Effect of dihydropyridine calcium channel blockers and glucocorticoids on the prevention and development of scleroderma renal crisis in an Italian case series

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ABSTRACT

Objectives. *Scleroderma renal crisis (SRC) is a relative rare yet dramatic event in the history of systemic sclerosis (SSc). Several factors that may precipitate or protect from the development of SRC have been described in previous case-control studies. To date, no attempt has been made to evaluate these factors in an observational fashion.*

Methods. *Retrospective data from 410 SSc patients with disease duration <5 years at referral were evaluated in an observational fashion for the development of hypertensive or normotensive SRC within 5 years from the first visit at our centre. Baseline characteristics as well as the use of steroids or dihydropyridine calcium-channel blockers (CCB) were analysed via the Cox regression method with time-dependent covariates.*

Results. *In the multivariate model the diffuse subset the disease (HR=5.728 CI_{95}=2.199–14.918, p<0.001) and the use of prednisone (HR=1.015, CI_{95}=1.004–1.026, p=0.006) resulted to be predictors for the development of SRC, with a risk to develop SRC increased by 1.5% for every mg of prednisone/day consumed the trimester prior SRC. Contrariwise, the risk to develop SRC was highly reduced in those who were prescribed CCBs (HR=0.094, CI_{95}=0.038–0.236, p<0.001).*

Conclusion. *Steroids exhibits a weak effect on the risk to progress toward SRC in our case series, whilst dihydrophyridines CCB appeared to be protective against that. Further larger prospective studies are warranted to better define the role of CCB in this setting or as a background therapy for SSc.*

Introduction

Systemic sclerosis (SSc) is a chronic systemic autoimmune disease characterised by vascular damage, immune deregulation and cutaneous and visceral fibrosis. Renal involvement is a relatively rare (1-6), yet dramatic event in the history of SSc, which is plagued by high morbidity and mortality rates (7). The use of angiotensin-converting enzyme (ACE)-inhibitors has consistently improved the prognosis of scleroderma renal crisis (SRC) (8), nonetheless 30–50% of patients is still expected to need permanent haemodialysis or to die within 5 years from the onset of kidney disease (9). In the vast majority of patients, SRC is characterised by the abrupt development of arterial hypertension, oliguria and rapidly progressive renal failure, however, 10% of subjects with a rapidly progressive deterioration of renal function may not experience hypertension at all (10).

The mechanisms implied in the pathogenesis of SRC are not completely understood, even though several lines of evidence suggest that vasculopathy plays a predominant role in the development and in the progression of the renal disease. Increased platelet aggregation, overt intimal hypertrophy and proliferation leading to the pathognomonic "onion bulb" lesions, are typically observed in renal biopsies from SRC patients (11). These factors associated with vascular hyper-reactivity are responsible for a decreased renal blood flow with consequent renal ischaemic damage. Hypoperfusion may eventually lead to the activation of the renin-angiotensin-aldosterone system, further enhancing vasoconstriction and hyperplasia, thus strengthening and amplifying a pathological vicious circle (12).

Although the etiology of SSc-associated kidney disease remains elusive, several studies have been conducted in the effort to determine which factors may precipitate/protect from the development of SRC. A disease duration of less than 4 years (1, 13), a diffuse

and rapidly progressive skin thickening (13, 14), a recent onset of anaemia or cardiac events – particularly pericardial effusion or congestive heart failure (13, 14) – and the presence of anti-RNA polymerase III antibodies were reported as clinical predictors of SRC (1). Furthermore, a number of therapies, including cyclosporine and corticosteroids, has been reported as a precipitant factors for SRC; nonetheless not all the studies on steroids provided univocal data and controversial results have been obtained reworking the data from randomised controlled clinical trials (14). Conversely, to date, only the presence of anti-centromere antibodies has been described as protective against the development of SRC (13), whilst no exhaustive analysis has ever been provided to clearly define the effect vasoactive drugs commonly used in the treatment of scleroderma vasculopathy (15) may have on the prevention of SRC.

In the present retrospective observational study we analysed the factors associated with the development of SRC. We particularly focused on the role of glucocorticoids in the effort to determine if a correlation between the consumed daily dose and SRC exists, evaluating, at the same time, the effect of vasodilators such as calcium-channel blockers (CCB) and iloprost, on the risk for SRC.

Material and methods

Patients

Four hundred and ten consecutive patients with a diagnosis of SSc, made according to the preliminary criteria for the classification of SSc proposed by the American College of Rheumatology (ACR) (16) and referred to our outpatient clinic between 1990 and 2011, were considered for the analysis. A previous survey in Italy reported that the incidence of SRC is higher in the first years of illness (2); accordingly, only patients with disease duration ≤5 years were included in the study. Disease duration was calculated from the first non-Raynaud feature clearly attributable to SSc (2). This inclusion criterion allowed us to select a population enriched for subjects at risk for SRC, dampening the possibility to produce a biased estimate of SRC-associated risk factors.

All the eligible patients were categorised as having the limited cutaneous (lcSSc) or the diffuse cutaneous (dcSSc) subset of the disease, according to LeRoy *et al*. (17), and the patients' autoantibody profile was determined by reviewing the patients' medical records. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep_2 cells (Kallestad, Chaska, MN, USA) using a standardised technique (18); extractable nuclear antigens (ENAs) were determined by a commercial enzyme-linked immunoassay (ELISA) (Diamedix, Miami, FL, USA). The data were observationally handled from the year of referral onward and we considered the following baseline characteristics for the analysis: the percentage values for the forced vital capacity (FVC) and for the diffusion lung capacity for carbon monoxide (DLco); the presence of a right ventricular systolic pressure (RVSP) ≥45 mmHg on echocardiography (19); the presence of muscle involvement, defined as weakness with elevated creatinine phosphokinase ≥2 times the laboratory upper normal values; the presence of systemic inflammation, defined as the presence of an erythrocyte sedimentation rate (ESR) ≥25 mm/h without evidence of concurrent infection (20) and the presence of digital ulcers, defined as the loss of surface epithelialisation (21).

Observation time was set to 5 years and it was divided in 3-month time-intervals (*e.g*. 20 trimesters). For each trimester, information about the occurrence of SRC, the average daily dose of prednisone and the use of dihydropyridine calcium-channel blockers or therapy with cyclic Iloprost (22) was retrieved. No data about other vasodilators were retrieved, because for clinical practice we do not prescribe them for the treatment of SSc-related Raynaud's phenomenon, consequently, the exposure to them in our patients is very low to allow for a reliable analysis.

Hypertensive and normotensive SRC were defined as reported by Teixera *et al*. (6). Hypertensive SRC was defined by rapidly progressive oliguric renal insufficiency with no other explanation and/or rapidly progressive hypertension occurring during the course of SSc. Normotensive SRC was defined by an increase in serum 50% over baseline or serum creatinine >20% of upper limit of normal and one of the following five features; proteinuria >2+ by dipstick, haematuria >2+ dipstick or >10 red blood cells/high-power field, thrombocytopenia, $<1*10^5$ cells/mm^3, haemolysis defined as anaemia not due to others causes and either schistocytes or other red blood cell fragments seen on blood smear, or increased reticulocyte count, or renal biopsy findings consistent with SRC.

All the patients referring at our clinic at the first visit gave written consent to allow the use of their data for epidemiological studies; the present research complies with the ethic rules of our hospital and was approved by the regulatory board of our institution.

Statistical analysis

Cox regression with time-dependent covariates was used to determine which factors were associated with the development of SRC (23). Firstly, univariate Cox regression was conducted and variables significant at the 0.01 level were further considered for multivariate analysis. Owing to the low number of events in our population, the 0.01 threshold was used instead of the canonical 0.05 threshold, so as to minimise Type I error. Before conducting multivariate Cox regression, we checked the occurrence of multicollinearity among the candidate features; in this case, the least explicatory variable was not included in the multivariate model. To conduct the multivariate Cox regression analysis, the forward stepwise procedure was chosen. Results from univariate and multivariate models are expressed as hazard ratios (HRs) with their relative 95% confidence intervals (CI_{95}) throughout the manuscript. For descriptive statistics, continuous variables are expressed as mean±standard deviation (SD), except when skewed; in the latter case, median and interquartile range (IQR) values are reported. The SPSS software version 18.0 (SPSS Inc, Chicago, IL, USA) was used to conduct all the statistical analyses.

Results

Baseline demographic and clinical characteristics of our population are reported in Table I. Twenty-two patients (5.4%) developed SRC within 5 years from referral; overall nineteen hypertensive SRC (86% of the total) were observed. The median time elapsed between the first visit at our centre and the onset of renal involvement was 9 months (IQR: 1.8–14 months); the overall median disease duration at the onset of SRC was 12 months (IQR: 6.7–34.8 months). Two hundred and twenty-one patients (53.9%) were treated with corticosteroids and 142 patients received cyclic therapy with iloprost (34.6%); the vast majority of the patients (n=368, 89.8%) had been treated with dihydropyridine CCB. Overall, exposure to CCBs was equal to 5498/6308 trimesters (87.2%), whilst steroids were used in 1993 out of 6308 trimesters analysed (31.6%), with a mean dose/trimester of 5.4 mg prednisone/day and a mean maximum dose/trimester of 17.4 mg prednisone/day. Steroids were used in 24 cases (10.9%) to treat myositis, in 74 cases to treat lung involvement (33.4%), in 65 cases to treat skin involvement (29.4%), in 24 cases to treat articular involvement (11%) and in the remaining cases to treat a miscellanea of conditions. No differences were observed between patients who received CCB and those who did not as far as any of the baseline clinical or demographic variables were concerned.

Table I. Demographics.

Variable	SSc (n=410)
Females, n (%)	364 (88.8%)
Age, yrs	51.6 ± 12.9
Disease duration, months	12 (4–28)
dcSSc, n (%)	105 (25.6%)
Autontibodies, n (%)	
ANA	388 (94.8%)
Scl70	162 (39.5%)
ACA	154 (37.6%)
RVSP >45mmHg, n (%)	14 (3.4%)
FVC, % predicted	95.3 ± 19
DLCO, % predicted	74.4 ± 20.7
Ulcers, n (%)	112 (27,4%)
CPK >2 UNL, n (%)	24 (5.9%)
ESR >25 mm/h, n (%)	123 (30%)

Clinical characteristics at referral. Values expressed as mean±standard deviation, except for disease duration (median and interquartile range) or where otherwise indicated. dcSSc: diffuse cutaneous systemic sclerosis (SSc); ANA: antinuclear antibodies; Scl70: anti-Topoisomerase I antibodies; ACA: anticentromere antibodies; RVSP: right-ventricular systolic pressure; FVC: forced vital capacity; DLco: diffusing capacity for carbon monoxide; CPK: creatine-phophokinase; UNL: upper normal laboratory values; ESR: erythrocyte sedimentation rate.

Table II. Factors associated with scleroderma renal crisis (SRC).

	Univariate analysis			Multivariate analysis		
Variabile	HR	CI_{95}	*p*-value	HR	CI_{95}	*p*-value
dcSSc	6.797	2.769–16.683	<0.001	5.728	2.199–14.918	<0.001
Male gender	5.13	2.147–12.261	0.001			NS
Prednisone, mg/day	1.028	1.018–1.038	<0.001	1.015	1.004–1.026	0.006
FVC, % of predicted*	0.961	0.93–0.973	0.001			
Disease duration (months)**	0.962	0.914–0.991	0.018			
Use of calcium-channel blockers	0.099	0.041–0.241	<0.001	0.094	0.038–0.236	<0.001
Presence of ACAs**	0.72	0.01–0.539	0.012			

Results from Cox regression analysis with time-dependent covariates. dcSSc: diffuse systemic sclerosis; FVC: forced vital capacity; ACAs: anti-centromere antibodies; HR: hazard ratios; CI_{95}: 95% confidence intervals or the HRs. *Not included in the multivariate model due to multicollinearity with dcSSc. **Not included in the multivariate model (*p*-values from univariate analysis above 0.01 screening threshold).

Significant results from univariate and multivariate Cox regression analysis with time-dependent covariates are reported in Table II; all the other studied covariates that do not appear into the table were not significantly associated with SRC occurrence. Overall, 55.6% of instances had a type II censoring, type I censoring rates were equal to 39%. Univariate analysis sorted out the following variables as significantly associated with the occurrence of SRC: the dcSSc subset (HR=6.797, CI_{95}=2.769–16.683), the male gender (HR=5.13, CI_{95}=2.147–12.261) and the use of steroids (HR=1.028, CI_{95}=1.018-1.038). The following variables were inversely associated with the occurrence of SRC: the percentage of FVC predicted values at baseline (HR=0.951, CI_{95}=0.93–0.979), the disease duration (HR=0.962, CI_{95}=0.935–0.991), the presence of anti-centromere antibodies (ACAs) (HR=0.072, CI_{95}=0.01–0.539) and the use of calcium-channel blockers (HR=0.099, CI_{95}=0.041–0.241).

The presence of ACAs and the disease duration were not included in the multivariate model, as they did not reach the prerequisite significance threshold; the multicolinearity testing showed a correlation between the percentage of FVC predicted values and the dcSSc subset, therefore the FVC was not included in the multivariate model. From the multivariate model the dcSSc subset (HR=5.728, CI_{95}=2.199–14.918) and the use of prednisone (HR=1.015, CI_{95}=1.004–1.026) resulted to be predictors for the development of SRC, whilst the use of CCB was associated with a reduced risk for that complication (HR=0.094, CI_{95}=0.038–0.236). Overall, the results indicate that the risk to develop SRC is increased by 1.5% for every mg of prednisone/day. Cumulatively, the exposure to CCB in subjects who did or did not experience SRC was thus distributed: 8/22 trimesters with SRC (36.4%), 5490/6286 trimesters without SRC (87.3%).

Discussion

SRC is one of the most dreadful complication of SSc and, even if the early use of ACE-inhibitors has dramatically improved SRC survival (8), its occurrence is still plagued by high morbidity and mortality rates. Several studies have been conducted in order to sort out the factors that may predict or protect from the occurrence of SRC. Amongst clinical and serological factors, corticosteroids have emerged as a relevant risk-factor for the development of SRC, with a 3-fold estimated risk for those who were treated with any dose of steroid, according to a previous report by Steen and co-workers (13). Nonetheless, reworking the data from the high-dose *versus* low-dose D-penicillamine in early diffuse systemic sclerosis trial (14), the association between corticosteroids consumption and SRC was

found to be less crucial: prednisone had a causative role only in presence of high skin scores and joint contractures together, exerting no effect when considered alone. In our study, the use of prednisone (or equivalent) was found to be associated with the occurrence of SRC both after univariate and multivariate analysis. Differently from previous studies, rather than establishing a cut-off value for the dose of corticosteroids, we treated prednisone as a continuous variable and handled it observationally in a time-dependent fashion; the resulting estimated risk for SRC in those who made use of prednisone *versus* those who did not was found to be rather weak (1.5% per mg/prednisione/day). A much higher risk for SRC was observed in patients with the dcSSc subset, in accordance with previous reports (2, 13, 14). Owing to the limited number of subjects included in our analysis, it was difficult to model for interaction terms and/or to conduct a stratified analysis to assess the risk for renal complications in those with the dcSSc who were given steroids. Nonetheless, even if in our population the risk of SRC associated with prednisone would appear negligible, it seems reasonable to be advise caution in prescribing high-dose steroids to dcSSc patients.

To date, no pharmacological therapy has proved effective in protecting against the risk of developing SRC in SSc patients. On the contrary, in our study, the use of CCB was found to be associated with a dramatic reduction in the risk for SRC. As for clinical practice, the vast majority of our patients (89.8%) is prescribed first or second generation dihydropyridines (mainly nifedipine, but also perdipine or amlodipine) during the course of their illness. Presently, it is not possible to define the precise mechanism by which this class of drugs may reduce the risk for SRC, even if it seems reasonable to speculate that this may be somewhat related to their vasoactive properties. CCBs block L-type calcium channels preferentially acting on the peripheral circulation and are considered a first-line therapy for the treatment of Raynaud's phenomenon (15). However, several studies also demonstrated that dihydropyridines act on the kidney vascular bed, selectively vasodilating the afferent arteriole with almost no effect on the efferent arteriole (24, 25). Preglomerular vasoconstriction, intimal fibrosis and endothelial tissue remodelling are prominent features that can be observed in the pathogenesis of scleroderma renal disease, decreasing kidney perfusion and leading to an impaired glomerular filtration rate (11, 12). In this critical situation, hypoperfusion activates the renin-angiotensin system, giving rise to angiotensin II levels in order to maintain a stable glomerular filtration rate via postglomerular arteriolar vasoconstriction. Dihydropyridines were shown to antagonise the vasocostrictive action exerted by angiotensin II on the preglomerular arteriole (26, 27), preserving its effect on the efferent arteriole and exerting thus a preferential augmentation of glomerular filtration rate and filtration fraction, eventually counteracting the trigger events of SRC. These peculiar properties of dhydropyiridines may also explain their observed capability of reducing renal sufferance secondary to cyclosporine therapy (27). Of interest and similarly to SRC, cyclosporine-induced nephrotoxicity is thought to be partially caused by vasoconstriction of the glomerular afferent arterioles (28).

Among the other analysed factors, the presence of ACAs in the univariate analysis confirmed its negative association with SRC as observed by Penn *et al*. (1); similarly to the English case-series, it is difficult to ascertain whether this inverse risk-association is due to a protective effect of ACAs or is somewhat related to the absence of RNA-polymerase III antibodies, a strong risk-factor for SRC in some populations (1, 29), albeit not in Italy (2), and whose occurrence is mutually exclusive with ACAs (29). The retrospective design of the study, dating back to the early 90's, indeed prevented us from a thorough collection of this datum. Penn *et al*. (1) identified a median of disease duration of 7.5 months in patients with renal complication and 69% of SCR developed within 1 year of the diagnosis of SSc. In our population, the median disease duration at SRC was 12 months and 73% of SRC occurred within 15 months from the diagnosis. Therefore, our observation confirms that SRC is a precocious manifestation in the disease course. In our population, 5.4% of patients develops SRC; this datum contrasts with the SRC incidence of 13% described in the American (5) or north European SSc-patients (1), but it is in accordance with previous studies performed in Mediterranean area (2, 4).

The low prevalence of SRC and the inclusion criteria we applied prevented us from observing a large number of cases in our population and, thus, to conduct more sophisticated analyses that might have better elucidated the role of steroids and CCB on the prevention or on the progression of SRC, as for instance, the search for interaction terms. Also, the low prevalence of SRC in our series and the need to avoid the risk to describe spurious results (*e.g*. to lower Type I error) was inevitably accompanied by a low power to detect significant effects from other factors described elsewhere (1, 13, 14). The number of events per predictor (EPV) variable we used in the multivariate analysis (*e.g*. 22/4=5.5) may suggest a significant departure from the empirical "rule of thumb" of 10 EPV and thus the presence of a significant bias in our analysis. Yet, as shown in (30), this rule is by far too conservative and the presence of 5–9 EPV is sufficient to keep low the bias in risk estimation and type I error rates as well as to adequately estimate confidence interval coverage.

As with any observational data, extreme caution should be applied in the evaluation of results, being the risk of the so-called "survivor bias", not negligible (31): patients who do not experience the event of interest may preferentially be exposed to treatment (*e.g*. steroids or CCB) leading to spurious associations. However, as noted in (32), this risk is negligible when Cox regression with time-dependent covariates is applied; indeed this technique, which we applied both in the univariate and multivariate analysis, is regarded as the optimal choice to address the survivor bias issue.

To our knowledge, this is the first time CCB emerged as a significant inversely related risk factor for the development

of SRC. It is nearly impossible to explain the divergence of our results from the two reports that have, at least partially, addressed the question (13, 14); a direct comparison between our and these studies is not feasible, due to the completely different methodological approach employed for the analysis (*e.g.* retrospective survival study with time-dependent covariates *vs.* case-control) and to the strikingly different exposure to this class of drugs.

Overall, even if our findings should be carefully gauged in light of the above-mentioned considerations and of the retrospective design of the study, our results may help to cast new light on the possible role of CCBs as a "background" therapy for SSc, besides their well-known vasodilating property used to counteract Raynaud's phenomenon symptoms. Indeed, renal-protection would add to a number of other possible systemic effects of CCBs already described in SSc case series such as the prevention of pulmonary hypertension in lcSSc patients (33), the amelioration of myocardial perfusion (34) and the prevention of left ventricular dysfunction (35). Larger observational studies are thus needed to confirm these preliminary findings, which are supported by the physiological properties of dihydropyridines.

References

1. PENN H, HOWIE AJ, KINGDON EJ *et al*.: Scleroderma renal crisis: patient characteristics and long-term outcomes. *QJM* 2007; 100: 485-94.
2. CODULLO V, CAVAZZANA I, BONINO C *et al*.: Serologic profile and mortality rates of scleroderma renal crisis in Italy. *J Rheumatol* 2009; 36: 1464-9.
3. SIMEON CP, ARMADANS L, FONOLLOSA V *et al*.: Survival prognostic factors and markers of morbidity in Spanish patients with systemic sclerosis. *Ann Rheum Dis* 1997; 56: 723-8.
4. VLACHOYIANNOPOULOS PG, DAFNI UG, PAKAS I, SPYROPOULOU-VLACHOU M, STAVROPOULOS-GIOKAS C, MOUTSOPOULOS HM: Systemic scleroderma in Greece: Low mortality and strong linkage with HLA-DRB1*1104 allele. *Ann Rheum Dis* 2000; 59: 359-67.
5. MAYES MD, LACEY JV JR., BEEBE-DIMMER J *et al*.: Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003; 48: 2246-55.
6. TEIXEIRA 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.
7. STEEN VD, MEDSGER TA: Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007; 66: 940-4.
8. STEEN VD, COSTANTINO JP, SHAPIRO AP, MEDSGER TA JR.: Outcome of renal crisis in systemic sclerosis: relation to availability of angiotensin converting enzyme (ACE) inhibitors. *Ann Intern Med* 1990; 113: 352-7.
9. STEEN VD, MEDSGER TA JR.: Long-term outcomes of scleroderma renal crisis. *Ann Intern Med* 2000; 133: 600-3.
10. STEEN VD: Scleroderma renal crisis. *Rheum Dis Clin North Am* 2003; 29: 315-33.
11. BATAL I, DOMSIC RT, MEDSGER TA, BASTACKY S: Scleroderma renal crisis: a pathology perspective. *Int J Rheumatol* 2010; 2010: 543704.
12. CANNON PJ, HASSAR M, CASE DB, CASARELLA WJ, SOMMERS SC, LEROY EC: The relationship of hypertension and renal failure in scleroderma (progressive systemic sclerosis) to structural and functional abnormalities of the renal cortical circulation. *Medicine* (Baltimore) 1974; 53: 1-46.
13. STEEN VD, MEDSGER TA JR, OSIAL TA JR, ZIEGLER GL, SHAPIRO AP, RODNAN GP: Factors predicting development of renal involvement in progressive systemic sclerosis. *Am J Med* 1984; 76: 779-86.
14. 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.
15. KOWAL-BIELECKA O, LANDEWÉ R, AVOUAC J *et al*.: EULAR recommendations for the treatment of systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group (EUSTAR). *Ann Rheum Dis* 2009; 68: 620-8.
16. PRELIMINARY CRITERIA FOR THE CLASSIFICATION OF SYSTEMIC SCLEROSIS (SCLERODERMA): Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutics Criteria Committee. *Arthritis Rheum* 1980; 23: 581-90.
17. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subset and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
18. BAYER PM, BAUERFEIND S, BIENVENU J *et al*.: Multicenter evaluation study on a new HEp2 ANA screening enzyme immune assay. *J Autoimmun* 1999; 13, 89-93.
19. MUKERJEE D, ST GEORGE D, KNIGHT C *et al*.: Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology* (Oxford) 2004; 43: 461-6.
20. SCUSSEL-LONZETTI L, JOYAL F, RAYNAULD JP *et al*.: Predicting mortality in systemic sclerosis: analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine* (Baltimore) 2002; 81: 154-67.
21. KORN JH, MAYES M, MATUCCI-CERINIC M *et al*.: Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum* 2004; 50: 3985-93.
22. SCORZA R, CARONNI M, MASCAGNI B *et al*.: Effects of long-term cyclic iloprost therapy in systemic sclerosis with Raynaud's phenomenon. A randomized, controlled study. *Clin Exp Rheumatol* 2001; 19: 503-8.
23. FISHER LD, LIN DY: Time-dependent covariates in the Cox proportional-hazards regression model. *Annu Rev Public Health* 1999; 20: 145-57.
24. HAYASHI K, NAGAHAMA T, OKA K, EPSTEIN M, SARUTA T: Disparate effects of calcium antagonists on renal microcirculation. *Hypertens Res* 1996; 19: 31-6.
25. LOUTZENHISER R, EPSTEIN M: Renal microvascular actions of calcium antagonists. *J Am Soc Nephrol* 1990; 1: S3-12.
26. LOUTZENHISER RD, EPSTEIN M, FISCHETTI F, HORTON C: Effects of amlodipine on renal hemodynamics. *Am J Cardiol* 1989; 64: 122I-127I.
27. RODICIO JL: Calcium antagonists and renal protection from cyclosporine nephrotoxicity: long-term trial in renal transplantation patients. *J Cardiovasc Pharmacol* 2000; 35:S7-11.
28. ANDREONI KA, O'DONNELL CP, BURDICK JF, ROBOTHAM JL: Hepatic and renal blood flow responses to a clinical dose of intravenous cyclosporine in the pig. *Immunopharmacology* 1994; 28: 87-94.
29. 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.
30. VITTINGHOFF E, MCCULLOCH CE: Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol* 2007; 165: 710-8.
31. VAN WALRAVEN C, DAVIS D, FORSTER AJ, WELLS GA: Time-dependent bias was common in survival analyses published in leading clinical journals. *J Clin Epidemiol* 2004; 57: 672-82.
32. SY RW, BANNON PG, BAYFIELD MS, BROWN C, KRITHARIDES L: Survivor treatment selection bias and outcomes research: a case study of surgery in infective endocarditis. *Circ Cardiovasc Qual Outcomes* 2009; 2: 469-74.
33. STEEN V, MEDSGER TA JR.: Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Arthritis Rheum* 2003; 48: 516-22.
34. VIGNAUX O, ALLANORE Y, MEUNE C *et al*.: Evaluation of the effect of nifedipine upon myocardial perfusion and contractility using cardiac magnetic resonance imaging and tissue Doppler echocardiography in systemic sclerosis. *Ann Rheum Dis* 2005; 64: 1268-73.
35. ALLANORE Y, MEUNE C, VONK MC *et al*.: Prevalence and factors associated with left ventricular dysfunction in the EULAR Scleroderma Trial and Research group (EUSTAR) database of patients with systemic sclerosis. *Ann Rheum Dis* 2010; 69: 218-21.