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Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study

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Nonstandard abbreviations: α-SMA: α-smooth muscle actin, DAS: disease activity score, dc-SSc: diffuse cutaneous systemic sclerosis, DLCO: diffusing capacity of the lung for carbon monoxide, FVC: forced vital capacity, GEE: generalized estimating equation, HAQ-DI: health assessment questionnaire disability index, LVEF: left ventricular ejection fraction, MMA: mixed models analysis, mRSS: modified Rodnan skin score, SF36: Study 36-item Short Form Health Survey, sPAP: systolic pulmonary arterial pressure,

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ABSTRACT

Objectives: We examined the safety and potential efficacy of rituximab in diffuse cutaneous systemic sclerosis (dc-SSc).

Methods: We conducted a 24 week open-label study in which 8 patients with dc-SSc received an infusion of 1000 mg rituximab administered at baseline and day 15, together with 100 mg methylprednisolone at each infusion. Assessment included CD19+ peripheral blood lymphocyte number, skin sclerosis score, indices of internal organ functioning, HAQ-DI, SF-36, and histopathological evaluation of the skin. This study is registered with ClinicalTrials.gov, number NCT00379431.

Results: Ritixumab induced effective B cell depletion in all patients (<5 CD19+ cells/µl blood). There was a significant change in skin score at week 24 ($p<0.001$). Also, significant improvements were measured in the dermal hyalinised collagen content ($p=0.014$) and dermal myofibroblast numbers ($p=0.011$). Two serious adverse events occurred, which were thought to be unrelated to the rituximab treatment.

Conclusions: Rituximab appears to be well-tolerated and may have potential efficacy for skin disease in dc-SSc.

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INTRODUCTION

Diffuse cutaneous systemic sclerosis (dc-SSc) has a poor prognosis. In recent years, clinical trials with B cell depleting agents, unveiled a role for B lymphocytes in the pathogenesis of several auto-immune diseases. Multiple elements point to a role for B cells in SSc pathogenesis.[1,2] Communications concerning the use of rituximab in this indication are scarce.[3,4]

In this study, we explored the safety and potential efficacy of B cell depletion with rituximab in patients with dc-SSc in an open-label trial, using clinical as well as histopathological endpoints. Data on efficacy were only used to generate hypotheses.

PATIENTS AND METHODS

Study design

Rituximab (1000 mg) together with 100 mg methylprednisolone was administered at day 1 and day 15. Skin biopsies were obtained at baseline and at 12 weeks. Indices of internal organ functioning, HAQ-DI and SF-36 were evaluated at week 0, week 2, week 12 and week 24. The protocol and patient informed consent forms were approved by the Ethics Committee of the Ghent University Hospital. The study ClinicalTrials.gov Identifier is : NCT00379431.

Study patients

Patients with dc-SSc, fulfilling the American College of Rheumatology preliminary criteria for SSc,[5] were screened at the Ghent University Hospital.

Inclusion criteria were: age older than 18 years, a disease duration (time passed since the first non-Raynaud's disease manifestation) of ≤4 years, a modified Rodnan skin score (mRSS) ≥14 or DAS ≥3.[6] Low-dose prednisolone (≤10 mg/day) was allowed, provided that the patients were on a stable dose at least 12 weeks before inclusion in the trial. All DMARDS (except methotrexate) were stopped 12 weeks before screening and were replaced by methotrexate 15 mg per week (unless contra-indicated). Exclusion criteria were: forced vital capacity (FVC) ≤50%, a diffusing capacity of the lung for carbon monoxide (DLCO) ≤40%, echocardiographically assessed left ventricular ejection fraction (LVEF) ≤40%, serious and uncontrolled coexisting diseases, infection, immunodeficiency, or a history of cancer.

Clinical measurements

Primary outcome was skin involvement assessed by the 17-site mRSS (0-3 scale), which was done by the same investigator throughout the study (VS). Lung involvement was assessed by high resolution computed tomography and pulmonary function tests. Cardiac involvement was assessed by echocardiography. Renal function was determined by the estimation of the creatinine clearance with the MDRD formula. All subjects completed the disability index of the Health Assessment Questionnaire Disability Index (HAQ-DI) and the Study 36-item Short Form Health Survey (SF36) to evaluate the influence on daily functioning and quality of life.[7]

Skin histology and histological assessment of skin involvement

Full-thickness skin biopsies (approximately 1.5 cm long and 0.5 cm wide) were surgically obtained at baseline and 12 weeks. Samples taken at 12 weeks were obtained within 1 cm of the original biopsy site. Skin was taken from the dorsal side of the forearm and the inner side of the upper arm. A normal reference set was included in the analysis. This set contained the skin biopsy from the inner upper arm of 9 persons who were referred for a lupus band test and in whom further evaluation excluded any specified autoimmune disease.

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Blinded Masson's trichrome and anti-α-SMA stained slides were scored 3 times by 2 independent observers (BV and JTVP). A score was assigned on a 10-cm VAS scale.[8] The average score was used for statistical analysis. The interrater correlations for hyalinised collagen and myofibroblast scores were 0.74 ($p<0.01$) and 0.86 ($p<0.01$) respectively. CD20 positive cells were counted in 10 randomly chosen fields (640 to 860 µm), which were orientated perpendicular to the epidermis.

Biochemistry

Screening for antinuclear antibodies was performed with indirect immunofluorescence on HEp-2000 cells (Immunoconcepts, Sacramento, CA, USA) at a 1:40 dilution with a FITC-labelled goat anti-human IgG (heavy and light chain). All samples were analyzed with INNO-LIA Update (Innogenetics NV, Zwijnaarde, Belgium). Serum anti-RNA polymerase III antibodies (MBL international, Woburn, MA, USA) and anti-topoisomerase I antibodies (Varelisa, Phadia GmbH, Freiburg, Germany) levels were quantified with a commercial ELISA kit. B-cell depletion was analysed by flowcytometry (FC500, Beckman Coulter, Fullerton, CA, USA).

Statistical Analysis

Mixed models analyses (MMA) with random intercept and slopes were used to evaluate changes of clinical parameters over time.[9] Histological data were analyzed by multilevel methods taking into account two levels: time and place of biopsy. For continuous data with normal or nearly normal distribution, MMA was used. Data with more skewed distributions and on-off phenomena were, after dichotomisation, analysed with generalized estimating equations (GEE) and a logit link function. GEE and MMA were calculated without imputation of missing data as these methods are the best way to handle missingness under the assumption of missingness at random.[9] Interreader correlations coefficients were calculated using Spearman's correlation coefficient. Differences between normal and diseased skin were analyzed by Mann-Whitney U.tests. All analyses were performed with SPSS 15.0 (Chicago, IL, USA).

Role of the funding source

This is an investigator initiated study.

RESULTS

Characteristics of the study subjects

Nine patients were screened and 8 were included in the trial. The patient characteristics are shown in table 1a.

The normal reference set for skin histology included 6 females and 3 males. Median age was 38 (range 16-68).

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Table 1 a Demographics of the study population

Patient number/ Sex/Age	Disease duration (months)	Organ involvement at baseline*					Past immune-based therapy	Concomitant medication	SSc specific anti-nuclear antibodies†
		Lung‡	Renal§	Heart‖	Musculo-skeletal¶	GI**			
1/F/55	9	+	+	+	+	+	D-penicillamine, oral corticosteroids, PUVA, etanercept, cyclophosphamide and methotrexate	Methotrexate 15mg IM/week and guanethedine	Anti-RNA-polymerase III
2/F/57	30	+	-	-	+	-	Salazopyrine, oral corticosteroids and methotrexate	Methotrexate 15mg IM/week and prednisolone 10 mg/day	Anti-topoisomerase I
3/M/69	11	-	+	-	+	-	-	-	Anti-RNA-polymerase III
4/M/50	34	-	-	+	+	-	Methotrexate and oral corticosteroids	Methotrexate 15 mg IM/week and methyl-prednisolone 8 mg/day	Anti-topoisomerase I
5/M/49	22	+	-	+	+	-	Methotrexate and oral corticosteroids	Methotrexate 25 mg IM/week and methyl-prednisolone 8 mg/day	Anti-topoisomerase I
6/F/54	9	+	-	-	+	-	Oral corticosteroids	Prednisolone 7,5 mg/day	Anti-topoisomerase I and anti-centromeric protein B
7/M/51	9	-	-	+	+	-	Methotrexate and oral corticosteroids	Methotrexate 15mg IM/week and prednisolone 10 mg/day	Anti-RNA-polymerase III
8/M/53	8	+	-	+	+	-	-	-	-

*All patients had truncal skin involvement.
†All patients were positive for antinuclear antibodies (indirect immunofluorescence on HEp-2000 cells).
‡Lung involvement was defined as alveolitis or fibrosis on HRCT (patient 1), restrictive lung function test (TLC ≤ 80%) (patients 2 and 5) or both (patients 6 and 8).
§Renal involvement was defined as antecedent scleroderma renal crisis (patient 3), proteinuria (≥0.3 g/l) or estimated GFR < 60 ml/min/1.73 m^2 (stage 3 of CKD) (patients 1 and 3).
‖Cardiac involvement was defined as conduction disturbances (patients 7 and 8), LVEF <55%, sPAP >40 mm Hg, pericardial effusion or diastolic dysfunction (patient 1, 3, 4, 5 and 8).
¶Musculoskeletal involvement was defined as joint contractures (patients 1, 2, 3, 4, 5, 6, 7 and 8), synovitis, muscle weakness (patients 1 and 4), or CK elevation.
**Gastro-intestinal involvement was only technically investigated when new clinical complaints were present, and defined as gastro-intestinal motility disturbance by barium swallow (patient 1), malabsorption, oesophageal stenosis, gastro-oesophageal reflux or intestinal pseudo-obstruction. Patients 2, 4, 5 and 8 were already on proton pump inhibitors before screening, and had no further investigations.

Safety and tolerability

Two serious adverse events, considered to be probably unrelated to the study drug, occurred. Patient 2 underwent coronary artery bypass surgery just before the 24 weeks evaluation. The post-operative condition of the patient made the 24 week evaluations impossible. Therefore this patient was excluded from the 24 weeks evaluation. Patient 3 reported a 2 day low-grade fever occurring 2 weeks after the second infusion of rituximab, for which he was hospitalized. Upon hospitalization, the fever had spontaneously subsided and no infectious focus could be found. The minor adverse events were: one infectious exacerbation of existing polyposis nasi and initiation of anti-hypertensive therapy, in patient 1, who had known hypertension; an episode of nausea, after initiation of rifampycine for prevention of exacerbation of latent

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tuberculosis (in patient 3), and one episode of non-infusion related nausea and of depressive mood (in patient 5).

Clinical efficacy

Over 24 weeks the clinical skin score improved from a mean mRSS of 24.8 (SD 3.4) to 14.3 (SD 3.5) (MMA: $p<0.001$), which is a mean percentage of improvement of 43%, and from a median of 24.5 to 15, which is a median percent of change of 40%. Parameters of internal organ involvement and functioning remained stable. These are depicted in table 1b.

Table 1b Changes in clinical and laboratory parameters in the study upon treatment with rituximab

Parameter	Statistic	Week 0 (n=8)		Week 12 (n=8)		Week 24 (n=7)	
Total Skin Score*	Mean, SD	24.8	3.4	19.4	5.4	14.3	3.5
	Median	24.5		18.0		15.0	
	Minimum, maximun	21.0	30.0	12.0	26.0	9.0	18.0
DLCO (% of normal)†	Mean, SD	73.3	22.7	68.5	22.1	73.0	18.1
	Median	60.5		60.0		64.0	
	Minimum, maximun	54.0	111.0	46.0	106.0	55.0	98.0
Lung Vital Capacity (% of normal)†	Mean, SD	92.8	8.6	88.5	12.9	88.3	9.3
	Median	92.5		92.5		91.0	
	Minimum, maximun	76.0	106.0	68.0	101.0	71.0	99.0
Forced Expiratory Volume (% of normal)†	Mean, SD	83.9	8.1	81.0	17.7	77.0	9.8
	Median	87.0		82.5		78.0	
	Minimum, maximun	71.0	94.0	49.0	104.0	66.0	93.0
Systolic Pulmonary Artery Pressure (mmHg)†	Mean, SD	31.0	4.0	28.0	5.3	30.0	3.7
	Median	30.0		26.0		29.5	
	Minimum, maximun	26.0	36.0	23.0	37.0	26.0	35.0
Left Ventricular Ejection Fraction (% of normal)†	Mean, SD	69.6	2.3	67.4	2.2	67.0	4.2
	Median	70.0		68.0		66.5	
	Minimum, maximun	66.0	72.0	64.0	70.0	63.0	72.0
Creatinine clearance (ml/min/1.73m^2)†	Mean, SD	83.0	32.5	80.2	30.0	74.2	20.2
	Median	87.1		81.0		78.2	
	Minimum, maximun	30.8	143.6	35.3	140.0	36.7	91.8
total SF36†	Mean, SD	41.2	15.1	41.2	21.5	51.1	22.4
	Median	40.9		31.0		39.7	
	Minimum, maximun	18.9	58.5	19.3	76.8	25.2	89.6
HAQ-DI†‡	Mean, SD	1.4	0.6	1.5	0.6	1.3	0.7
	Median	1.3		1.4		1.1	
	Minimum, maximun	0.8	8.3	0.6	8.3	0.3	7.9
Disease Activity Score*	Mean, SD	4.5	1.9	2.3	1.5	1.1	0.8
	Median	4.5		2.0		1.0	
	Minimum, maximun	1.5	7.5	0.0	5.0	0.0	2.0

*$p<0.05$ †$p>0.05$ ‡The HAQ-DI was calculated on 19 questions. DLCO: diffusion capacity of the lung for carbon monoxide, SF36: Study 36-item Short Form Health Survey, HAQ-DI: health assessment questionnaire disability index.

Skin histology

The myofibroblast and hyalinised collagen scores could not be determined in one baseline patient sample, as well as in one normal reference sample due to technical reasons. Hyalinised collagen content and myofibroblast positivity were increased in the upper arm of the SSc patients at baseline compared to the normal reference set. The mean hyalinised

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collagen score was 60 (SD 9.5) versus 7.1 (SD 7.2) ($p<0.001$). Myofibroblast positivity was 4/7 versus 0/8 (χ^2-test: $p=0.013$).
After the treatment, the hyalinised collagen content decreased in the upper arm from 60 (SD 9.5) at baseline to 28 (SD 21) at week 12, and in the forearm from 62 (SD 22) at baseline to 41 (SD 25) at 12 weeks (MMA: $p=0.014$). Myofibroblast positivity decreased in the upper arm from 4/7 to 2/7 and in the forearm from 8/8 to 6/8 (GEE: $p=0.011$) (figure 1).
B cells, which were immunostained with an antibody directed against an intracytoplasmatic epitope of the CD20 molecule,[10] could be detected at baseline in the dermis of the forearm in 4 out of 8 patients and in the dermis of the upper arm in 3 out of 8 patients. In the normal reference set, B cells were present in 1 out of 9 subjects. After rituximab treatment, in none of the patients B cells could be detected in their dermis (GEE: $p=0.03$) (figure 1). All 8 patients had effective depletion of CD 19+ B cells in the peripheral blood at week 2 (<5 CD19+ lymphocytes/µl). Anti-topoisomerase I and anti-RNA polymerase III antibody titers did not change significantly over the study period.

DISCUSSION

Our trial was a safety study of rituximab in dc-SSc. As the serious adverse events were probably unrelated to the study medication and as we had no unexpected other adverse events, we state that rituximab was well tolerated. Even though the use of steroids caused no renal crises, one should always consider its potential harmful effects in this patient group.[11, 12] Skin score dropped 10.5 points at week 24 (mean at baseline was 24.8), which largely exceeds the minimal clinically relevant treatment effect estimate for the skin as provided by a recent Delphi exercise,[13] and which is more than can be expected as spontaneous improvement in patients with similar disease duration.[14] This leads us to hypothesise that rituximab may be a potential efficacious drug in the treatment of dc-SSc, although one must emphasize this is an open trial. Encouragingly, blinded histopathological analysis of the skin led us to postulate in the same direction. Parameters of internal organ involvement remained stable, but further follow up is needed before drawing any conclusions.

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COMPETING INTEREST

All authors declare that the answer to the questions on the competing interest form are all No and therefore have nothing to declare.

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REFERENCES

1. Sakkas LI, Chikanza IC, D Platsoucas C. Mechanisms of Disease: the role of immune cells in the pathogenesis of systemic sclerosis. Nat Clin Pract Rheumatol. 2006; 2:679-685.
2. Walker UA, Tyndall A, Czirjak L, Denton C, Farge-Bancel D, Kowal-Bielecka O, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. Ann Rheum Dis. 2007; 66:754-763.
3. Lafyatis R, Kissin E, Viger K, Merkel P, Simms R. Rituximab treatment for patients with diffuse cutaneous systemic sclerosis - A phase I study. Arthritis Rheum. 2006; 54:S523-S524.
4. Lombardi S, Quartuccio L, Franzolini N, Salvin S, Sacco S, Di Poi E, et al. Rituximab for the long term treatment of severe cutaneous involvement in systemic sclerosis. Arthritis Rheum. 2008; 58:S822-S823.
5. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum. 1980; 23:581-590.
6. Valentini G, Della Rossa A, Bombardieri S, Bencivelli W, Silman AJ, D'Angelo S, et al. European multicentre study to define disease activity criteria for systemic sclerosis. II. Identification of disease activity variables and development of preliminary activity indexes. Ann Rheum Dis. 2001; 60:592-598.
7. Rannou F, Poiraudeau S, Berezne A, Baubet T, Le-Guern V, Cabane J, et al. Assessing disability and quality of life in systemic sclerosis: Construct validities of the Cochin Hand Function Scale, Health Assessment Questionnaire (HAQ), systemic sclerosis HAQ, and Medical Outcomes Study 36-Item Short Form Health Survey. Arthritis Rheum. 2007; 57:94-102.
8. Kissin EY, Merkel PA, Lafyatis R. Myofibroblasts and hyalinized collagen as markers of skin disease in systemic sclerosis. Arthritis Rheum. 2006; 54:3655-3660.
9. Fitzmaurice GM, Laird NM, Ware JW. Applied Longitudinal Analysis. Hoboken: John Wiley & Sons, Inc., 2004.
10. Teng YKO, Levarht EWN, Hashemi M, Bajema IM, Toes REA, Huizinga TWJ, et al. Immunohistochemical analysis as a means to predict responsiveness to rituximab treatment. Arthritis Rheum. 2007; 56:3909-3918.
11. Steen VD, Medsger TA, Jr. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. Arthritis Rheum. 1998; 41:1613-1619.
12. DeMarco PJ, Weisman MH, Seibold JR, Furst DE, Wong WK, Hurwitz EL, 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; 46:2983-2989.
13. Gazi H, Pope JE, Clements P, Medsger TA, Martin RW, Merkel PA, et al. Outcome measurements in scleroderma: results from a delphi exercise. J Rheumatol. 2007; 34:501-509.
14. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta 1 antibody therapy in systemic sclerosis - A multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007; 56:323-333.

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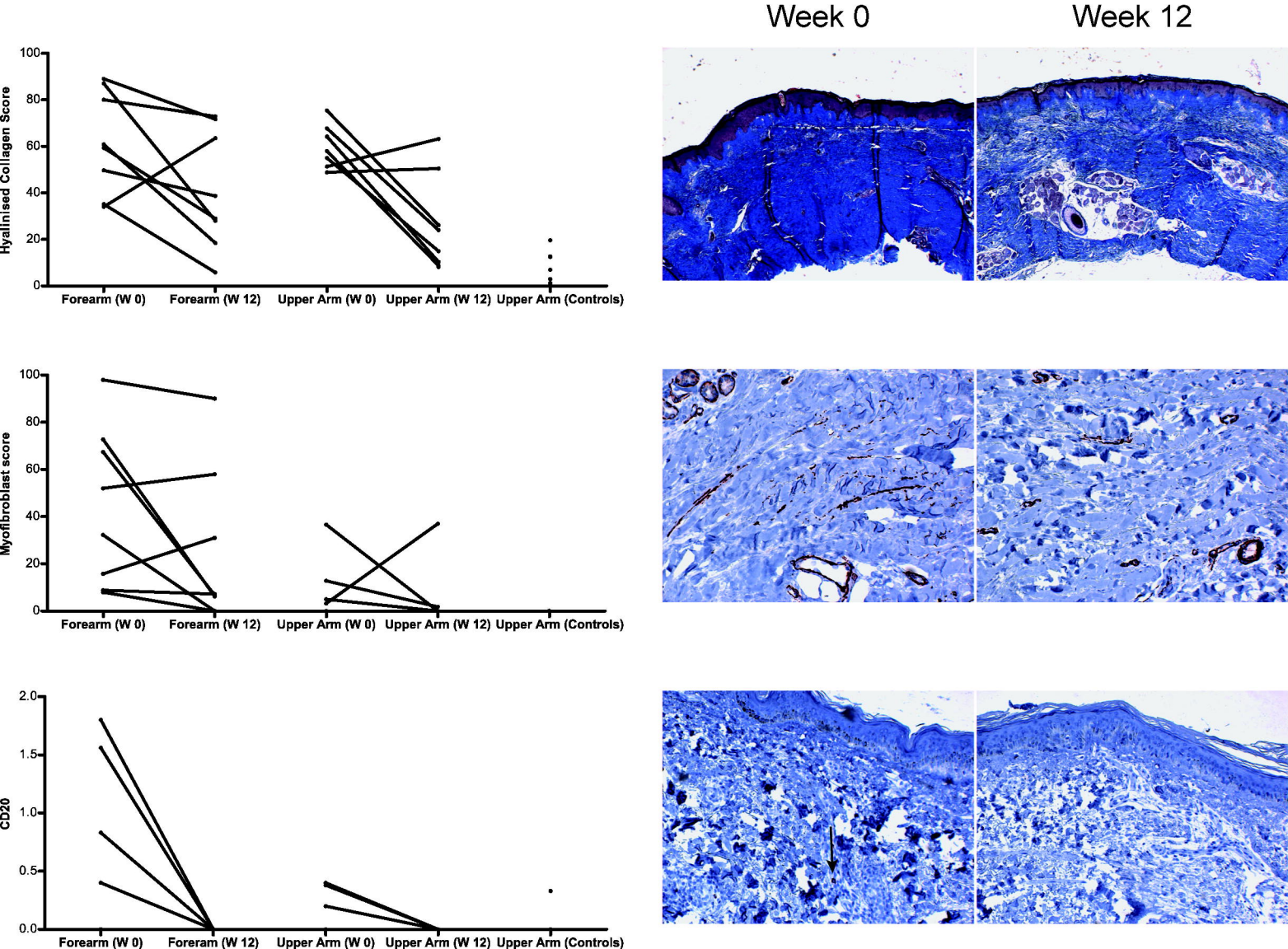
FIGURE LEGENDS

Figure 1 Hyalinized collagen content (Masson's trichrome staining, upper panel), myofibroblast score (α-smooth muscle actin staining, middle panel) and B lymphocyte numbers (CD20 staining, lower panel) in the skin before and 12 weeks after rituximab treatment. Representative pictures are shown (upper panel original magnification 40X, middle panel original magnification 400X, lower panel original magnification 100X). W: week.


Week 0
Week 12
Hyalinised Collagen Score
100
80
60
40
20
0
Forearm (W 0)
Forearm (W 12)
Upper Arm (W 0)
Upper Arm (W 12)
Upper Arm (Controls)
Myofibroblast score
100
80
60
40
20
0
Forearm (W 0)
Forearm (W 12)
Upper Arm (W 0)
Upper Arm (W 12)
Upper Arm (Controls)
CD20
2.0
1.5
1.0
0.5
0.0
Forearm (W 0)
Foreram (W 12)
Upper Arm (W 0)
Upper Arm (W 12)
Upper Arm (Controls)