CONCISE REPORT

Germinal centres in diagnostic labial gland biopsies of patients with primary Sjögren’s syndrome are not predictive for parotid MALT lymphoma development

Erlin A Haacke,1,2 Bert van der Vegt,2 Arjan Vissink,3 Fred K L Spijkervet,3 Hendrika Bootsma,1 Frans G M Kroese1

ABSTRACT

Objective Patients with primary Sjögren’s syndrome (pSS) have an increased risk of developing non-Hodgkin’s lymphoma (NHL), particularly parotid gland mucosa-associated lymphoid tissue (MALT) lymphomas. Presence of germinal centres (GCs) in labial gland biopsies has been suggested as predictive factor for NHL. We assessed whether presence of GCs is increased in labial gland biopsies from patients with pSS who developed parotid MALT lymphoma, the dominant NHL-subtype in pSS, compared with patients with pSS who did not develop lymphoma.

Methods Eleven labial gland biopsies from patients with pSS that were taken prior to parotid MALT lymphoma development were compared with biopsies of 22 matched pSS controls (1:2) who did not develop lymphoma. Biopsies were evaluated for GCs (H&E and Bcl6).

Results Labial gland biopsies of pSS MALT lymphoma patients, revealed GCs in 2/11 (18%) H&E sections and 3/11 (27%) Bcl6 stained sections. In controls, GCs were present in 4/22 (18%) of H&E sections and 5/22 (23%) of Bcl6 stained sections.

Conclusion Presence of GCs in labial gland biopsies does not differ between patients with pSS that develop parotid MALT lymphoma and patients with pSS who do not develop lymphoma. The presence of GCs in labial gland biopsies is therefore not a predictive factor for pSS-associated parotid MALT lymphomas.

INTRODUCTION

Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease, in which salivary and lacrimal glands are affected by a chronic inflammatory process, which leads to dryness of mouth and eyes.1 Histopathologically, this inflammatory process is characterised by a periductal lymphoid infiltrate in the glandular parenchyma.2 In roughly one quarter of the patients with pSS, germinal centres (GCs) can be found within these lymphoid infiltrates reflecting the B-cell hyperactivity that characterises the disease.3,4 Although the clinical significance of these GCs remains to be elucidated, the presence of GCs in the glandular tissue of patients with pSS is generally associated with more severe clinical disease as reflected by a higher focus score (FS), increased presence of anti-SSA/Ro (52 kD + 60 kD) and anti-SSB/La autoantibodies and elevated levels of proinflammatory cytokines in the blood.5

A serious complication of pSS is the 5%–10% lifetime risk of developing non-Hodgkin’s B-cell lymphomas (NHL).6 The most common subtype NHL in pSS is the mucosa-associated lymphoid tissue (MALT) lymphoma.5–7 These MALT lymphomas preferentially arise in the parotid glands and account for >60% of the lymphomas arising in patients with pSS.6–8 Which patients with pSS will develop NHL is largely unknown, but several predictors have been identified including disease activity, persistent glandular enlargement, lymphadenopathy, palpable purpura, anti-Ro/anti-La antibodies, rheumatoid factor, lymphopaenia, declined C3 or C4 levels, cryoglobulinaemia and an FS ≥3 in the labial gland biopsy.9–11 Presence of GCs in diagnostic labial gland biopsies has also been proposed as a predictive factor for the development of NHL. However, in the study underlying this assumption, all subtypes of NHL were taken into account, including NHL subtypes not typically associated with pSS, such as follicular lymphoma and T-cell lymphoma.12 For this reason, we explored the predictive role of GCs in labial gland biopsies from patients with pSS for parotid gland MALT lymphomas.

MATERIALS AND METHODS

Patients

From 56 patients with pSS diagnosed with parotid MALT lymphoma, we were able to acquire labial gland biopsies of 11 patients taken at diagnosis of pSS, before (median 4.0, IQR 1.5–6.1 years) lymphoma diagnosis (table 1). Labial gland biopsies from 22 pSS patients with an NHL free follow-up (median 12.0, IQR 6.3–16.8 years) served as controls (see online supplementary table S1). Matching of control pSS patients (1:2) was based on age at diagnosis of pSS and the presence of SSA autoantibodies. Patients were frequency-matched within three age groups: patients diagnosed with pSS at an age of ≤40, between 40 and 60 and ≥60 years. All patients were clinically diagnosed as pSS and retrospectively fulfilled the ACR-EULAR(American College of Rheumatology - European League Against Rheumatism - classification) criteria13 at time of diagnosis. Of the 33 included patients, 32 also fulfilled the AECG-criteria at time of diagnosis. Of one pSS patient this is uncertain due to missing sialometry and ocular examination.

Histopathological assessment of diagnostic salivary gland biopsies

Diagnostic labial salivary gland biopsies were formalin fixed, paraffin embedded and sectioned at 3µm thickness. Serial sections were stained
with H&E, and immunohistochemically for B-cell lymphoma six protein (Bcl6, clone GI191E/A8, Ventana, Illkirch, France) and CD45 (clone 2B11+PD7/26, Ventana, Illkirch, France). Staining was performed on a Ventana Benchmark platform as previously described. In H&E stained sections, FS, lymphoepithelial lesions (LELs) and GCs were assessed. FS was based on the number of clusters of ≥50 lymphocytes (foci)/4 mm² parenchyma. In case of multiple large confluent foci, an arbitrarily defined number of ≥50 lymphocytes was used. LELs were defined as a striated duct with lymphocytes within its basement membrane. GCs were defined as a clearly visible lighter area in a lymphocytic infiltrate containing cells usually present in classical GCs: follicular dendritic cells (FDCs), centrocytes, centroblasts and macrophages. Since detection of GCs is difficult in H&E stained sections, we observed an identical percentage of GCs in both groups: 27% for patients with prelymphoma and 23% for non-lymphoma pSS patients. Although the two groups of patients with pSS who did not develop parotid MALT lymphomas (nor any other type of NHL), GCs were detected in 4/22 (18%) diagnostic labial gland biopsies in H&E stained sections and in 5/22 (23%) of Bcl6 stained sections (table 2). This proportion was comparable with that seen in patients with pSS who did develop parotid MALT lymphoma.

Since FS ≥3 has been suggested as predictive factor for NHL development, we compared FS and relative area of CD45+ infiltrate in prelymphoma labial gland biopsies and biopsies from control pSS patients. FS did not differ between both groups (Mann-Whitney U test, p=0.204). The percentage of biopsies with FS ≥3 was even higher in the control group (36% vs 27%). The relative area of CD45+ lymphocytic infiltrate, however, tended to be higher in the prelymphoma labial gland biopsies than in the controls (table 2, online supplementary figure 1).

## DISCUSSION

This study shows that the presence of GCs does not differ between diagnostic labial gland biopsies from patients with pSS who did develop parotid MALT lymphoma and patients with pSS who did not develop such lymphoma. In H&E stained sections, we observed an identical percentage of GCs in both categories of patients (18%). With a more sensitive and specific method to identify GCs, viz. staining for the GC B-cell associated transcription factor Bcl6, a slightly higher incidence of GCs was seen in both groups: 27% for patients with prelymphoma and 23% for non-lymphoma pSS patients. Although the two groups of patients with pSS are rather small, the percentages of GCs are similar to those reported for labial gland biopsies among the general pSS population.

Based on a large number of studies, Risselada et al reported that the mean weighted percentage of GCs in labial gland biopsies of patients with pSS was 25.1%±5.0% (range 18.3%–33%) in H&E stained sections. Since there was no difference in the occurrence of GCs in labial gland biopsies of patients with pSS prior to parotid MALT lymphoma development and the matched pSS controls as well as with the general pSS population, we conclude that presence of GCs in labial biopsies is not likely predictive for parotid MALT lymphoma development.

Other studies that examined the predictive value of GCs in NHL development did not restrict themselves to MALT lymphoma.
Table 2 Patient characteristics and histological results of diagnostic labial gland biopsies from pSS patients developing parotid MALT lymphomas and control labial gland biopsies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Labial biopsies prior to parotid MALT lymphoma (n=11)</th>
<th>Labial biopsies from patients with pSS without lymphoma (n=22)</th>
<th>p Value Mann-Whitney U test (MWU) or Fisher’s exact test (FT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female n (%)</td>
<td>11/11 (100)</td>
<td>20/22 (91)</td>
<td>0.542 (FT)</td>
</tr>
<tr>
<td>Age (year, mean (SD))</td>
<td>47.5 (14.0)</td>
<td>48.7 (17.2)</td>
<td>0.638 (MWU)</td>
</tr>
<tr>
<td>Anti-SSA positive, n (%)</td>
<td>11/11 (100)</td>
<td>22/22 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Anti-SSB positive, n (%)</td>
<td>7/11 (64)</td>
<td>13/22 (59)</td>
<td>1.000 (FT)</td>
</tr>
<tr>
<td>Anti-RF positive, n (%)</td>
<td>11/11 (100)</td>
<td>19/22 (86)</td>
<td>0.534 (FT)</td>
</tr>
<tr>
<td>Anti-ANA positive, n (%)</td>
<td>11/11 (100)</td>
<td>21/22 (96)</td>
<td>1.000 (FT)</td>
</tr>
<tr>
<td>Δ* pSS-lymph (year), median (IQR)</td>
<td>4.0 (1.5–6.1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FS, median (IQR)</td>
<td>1.8 (0.8–4.0)</td>
<td>2.7 (1.4–3.5)</td>
<td>0.204 (MWU)</td>
</tr>
<tr>
<td>A ≥3, n (%)</td>
<td>3/11 (27)</td>
<td>8/22 (36)</td>
<td>1.000 (FT)</td>
</tr>
<tr>
<td>Area CD45 (%, median (IQR))</td>
<td>20.3 (7.4–27.7)</td>
<td>12.7 (9.4–19.1)</td>
<td>0.143 (MWU)</td>
</tr>
<tr>
<td>LELs based on H&amp;E, n (%)</td>
<td>7/11 (64)</td>
<td>13/22 (59)</td>
<td>1.000 (FT)</td>
</tr>
<tr>
<td>GC based on H&amp;E, n (%)</td>
<td>2/11 (18)</td>
<td>4/22 (18)</td>
<td>1.000 (FT)</td>
</tr>
<tr>
<td>GC based on BcI6, n (%)</td>
<td>3/11 (27)</td>
<td>5/22 (23)</td>
<td>1.000 (FT)</td>
</tr>
</tbody>
</table>

Δ*pSS-lymph: time between diagnosis of pSS and parotid MALT lymphoma. 
Δ*pSS-FU: time between diagnosis of pSS and last follow-up. 
FU, follow up; BcI6, B-cell lymphoma 6 protein; FS, focus score; GC, germinal centre; FT, Fisher’s exact test; LEL, lymphoepithelial lesions; MALT, mucosa-associated lymphoid tissue; MWU, Mann-Whitney U test; pSS, primary Sjögren’s syndrome; RF, rheumatoid factor.

In conclusion, there are no indications that the occurrence of GCs in diagnostic labial salivary gland biopsies, prior to NHL development, is increased in patients with pSS who developed parotid MALT lymphoma. Thus, in our opinion, labial salivary gland GCs of patients with pSS are not likely a predictive factor for parotid MALT lymphoma development. Nevertheless, their presence might be of clinical relevance for stratification of pSS patients regarding treatment options. For this reason, uniform histopathological criteria for the assessment of GCs are eagerly awaited.

Acknowledgements The authors would like to thank the following pathology departments from the Netherlands for providing residual biopsy material: Pathology Friesland (Leeuwarden), Pathology Gelre Hospital (Apeldoorn), Pathology Rijnstate (Arnhem), Pathology Martini Hospital (Groningen), Pathology University Medical Center Utrecht (Utrecht) and Laboratory Pathology and Medical Microbiology (Eindhoven).

Contributors Study concept and design: EAH, FGMK, BvdV, HB and AV. Patient recruitment: HB and AV. Patient biopsy sampling: FKLS. Data collection: EAH and BvdV. Data analysis and interpretation: EAH, FGMK, BvdV, AV, FKLS and HB. The first manuscript was written by EAH and FGMK. All authors critically reviewed the manuscript and approved the final version to be published.

Basic and translational research

Competing interests None declared.

Ethics approval METc (University Groningen). Study registration number: 2014/211.

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REFERENCES

Correction: How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition


Figure 1 was corrected online but the incorrect version appeared in the August print issue.

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Ann Rheum Dis 2017;76:1784. doi:10.1136/annrheumdis-2016-210511corr1

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*Ann Rheum Dis* 2017 76: 1781-1784 originally published online July 14, 2017

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