In primary Sjögren's syndrome high absolute numbers and proportions of B cells in parotid glands predict responsiveness to rituximab as defined by ESSDAI, but not by SSRI

With great interest we have read the letter to the editor by Cornec *et al*[1] regarding our paper 'Towards personalised treatment in primary Sjögren's syndrome (pSS): baseline parotid histopathology predicts responsiveness to rituximab treatment'.[2] In essence, we showed in our paper that absolute numbers of CD20+ cells/mm^2 of parenchyma of parotid gland tissue are predictive for the responsiveness of patients with pSS to rituximab (RTX) treatment. Cornec *et al* argue that there is a discrepancy in outcomes presented in their study and our study,[1] as they observed that a high proportion of minor salivary gland B cells predict the absence of a clinical response to RTX.[3] As we will show and explain here, there is no inconsistency between the two studies and most of the apparent discrepancy is likely the result of differences in how the tissues are analysed and how the disease activity is established.

ABSOLUTE NUMBERS VERSUS PROPORTIONS OF B CELLS AND TECHNIQUE APPLIED

A major difference in the two studies is how B cells are assessed in tissue sections of salivary gland biopsies of patients with pSS before (and after) RTX treatment. We measured absolute numbers of CD20+ B cells/mm^2 of parenchyma, while Cornec *et al* assessed the proportion of B cells.[1 3] Obviously, even when there is a change in absolute numbers of B cells in the tissue, the B/T cell ratio still can remain the same. Thus, although higher numbers of B cells, do not need to be reflected per se in higher proportions of B cells, we also found in our study that patients with higher absolute numbers of B cells in the glandular tissue, had a higher B/B+T cell ratio. Furthermore, responders to RTX, as defined by a decrease in European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) score of ≥3 (ΔESSDAI≥3) at 12 weeks after treatment compared with baseline,[4] had a higher B/B+T cell ratio compared with non-responders (figure 1).

Nevertheless, there are some technical differences between the two studies that warrant some attention. We counted the absolute

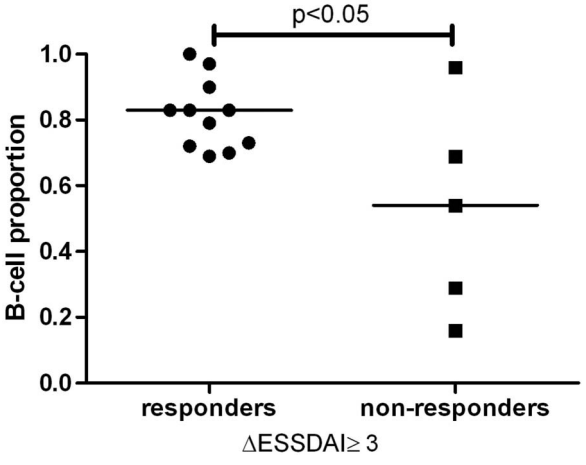

Figure 1 Proportions of baseline CD20+ cells in clinical responders (n=11) and non-responders (n=5) as defined by European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI), p<0.05. Horizontal lines indicate median values.

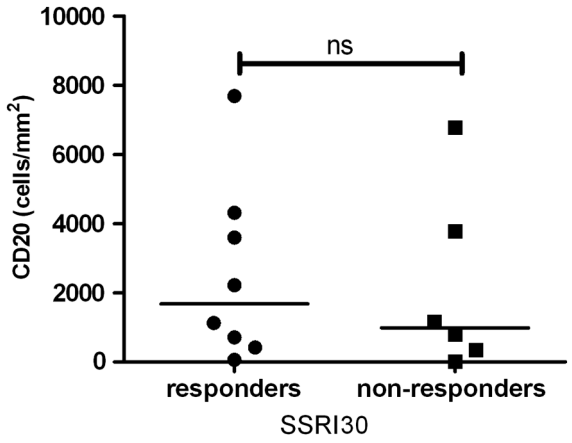

Figure 2 Numbers of baseline CD20+ cells/mm^2 in clinical responders (n=8) and non-responders (n=6) as defined by Sjögren's Syndrome Response Index (SSRI). Horizontal lines indicate median values, ns: non-significant.

number of cells with HistoQuest software (V.3.5.3.0171, Tissuegnostics, Vienna, Austria), a well known and widely used image analysis software package within pathology. Since parotid gland biopsies include areas of fat and fibrous tissue and intraparenchymal lymph nodes, we excluded these areas manually from the analysis, in order to increase the accuracy of the data. We would like to emphasise that, as stated in the section on 'Immunohistochemical analysis' in the materials and methods of our article,[2] the whole slide (except from fat and fibrous tissue) was evaluated and areas of interest were not electively chosen, as implied by Cornec *et al*.[1] The methodology used by Cornec *et al* is based on digital pixel counting procedure developed by Costa *et al*.[1 3 5] We have some concerns about this method. First, with the method of Costa *et al* extraglandular areas are not excluded from the tissue specimen studied,[5] which has the risk to include in the counting infiltrating cells located in areas of non-interest, for example, intraparenchymal fat tissue, fibrous tissue and perineural tissue. Second, although the number of pixels is reported to correlate to the manually counted cells,[5] the exact number of pixels corresponding to one cell remains unknown. Moreover, although the B-cell proportions assessed with the method of Costa *et al* correlate moderately to focus scores,[5] the focus score does not give an indication about the severity of inflammation, as discussed in our paper.[2] As a consequence, when the area covered by a single focus at baseline is rather large, a clinically relevant decrease in the inflamed area is not necessary reflected by a decrease in focus score. Apparently, the HistoQuest method of counting the absolute number of cells is more precise in these aspects. Thus, it would be interesting to know whether the results reported by Cornec *et al* change if biopsies would have been analysed with the HistoQuest software approach.[3]

ESSDAI VERSUS SJÖGREN'S SYNDROME RESPONSE INDEX

Another factor that contributes significantly to the apparent difference between the two studies concerns the way disease activity has been defined. Cornec *et al* pose that the discrepancy in outcomes can partially be attributed to ineffectiveness of RTX in improving systemic involvement as measured by ESSDAI.[1] This prompted them to develop and use the Sjögren's Syndrome Response Index (SSRI), an index reflecting mainly the objective and subjective sicca symptoms.[6] When we applied the SSRI to classify patients as responder or non-responder, we were unable to detect any difference in our data in baseline CD20+ B-cells/

Table 1 Comparison of two studies

Study	Cornec *et al*[3]	Delli *et al*[2]
Outcome	Proportion of B cells	Absolute number of CD20+B-cells/mm^2 parenchyma
Software	Digital pixel counting software, developed by the same team[5]	HistoQuest software, V.3.5.3.0171, Tissuegnostics, Vienna, Austria
Tool for measuring response to RTX	SSRI[6]	ESSDAI[4]
Salivary gland	Minor salivary glands	Parotid gland
General features	▶ Baseline ESSDAI:10 ▶ Median age: 50.4 and 54.8 (±9.5 and ±13.8) ▶ Baseline salivary gland biopsy positivity: 64% ▶ Baseline anti-SSA positivity: 80%	▶ Baseline ESSDAI: 8 ▶ Median age: 43 (±11 years) ▶ Baseline salivary gland biopsy positivity: 100% ▶ Baseline anti-SSA positivity: 100%

ESSDAI, European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; RTX, rituximab; SSRI, Sjögren's Syndrome Response Index.

mm^2 parenchyma between responders and non-responders (figure 2). Importantly, the agreement between ESSDAI and SSRI in defining responders in our study was rather poor (κ=0.25, percentage of agreement 64%; data not shown). Based on these findings we conclude that it is evident that ESSDAI and SSRI measure different outcomes; the ESSDAI focuses on systemic disease activity and the SSRI mainly on sicca-related complaints. In this respect, it is also worth mentioning that in our placebo treated patients, SSRI characterised 40% of the patients as responders, while ESSDAI only 11%. ESSDAI has been proven to be sensitive to measure the change in disease activity after therapeutic interventions and also showed that RTX was effective in our double-blind placebo-controlled RTX trial.[4 7–10] Thus, further validation is necessary for the SSRI.

DIFFERENCES IN GENERAL FEATURES

In addition to these two main aspects that result in the apparent discrepancies between the two studies, there are also some other differences that may influence differences in outcomes.

Baseline ESSDAI: although the baseline ESSDAI scores were rather similar between the two studies (eight in our study and 10 in the TEARS study) only in our study the ESSDAI was prospectively evaluated.[11 12] In the TEARS study, the ESSDAI was retrospectively evaluated.

Baseline salivary gland positivity: another major difference between the two studies is the positivity of salivary gland biopsy of the included patients; all patients in our study had a positive parotid gland biopsy at baseline, while only 64% of the patients included in the study by Cornec *et al* had a positive minor salivary gland biopsy.[1] When excluding patients with a negative minor salivary gland biopsy from the study by Cornec *et al*, the median proportion of B cells in responders would have been probably higher than in non-responders, which is in agreement with the conclusion of our study.

Parotid versus *minor salivary gland biopsy:* the different histopathological characteristics observed in parotid and minor salivary gland biopsies complicate the comparison as, for example, the B/T-cell ratio differs greatly. Minor salivary glands of healthy controls may have a physiological infiltrate that consists mainly of T-lymphocytes (and plasma cells), while parotid salivary gland tissue of healthy controls shows rarely a lymphocytic infiltrate. Although those differences have been shown by Pijpe *et al*,[13] there is still a need for larger studies focusing on the inherent differences in the histopathological characteristics of parotid and minor salivary gland tissue in both patients with pSS and healthy controls.

SALIVARY GLAND ULTRASOUND

Like Cornec *et al*, we also feel that ultrasound has merit in the diagnosis and assessment of the disease activity of pSS.[1 14 15] However, before making salivary gland ultrasound a standard in pSS diagnostics, disease monitoring and treatment evaluation, there are several questions that need to be answered first, that is, the reliability of ultrasound in the evaluation of changes that occur in the major salivary glands of patients with pSS, and the validity of ultrasound to detect the histopathological changes occurring in the parotid tissue of patients (suspected) with pSS (in particular direct comparison of ultrasonographical and histopathological features).

From the above-mentioned, it may be concluded that our study and the study of Cornec *et al* differ in some respect,[2 3] but do not present contradicting results. Most likely differences in assessment of patients' responsiveness to RTX treatment by using different methods and techniques lead to different results and apparent differences (table 1). Probably, by combining theirs and our analyses we might even be able to more efficiently select patients at baseline who probably will benefit from RTX treatment.

Konstantina Delli,[1] Erlin A Haacke,[2,3] Frans GM Kroese,[2] Rodney P Pollard,[1] Stephan Ihrler,[4] Bert van der Vegt,[3] Arjan Vissink,[1] Hendrika Bootsma,[2] Frederik KL Spijkervet[1]

[1]Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[2]Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[3]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[4]Laboratory for Dermatohistology and Oral Pathology, Munich, Germany

Correspondence to Dr Konstantina Delli, Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Hanzeplein 1, Groningen 9713 GZ, The Netherlands; k.delli@umcg.nl

Twitter Follow Konstantina Delli at @KonstantinDelli

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Delli K, Haacke EA, Kroese FGM, *et al*. *Ann Rheum Dis* 2016;**75**:e34.

Received 19 February 2016
Accepted 21 February 2016
Published Online First 9 March 2016

▶ http://dx.doi.org/10.1136/annrheumdis-2016-209300

Ann Rheum Dis 2016;**75**:e34. doi:10.1136/annrheumdis-2016-209317

REFERENCES

1 Cornec D, Costa S, Devauchelle-Pensec V, *et al*. Do high numbers of salivary-gland infiltrating B cells predict better or worse outcomes after rituximab in patients with primary Sjögren's syndrome? *Ann Rheum Dis* 2016;75:e33.
2 Delli K, Haacke EA, Kroese FG, *et al*. Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment. *Ann Rheum Dis* 2016; Published Online First: 12 Jan 2016. doi: 10.1136/annrheumdis-2015-208304
3 Cornec D, Costa S, Devauchelle-Pensec V, *et al*. Blood and salivary-gland BAFF-driven B-cell hyperactivity is associated to rituximab inefficacy in primary Sjögren's syndrome. *J Autoimmun* 2016;67:102–10.
4 Seror R, Bootsma H, Saraux A, *et al*. Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). *Ann Rheum Dis* 2016;75:382–9.
5 Costa S, Schutz S, Cornec D, *et al*. B-cell and T-cell quantification in minor salivary glands in primary Sjögren's syndrome: development and validation of a pixel-based digital procedure. *Arthritis Res Ther* 2016;18:21.
6 Cornec D, Devauchelle-Pensec V, Mariette X, *et al*. Development of the Sjögren's Syndrome Responder Index, a data-driven composite endpoint for assessing treatment efficacy. *Rheumatology (Oxford)* 2015;54:1699–708.
7 Meiners PM, Arends S, Brouwer E, *et al*. Responsiveness of disease activity indices ESSPRI and ESSDAI in patients with primary Sjögren's syndrome treated with rituximab. *Ann Rheum Dis* 2012;71:1297–302.
8 Meiners PM, Vissink A, Kroese FG, *et al*. Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). *Ann Rheum Dis* 2014;73:1393–6.
9 Meiners PM, Arends S, Meijer JM, *et al*. Efficacy of retreatment with rituximab in patients with primary Sjögren's syndrome. *Clin Exp Rheumatol* 2015;33:443–4.
10 Moerman RV, Arends S, Meiners PM, *et al*. EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI) is sensitive to show efficacy of rituximab treatment in a randomised controlled trial. *Ann Rheum Dis* 2014;73:472–4.
11 Meijer JM, Meiners PM, Vissink A, *et al*. Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2010;62:960–8.
12 Devauchelle-Pensec V, Mariette X, Jousse-Joulin S, *et al*. Treatment of primary Sjögren syndrome with rituximab. A randomized trial. *Ann Intern Med* 2014;160:233–42.
13 Pijpe J, Kalk WWI, van der Wal JE, *et al*. Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjögren's syndrome. *Rheumatology (Oxford)* 2007;46:335–41.
14 Delli K, Dijkstra PU, Stel AJ, *et al*. Diagnostic properties of ultrasound of major salivary glands in Sjögren's syndrome: a meta-analysis. *Oral Dis* 2015;21:792–800.
15 Jousse-Joulin S, Milic V, Jonsson MV, *et al*. Is salivary gland ultrasonography a useful tool in Sjögren's syndrome? A systematic review. *Rheumatology (Oxford)* 2015; Published Online First: 14 Dec 2015. doi: 10.1093/rheumatology/kev385