Can ultrasound of the major salivary glands assess histopathological changes induced by treatment with rituximab in primary Sjögren's syndrome?

With great interest we have read the recent publication by Fisher *et al.*,¹ entitled 'Effect of rituximab on a salivary gland ultrasound score in primary Sjögren's syndrome: results of the TRACTISS randomised double-blind multicenter substudy' in which the authors demonstrated a significant improvement in total ultrasound score (TUS) after rituximab treatment compared with placebo at weeks 16 and 48 in 52 patients with primary Sjögren's syndrome (pSS).

We and others have shown that treatment with rituximab, a chimeric anti-CD20 monoclonal antibody, affects the histopathology of the salivary glands and results in a decrease in area of lymphocytic infiltrate in the labial and parotid glands.²⁻⁵ This reduction in infiltrated area, that is, up to 50%, is mainly caused by B cell depletion.² Rituximab treatment also causes a significant loss of germinal centres.² Interestingly, rituximab treatment also leads to a significant restoration of the ductal epithelium glandular tissue itself. This is illustrated by a significant decrease in number and severity of lymphoepithelial lesions (LELs) in parotid gland tissue 12 weeks after rituximab treatment.^{2,6} The normalisation of the epithelium appears to be a direct consequence of depletion of intraepithelial B cells.⁷

Significant improvement of the parotid gland ultrasound score of patients with pSS has been observed before in another randomised controlled trial (RCT) with rituximab, the Tolerance and Efficacy of Rituximab in Primary Sjögren's Syndrome (TEARS) study.⁸ This is an RCT with rituximab versus placebo, performed by a French group. Together with the improvement of the histopathology of the gland, it might not be a surprise that Fisher *et al.*¹ also observed a significant improvement in TUS. This newly developed scoring system is composed of five domains: echogenicity, consistency, definition, glands involved and hypoechoic foci size. In the study by Fisher *et al.*¹, the difference in TUS between rituximab and placebo-treated patients was only attributed to the 'definition' domain. This domain addresses the visibility of the salivary gland posterior border. Other studies have shown that this domain is hard to assess,^{1,9} which is also supported by the accompanying low interobserver reliability.^{10,11} None of the other components showed a significant improvement at weeks 16 and 48 after rituximab treatment in patients with pSS.¹ In a previous study, we have shown that the presence of hypoechoic areas is the most important component in the evaluation of pSS.¹² Hypoechoic areas contribute to several domains scored when using the ultrasound scoring system applied by Fisher *et al.*¹ which include echogenicity, consistency and hypoechoic foci size. Although no significant improvement was found in these ultrasonographic components, there seems to be a tendency that the consistency domain does somewhat improve after rituximab treatment.¹ On the basis of the observed histopathological changes seen in parotid glands after rituximab treatment, however, it might have been expected that more domains of the TUS would have been improved.

The question becomes now why Fisher *et al.*¹ in our opinion, observe only minimal changes in ultrasound score after rituximab treatment. First, as long as we do not exactly know which histopathological parameters in salivary glands are being measured by ultrasound, it is hard to understand the effects of treatment on the ultrasound scores. While in the TEARS study, a good

association between focus score in the labial gland biopsy and their ultrasound score was found, our group found only fair to moderate associations between the Hoyer ultrasound score¹³ and focus score in parotid and labial salivary gland biopsies, respectively.^{14,15} The focus score is not appropriate to measure the local effect of rituximab on the periductal lymphocytic infiltration.² Moreover, a focus score does not reflect the size of the infiltrate and the inflamed area can be considerably reduced as a consequence of treatment, while the number of foci does not or only slightly decrease. Indeed, after rituximab treatment, the focus score was not altered, although there was a reduction in the size of the periductal infiltrate.² Taking this limitation of the focus score into account, other measurements, such as the relative area of infiltrating CD45+ cells, the number of B cells/mm² of glandular parenchyma, the presence and stage of LELs as well as the presence of germinal centres, more accurately define the magnitude of the inflammatory status of the gland.² Since these histopathological features are too small to be represented by the hypoechoic areas, it is not surprising that only minor changes in TUS were observed by Fisher *et al.*¹ Apparently, the change in the area of the inflammatory infiltrates might not be measured directly by the change in percentage of hypoechoic areas seen on ultrasound.

Second, another possible explanation why no more domains of the TUS change after rituximab treatment is that TUS is not sensitive enough to detect the histopathological changes. The consistency component is scored on a categorical scale, based on the ultrasonographers' estimate of the amount of hypoechoic areas present. It is questionable whether the decrease in the glandular area occupied by infiltrating cells is reflected by a similar change in the ultrasound image. In other words, it is uncertain whether the applied scoring system (TUS) is sensitive enough to detect this change. With only a few categories, there has to be a relative large change in the amount of hypoechoic areas present in the ultrasonographic image of a particular salivary gland to result in a different score. Furthermore, with regard to the ultrasonographic scoring, there are some other points to keep in mind. For example, ultrasound scoring was done live, but for an unbiased evaluation, blinded scoring is warranted. In addition, as already pointed out by Fisher *et al.*,¹ the ultrasound devices between the different centres were not standardised and intraobserver and interobserver reliability were not assessed. This last point is particularly important since previously there was more inconsistency in scoring the severity of the ultrasonographic characteristics¹⁰ as used in the study of Fisher *et al.*¹ On the other hand, the ultrasonographers were able to reliably identify in which patients the ultrasonographic characteristics support the diagnosis of pSS.¹⁰

Finally, the most simple explanation for the minor TUS changes is that the TRACTISS study, a randomised double blind placebo controlled clinical trial of Anti-B-Cell Therapy in patients with primary Sjögren's Syndrome, failed to meet its primary endpoint, that is, a change of 30% in oral dryness or fatigue on the visual analogue scale.⁸ Taking this into account, we should have been surprised if major differences in TUS were found. Another important point to mention is that in the TRACTISS study,⁸ the effect of rituximab treatment on the histopathology of the glandular tissue itself has not been studied, nor is any information given about how many patients had a positive salivary gland biopsy at the start of the study. Because of this, it is impossible to make a statement about whether or not the right subgroup of patients was included in the first place, to detect changes in TUS, as it appears that rituximab shows efficacy in a subgroup of patients with pSS.¹⁶

In conclusion, rituximab treatment leads to significant improvements in histopathological characteristics of salivary gland biopsies. This improvement was not reflected by a similar improvement in the amount of hypoechoic areas identified with ultrasound by Fisher *et al.*,¹ but this could be due to several reasons, that is, ultrasound is not sensitive enough to detect the histopathological differences or does not reflect the changes seen in the biopsies and the TRACTISS study was a negative study, perhaps due to the chosen inclusion criteria. In order to make a statement about whether or not ultrasound is the right tool to measure treatment effect, the sensitivity to change should be determined in positive clinical trials. At this point, the baseline histopathological characteristics of the parotid gland should be used to predict the effect of rituximab therapy and to help us to determine a personalised treatment approach.

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