Combination therapy with rituximab and mycophenolate mofetil in systemic sclerosis. A single-centre case series study

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ABSTRACT

Objective. *To describe a single centre experience using combination therapy with rituximab (RTX) and mycophenolate mofetil (MMF) in a prospective series of systemic sclerosis (SSc) patients with pulmonary and cutaneous involvement, rapidly progressive or resistant to conventional therapy.*

Methods. *RTX was administered in two different regimens (1000 mg fortnightly x 2 or 375 mg/m²/week for 4 consecutive weeks) at baseline and after 6 months, associated with MMF 2000 mg/day continuously. Cutaneous fibrosis was evaluated assessing modified Rodnan Skin Score (mRSS) and pulmonary involvement was evaluated performing pulmonary function tests, diffusing lung capacity for carbon monoxide and chest high-resolution computed tomography (HRCT). The radiological extension of the interstitial lung disease (ILD) at HRCT, was assessed with the conventional visual reader-based score (CoVR) and with a computerised-aided method (CaM) using a DICOM software.*

Results. *Eighteen SSc patients underwent combination therapy (F/M: 10/8, median age 51 years, median duration of disease 27 months). Data from fifteen patients were available at 12-month follow-up. The mRSS showed a significant improvement; a significant increase in forced vital capacity and forced expiratory volume in the first second were also observed. In addition, a significant reduction of the extension of ILD was detected when evaluated with CaM. No serious adverse events were observed during the follow-up period.*

Conclusion. *Despite preliminary results and limited to a small number of patients, our data suggest that therapy with RTX and MMF is well tolerated, safe, and potentially effective for cutaneous and pulmonary involvement in SSc.*

Introduction

Systemic sclerosis (SSc) is a chronic systemic autoimmune disease characterised by microangiopathy, increased deposition of extracellular matrix developing progressive fibrosis, both in the skin and in visceral organs (1). SSc-interstitial lung disease (ILD) represents the clinical manifestation that dictates prognosis, but the few therapeutic options available are characterised by modest results. Cyclophosphamide (CYC) has shown efficacy in the treatment of SSc-ILD despite questionable clinical benefit and remarkable side effects (2). During the last few years, two different drugs were proposed for SSc therapy: rituximab (RTX) and mycophenolate mofetil (MMF). RTX was firstly used for $CD20^+$ B-cell non-Hodgkin lymphomas but it plays a well-recognised role in the treatment of several autoimmune diseases. It has been proposed as a candidate therapy for SSc, since several evidences suggest that B cells may have a possible pathogenic role in this disease (3). Our group demonstrated that a single course of RTX reduced scleroderma fibroblast activation in vitro and the serum levels of anti-PDGFR stimulatory autoantibodies, providing evidence for a role of B cells in scleroderma pathogenesis (4). After promising results of pilot trials, the EUSTAR group published the first multicentre, nested, case-controlled study with RTX in SSc; researchers described an improvement in fibrosis reporting stable lung function parameters in SSc-ILD patients, with an acceptable safety profile (5). MMF is an immunosuppressant with anti-proliferative effects on inflammatory cells, especially on T cells modulation. Meta-analysis and systematic reviews of the current evidence provides a valid appraisal of the safety and the efficacy profile of MMF

in SSc-ILD patients (6). In a multicentre randomised clinical trial, aimed to compare the functional effect and tolerability of MMF with oral CYC in patients with SSc-ILD, both resulted in significant improvements in lung function parameters over the 2-year course of the study, although MMF was better tolerated and associated with less toxicity (7). Moreover, MMF showed a significant improvement in skin fibrosis in patients with diffuse cutaneous SSc, comparable with CYC (8).

Our purpose was to treat SSc patients with cutaneous and lung involvement, with a combination therapy based on RTX and MMF, which may offer a multiple-pathway targeted therapy, evaluating effects on skin fibrosis and SSc-ILD.

Patients and methods

We report data about 18 patients referred to our clinic from September 2013 to December 2015. Patients were considered for treatment if affected by SSc (9), aged ≥18 y/o, with diffuse or limited cutaneous form and with pulmonary involvement. Cutaneous involvement was defined according to Le Roy *et al*. criteria. Pulmonary involvement was defined considering ground-glass and/or honeycombing involving at least 10% of the lung and/or reduction in forced vital capacity (FVC) of at least 15% without any other concomitant lung disease. The agreement to use effective contraceptive methods was a mandatory requirement. Patients were enrolled consecutively; we did not consider for treating patients that presented contraindications for a long-term immunosuppressive therapy (history of HBV, HCV or TB infection; history cancer). None of the patients had the diagnosis of pulmonary hypertension, according to ESC/ERS 2009 Guidelines. Authorisation to treatment was requested from time to time for each patient at local Ethics Board (Comitato Etico Regione Marche, Italy) and a written informed consent was obtained from all patients. To obtain the better patient's compliance, RTX were administered with intravenous infusion of in two different regimens: 1000 mg fortnightly x 2 or 375 mg/m2 four weekly pulses, at baseline and at 6 months. The infusion regimen was discussed with every patient during the informed consent explanation, and the choice was taken together with the patient and his/her caregiver (details are showed in Table I). Prior to RTX infusions all patients received pretreatment according to the label recommendations. After the last infusion of RTX was started MMF 500 mg, gradually increased, till the final daily dose of 2000 mg. Patients received anti-infective prophylaxis with Valacyclovir 1000 mg and Cotrimoxazole 160/800 mg alternate days for all the period of follow-up. Skin fibrosis was evaluated using the modified Rodnan Skin Score (mRSS), performed by the same experienced assessor at baseline and after 3, 6 and 12 months (10). Standard pulmonary function tests (PFTs) and single-breath diffusing capacity were performed, at baseline, at 6 months and at 12 months in all patients, including FVC, forced expiratory volume in the first second (FEV_1), and diffusing capacity of carbon monoxide (DLCO). All patients had a chest high-resolution computed tomography (HRCT) performed at baseline, at 6 months and at 12 months. The extension of pulmonary fibrosis (PF) was analysed by semi-quantitative visual reader-based scores (CoVR) and quantitative computerised-aided methods (CaM) using OsiriX software as described in previous experiences (11-13). All patients underwent volumetric thin-section CT examinations using a CT 64 GE light Speed VCT power scanner; details are shown in Table II. The parenchymal abnormalities on HRCT were coded and scored by two independent readers, blinded with respect the results, according to Warrick *et al*. (14). The severity and extent of disease were then calculated as total HRCT score (range from 0 to 30). The HRCT examinations were randomised and blinded reviewed by a radiologist expert in ILD reporting. The CaM quantification process was conducted according to previously proposed protocol (11, 13). Non-parametric data were analysed by Friedman's test. Analysis was performed using GraphPad® Prism v .5 software.

Results

Patients' demographics (median age: 51 years, gender F/M: 10/8), disease duration from the first non-Raynaud's symptom (median: 27 months), disease subset (limited/diffuse: 8/10) refers to all 18 enrolled patients; details are showed in Table I. Concomitant therapy was: low dose steroids (four patients, less than 10 mg/day prednisone for arthralgia), Bosentan 125 mg bid (one patient, for acral ulcers), Iloprost or Alprostadil (all patients, monthly infusions for Raynaud's symptomatic phenomenon). Three patients withdrew from the study: the first was lost at follow-up, the second had a minor infusion reaction which led to RTX discontinuation, and the third died six months after the beginning of therapy (he stopped MMF after one month), for sudden cardiac arrest happened during a progressive and refractory heart failure event. Therefore, the 12-month analysis includes the 15 patients who completed follow-up. The improvement of skin fibrosis (Fig. 1A) was evident after 6 months of treatment with a significant decrease of mRSS value (p=0.0376), that was confirmed at the 12th month evaluation, compared with the baseline score: mRSS (mean±SD) fall from 17.6±12,5 to 11.1±6,6 (p=0.0036); it was more remarkable in patients with diffuse cutaneous SSc, as expected. Moreover, after twelve months, there was a significant increase of FVC (p=0.0093) and FEV_1 (p=0.0061) compared with baseline, while DLCO values remained stable (p=0.3375). (Fig. 1B, C, D). The improvement of lung function tests was already detectable after six months, with a significant increase for FVC (p=0.0197) and FEV_1 (p=0.0278); details are showed in Table II. The HRCT scan showed a significant improvement, evaluated with quantitative CaM analysis (p=0.0331), while the semiquantitative Warrick score showed a not significant trend toward improvement, between baseline and at 12th month (Fig. 1 E, F). Patients' HRCT details and the descriptive statistics are summarised in Table II. No serious adverse events were observed. In two patients the MMF dose was reduced for mild neutropenia; in one pa-

Table I. Patients' baseline demographic and clinical features.

ID Patient	Gender F/M	Age (yr)	Body Surface Area (mq)	Disease duration (months)	SSc autoantibodies positive*	Subset SSc	Rituximab regimens	Previous immunosuppressive therapy
Pt A	F	35	1,75	10	Scl 70	limited	1000mg fortnightly x 2	None
Pt B §	M	30	1,85	12	Scl 70	diffuse	1000mg fortnightly x 2	CYC, Imatinib
Pt C	F	52	1,72	15	ANA granular	limited	1000mg fortnightly x 2	None
Pt D	M	72	1,70	12	ANA granular	diffuse	1000mg fortnightly x 2	None
Pt E	M	62	1,86	14	Scl 70	limited	1000mg fortnightly x 2	None
Pt F	F	63	1,60	10	Scl 70	diffuse	1000mg fortnightly x 2	None
Pt G	F	61	1,74	70	Scl 70	diffuse	1000mg fortnightly x 2	CYC
Pt H	F	63	1,80	12	Scl 70	diffuse	1000mg fortnightly x 2	None
Pt I	M	55	1,82	96	Scl 70	diffuse	1000mg fortnightly x 2	CYC
Pt J	M	41	1,94	16	Scl 70	limited	1000mg fortnightly x 2	None
Pt K	F	64	1,71	24	Scl 70	diffuse	1000mg fortnightly x 2	None
Pt L	F	50	1,74	24	Scl 70	diffuse	375 mg/mq x 4 weeks x 2	None
Pt M	F	58	1,69	36	Scl 70	limited	375 mg/mq x 4 weeks x 2	None
Pt N	M	49	1,76	48	Scl 70	diffuse	1000mg fortnightly x 2	None
Pt O	M	40	1,87	60	Scl 70	limited	375 mg/mq x 4 weeks x 2	CYC
Pt P	F	49	1,78	12	Scl 70	diffuse	375 mg/mq x 4 weeks x 2	None
Pt Q §	M	22	1,82	18	Scl 70	limited	Not completed **	None
Pt R §	F	22	1,70	8	Scl 70	limited	1000mg fortnightly x 2	None

*Autoantibodies were measured and interpreted according to local standards; **Infusion reaction; §Patients lost during follow-up period; CYC: cyclophosphamide.

Table II. Pulmonary function test results and high resolution computed tomography evaluation performed by visual reader-based score (CoVR) compared with the computer-aided evaluation (CaM) for pulmonary fibrosis. Scans were obtained at full inspiration in the supine position. Scanning parameters: 120 kV, 300 mAs, acquisition time 0.8 s, slice thickness 1 mm with 0.6 mm reconstructions and the smallest possible field of view (FOV) covering both lungs. The scans were viewed with a window level of -600 Hounsfield units (HU) and width of 1600 HU. Data are evaluated at baseline and at 6th and 12th month in all patients.

					CoVR Score			CaM Score		
ID Patient	FVC baseline (% pred)	FVC 12 months (% pred)	DLCO baseline (% pred)	DLCO 12 months (% pred)	Score baseline	Score 6 months	Score 12 months	% fibrosis baseline	% fibrosis 6 months	% fibrosis 12 months
Pt A	64	102	48	77	15	8	8	23.3	11.2	10.7
Pt C	79	76	70	61	12	12	12	24.9	22.6	21.4
Pt D	78	89	81	70	11	11	11	7.8	6.6	7.1
Pt E	90	96	81	72	11	11	11	13.9	10.5	8.7
Pt F	120	124	60	67	11	10	11	12.5	7.3	10.9
Pt G	67	79	66	42	26	26	26	20.4	20.3	20.1
Pt H	109	124	95	108	15	15	14	16.1	16.5	11.9
Pt I	82	87	63	59	21	20	21	20.7	13.1	15.1
Pt J	99	92	75	67	10	9	11	15.9	17.1	12.7
Pt K	87	85	53	52	20	20	20	15.2	16.1	16.4
Pt L	82	103	41	50	16	14	14	20.6	12.6	8.9
Pt M	91	96	61	66	21	21	21	22.1	19.3	20.1
Pt N	73	74	47	39	9*	13	16	14.7*	29.6	29.7
Pt O	61	61	43	47	27	27	27	35.4	33.4	30.1
Pt P	54	58	34	20	15	15	15	22.3	22.2	28.4
Median [IQR]	82 [26]	89 [26]	61 [28]	61 [23]	15 [10]	14 [9]	14 [10]	20.5 [8.2]	16.5 [11]	15.1 [10.7]
mean ±SD	82.4±17.3	89.7±18.6	61.2±16.6	59.8±19.4	16.0 ±5.8	15.5 ±6.0	15.9 ±5.8	19.7±6.9	17.2±7.6	16.8±7.8

*We considered for the study a recent chest CT scan, already available, although performed without high resolution examination.
FVC: forced vital capacity; DLCO: diffusing capacity of carbon monoxide.

tient was discontinued the prophylaxis with cotrimoxazole for mild macrocytic anaemia.

Discussion

SSc remains a challenging disease, without therapies of long-term proven efficacy. Evidences available upon the therapeutic use of RTX in diffuse cutaneous SSc, are largely based on one observational study (5). In a recent series of 14 patients, improvement or stabilisation of pulmonary disease was also observed in patients with long-standing SSc-associated ILD (15). In our experience, a potential benefit was observed in cutaneous involvement, but temporary and requiring retreatment after a mean of six months (4). Therefore, we decided to repeat RTX after 6 months preventing B lymphocytes reappraisal and to add a second immunosuppressive drug, targeting also T lymphocytes. The effects on pul-

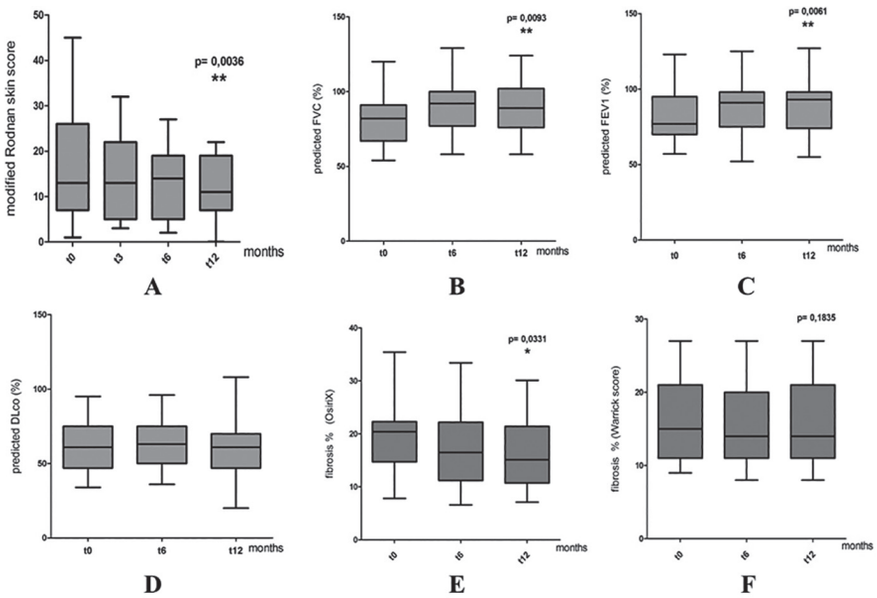

Fig. 1. Effects of the combination therapy: box plots are representative of the median value and the interquartile ranges. The mRSS at baseline, 3rd, 6th and 12th month showed a significant improvement in skin thickening after 12 months (**A**). Pulmonary function tests at baseline, 6th and at 12th month showed a significant increase in FVC and in FEV1 and substantially stable values for DLCO (**B**,**C**,**D**). Pulmonary fibrosis on high resolution computed tomography scans at baseline, 6th and at 12th month evaluated with computer-aided evaluation ("OsiriX" as reported in the plot) (**E**) showed a significant reduction of lung fibrosis at 12 months whereas the Warrick score (**F**) showed trend toward improvement. mRSS: modified Rodnan Skin Score; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; DLCO: diffusing capacity of carbon monoxide.

monary function were remarkable, witnessed by improvement in FVC and in FEV_1; DLCO seems to be not affected. The HRCT pattern was analysed with two different methods. The first one consists of a semiquantitative standardised method, but the results did not show significant improvement. The second one, is an automatic computer-based assessment, which reported a significant reduction in the quantification of pulmonary fibrosis; the reason may be found in its better sensitivity to appreciate also minimal changes. As previously described, CaM may assist analysis of lung HRCT: it provides an objective method that supplements the subjective visual-based grading of the extent of ILD and to achieve precise and reader-independent quantification (11, 13). Taken together, the data seems to show that the combined therapy may be able to improve pulmonary involvement or at least to stabilise it, also considering that most of the patients had an early stage disease or have failed previous cyclophosphamide therapy. Concerning the significant reduction in skin fibrosis, our results are aligned with the current literature evidences (8). The two drugs association was well tolerated, without significant adverse events. The low rate of infections may be consequent to the use of antimicrobial and antiviral prophylaxis. Obviously, the study has several limitations: the small number of patients in a monocentric cohort, no control group and the limited follow-up period to 12 months. In conclusion, the association of RTX plus MMF seems to be a promising strategy for SSc, both in the pulmonary and cutaneous involvement. Adequate studies are awaited to assess the efficacy and safety of the combined therapy.

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