Persistence of focal lymphocytic sialadenitis in patients with primary Sjögren's syndrome treated with rituximab: a possible role for glandular BAFF

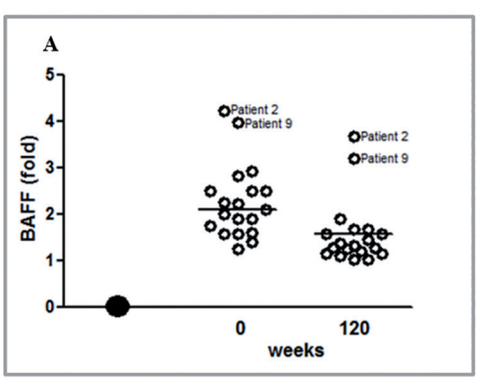
Sirs,

Over the past ten years, several studies demonstrated that rituximab (RTX) is effective in the treatment of primary Sjögren's syndrome (pSS) (1). In this context, we recently provided evidence for long-term efficacy and safety of RTX in early active pSS patients with systemic, extra-glandular involvement (2). pSS patients included in our cohort were treated with 2 infusions of 1,000 mg of RTX at day 1 and day 15 to complete a course of therapy and the course was repeated every 24 weeks, for a total of six courses (2). RTX induced a significant reduction of the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) in all the RTX-treated patients. To note, the focus score (FS) was <1 at week 120 in 17 out of 19 RTX-treated patients (89.4%), suggesting that RTX treatment may reverse the specific focal lymphocytic sialadenitis (FLS) to a non-specific chronic sialadenitis pattern or to a full restoration of minor salivary gland (MSG) architecture. Furthermore, we observed that the number of germinal centre (GC)-positive ($^{+ve}$) biopsies dropped from 52.6% to 5.2% in RTX-treated patients, and after 120 weeks only 1 out of 19 patients still displayed GCs. On the

Table I. Histological characteristics of RTX-treated pSS patients.

	Baseline (pre-RTX treatment)		Week 120 (post-RTX treatment)	
	Focus Score	Presence of GC	Focus Score	Presence of GC
Patient 1	1	-	0	-
Patient 2	3.4	+	2.8	+
Patient 3	2	-	0	-
Patient 4	2.3	+	0	-
Patient 5	2	+	0	-
Patient 6	2.2	+	0	-
Patient 7	1	-	0	-
Patient 8	1	-	0	-
Patient 9	2.2	+	1.7	-
Patient 10	1.3	-	0	-
Patient 11	1	-	0	-
Patient 12	3	+	0	-
Patient 13	1.7	+	0	-
Patient 14	1	+	0	-
Patient 15	2.5	+	0	-
Patient 16	1.3	-	0	-
Patient 17	3.2	+	0	-
Patient 18	1	-	0	-
Patient 19	1.8	-	0	-

RTX: rituximab; pSS: primary Sjögren's syndrome; GC: germinal centre; +: positive; -:negative; BAFF: B-cell activating factor.



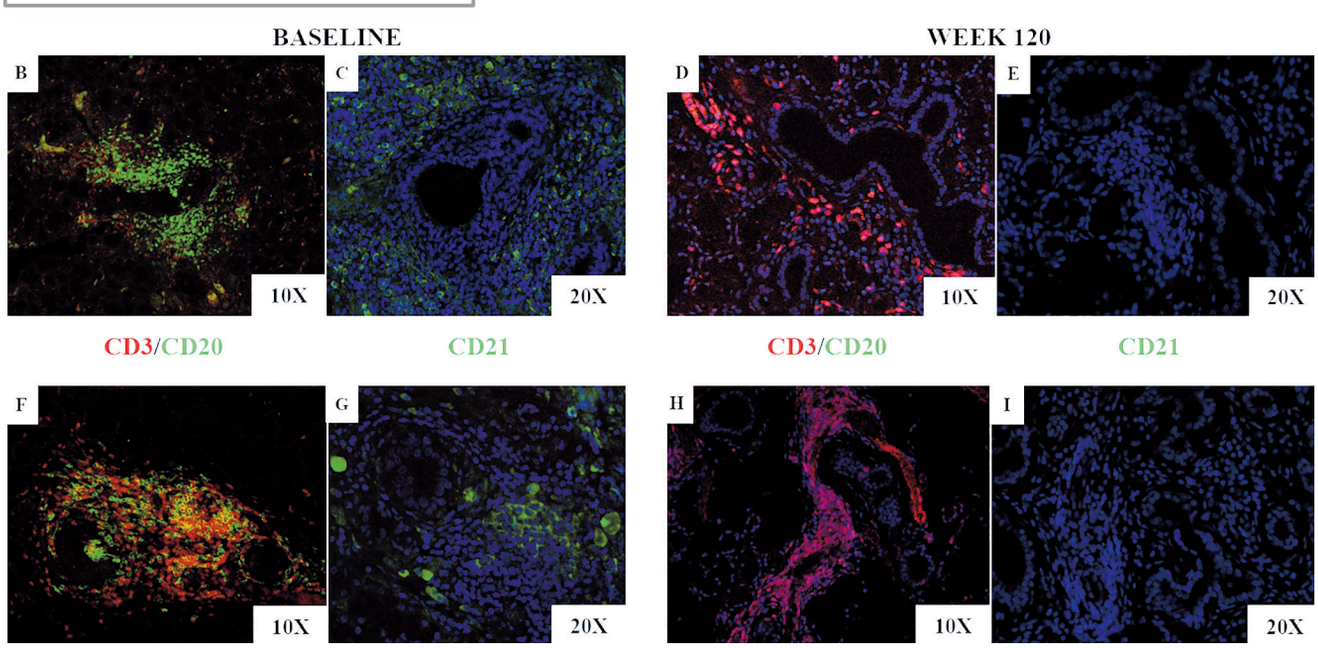


Fig. 1. Molecular analysis of minor salivary gland (MSG) biopsies in the rituximab-treated group. Relative mRNA quantification of B-cell activating factor (BAFF) was assessed by quantitative real-time polymerase chain reaction in MSGs obtained from 19 patients with primary Sjögren's syndrome (pSS) before and after treatment with rituximab (A). Five subjects with sicca symptomatology but no clinical or serological features of pSS acted as controls. Levels are expressed as the fold increase or decrease of BAFF mRNA in pSS-MSGs compared to normal MSGs.
Histological analysis of minor salivary gland (MSG) biopsies in a patient with glandular response to Rituximab (B-C and D-E) and in patient $^{\#}$9 (F-G and H-I), at baseline and week 120. 10X and 20X, level of magnification of the corresponding picture.

contrary, no significant differences were observed in the DMARD-treated group. Finally, although we observed a significant reduction of glandular mRNA expression of lymphotoxin (LT)α, LTβ, CXCR4 and CXCR5 in RTX-treated patients, an abnormal local micro-environment, favoring B-cell activity, persisted, due to the unchanged levels of B-cell activating factor (BAFF).

On this basis, we attempted to identify any correlations between the glandular BAFF levels, before and after treatment, and the disappearance of FLS and GCs in the RTX-treated group.

The histological characteristics of the patientsare shown in Table I. At baseline, when pSS patients were divided according to the presence or absence of GC-like structures in MSGs, we observed that GC^{+ve} patients displayed higher levels of BAFF when compared to GC-negative ($^{-ve}$) patients (mean values ± standard errors of the mean: 2.8±0.3 and 1.3±0.3 respectively, $p<0.001$, Mann Whitney U-test). At week 120, Patient #2 and #9 still displayed a FS >1 (2.8 and 1.7, respectively), the former showing GC-like structures, despite the therapy with RTX. Interestingly, the same two patients displayed the highest BAFF levels at baseline and after 120 weeks (Fig. 1). Both patients, at the time of the inclusion in the study, showed, as extraglandular manifestations, intermittent fever, lymphadenopathy, oligoarthritis, anemia of chronic inflammation, and hypergammaglobulinemia. Patient #2 showed also an important parotid swelling, while patient #9 a limited cutaneous vasculitis.

Although conclusive data in a larger population are required to confirm our observation, these preliminary results suggest that higher glandular BAFF levels at baseline may predict the persistence of FLS, independently of the clinical response. Increased BAFF levels were observed in serum, saliva, and MSGs of pSS patients, especially those with GCs (3-6). A correlation between serum levels of BAFF and autoantibodies (7), as well as lymphoproliferative complications has been reported in pSS (8). Moreover, the evidence that in patients with higher serum BAFF levels, B-cell recovery occurs sooner after RTX therapy (9), and tissue/systemic BAFF over-expression may be implicated in RTX-resistance of pSS-associated MALT lymphoproliferation (10), fits with our observations. On this basis, we speculate that disease heterogeneity, including different glandular BAFF expression, may partially explain the conflicting results concerning RTX treatment outcome, and underline the potential role of BAFF targeting therapy in pSS (11).

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Competing interests: none declared.

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