 5-year follow-up study. Renal function was evaluated at 6-month intervals in 28 cases; two patients, living in other Italian regions, underwent annual evaluation.

All patients were instructed to perform 24-h urine collection and to note the exact start and end times. They were invited to drink at least 1500 ml of water during the collection day. Urinary volume was accurately measured in the hospital and all blood and urinary samples were analysed by the same laboratory.

In all patients, CCr (autoanalyser), 24 h albuminuria (immunonephelometry) and 24 h total urinary protein excretion (protein-dye binding method) were evaluated. Microalbuminuria was defined as urinary albumin excretion of between 30 and 300 mg/day and clinical proteinuria as total urinary protein excretion exceeding 150 mg/day, the upper limit of physiological proteinuria.[22] CCr values were corrected to the standard body surface area (BSA) of 1.73 m^2.

At the end of each medical visit, BP was measured twice by a standard mercury sphygmomanometer, values averaged and individual mean values registered. If high BP values were observed, the patient was submitted to two additional medical tests (evaluation of serum creatinine and urea) within 1 month.

During follow-up, therapy for SSc or associated conditions was decided on for all patients according to their clinical features.

Treatment included intravenous infusions of iloprost (once a month, six cases, four with abnormal RFR) or prostaglandin E (2–4 times a month, 12 cases, eight with abnormal RFR), transdermal nitroglycerine (14 cases) or low-dose calcium antagonists (nine cases) for Raynaud's phenomenon. In those patients developing arterial hypertension, hypotensive treatment was based on ACE inhibitors, AT1-receptor blockers and calcium channel blockers.

No patient was treated with corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), cyclosporine or penicillamine. The two patients with Sjogren syndrome were taking hydroxychloroquine, and one patient was being treated with levothyroxine.

Two female patients with lcSSc, 44 and 47 years of age, one with normal and one with abnormal RFR, were lost to follow-up after 18 and 24 months, respectively, because of a diagnosis of lung cancer in the first case and of breast cancer in the second. Until drop-out, CCr and BP remained substantially unchanged in both patients.

Twenty-eight patients completed the follow-up study. Their clinical characteristics at admission are reported in table 1.

Statistical analysis

Renal and BP data obtained at admission and at the end of the follow-up period were used for the analysis. The statistical significance of the differences between means was evaluated by Student's t test for unpaired data and, when indicated, by the Mann–Whitney U-test. A p level <0.05 was considered statistically significant.

Linear regression analysis was used to evaluate the influence of various parameters potentially affecting the observed renal function changes. The assumption of normality of the distribution of the dependent variable was verified by the Kolmogorov–Smirnoff test.

RESULTS

The results are summarised in tables 2 and 3.

At admission, patients with normal RFR did not differ from those with abnormal RFR regarding age, disease duration, serum creatinine or CCr. Patients with abnormal RFR had higher mean systolic and diastolic BP than patients with normal RFR, although no patient had BP above 140/90 mm Hg.

Basal echocardiographic data excluded systolic and diastolic left ventricular dysfunction in all patients. Two patients with interstitial lung disease had mild (38 and 39 mm Hg) asymptomatic Doppler-calculated elevation of pulmonary arterial pressure (PAP).[23]

During follow-up, none of the patients developed SRC.[24] Serum creatinine showed only limited variation, always remaining below

Table 1 Clinical characteristics of systemic sclerosis patients at admission

No. cases	28
Males/females	4/24
Clinical subset (lcSSc/dcSSc)	20/8
Age (years)	49.7±10.8
Disease duration at admission (months)	23.7±18.9
Oesophageal involvement	18
Lung involvement	13
Pulmonary hypertension	2
Systolic blood pressure (mm Hg)	125.3±13.2
Diastolic blood pressure (mm Hg)	76.2±6.8
Serum creatinine (mg/dl)	0.87±0.14
Creatinine clearance (ml/min×1.73 m^2 BSA)	94.2±16.2
Albuminuria (mg/day)	13.0±6.6
Total proteinuria (mg/day)	51.0±19.5

Data are expressed as means±SD.
BSA, body surface area; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis.

Table 2 Clinical characteristics of systemic sclerosis patients with normal and abnormal renal functional reserve at admission

	Normal RFR	Abnormal RFR	p Value
No. cases	9	19	
Males/females	2/7	2/17	
Clinical subset (lcSSc/dcSSc)	7/2	13/6	
Age (years)	48.1±11.4	50.4±10.5	NS
Disease duration at admission (months)	30.5±22.6	20.7±17.2	NS
Antinuclear antibodies	6 (2 dc)	18 (6 dc)	
Anti centromere	3 (0 dc)	11 (0 dc)	
Anti topoisomerase I	2 (1 dc)	6 (5 dc)	
Oesophageal involvement	5 (2 dc)	13 (3 dc)	
Lung involvement	2 (2 dc)	11 (6 dc)	
Pulmonary hypertension	0	2 (1 dc)	
Systolic blood pressure (mm Hg)	115.3±15.0	130.0±12.4	p<0.011
Diastolic blood pressure (mm Hg)	68.8±6.8	79.8±6.8	p<0.0005
Serum creatinine (mg/dl)	0.94±0.14	0.83±0.14	NS
Creatinine clearance (ml/min×1.73 m^2 BSA)	90.5±16.0	96.0±13.3	NS
Albuminuria (mg/day)	14.2±7.9	12.5±6.1	NS
Total proteinuria (mg/day)	49.8±19.2	51.6±20.0	NS

Data are expressed as means±SD.
BSA, body surface area; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; RFR, renal functional reserve.

Table 3 Comparison of renal functional changes and related clinical signs in systemic sclerosis patients with normal and abnormal renal functional reserve after a 5-year follow- up

	Normal RFR	Abnormal RFR	p Value
Serum creatinine (mg/dl)	0.86±0.15	0.93±0.16	NS
Creatinine clearance (ml/min×1.73 m^2 BSA)	87.9±14.2	81.4±18.2	NS
Absolute CCr change with respect to basal values (ml/min)	−2.59±3.32	−14.6±9.3	p<0.001
Percent CCr change with respect to basal values (%)	−2.59±3.78	−15.4±9.5	p<0.0007
Annual rate of CCr reduction (ml/min)	0.52±0.66	−2.92±1.9	p<0.001
Median	−0.62	−2.56	
Maximum systolic blood pressure values observed during follow-up (mm Hg)	120.6±12.0	143.3±17.9	p<0.002
Maximum diastolic blood pressure values observed during follow-up (mm Hg)	73.1±5.9	86.2±9.8	p<0.002
Patients developing systemic hypertension (>140 and/or >90 mm Hg)	0/9	10/19	
Albuminuria (mg/day)	10.3±4.3	21.1±37	NS
Median (range)	10 (8–21)	10 (4–141)	
Patients developing microalbuminuria	0/9	2/19	
Total proteinuria (mg/day)	48.0±20.8	63.6±45.4	NS
Median (range)	44 (28–92)	48 (31–194)	
Patients developing clinical proteinuria	0/9	2/19	

Data are expressed as means±SD.

1.2 mg/dl in all but one patient. In this case, serum creatinine gradually increased, reaching a value of 1.2 mg/dl only in the last year of follow-up (30% increase with respect to basal value).

Patients with abnormal RFR showed a significant reduction in CCr, while this parameter remained substantially unchanged in patients with normal RFR.

Thirteen of 19 patients (68.4%) with abnormal RFR showed a CCr reduction rate (CCrRR) of 2 ml/min/year or more; CCr decreased at or below 70 ml/min×1.73 m^2 BSA in eight cases. Individual mean annual CCrRR are shown in figure 1.

Ten (two dcSSc, eight lcSSc) of 19 patients (52.6%) with abnormal RFR developed systemic hypertension during follow-up, while all patients with normal RFR remained normotensive. In line with current guidelines,[25] hypertension was confirmed on two or three different occasions before starting treatment. In most cases, the BP increase occurred after 2–3 years of follow-up (mean 31.8 months, range 18–48 months). No patient showed retinal signs of accelerated hypertension; the maximum registered values were 178/104 mm Hg in one patient. In hypertensive patients, BP elevation was classified as grade 1 (≤159/99 mm Hg, six cases) or grade 2 (≤179/109 mm Hg, four cases).[25]

Patients developing hypertension were treated with ACE inhibitors (three cases), dihydropiridine calcium channel blockers (two cases), a combination of ACE inhibitors and calcium channel blockers (two cases) or a combination of AT1-receptor blockers and calcium channel blockers (two cases). Only one patient failed to regularly take the hypotensive medication. In nine patients with good compliance with treatment, BP was quickly restored to normal values.

The observed CCrRR was >2 ml/min/year in eight patients with hypertension and in five patients who remained normotensive during follow-up. Two hypertensive and four normotensive patients had a CCrRR of <2 ml/min/year.

Linear regression analysis showed final CCr was significantly related to basal systolic BP values (r=–0.4704; p<0.043) and maximum systolic (r =–0.6723 p<0.0017) and diastolic (r=–0.4702 p< 0.043) BP values observed during follow-up. Also the percent CCr reduction with respect to the basal value observed at the end of follow-up was significantly dependent on the maximum systolic BP registered during follow-up. The highest CCrRR (7.4 ml/min/year) was observed in the patient

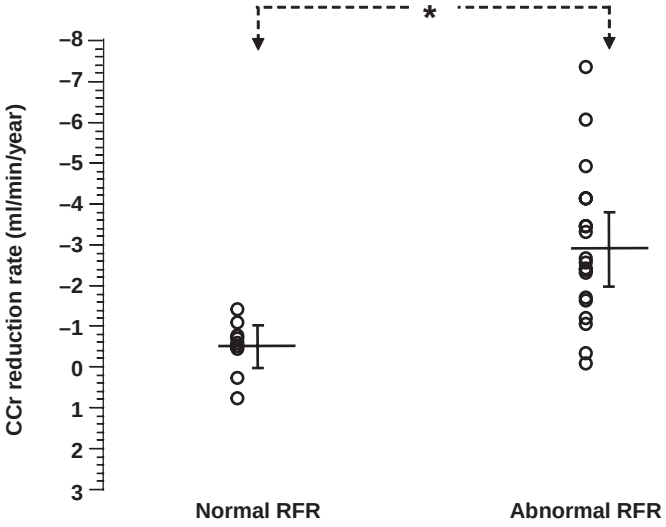

Figure 1 Individual and group values of mean annual creatinine clearance reduction rate in patients with normal and abnormal renal functional reserve. CCr, creatinine clearance; RFR, renal functional reserve *p<0.001.

with uncontrolled hypertension because of poor adherence to therapy.

Two patients with lcSSc, abnormal RFR and systemic hypertension developed microalbuminuria (141 and 108 mg/day) and clinical proteinuria (172 and 194 mg/day). In one of them, with a mean CCrRR of 1.6 ml/min/year, initial left ventricular hypertrophy with mild diastolic dysfunction and normal systolic function was found; no echocardiographic changes were demonstrated in the remaining patients.

One hypertensive patient with lung involvement (lcSSc, abnormal RFR, mean CCrRR 7.4 ml/min/year) developed mild (39 mm Hg) pulmonary hypertension; PAP remained substantially unchanged in the two patients with mild pulmonary hypertension at admission. Interstitial lung disease was demonstrated in three additional patients, one with normal and two with abnormal RFR.

DISCUSSION

In SSc, renal involvement other than SRC has not been extensively studied; however, clinical and experimental data indicate chronic renal damage in this disease.[26]

Our data do not support the view that chronic renal involvement in SSc is infrequent and generally does not result in clinically relevant renal functional impairment.[27 28]

Although the renal function of all patients with normal RFR remained substantially unchanged, a significant CCr reduction was observed in about two thirds of patients with abnormal RFR, who had, at admission, even somewhat higher (although not significant) CCr than patients with normal RFR. Patients with abnormal RFR showed a rate of renal functional decline over time that was at least double that expected in relation to age-related reduction in GFR.[29]

In 5 years, eight patients reached CCr values below 70 ml/min. A CCr of 70 ml/min is roughly equivalent to a true GFR of 60 ml/min,[30] a limit that is generally accepted to denote patients with clinically relevant renal functional derangement.[31 32]

The use of CCr for estimating renal filtration may be a limitation of our study as a more reliable estimate of GFR can be obtained by methods based on the intravenous administration of exogenous substances such as inulin (the gold standard), radioisotopic filtration markers (^{125}I-iothalamate, ^{51}Cr-EDTA, ^{99}Tc-DTPA) or low osmolar contrast medium (iohexol).[33] However, CCr is comparable to inulin for revealing and quantifying the activation of RFR[34]; moreover, CCr does not require administration of radioactive substances, possibly dangerous when the same patient has to be submitted to multiple tests. Finally, CCr is easily measured with standard laboratory equipment (iohexol requires HPLC). In this follow-up study, overestimation of true GFR due to tubular secretion of creatinine and interindividual variation in creatinine tubular secretion should not represent a critical problem because CCr was used to evaluate filtration changes at the individual level.

Immunological causes of kidney disease, in particular ANCA-related glomerulopathy,[35 36] were ruled out in our patients on the basis of clinical data and immunological profiles.

The assay for anti-RNA polymerase antibodies, which are associated with the risk of developing SRC,[37] is not available in our laboratory. However, their presence may be suggested by positive fine speckled antinuclear antibodies in the absence of antibodies against extractable nuclear antigens.[38] In our cases, this particular immunological pattern was found in two dcSSc patients, one with normal and one with abnormal RFR.

None of our patients had significant stenosis of the renal arteries. No patient had significant systolic or diastolic left ventricular dysfunction and only three of 13 patients with abnormal RFR and CCrRR >2 ml/min/year showed mild asymptomatic elevations in PAP. Thus, it seems unlikely that the observed renal findings are due to these factors.

In our opinion, the prevalence of the chronic form of renal involvement in SSc is currently underestimated for at least two reasons. The first is the use of serum creatinine concentrations to define renal involvement. Patients with SSc often have reduced muscular mass, which may provoke the so-called 'creatinine defect', leading to false normal creatinine levels despite the existence of a GFR defect. In our series, only one patient reached, during follow-up, creatinine values of 1.2 mg/dl, below the level of 1.3 mg/dl indicated as the cut-off value for the definition of mild renal involvement.[39] Even the use of mathematical formulae to indirectly estimate GFR from serum creatinine, age and other anthropometric and laboratory parameters, has proven to be more reliable than serum creatinine concentration alone to identify those SSc patients with reduced GFR.[40]

The second reason is related to the rate of progression of chronic renal damage in SSc, which is generally slow and clinically silent until it reaches very advanced functional derangement.

However, the underlying renal defect may become clinically relevant in conditions further reducing renal perfusion, as occurs in heart failure, when vomiting or diarrhoea provokes hypovolemia, or when the patient is treated with NSAIDS, diuretics or cyclosporine A.

At baseline, patients with abnormal RFR had higher (although in the normal range) BP levels than patients with normal RFR. During follow-up, more than half of these patients developed systemic hypertension, which appeared to be linked to renal functional changes; two hypertensive patients developed microalbuminuria, which may reflect vascular injury.[41]

A possible question is whether all patients developing hypertension and renal function reduction are actually cases of averted SRC because of early hypotensive treatment including, in the majority of cases, drugs acting on the renin–angiotensin system. However, several considerations argue against this hypothesis: we observed only mild to moderate BP elevations, without associated rapid worsening of renal function parameters. Only two out of six patients with dcSSc developed hypertension, while eight of 13 lcSSc patients became hypertensive. A CCrRR of more than 2 ml/min/year was also found in five of nine patients who remained normotensive during the follow-up period.

The lack of clinical characteristics of SRC and, in particular, of an active urinary sediment discouraged us from submitting patients to invasive procedures during the study. However, renal biopsy findings could define the histological features underlying the observed renal functional changes.[42] We are planning to carry out renal biopsies at least in patients with the highest CCrRR.

RFR is blunted in conditions characterised by glomerular hyperfiltration, and this functional adaptive change results in progressive glomerular damage, with loss of nephrons, renal functional decline, systemic hypertension and proteinuria.[43 44] The development of systemic hypertension accelerates the progression of renal damage, although hypotensive treatment can slow renal functional decline in all glomerular nephropathies.[45]

We can hypothesise that the hypotensive treatment has slowed renal function decline in our hypertensive SSc patients, because the patient with poor compliance and persistent high BP showed the highest CCrRR during follow-up.

It is noteworthy that the four patients with abnormal RFR who had been regularly treated with monthly intravenous iloprost infusions did not show accelerated renal function decline. A possible renal protective effect of iloprost is suggested by the demonstration of a long-lasting reduction in the resistance index of interlobular and cortical renal arteries in iloprost-treated SSc patients.[46] A more recent retrospective study, however, has raised doubts about a beneficial effect of iloprost on the evolution of internal organ damage in SSc, as far as active interstitial lung disease, pulmonary arterial hypertension, incidence of SRC and mortality are concerned.[47] The present study was not designed to explore a renal protective effect of iloprost, but the finding of a preserved renal function in iloprost-treated SSc patients with abnormal RFR deserves to be investigated in the future by a specifically designed prospective study.

In conclusion, our data confirm that the lack of activation of RFR is an early sign of renal involvement in SSc. The 5-year follow-up study demonstrates that SSc patients, with apparently normal renal function at usual clinical evaluation but abnormal RFR, are at increased risk of developing systemic hypertension and a clinically evident renal filtration defect, and that the progression of renal functional derangement is accelerated if systemic hypertension is not adequately controlled, as it generally occurs in kidney disease.

BSA, body surface area; CCr, creatinine clearance; RFR, renal functional reserve.

Competing interests None.

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