

## Specific Antinuclear Antibody Level Changes after B Cell Depletion Therapy in Systemic Sclerosis Are Associated with Improvement of Skin Thickening

To the Editor:

B cell depletion therapy using rituximab (RTX) has emerged as a promising therapy in systemic sclerosis (SSc). Our group reported a clinical benefit on skin thickening when RTX ( $2 \times 1000$  mg) in combination with methylprednisolone (100 mg) was administered at months 0 and 6<sup>1</sup>. The rationale behind the use of B cell depletion in SSc treatment is based on the growing evidence that B cells play a role in the pathogenesis of the disease<sup>2,3</sup>. In addition, SSc-associated antinuclear antibody (ANA) are present in the majority of patients, but are generally accepted to be rather static markers, primarily important in diagnosis rather than in followup<sup>4</sup>. Moreover, conflicting results have been published<sup>5</sup> on the change in disease-specific ANA titers in relation to clinical response in other connective diseases (e.g., systemic lupus erythematosus and rheumatoid arthritis) after RTX. To our knowledge, no studies in SSc are available documenting the longterm effect of RTX on these autoantibody levels.

In this letter we report the 2-year serologic followup data (months 0, 3, 6, 12, 15, 18, and 24) on the same 8 patients with SSc with diffuse skin involvement (dcSSc) who were included in our initial RTX studies<sup>1,6</sup>. The idea was to evaluate the relationship between SSc-ANA level changes and clinical response. To document this latter relationship, we performed specific SSc-ANA analysis [anticentromere B, antitopoisomerase I, and anti-RNA polymerase III immunoglobulin G (IgG)] on all serial serum samples (months 0, 3, 6, 12, 15, 18, and 24) of each patient using fluorescent enzyme immunoassay on the Immunocap 250 system (EliA, ThermoFisher Scientific)<sup>7</sup>. On the same serum samples, Epstein-Barr virus (EBV) IgG and total IgG levels were also determined using ELISA (Enzygnost, Siemens Healthcare Diagnostics Products GmbH) and nephelometry on the BNII analyzer (N Antisera to human immunoglobulins IgG, Siemens Healthcare Diagnostics Products GmbH), respectively. For the specific ANA analysis, all timepoint samples of 1 patient were performed in duplicate in the same run to avoid inter-run variability<sup>5</sup>.

Seven patients had SSc-ANA [3 patients had antitopoisomerase I, 3 patients had anti-RNA polymerase III, and 1 patient (number 6) showed double reactivity with anticentromere B and antitopoisomerase I]. Mean levels of topoisomerase I antibodies and RNA-polymerase III antibodies were 717 (SD 1179) and 134 (SD 107) EliA units/ml, respectively. Of particular interest for our report, we previously documented for the same patient group a clinically and statistically significant change in skin score [from a mean modified Rodnan skin score (mRSS) of 24.8, SD 3.4 at baseline to 13.6, SD 5.6 at Month 24]<sup>1</sup>. In our current study, we observed a disproportional effect of RTX on the different types of (auto)antibodies, with the SSc-ANA levels clearly more affected than the levels of the protective antibodies (see Figure 1 for the evolution of the antibody titers). There was a statistically significant overall decrease of the SSc-ANA levels over the 2-year followup period (mixed model analysis  $p = 0.001$ ; see Appendix 1 for details on the statistical analysis), with a mean decrease of 50% (95% CI 12–72) at Month 24 compared with baseline. A significant decrease compared with baseline was also shown for each timepoint in between starting from Month 12 onward (Table 1). Comparable results were found when the SSc-ANA levels were corrected for total IgG [ratio; overall decrease, mixed model analysis  $p = 0.008$ ; mean percentage decrease at Month 24 compared with baseline 48% (95% CI 9–72), mixed model analysis  $p = 0.003$ ]. In contrast, no statistically significant evolution of total IgG and EBV IgG could be shown at most timepoints compared to baseline (Table 1).

Because both variables showed a significant decrease after a 2-year period following RTX therapy (see above), the association between the SSc-ANA levels and the mRSS over time was also evaluated. Appendix 2 shows the evolution of the mRSS, the SSc-ANA titers, and the total IgG titers for the individual patients over time as expressed by the percentage decrease of baseline values. The percentage decrease in mRSS and

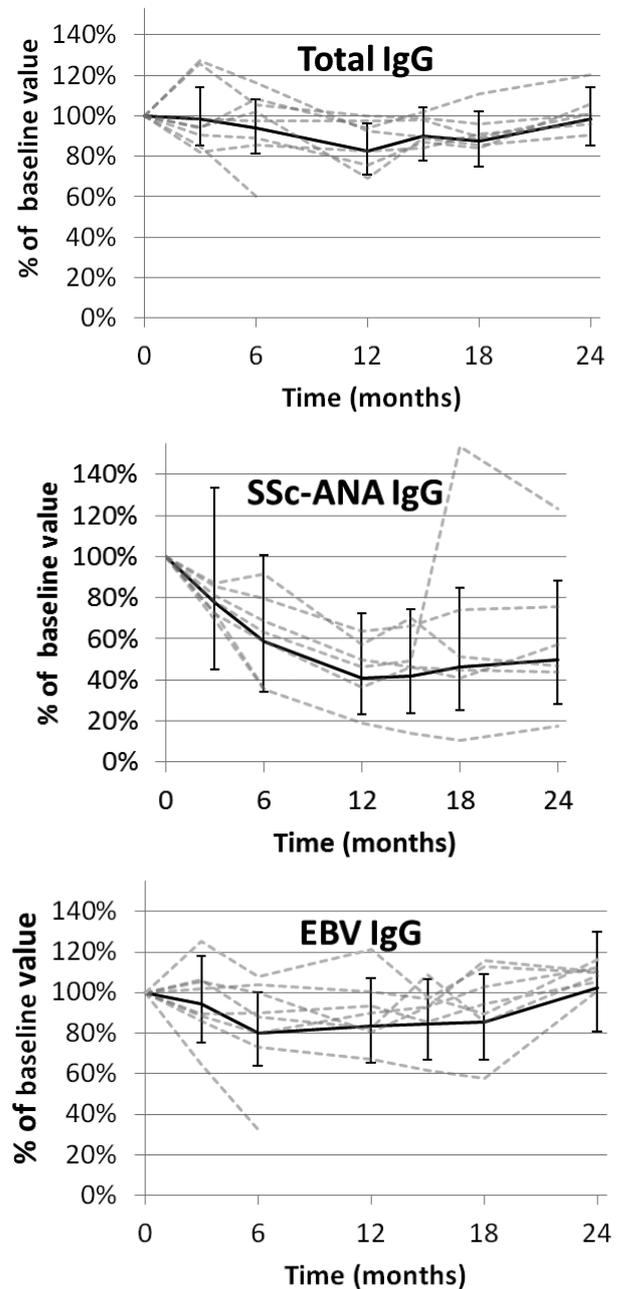


Figure 1. Evolution of the antibody titers compared with their baseline values over time. The dashed lines represent the evolution of the antibody titers (total IgG, SSc-associated ANA IgG, and EBV IgG) over time (mos) of the individual patients. The full line and error bars represent the estimated means and the 95% CI obtained in the mixed model analysis for each timepoint compared with baseline. IgG: immunoglobulin G; SSc: systemic sclerosis; ANA: antinuclear antibodies; EBV: Epstein-Barr virus.

SSc-ANA levels had similar kinetics in the individual patients, and the extent of mean percentage decrease in mRSS (45% at Month 24 vs baseline) and autoantibody level (50% at Month 24 vs baseline) were comparable. Moreover, there was a significant association between the percentage decrease of the mRSS and the percentage decrease of the SSc-ANA level at a given timepoint (mixed model analysis  $p = 0.007$ ). Contrarily, no significant association between the total IgG and the mRSS could be shown (mixed model analysis  $p = 0.063$ ).

Table 1. Mixed model analysis on the antibody measurements upon treatment with rituximab.

Variable	Statistics, Mixed Model Analysis	Timepoints, Mos						p, Mixed Model Analysis <sup>†</sup>
		3	6	12	15	18	24	
IgG, g/l	Mean % compared with baseline	99	94	82	90	87	99	<b>0.010</b>
	95% CI*	85–114	81–108	71–96	78–104	75–102	85–114	
	p <sup>#</sup>	0.798	0.217	<b>0.001</b>	0.051	0.020	0.785	
SSc-ANA IgG, U/ml	Mean % compared with baseline	78	59	41	42	46	50	<b>0.001<sup>‡</sup></b>
	95% CI*	45–134	34–101	23–72	24–74	25–85	28–88	
	p <sup>#</sup>	0.200	0.009	<b>0.000</b>	<b>0.000</b>	<b>0.001</b>	<b>0.002</b>	
SSc-ANA IgG corrected for total IgG, ratio	Mean % compared with baseline	79	63	50	47	53	52	<b>0.008<sup>‡</sup></b>
	95% CI*	45–138	36–109	28–89	26–85	29–99	28–91	
	p <sup>#</sup>	0.243	0.025	<b>0.002</b>	<b>0.001</b>	<b>0.007</b>	<b>0.003</b>	
EBV IgG, U/ml	Mean % compared with baseline	94	80	84	84	85	103	<b>0.031</b>
	95% CI*	75–118	64–100	65–107	67–107	67–109	81–130	
	p <sup>#</sup>	0.473	0.009	0.050	0.052	0.080	0.772	
EBV IgG corrected for total IgG, ratio	Mean % compared with baseline	96	85	101	94	98	104	0.114
	95% CI*	80–116	71–102	83–124	77–113	80–119	86–126	
	p <sup>#</sup>	0.588	0.019	0.852	0.351	0.737	0.576	

<sup>†</sup> Mixed model analysis performed on the ln transformed values, a statistical significance level of 0.05 was used; <sup>‡</sup> for patient 6 (2 SSc-ANA detected), only antitopoisomerase I levels were included in the mixed model analysis. <sup>#</sup> Pairwise comparison versus baseline: p values in bold face are considered statistically significant (p < 0.0083, Bonferroni correction for multiple comparisons). \* Bonferroni corrected 95% CI. IgG: immunoglobulin G; SSc: systemic sclerosis; ANA: antinuclear antibodies; EBV: Epstein-Barr virus.

We observed a selective decline in SSc-ANA levels in dcSSc, suggesting that the B cell clones responsible for the production of these antibodies are particularly more sensitive for B cell depletion therapy. Moreover, the effect of RTX on the SSc-associated humoral immunity was associated with skin improvement. Further, large and longterm prospective studies will clarify the potential clinical added value of monitoring of the SSc-ANA levels after RTX treatment.

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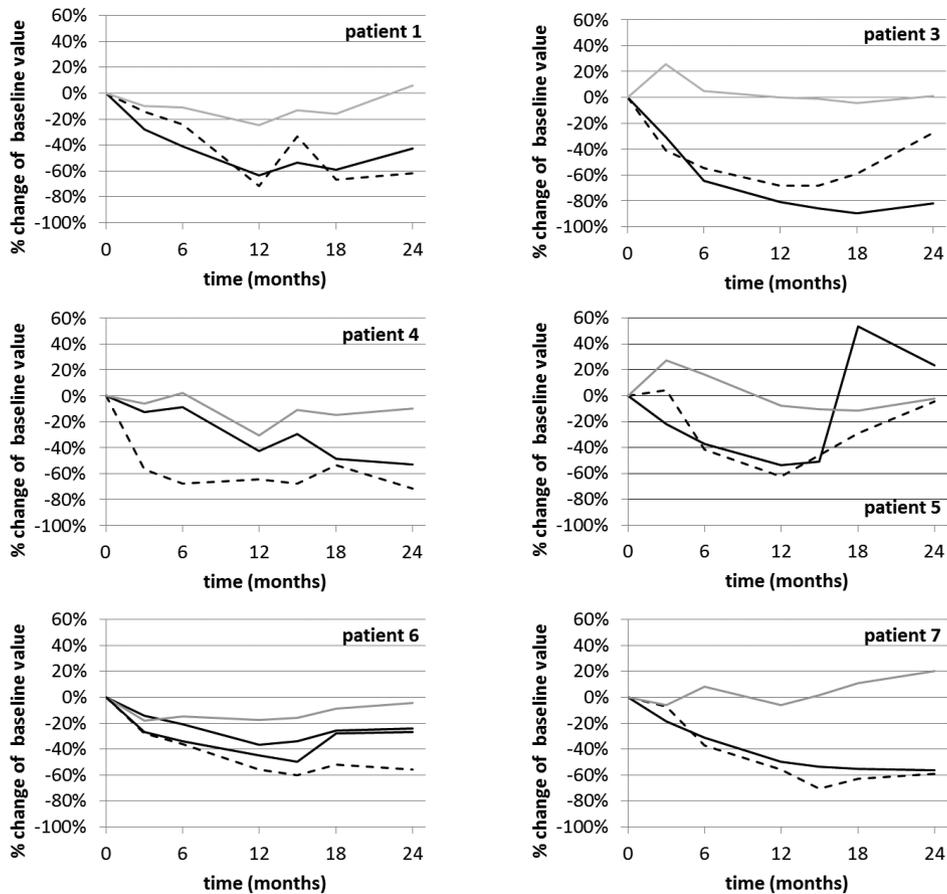
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**APPENDIX 1.** Statistical analysis.

Mixed model analysis with random intercept for patient was used to evaluate the serologic changes over time and the association between percentage decrease in systemic sclerosis (SSc)-associated antinuclear antibody (ANA) levels and clinical activity (as expressed by the modified Rodnan skin score). When appropriate, heterogeneous variances over time were allowed. In case of double SSc-associated ANA reactivity, only 1 antibody per patient was included in the analysis (randomly selected). Mixed model analysis on the serologic changes over time was applied on ln-transformed values. All analyses were performed using SPSS 21 (SPSS Inc.). A statistical significance level of 0.05 was used. In case of multiple testing, the Bonferroni correction was applied.

**APPENDIX 2.** Percentage change compared to baseline value of the mRSS, the SSc-ANA titers, and the total IgG in the individual patients. The dashed lines represent the percentage change of the mRSS. The full black lines represent the percentage change of the SSc-ANA titers. The full grey lines represent the percentage change of the total IgG. For patient 6, the percentage change of both SSc-ANA is shown. Patient 2 (no mRSS after Mo 3 and no antibody data after Mo 6) and patient 8 (no SSc-ANA detected) are excluded from the figure. mRSS: modified Rodnan skin score; SSc-ANA: systemic sclerosis-associated antinuclear antibody; IgG: immunoglobulin G.



## Corrections

### **Specific Antinuclear Antibody Level Changes after B Cell Depletion Therapy in Systemic Sclerosis Are Associated with Improvement of Skin Thickening**

Bonroy C, Smith V, Deschepper E, De Keyser F, Devreese K. Specific antinuclear antibody level changes after B cell depletion therapy in systemic sclerosis are associated with improvement of skin thickening (letter). *J Rheumatol* 2016;43:247-9. The author byline should have included the following statement: Carolien Bonroy and Vanessa Smith contributed equally to this work and are co-first authors. We regret the error.

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