# Two-year Results of an Open Pilot Study of a 2-treatment Course with Rituximab in Patients with Early Systemic Sclerosis with Diffuse Skin Involvement

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*ABSTRACT.* ***Objective.*** To study safety and potential efficacy of a 2-treatment course (month 0/6) with rituximab (RTX) in early diffuse systemic sclerosis (dcSSc).

***Methods.*** Two years' followup (open-label study) was done of 8 patients with early dcSSc. Patients received an infusion of 1000 mg RTX 2 times at months 0 and 6, with 100 mg methylprednisolone. Clinical measurements, Disease Activity Score, functional status, and CD19+ peripheral blood count were performed at months 0, 3, 6, 12, 15, 18, and 24 and histopathological evaluation of the skin at months 0, 3, 12, and 24.

***Results.*** There was a clinically significant change in skin score, with a mean Modified Rodnan skin score of 24.8 at baseline (SD 3.4) and 13.6 at Month 24 [SD 5.6; mixed models analyses (MMA) $p < 0.0001$] and a significant decrease in Disease Activity Score (DAS), with a median of 4.5 at baseline (range 1.5–7.5) and 0.5 at Month 24 (range 0.0–5.5; MMA $p < 0.0001$). Indices of internal organ involvement remained stable throughout the study. RTX induced effective B cell depletion at baseline and Month 6 (< 5 CD19+ cells/$\mu$l blood). The blindly assessed hyalinized collagen score changed significantly over time (MMA $p = 0.009$), with a mean of 69.3 at baseline (SD 22.8) and 33.1 at 24 months (SD 27.0). Five serious adverse events were considered unrelated to the RTX treatment.

***Conclusion.*** A 2-treatment course (months 0/6) with RTX appears to be well tolerated and may have potential efficacy for skin disease and stabilization of internal organ status in early dcSSc. Clinical Trials Registration NCT00379431. (First Release Nov 1 2012; J Rheumatol 2013;40:52–7; doi:10.3899/jrheum.120778)

*Key Indexing Terms*
DIFFUSE SYSTEMIC SCLEROSIS RITUXIMAB B LYMPHOCYTES

Systemic sclerosis (SSc) is a multisystemic autoimmune disease characterized by fibrosis of skin and internal organs, generalized microvasculopathy, and antibody response against various cellular antigens. SSc has a high morbidity and mortality, and to date no treatment has been shown through randomized controlled trials to halt the natural progression of the disease[1]. Rituximab (RTX), a monoclonal chimeric antibody against CD20 that depletes peripheral B cells, has been proposed as a therapy because there is growing evidence that B cells play a role in the pathogenesis of SSc[2,3,4,5,6,7,8,9,10,11,12].

The safety and potential benefits of RTX in SSc have been evaluated[13,14,15,16,17]. But to date a 2-treatment course (months 0/6) of RTX in early diffuse SSc has not been investigated.

Severe organ involvement occurs early in the course of diffuse cutaneous SSc (dcSSc) and has a bad prognosis. Survival of the first years of the disease is associated with an improved outcome. Therapies that may help the patient to overcome this early period seem warranted[1,18].

Against this background, we conducted an open-label trial of a 2-treatment course (months 0/6) with RTX in patients with early dcSSc. The 6-month followup data have been reported[16]. The results supported RTX as safe in early dcSSc and suggested that it reduced the skin score clinically and statistically significantly, a finding supported by blinded histopathological analysis of the skin[16].

We report here our 2-year followup data.

## MATERIALS AND METHODS

*Study design.* Our study was an open-label therapeutic trial. RTX (1000 mg) with methylprednisolone (100 mg) was administered at Week 0 and

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Week 2 and readministered at Week 26 and Week 28. Indices of internal organ functioning, the Health Assessment Questionnaire Disability Index (HAQ-DI), and the Medical Outcome Study Short-Form 36 (SF-36) were evaluated at months 0, 3, 6, 12, 15, 18, and 24. Peripheral B cell counts were performed at the same timepoints. In addition, B cell counts were also analyzed 2 weeks after baseline and at the Month 6 infusion with RTX. Histopathology was performed at 0, 3, 12, and 24 months. The protocol and patient informed consent form were approved by the Ethics Committee of Ghent University Hospital and are in accord with the Declaration of Helsinki. Written consent was obtained from all patients.

*Study patients*. Patients with dcSSc (fulfilling the American College of Rheumatology preliminary criteria for SSc) were screened at Ghent University Hospital. Inclusion criteria were age older than 18 years, disease duration (time passed since the first non-Raynaud disease manifestation) ≤ 4 years, a modified Rodnan skin score (MRSS) ≥ 14, or a Disease Activity Score (DAS) ≥ 3. Low-dose prednisolone (≤ 10 mg/day) was allowed, provided that the patients were taking a stable dose at least 12 weeks before inclusion in the trial. All disease-modifying antirheumatic drugs (except methotrexate) were stopped 12 weeks before screening and were replaced by methotrexate 15 mg per week (unless contraindicated). Exclusion criteria included endstage internal organ involvement, defined as forced vital capacity (FVC) ≤ 50%, diffusing lung capacity for carbon monoxide (DLCO) ≤ 40%, and echocardiographically assessed left ventricular ejection fraction ≤ 40%. Other exclusion criteria were serious and uncontrolled coexisting diseases, infection, immunodeficiency, and a history of cancer.

*Clinical measurements*. The primary outcome was skin involvement assessed by the 17-site MRSS (0–3 scale), done by the same investigator throughout the study. Lung involvement was assessed by high-resolution computed tomography and pulmonary function tests. Cardiac involvement was assessed by echocardiography. Renal function was determined by estimation of creatinine clearance with the Modification of Diet in Renal Disease formula. All subjects completed the HAQ-DI and the SF-36 to evaluate the influence on daily functioning and quality of life.

*Serology and flow cytometry*. Screening for antinuclear antibodies was performed as described[16]. B cell depletion (anti-CD19, clone SJ25C1, BD Biosciences) was analyzed by flow cytometry (FC500, Beckman Coulter).

*Skin histology*. Full-thickness skin biopsies from the dorsal side of the forearm (about 1.5 cm long and 0.5 cm wide) were surgically obtained at baseline and months 3, 12, and 24. Followup samples were obtained within 3 cm of the original biopsy site. Scoring was performed as described[16,19,20]. Blinded Masson's trichrome and anti-α-smooth muscle actin-stained slides were scored twice by 1 observer. A score was assigned on a 10-cm visual analog score scale. The average score was used for statistical analysis. CD20-positive cells were counted in 10 randomly chosen fields (640–860 mm), which were oriented perpendicular to the epidermis.

*Statistical analysis*. Mixed models analyses (MMA) with random intercept for patient were used to evaluate changes in clinical measurements and continuous histopathological measurements (hyalinized collagen score) over time. Changes in proportions of myofibroblast and B cell positivity over time were assessed by means of Cochran Q-tests and McNemar tests for comparisons versus baseline at different timepoints. All analyses were performed with SAS 9.2 (SAS Institute Inc.) and SPSS 19 (SPSS Inc.). A statistical significance level of 0.05 was used. In case of multiple testing, the Holm-Bonferroni correction was applied[21].

## RESULTS

*Patient characteristics*. All consecutive patients were diagnosed with early dcSSc (with a disease duration ≤ 3 years as described[16,22]). Patient characteristics are given in Table 1.

*Safety and tolerability*. Five serious adverse events (SAE) were observed (Table 2) and were considered probably unrelated to the study medication. Two of them [a coronary artery bypass graft (SAE 1) and an episode of noninfectious subfebrility (SAE 2)] were reported in the 6-month followup data[16]. Patient 2, who had undergone coronary arterial bypass grafting during the first 6 months of followup and who received no Month 6 treatment with RTX, died (SAE 3) 4 months after surgery because of severe sepsis, presumably through infection of the portacath. Patient 5 developed a secondary infection of a digital ulcer (SAE 4), leading to admission and administration of intravenous antibiotics. Because SAE 3 and 4 occurred, respectively, at Month 10 and Month 24, 10 and 18 months after administration of

*Table 1*. Characteristics of patients (n = 8).

| Characteristic | |
|---|---|
| Age, yrs, median (range) | 38 (49–69) |
| Sex (women/men) | 3/5 |
| SSc-specific antibodies | 7/8 |
| Anti-RNA polymerase II | 3/8 |
| Antitopoisomerase I | 3/8 |
| Antitopoisomerase I and anticentromeric protein B | 1/8 |
| Disease duration*, mo, median (range) | 10 (8–34) |
| MRSS, mean ± SD | 24.8 ± 3.4 |
| Lung involvement | |
| HRCT (alveolitis or fibrosis) | 3/8 |
| TLC ≤ 80% | 4/8 |
| FVC < 70% | 0/8 |
| DLCO < 70% | 5/8 |
| Cardiac involvement** | 5/8 |
| Musculoskeletal involvement*** | 8/8 |
| Gastrointestinal involvement† | 1/8 |
| History of renal crisis†† | 1/8 |

* Disease duration from onset of first non-Raynaud phenomenon. ** Defined as conduction disturbances (2 patients), left ventricular ejection fraction < 55%, systolic pulmonary artery pressure > 40 mm Hg, pericardial effusion or diastolic dysfunction (5 patients). *** Defined as joint contractures (all patients). † Technically investigated only when new clinical complaints were present, and defined as gastrointestinal motility disturbance by barium swallow (1 patient), malabsorption, esophageal stenosis, gastroesophageal reflux, or intestinal pseudoobstruction. †† Antecedent scleroderma renal crisis (1 patient). MRSS: modified Rodnan Skin Score; SSc: systemic sclerosis; HRCT: high-resolution computed tomography; TLC: total lung capacity; FVC: forced vital capacity; DLCO: diffusing lung capacity for carbon monoxide.

*Table 2*. Summary of deaths and serious adverse events (SAE).

| | |
|---|---|
| No. deaths | 1* |
| No. patients experiencing an SAE | 3 |
| Total no. SAE | 5 |
| SAE 1. Coronary arterial bypass grafting*,** | 1 |
| SAE 2. Episode of noninfectious subfebrility**,*** | 1 |
| SAE 3. Sepsis leading to death* | 1 |
| SAE 4. Secondary infection of digital ulcer | 1 |
| SAE 5. Episode of hyperventilation*** | 1 |

* Patient 2. ** Described in the 6-month report of this study[16]. *** Patient 3.

RTX, and no neutropenia or hypogammaglobulinemia were observed, these SAE were deemed probably unrelated to the study medication. Patient 3 was hospitalized because of shortness of breath (SAE 5), probably due to hyperventilation (there were suggestive findings on arterial blood gas analysis and after exclusion of alternative diagnosis, including pulmonary embolism).

No unexpected minor adverse events occurred (Table 3).

*Clinical efficacy*. Evaluation of internal organ involvement and functioning is described in Table 4. The clinical skin score improved steadily over 2 years. There was a statistically and clinically significant decrease in MRSS, with a mean MRSS of 24.8 (SD 3.4) at baseline versus 13.6 (SD 5.6) at Month 24 (MMA $p < 0.0001$), a mean percentage improvement of 45%, and a median of 24.5 at baseline versus 11.0 at Month 24, an improvement of 55%. This applies also to each timepoint in between (months 0, 3, 6, 12, 15 and 18) versus baseline, even after Holm-Bonferroni correction for multiple testing.

There was also a statistically and clinically significant decrease in DAS, with a median of 4.5 at baseline (range 1.5–7.5) and 0.5 at Month 24 (range 0.0–5.5; MMA $p < 0.0001$). This also applies to each timepoint versus baseline, even after Holm-Bonferroni adjustments.

FVC showed a statistically but not clinically significant overall decrease, with a mean FVC 92.8% of normal at baseline (SD 8.6) versus 84.7% of normal at Month 24 (SD 13.3; MMA $p = 0.047$). After Holm-Bonferroni correction, no statistically significant change was noted at any timepoint versus baseline. DLCO remained stable over the 2 years of followup.

There was no significant change in systolic pulmonary arterial pressure (sPAP), with a mean sPAP of 31.0 mm Hg at baseline (SD 4.0) versus 30.6 mm Hg at Month 24 (SD 4.3; MMA $p = 0.717$). No significant alterations occurred on electrocardiograms.

*Table 3*. Summary of minor adverse events.

| | |
|---|---|
| No. patients experiencing a minor adverse event | 4 |
| Total no. minor adverse events | 14 |
| Infectious episodes | 4 |
| Upper airway | 3 |
| Dermatological | 1 |
| Gastrointestinal manifestations | 4 |
| Pyrosis | 1 |
| Nausea | 2 |
| Stomatitis | 1 |
| Cardiovascular manifestations | 2 |
| Arterial hypertension | 1 |
| Arterial thrombosis | 1 |
| Pulmonary manifestations | 1 |
| Noninfectious exacerbation of COPD | 1 |
| Neuropsychiatric manifestations | 1 |
| Depression | 1 |
| Musculoskeletal manifestations | 2 |
| Rotator cluff tendinitis | 2 |

COPD: chronic obstructive pulmonary disease.

During followup, no renal crisis occurred, nor were there signs of progressive gastrointestinal disease. Indices of global health and functionality, as measured with HAQ-DI and total SF-36, remained stable.

*Flow cytometry*. B cell depletion was induced effectively in all patients at baseline (< 5 CD19+ cells/$\mu$l blood). At Month 6, B cells in the peripheral blood of 5 out of 7 patients were still depleted (Patient 2 was withdrawn before the 6-month evaluation). In the 2 patients who showed no B cell depletion at Month 6, readministration of RTX induced B cell depletion again, and B cells gradually increased.

*Skin histology*. The mean hyalinized collagen score changed significantly over time (MMA $p = 0.009$), with a mean of 69.3 at baseline (SD 22.8) and 33.1 at 24 months (SD 27.0; Figure 1, Table 5). It decreased from baseline until Month 12 and seemed to rise again at Month 24 versus Month 12 (although this rise was not statistically significant). Myofibroblasts were present at baseline in all patients. As depicted in Table 5, myofibroblast positivity decreased significantly over time (Cochran Q, $p = 0.005$), to 4/8 at Month 3 and 1/7 at months 12 and 24 (Figure 2). B cells decreased significantly over time (Cochran Q, $p = 0.031$). They were present in the skin at baseline in 4 out of 8 patients and in none of the patients at 3 months and 12 months. In 1 patient, B cells reappeared in the skin of the forearm at 24 months (Figure 3).

## DISCUSSION

To our knowledge, this is the first report of 2-year followup data of an open pilot study of a 2-treatment course (months 0 and 6) of RTX in patients with early dcSSc. The previously reported 6-month followup data of our study suggested that RTX was well tolerated and could safely be administered in patients with early dcSSc[16]. The 2-year followup data confirm this finding, as does work from other groups[13,14,15,17].

Our previous results also demonstrated a significant decline in clinical skin score. At 2 years, a further decrease is observed, with a drop in MRSS of 11.2 points at Month 24 versus baseline. This largely exceeds the minimal clinically relevant treatment effect estimate for the skin as provided by a Delphi exercise[23], and is more than can be expected as a spontaneous improvement in patients with similar disease duration[24]. These findings are promising, as Steen and Medsger showed that in patients with early dcSSc who showed improvement of skin score during 2 years of followup, survival was significantly better than in patients who had no such improvement[18]. The mean improvement in MRSS in our study (45% at Month 24 vs baseline) is comparable to that reported by Steen and Medsger. Moreover, the finding is backed by a blindly evaluated skin histopathology, which shows a similar

*Table 4*. Changes in clinical and laboratory measurements in the study upon treatment with rituximab.

| Variable | Statistic | 0M, n = 8 | | 3M, n = 8 | | 6M, n = 7 | | 12M, n = 7 | | 15M, n = 7 | | 18M, n = 7 | | 24M, n = 7 | | p (MMA) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MRSS | Mean, SD | 24.8 | 3.4 | 19.4* | 5.4 | 14.3*** | 3.5 | 10.8*** | 4.6 | 10.0*** | 2.6 | 10.8*** | 2.6 | 13.6*** | 5.6 | < 0.0001 |
| | Median | 24.5 | | 18.0 | | 15.0 | | 10.5 | | 9.0 | | 11.0 | | 11.0 | | |
| | Min, max | 21.0 | 30.0 | 12.0 | 26.0 | 9.0 | 18.0 | 6.0 | 19.0 | 7.0 | 14.0 | 7.0 | 14.0 | 8.0 | 23.0 | |
| DLCO (% of normal) | Mean, SD | 73.3 | 22.7 | 68.5 | 22.1 | 73.0 | 18.1 | 74.7 | 18.3 | 75.3 | 19.1 | 75.2 | 23.8 | 72.6 | 15.8 | 0.549 |
| | Median | 60.5 | | 60.0 | | 64.0 | | 74.0 | | 64.0 | | 72.0 | | 68.0 | | |
| | Min, max | 54.0 | 111.0 | 46.0 | 106.0 | 55.0 | 98.0 | 56.0 | 96.0 | 58.0 | 105.0 | 50.0 | 104.0 | 54.0 | 98.0 | |
| FVC (% of normal) | Mean, SD | 92.8 | 8.6 | 88.5 | 12.9 | 88.3 | 9.3 | 89.2 | 13.7 | 94.4 | 10.1 | 89.8 | 12.0 | 84.7† | 13.3 | 0.047 |
| | Median | 92.5 | | 92.5 | | 91.0 | | 92.5 | | 96.0 | | 92.0 | | 88.0 | | |
| | Min, max | 76.0 | 106.0 | 68.0 | 101.0 | 71.0 | 99.0 | 64.0 | 102.0 | 76.0 | 105.0 | 68.0 | 104.0 | 61.0 | 98.0 | |
| TLC (% of normal) | Mean, SD | 83.0 | 10.5 | 82.1 | 12.7 | 83.1 | 13.4 | 83.2 | 9.3 | 83.9 | 16.5 | 84.5 | 12.6 | 78.9 | 16.7 | 0.485 |
| | Median | 81.0 | | 82.5 | | 90.0 | | 85.0 | | 91.0 | | 86.0 | | 74.0 | | |
| | Min, max | 64.0 | 97.0 | 61.0 | 100.0 | 62.0 | 97.0 | 67.0 | 91.0 | 54.0 | 103.0 | 63.0 | 98.0 | 51.0 | 101.0 | |
| FEV (% of normal) | Mean, SD | 83.9 | 8.1 | 81.0 | 17.7 | 77.0 | 9.8 | 79.5 | 13.5 | 85.6 | 12.0 | 78.2 | 10.6 | 73.4† | 13.7 | 0.0286 |
| | Median | 87.0 | | 82.5 | | 78.0 | | 84.0 | | 84.0 | | 78.5 | | 75.0 | | |
| | Min, max | 71.0 | 94.0 | 49.0 | 104.0 | 66.0 | 93.0 | 62.0 | 96.0 | 70.0 | 103.0 | 65.0 | 95.0 | 55.0 | 99.0 | |
| SF-36 | Mean, SD | 41.2 | 15.1 | 41.2 | 21.5 | 51.1 | 22.4 | 45.2 | 22.8 | 47.9 | 21.3 | 51.0 | 19.7 | 45.5 | 21.0 | 0.192 |
| | Median | 40.9 | | 31.0 | | 39.7 | | 45.4 | | 39.8 | | 50.6 | | 43.6 | | |
| | Min, max | 18.9 | 58.5 | 19.3 | 76.8 | 25.2 | 89.6 | 17.3 | 70.9 | 22.4 | 80.5 | 29.0 | 73.8 | 19.9 | 79.9 | |
| HAQ-DI | Mean, SD | 1.4 | 0.6 | 1.5 | 0.6 | 1.3 | 0.7 | 1.3 | 0.8 | 1.3 | 0.6 | 1.2 | 0.8 | 1.3 | 0.7 | 0.464 |
| | Median | 1.3 | | 1.4 | | 1.1 | | 1.5 | | 1.0 | | 1.1 | | 1.6 | | |
| | Min, max | 0.8 | 2.1 | 0.6 | 2.5 | 0.3 | 2.1 | 0.1 | 2.0 | 0.4 | 2.0 | 0.1 | 2.3 | 0.4 | 2.0 | |
| DAS | Mean, SD | 4.5 | 1.9 | 2.3* | 1.5 | 1.1*** | 0.8 | 0.8*** | 1.0 | 1.1*** | 1.1 | 1.1*** | 1.1 | 2.1* | 2.4 | < 0.0001 |
| | Median | 4.5 | | 2.0 | | 1.0 | | 0.3 | | 1.0 | | 1.0 | | 0.5 | | |
| | Min, max | 1.5 | 7.5 | 0.0 | 5.0 | 0.0 | 2.0 | 0.0 | 2.5 | 0.0 | 2.5 | 0.0 | 2.5 | 0.0 | 5.5 | |
| CLcr, ml/min/1.73m$^2$ | Mean, SD | 83.0 | 32.5 | 80.2 | 30.0 | 74.2 | 20.2 | 71.1 | 19.8 | 68.6 | 17.3 | 68.5 | 22.9 | 71.0 | 19.5 | 0.386 |
| | Median | 87.1 | | 81.0 | | 78.2 | | 78.4 | | 74.5 | | 74.8 | | 79.0 | | |
| | Min, max | 30.8 | 143.6 | 35.3 | 140.0 | 36.7 | 91.8 | 34.0 | 86.9 | 35.4 | 87.2 | 31.9 | 96.0 | 32.0 | 86.7 | |
| sPAP, mm Hg | Mean, SD | 31.0 | 4.0 | 28.0 | 5.3 | 30.0 | 3.7 | 28.3 | 4.7 | NA | NA | NA | NA | 30.6 | 4.3 | 0.717 |
| | Median | 30.0 | | 26.0 | | 29.5 | | 28.0 | | NA | | NA | | 31.0 | | |
| | Min, max | 26.0 | 36.0 | 23.0 | 37.0 | 26.0 | 35.0 | 23.0 | 36.0 | NA | NA | NA | NA | 23.0 | 35.0 | |
| LVEF, % of normal | Mean, SD | 69.6 | 2.3 | 67.4 | 2.2 | 67.0 | 4.2 | 64.6† | 4.1 | NA | NA | NA | NA | 59.7** | 6.8 | 0.001 |
| | Median | 70.0 | | 68.0 | | 66.5 | | 67.0 | | NA | | NA | | 59.0 | | |
| | Min, max | 66.0 | 72.0 | 64.0 | 70.0 | 63.0 | 72.0 | 57.0 | 68.0 | NA | NA | NA | NA | 55.0 | 74.0 | |

Significance of all values versus baseline: † $p < 0.05$; * $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$. M: month; MMA: mixed model analysis; MRSS: modified Rodnan Skin Score; DLCO: diffusion capacity for carbon monoxide; FVC: forced vital capacity; TLC: lung total capacity; FEV: forced expiratory volume; SF-36: Medical Outcome Study Short-Form 36; HAQ-DI: Health Assessment Questionnaire Disability Index; DAS: Disease Activity Score; CLcer: creatinine clearance; sPAP: systolic pulmonary artery pressure; LVEF: left ventricular ejection fraction; NA: not applicable, because echocardiography was performed only at months 0, 3, 6, 12, and 24.

decline in hyalinized collagen content (52% at Month 24 vs baseline).

Further, there was no clinically significant progression in internal organ involvement, while normally the largest prevalence of progressive organ involvement is to be expected in this patient group[1]. This may suggest disease-modifying properties of RTX. This is promising because the literature shows that patients with early dcSSc who do not develop severe organ involvement within the first 3 years from disease onset have a better 9-year cumulative survival rate than those who do (72% vs 38%; $p < 0.0001$)[25].

Our study suggests that RTX is well tolerated and relatively safe, reduces the clinical skin score significantly, and may be effective in stabilizing internal organ involvement, in patients with early dcSSc. Because there is an urgent need for a safe and efficacious treatment for this patient group, our findings warrant adequately powered placebo-controlled clinical trials in larger patient groups and with longer evaluation periods, to further evaluate the safety, efficacy, optimal treatment regimen, and survival benefit of treatment with RTX in dcSSc.


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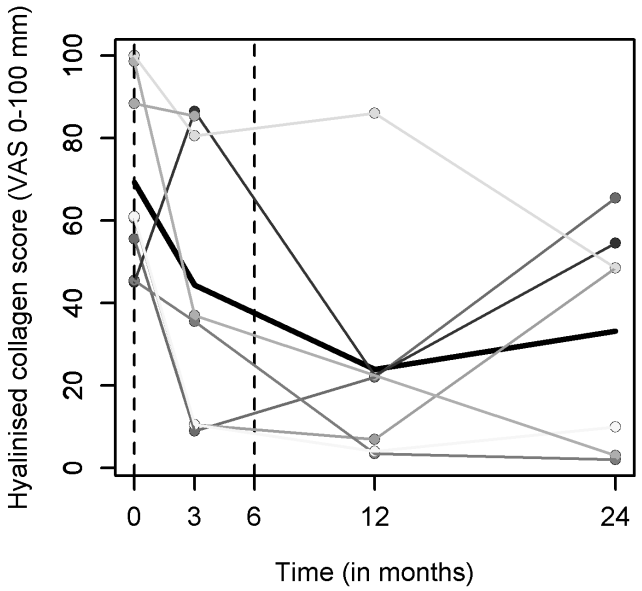


*Figure 1*. Hyalinized collagen content in the skin before and after 2-treatment course (months 0 and 6) with rituximab. Thick black line represents the mean/median.

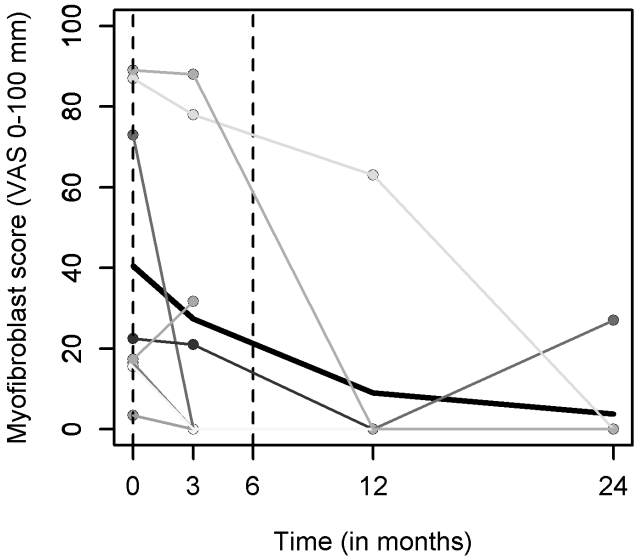


*Figure 2*. Myofibroblast score in the skin before and after 2-treatment course (months 0 and 6) with rituximab. Thick black line represents the mean/median.

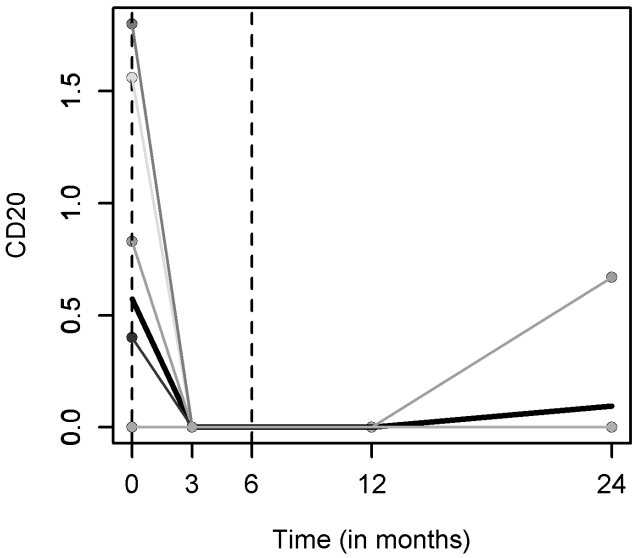


*Figure 3*. Lymphocyte numbers (CD20 staining) in the skin before and after 2-treatment course (months 0 and 6) with rituximab. Thick black line represents the mean/median.


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*Table 5*. Changes in skin histopathological measures (dorsal side of forearm).

| Variable | 0M, n = 8 | | 3M, n = 8 | | 12M, n = 7 | | 24M, n = 7 | | p |
|---|---|---|---|---|---|---|---|---|---|
| Hyalinized collagen score, mean, SD | 69.3 | 22.8 | 44.4<br>p = 0.05 | 34.7 | 23.9*<br>p = 0.002 | 28.8 | 33.1†<br>p = 0.025 | 27.0 | 0.009<br>(MMA) |
| Myofibroblast positivity, frequency (%) | 8/8 (100) | | 4/8 (50)<br>p = 0.125** | | 1/7† (14)<br>p = 0.031** | | 1/7† (14)<br>p = 0.031** | | 0.005 (Cochran) |
| B cell positivity, frequency (%) | 4/8 (50) | | 0/8 (0)<br>p = 0.125** | | 0/7 (0)<br>p = 0.125** | | 1/7 (14)<br>p = 0.250** | | p = 0.031 (Cochran) |

MMA: mixed model analysis. Significance of all values versus baseline: † $p < 0.05$; * $p < 0.01$; ** McNemar test (vs baseline).

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