Do high numbers of salivary gland-infiltrating B cells predict better or worse outcomes after rituximab in patients with primary Sjögren's syndrome?

We read with great interest the recent contribution by Delli *et al*,[1] which aimed at determining whether salivary gland histopathology could predict the response to rituximab in patients with primary Sjögren's syndrome (pSS). These authors concluded that a high number at pretreatment of $CD20^+$ B cells/mm^2 of parotid gland parenchyma predicted better responsiveness of patients with pSS to rituximab treatment. Interestingly, we have recently published an article that concluded the opposite: a high proportion of minor salivary gland (MSG) B cells predicted an absence of a clinical response to rituximab.[2]

How to assess this discrepancy and try to reconcile these divergent conclusions? This debate is important and timely, since, besides its indisputable diagnostic value,[3] salivary gland histopathology is increasingly considered to be a useful tool to assess the effects of treatments in clinical trials.[4 5] Furthermore, despite promising open-labelled studies and two small randomised trials,[6] the two large randomised controlled trials TEARS[7] and TRACTISS[8] (main results of which were revealed at the 2015 American College of Rheumatology meeting) did not demonstrate the superiority of rituximab over placebo on their primary end points. However, post-hoc analyses of the TEARS study showed that the effect of rituximab in patients with pSS was highly heterogeneous, and suggested that a subset of patients responded to the treatment.[9–11] Therefore, the determination of predictive factor for the response to rituximab is primordial for the design of future studies.

The report by Delli *et al* and ours agree on the effect of rituximab on salivary gland-infiltrating B cells. A sharp decline in B-cell infiltration at 12 weeks after rituximab infusion was observed in both studies. In addition, we also observed that this depletion of target tissue B cells was transient: at 24 weeks after the infusion, the patients who had already experienced peripheral blood B-cell reconstitution now had increased salivary gland B-cell infiltration.[2]

Several points may explain the divergent results between those of Delli *et al* and ours on the predictive value of baseline salivary gland B-cell quantification.

First, the patients included in the TEARS trial[7] and in the randomised controlled study from the Gröningen group[12] presented with a few differences in general features probably attributable to slightly different inclusion criteria. Although many variables were similar (all patients fulfilled the American-European Consensus Group criteria; disease duration was quite the same (~5 years); mean unstimulated salivary flow was ~0.1 mL/min in both these studies), patients in the TEARS trial were ~10 years older; mean European League against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) was 8 in the Gröningen study, whereas it was 10 in the TEARS study; and the frequencies of anti-SSA positivity and abnormal salivary gland histology were, respectively, ~80% and 90% in the TEARS trial compared to 100% for both in the Gröningen study (as they were an additional inclusion criteria).

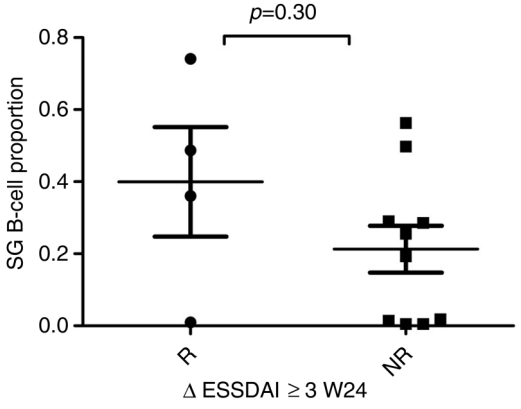

Figure 1 Comparison of baseline salivary-gland B-cell proportion between responders and non-responders using ESSDAI as an endpoint. ESSDAI, European League against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index; SG, salivary gland.

Second, different end points were used to determine the response to rituximab in our analyses. Delli *et al* used a decrease of ESSDAI of at least three points as a definition for a clinically significant response to treatment, as recently suggested.[13] Using this definition, 69% of patients (11/16) in the rituximab arm were considered responders in their study. In our study, only 29% of the patients (4/14) would be considered responders using this definition. Figure 1 shows the absence of predictive value of baseline salivary gland B-cell proportion in our study using this definition of a response. Furthermore, the TEARS study has shown that rituximab was not effective in improving systemic involvement measured by the ESSDAI.[7 10] In contrast, we used the Sjögren's Syndrome Responder Index (SSRI), which is based on improvement in symptoms (mouth and eye dryness and fatigue) and objective measures (salivary flow and erythrocyte sedimentation rate). Because the data from the Groningen study were used to confirm the performance of the SSRI to detect rituximab efficacy,[10] it would be interesting to know whether the use of the SSRI to define a response could change the conclusion of the article by Delli *et al*.

Third, the tissues examined in the two studies differed: Delli *et al* used sequential parotid biopsies, whereas we performed labial MSG biopsies. Except for the group from Gröningen, very few groups worldwide routinely use parotid biopsy, which requires the intervention of a trained surgeon. The differences between these two tissue types is ill-defined.[14] The presence of lymphoepithelial lesions is more commonly observed in parotid tissue than in labial MSG; parotid tissue may contain physiological lymphoid tissue between lobules, including lymphoid follicles even in healthy individuals; and parotid acini are purely serous whereas in labial MSG acini are both mucinous and serous. A theoretical advantage of parotid biopsy is that the same gland can be analysed sequentially. However, it seems that the analysis of sequential biopsies of the same gland is not reproducible over time, as illustrated by the apparent variability (in terms of number of lymphoepithelial lesions, relative area of infiltrates, number of germinal centres and of B cells) between baseline and 12 weeks biopsies reported in the placebo group in the study by Delli *et al*. New prospective studies that compare parotid and MSG biopsies are required.

Fourth, technical considerations could affect comparisons between these two studies. Histopathological analysis of the salivary glands is examiner-dependent, and assessment of different tissue features may vary significantly between pathologists.[15] To circumvent this issue, both our studies used automated methods to analyse immunostained slides and quantify B-cell and T-cell infiltration. However, notable differences exist between our methods and the presentation of the results. Our method was recently published[16] and is based on an automated count of stained pixels corresponding to B and T cells on the same whole double-immunostained MSG slides. Therefore, the entirety of

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the gland is analysed, without the need of defining manually any 'region of interest', which brings an inevitable variability to the test. We have shown that this digital method is highly correlated with a manual count of stained cells by the pathologist, with the major advantage of a fast analysis of a whole tissue section. We presented the results as the proportion of B cells among total lymphocytic infiltration, which takes into account the variations of T-cell infiltrate.[16] The results of Delli *et al* are surprising in that they report a very important predominance of B cells within the lymphocytic infiltrate, whereas in our series as well as in others B cells never represent more than 50% of infiltrating lymphocytes in MSG even in patients with the highest degrees of inflammation.[16 17] When we compute this proportion using the median values given in table 1 of this article (B-cell proportion being the number of B cells divided by the number of B cells+the number of T cells), in the placebo group, B-cell proportion would be around 76% at baseline and 68% at week 12, and in the rituximab group it would be around 78% at baseline and 66% at week 12. Thus, instead of the absolute number of B cells, it would be very informative to know B-cell proportion among infiltrating lymphocytes in the parotid glands of the patients included in the study by Delli *et al* and whether this proportion is affected by rituximab and/or display the same predictive value for the response to rituximab.

Fifth, we included other potential biomarkers in our analyses, and reported that the proportion of salivary gland B cells was correlated with several other markers for B-cell hyperactivity and, notably, with the serum level of BAFF, which was also negatively associated with the response to treatment. Since BAFF levels were also measured in patients in the Gröningen study,[18] it would be interesting to know whether BAFF level is also associated with the number of parotid B cells as assessed by the procedure described by Delli *et al*, and with the response to rituximab (either positively or negatively).

Finally, some of the patients included in the TEARS trial underwent salivary gland ultrasonography (SGUS) at inclusion.[9] We observed that the SGUS score was highly correlated with the MSG focus score, and that the severity of parotid involvement assessed by SGUS was also predictive of rituximab inefficacy (Cornec *et al*, manuscript submitted). This observation strengthens the validity of our conclusion that a single course of rituximab is probably insufficient to improve the disease in patients with pSS having the greatest B-cell hyperactivity and salivary gland involvement.

This debate stresses out the need to define robust and reproducible biomarkers in pSS, which would be useful to better understand the pathological processes of the disease and to assess the response to different therapies. The numerous clinical trials currently ongoing will hopefully bring new therapeutic options for this orphan condition.

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