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Standardisation of the detection of germinal centres in salivary gland biopsies of patients with primary Sjögren's syndrome is needed to assess their clinical relevance

We thank Alunno *et al*¹ for their comments, as a response to our recent publication² in which we describe that, in contrast to the prevailing view, germinal centres in diagnostic labial gland biopsies are not predictive for the development of mucosa-associated lymphoid tissue (MALT) lymphoma in parotid salivary glands in patients with (primary) Sjögren's syndrome (pSS). As we² and others also noted before,^{3–6} Alunno *et al*¹ underpin in their comments the need for standardisation of the detection method of germinal centres in salivary gland biopsies in patients with pSS. Germinal centres are structures that arise in B-cell follicles of secondary lymphoid organs as a response to antigenic stimulation. In these structures, high-affinity memory B-cells are generated, as a consequence of an intimate interplay between B-cells, follicular helper T cells (T_{FH}) and immune-complex presenting follicular dendritic cells (FDCs). Germinal centres can also be found within B-cell areas of ectopic (tertiary) lymphoid tissue that develops in target tissues of various rheumatic autoimmune diseases, including pSS.⁷ While detection in secondary lymphoid tissue is usually relatively simple, recognition of these structures in salivary gland biopsies of patients with pSS is generally more difficult. In particular in H&E stained sections, striated ducts infiltrated with lymphocytes (lymphoepithelial lesions), can erroneously be mistaken for germinal centres (see figure 1). As discussed before,⁵ we proposed for this reason to use staining for transcription factor B-cell lymphoma (Bcl)-6 to identify germinal centres (figure 1). Bcl-6 is consistently expressed at high levels by germinal centre B-cells and is commonly applied by pathology laboratories for lymphoma subtyping. In our study,² we observed slightly more germinal centres in Bcl-6 stained sections of pSS salivary gland biopsies compared with H&E stained sections, which was due to the fact that also small

germinal centres (defined as clusters of ≥ 5 Bcl-6⁺ B-cells) could easily be detected. Larger cohorts of salivary gland biopsies are obviously needed to establish the higher sensitivity and specificity of Bcl-6 stained sections, compared with H&E salivary gland stained sections.

FDCs are not only essential for the generation and function of germinal centres but are, as CXCL13 producing cells, also critically involved in the maintenance of the spatial organisation of the ectopic lymphoid tissue into segregated B-cell and T-cell areas.⁸ Indeed, the mere presence of FDC networks, as revealed by CD21 staining, in salivary gland lymphoid tissue of patients with pSS does not imply that germinal centres are also present.⁹ Staining for (the long isoform of) CD21 is thus not appropriate for identification of germinal centres. Nevertheless, CD21 staining may be useful to discriminate unorganised infiltrates from organised ectopic lymphoid tissue. Besides proper detection of structures, we like to stress here that the proper nomenclature should be used in all histopathological studies and that ectopic lymphoid tissue is not equivalent with ectopic germinal centres. We propose the following levels of organisation of the infiltrating lymphoid cells: (1) unorganised lymphocytic focus (CD21– Bcl-6–), (2) organised lymphocytic focus (ectopic lymphoid tissue) without germinal centres (CD21+ Bcl-6–) and (3) organised lymphocytic focus (ectopic lymphoid tissue) with germinal centres (CD21+ Bcl-6+).

An important question is whether identification of germinal centres is clinically relevant and has an additional value over the already collected serological and clinical data. Germinal centres are observed in diagnostic (minor) salivary gland biopsies of approximately 25% of the patients with pSS.¹⁰ Patients with germinal centres in their diagnostic biopsies seem to have more severe disease as witnessed by the presence of autoantibodies (rheumatoid factor, anti-SSA, anti-SSB), hypergammaglobulinaemia, increased levels of local and systemic proinflammatory cytokines and extraglandular manifestations.^{10 11} Presence of germinal centres has also been associated with a higher risk of non-Hodgkin's lymphoma development.¹² This latter association has been used frequently to justify the evaluation of germinal

Figure 1 Histopathological evaluation of germinal centres in parotid salivary glands of pSS patients.⁵ Consecutive formalin fixed-paraffin embedded parotid gland sections from two patients with pSS were stained for H&E, Bcl-6, CD21 and for CD20 (brown) plus cytokeratin (CK) 8+18 (red). The CK staining was used to identify epithelial cells. Lymphoepithelial lesions (composed of epithelial cells and lymphoid cells) are marked by an asterisk and can be mistaken for germinal centres in H&E stained sections. The H&E stained and the Bcl6 stained micrographs of the lower panels are from our reference.

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centres in minor salivary gland biopsies. However, importantly, only one of the seven non-Hodgkin lymphomas observed in the pSS cohort of the study of Theander *et al*¹² was a parotid MALT lymphoma, that is, the type of lymphoma that is characteristically associated with pSS. In our retrospective study,² we could not demonstrate that the presence of well-defined germinal centres in diagnostic minor salivary gland biopsies (both H&E and Bcl-6-defined) was also predictive for the development of parotid MALT lymphoma. We agree with Alunno *et al*¹ that histopathological analysis of salivary gland biopsies from larger cohorts of patients scored using standardised protocols and with well-defined definitions are needed to assess the clinical relevance of the presence of germinal centres in salivary gland biopsies of patients with pSS.

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