Primary Sjögren’s Syndrome
Delli, Konstantina

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Towards personalized treatment in primary Sjögren’s syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment

Konstantina Delli1*, Erlin A Haacke2-3*, Frans GM Kroese2, Rodney P Pollard1, Stephan Ihrler4, Bert van der Vegt1, Arjan Vissink1, Hendrika Bootsma2, Frederik KL Spijkervet1

Affiliations
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 & Oral Pathology, Munich, Germany

* Authors contributed equally to the paper

Abstract

Objectives: The aims of this study were (1) to assess the effect of rituximab (RTX; anti-CD20) treatment in primary Sjögren's syndrome (pSS) patients based on sequential parotid biopsies obtained in a placebo-controlled, randomized clinical trial, and (2) to assess the prognostic value of the histological characteristics of parotid gland tissue with regard to responsiveness to RTX treatment.

Methods: In a double-blinded, placebo-controlled trial, sequential parotid gland biopsies were taken from 20 RTX-treated and 10 placebo-treated pSS patients, at baseline and 12 weeks after treatment. The relative amount of lymphocytic infiltrate (stained for CD45), absolute number of T-cells and B-cells per mm² parenchyma (stained for CD3 and CD20, respectively), focus score, number of germinal centers and of lymphoepithelial lesions per mm² of parotid gland parenchyma were assessed. Histopathological data were compared between clinical responders (decrease in European League Against Rheumatism EULAR Sjögren’s Syndrome Disease Activity Index (ESSDAI) score of ≥3 at 12 weeks compared to baseline) and non-responders (change in ESSDAI <3) to RTX treatment.

Results: In RTX-treated patients, a significant reduction in the number of CD20+ B-cells/mm² parenchyma was observed, while no such reduction was observed in placebo-treated patients. The number of CD3+ T-cells/mm² in parenchyma did not change in either group. Furthermore, the number and the severity of lymphoepithelial lesions/mm² and number of germinal centers/mm² were significantly reduced in RTX-treated patients, but did not change in placebo-treated patients. When comparing the pre-treatment characteristics of clinical responders with non-responders, the median number of CD20+ B-cells/mm² parenchyma at baseline was significantly higher in responders (1871 versus 353 cells/mm², p<0.05). Other histopathological baseline characteristics were not predictive for response to RTX treatment.

Conclusion: RTX treatment in pSS leads to a major reduction of lymphocytic infiltration and to fewer B-cells, germinal centers and lymphoepithelial lesions in parotid gland parenchyma. A high pre-treatment number of CD20+ B-cells/mm² parotid gland parenchyma predicts better responsiveness of pSS patients to RTX treatment. Pre-treatment parotid gland histopathological characteristics could therefore contribute to a more personalized treatment approach to pSS.

Introduction

Primary Sjögren’s syndrome (pSS) is a common rheumatic disease, with a prevalence of 60.8 (95% CI: 43.7 to 77.9) cases per 100,000 inhabitants in the total population [1]. pSS commonly affects salivary and lacrimal glands, resulting in a sensation of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Although the exact pathogenic mechanism has not been fully elucidated, in patients with pSS the minor and major salivary glands are characteristically infiltrated by mononuclear lymphoid cells, which form periductal foci. The classic glandular lesion is composed of a lymphoid infiltrate of T and B lymphocytes, whose distribution may vary according to lesion severity [2]. A central role is attributed to B-cells, which tend to be hyperactive [3]. pSS patients have an increased risk of developing lymphoproliferative diseases, which is about 4% during the first 5 years, 10% at 15 years and 18% after 20 years post-diagnosis [4]. Consequently, about 7.5% of patients with pSS develop malignant B-cell lymphoma. In 48-75% of these cases, this is the mucosa-associated lymphoid tissue (MALT) type of lymphoma [5-7]. Most commonly, these lymphomas arise in the parotid glands. The assumed role of hyperactive B-cells in the pathogenesis of pSS is supported by the observed beneficial objective and subjective clinical effects of B-cell depletion by rituximab (RTX), a chimeric monoclonal antibody that binds to the B-cell surface antigen CD20 [8-16]. Significant response was observed in most trials, except from one large randomized clinical trial, the TEARS study [17]. Posthoc application of the Sjögren’s syndrome response index (SSRI) showed also significant response rate difference between RTX and placebo in TEARS [18]. Because there are some concerns about the efficacy of rituximab, the TRACTISS study is aiming to provide evidence whether rituximab improves the clinical outcomes [19]. The final results of the TRACTISS study, including a subanalysis on responders and non-responders, are eagerly awaited.

In a previous open-label phase II study, based on sequential parotid biopsies of 5 pSS patients, we showed that RTX treatment might result in restoration of secretory tissue at a glandular level in responding patients [20]. In that study we observed a reduction of the lymphocytic infiltration with partial to complete loss of germinal centers (GC) and redifferentiation of lymphoepithelial lesions (LEL) to regular stiated ducts. However, major limitations of the study by Pijpe et al. were the small number of patients and lack of a placebo group [20]. Therefore, the aims of the current study were (1) to assess the effect of RTX treatment in pSS patients based on sequential parotid biopsies obtained in a placebo-controlled, randomized clinical trial, and (2) to assess the prognostic value of the histological characteristics of parotid gland tissue with regard to responsiveness to RTX treatment.
Materials and methods

Patients

Thirty patients with pSS were treated in a randomized double-blinded placebo-controlled trial on days 1 and 15 with either 1000 mg RTX i.v. (Roche, Woerden, the Netherlands; n = 20) or placebo i.v. (n = 10) at the University Medical Center Groningen, the Netherlands, as described before [14]. All patients fulfilled the American European Consensus Group (AECG) criteria for pSS [21]. To minimise side effects (infusion reactions, serum sickness), all patients, including the placebo treated patients, were pre-medicated with methylprednisolone (100 mg i.v.), acetaminophen (1000 mg p.o.) and clemastine (2 mg i.v.), and received 60 mg oral prednisone on days 1 and 2, 30 mg on days 3 and 4, and 15 mg on day 5 after each infusion.

An incisional biopsy was taken under local anesthesia from the same parotid gland before and 12 weeks after therapy [22]. The European League Against Rheumatism (EULAR) Sjögren’s Syndrome Disease Activity Index (ESSDAI) [23,24] was assessed at the same time points by two experienced rheumatologists, who were blinded to the treatment group, in order to evaluate possible systemic complications [16]. With regard to response to RTX treatment, patients were categorized into two groups: clinical responders, if the decrease in ESSDAI was 3 points or more at 12 weeks after treatment compared to baseline, and clinical non-responders, if the change of ESSDAI was less than 3 points. The cut-off of 3 points was chosen because this difference indicates a clinically relevant effect [25]. All patients provided informed consent in accordance with the ethics committee of the University Medical Center Groningen (METC approval: 05.229).

Histopathologic analysis

Parotid gland tissue biopsies were fixed in 4% formaldehyde, embedded in paraffin, cut at a thickness of 3 μm, and stained with hematoxylin and eosin. The focus score (defined as ≥50 lymphocytes per 4 mm² glandular tissue), and the number of GC/mm² parotid gland parenchyma were determined. GC were defined as a clearly visible lighter area in a lymphocytic infiltrate containing cells that are usually present in classical germinal centers, like follicular dendritic cells, centrocytes or centroblasts and macrophages. LEL are expressed in LEL/mm² of parotid gland parenchyma, excluding intraparenchymal connective and fat tissue. LEL were evaluated in immunohistochemically stained tissue sections, stained for CD20 as B-lymphocytes predominate in the LEL. A LEL was defined as a cross section of a striated duct with infiltration of CD20+ B-cells within the basement membrane combined with hyperplasia of the epithelium. To evaluate regeneration of the ducts, LEL were subcategorized into three stages (Figure 1A [20]): stage 1) LEL affecting less than 50% of the epithelium of the striated duct (partial LEL); stage 2) LEL affecting between 50% and 100% of the epithelium of the striated duct (developed LEL); (3) LEL with fully circumferential affected epithelium without lumen (occluded LEL).
Immunostaining was utilized for the analysis of lymphocytic infiltrate and was performed as follows. Parotid glands were fixed in 4% buffered formaldehyde, embedded in paraffin wax and sectioned into 4-μm-thick serial sections. Sections were stained after deparaffinisation, pre-treatment with Ultra CC1 (Ventana Medical Systems, Inc, USA), antigen retrieval and endogenous peroxidase blocking using the Benchmark machine. Sections were immunohistochemically stained with anti-CD45 (dilution 1:25, Dako, Heverlee, Belgium, clone 2B11+PD7/26), anti-CD79a (dilution 1: 100, Dako, Heverlee, Belgium, clone JCB17), anti-CD20 (dilution 1:200, Dako, Heverlee, Belgium, clone L-26) and anti-CD3 (dilution 1:20, Monosan, Uden, the Netherlands, clone PS-1) antibodies. The sections were then treated with peroxidase-labelled secondary antibody and visualized with the chromogen DAB (3,3’ Diaminobenzidine) solution.

The relative amount of CD45 positive lymphocytic infiltrates was assessed in relation to the total amount of tissue parenchyma by morphometry with use of ImageJ software (v1.46). Using HistoQuest software, version 3.5.3.0.171, two markers were created, the DAB master marker (CD20) and the hematoxylin non-master marker (nucleus). For the master marker, multiple reference shade was set on 8 with a background threshold range of 5-255. Ring mask and identified cell mask were used. By using a color picker, the shade was chosen directly from a positively stained CD20 cell. One whole section was analyzed excluding intraparenchymal connective and fat tissue leaving multiple regions of interest (ROIs). For the assessment of CD20+ cells, scattergrams were created for each ROI, allowing the visualization of corresponding positive cells in the source ROI, using the real-time back-gating feature. To correct false events, a specific gate according to cell size and intensity of CD20 staining was defined and applied to all analyzed samples. CD20+ cells were quantified according to the selected marker and gate. By using the real-time back-gating feature, automatically counted CD20+ cells were visualized and controlled. The CD20+ cell count (number of cells/mm²) for each analyzed ROI was obtained. The same procedure was followed for CD3+ cell count.

<table>
<thead>
<tr>
<th>Placebo (n=9)</th>
<th>RTX-treated (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>baseline</strong></td>
<td><strong>week 12</strong></td>
</tr>
<tr>
<td><strong>baseline</strong></td>
<td><strong>week 12</strong></td>
</tr>
<tr>
<td>Focus score</td>
<td>1.63 (0.84-3.27)</td>
</tr>
<tr>
<td>LELs/mm²</td>
<td>0.77 (0.38-1.05)</td>
</tr>
<tr>
<td>GCs/mm²</td>
<td>0.06 (0-0.23)</td>
</tr>
<tr>
<td>CD45 (%)</td>
<td>15.2 (5.86-16.62)</td>
</tr>
<tr>
<td>CD20+ cells/mm²</td>
<td>2708 (1469-4395)</td>
</tr>
<tr>
<td>CD3+ cells/mm²</td>
<td>863 (359-1453)</td>
</tr>
</tbody>
</table>

**Immunohistochemical analysis**

**Statistical analysis**

Analysis was carried out with IBM SPSS Statistics 20 (SPSS, Chicago, Illinois, USA). Mann-Whitney U test was used to compare differences between the RTX and placebo therapy.
bo groups or between clinical responders and non-responders. Wilcoxon signed-rank test was used to compare differences over time within groups. Spearman’s correlation coefficient was used to analyze the relationship between histopathology and ESSDAI. Correlations ($\rho$) <0.3 were interpreted as a poor association, 0.3–0.6 as moderate, 0.6–0.8 as good and >0.8 as excellent [15]. P-values <0.05 were considered as statistically significant. Power analysis was performed with Statistical Power Calculator (DDS Research, Washington DC, USA).

## Results

From the total group of 30 patients, five patients had to be excluded from histopathological analysis, due to serum sickness (n=1, RTX-group) or insufficient biopsy material (n=3, RTX-group); one patient dropped out of the study (placebo group). Thus, complete evaluation could be performed of parotid gland biopsies taken from 16 RTX treated patients and 9 placebo-treated patients.

### i. Lymphocytic infiltrate in parotid glands

No differences at baseline between the RTX-treated group and the placebo-treated group were found regarding the focus score, relative area of CD45 staining, numbers of CD20 $^+$ B-cells and CD3 $^+$ T-cells and proportion of biopsies containing GC (data not shown).

After treatment, the focus score did not change significantly in either the RTX-treated group or the placebo-treated group (Table 1). However, CD45 staining demonstrated a significant decrease of the relative area of infiltrates at 12 weeks after RTX treatment. In the placebo group no change was observed between baseline values and 12-week post-treatment values (Figure 2, Table 1).

By counting the number of CD20 $^+$ cells/mm$^2$ of parenchyma, a significant decrease was observed in the number of B-cells (1172 versus 355 cells/mm$^2$, $p=0.001$) in the glandular tissue at 12 weeks after RTX treatment compared to baseline (Figure 3, Table 1). In the placebo-treated group, the number of CD20 $^+$ /mm$^2$ of parenchyma of the parotid glands at week 12 was not statistically different from the number of CD20 $^+$ cells at baseline (Table 1).

The number of CD3 $^+$ cells/mm$^2$ of parenchyma remained unaffected after 12 weeks both in the placebo and RTX-treated group (Table 1).

GC were present at baseline in 67% and 68% of the parotid glands of the placebo and RTX-treated patients, respectively. RTX treatment resulted in a significant decrease in the total number of GC/mm$^2$ (Figure 4, Table 1). Twelve out of 16 parotid glands (75%) were completely devoid of GC 12 weeks after treatment with RTX. In the placebo group, no significant difference was observed in the number of GC/mm$^2$ between baseline levels and 12 weeks after treatment.

### ii. Lymphoepithelial lesions

At baseline, no differences were observed in the presence of LEL in the parotid gland parenchyma between the group of RTX-treated patients and the placebo-treated patients (data not shown). In the RTX-treated group, a significant decrease in the total number of LEL/mm$^2$ was observed after 12 weeks of treatment (Figure 1B, Table 1). In 6 out of 16 patients (38%), LEL were completely absent after RTX treatment. In the placebo group, no significant change was observed in the amount of LEL/mm$^2$ after 12 weeks (Figure 1B, Table 1). Besides the number of LEL/mm$^2$, the severity of the lesions also appeared to decrease; all stages of LEL seemed to transform to a less severe stage. Detailed data regarding the presence of all stages of LEL/mm$^2$ in placebo and RTX-treated patients at week 0 and week 12 is presented in Figure 1C.

### Histopathology and ESSDAI

Of the 16 patients that were treated with RTX, 11 patients (69%) improved by 3 or more ESSDAI points and were therefore considered to be clinical responders.
Furthermore, in RTX treated patients the change in ESSDAI correlated with the change in the number of CD20⁺ cells/mm² of parenchyma (\(\rho=0.706\) and \(p<0.05\)). No other statistically and clinically significant correlations were found for the changes between baseline and 12 weeks.

Discussion

We demonstrated that RTX treatment significantly reduced the overall lymphocytic infiltrate with a major loss of the B-cell component and number of GC/mm² of parotid gland parenchyma in pSS patients. In addition, a major reduction of the quantity and severity of LEL was apparent, reflecting significant restoration of the striated ducts.

RTX treatment results in a considerable decrease in the number of B-cells in the parotid gland tissue. Although this is reflected by a decrease in the amount of infiltrate, as measured by staining for CD45, this is not manifested by a decrease in the focus score. This apparent discrepancy can be explained by the fact that the foci also contain high numbers of T-cells, which may outnumber the number of B-cells [2], and which are not affected in significant numbers by RTX treatment. The focus score is therefore not an appropriate criterion to measure the local effect of RTX on the periductal lymphocytic infiltration. Although RTX treatment results in the almost complete absence of B-cells in the peripheral blood of patients with pSS [26], this is thus not accompanied by a complete loss of B-cells in parotid salivary gland tissue. These results are in line with other studies in pSS [20,28] and rheuma-
toid arthritis [29-31] showing a certain degree of persistence of B-cells in the local tissue after RTX treatment. In contrast, Devauchelle-Pensec et al. reported a total depletion in B-cells in labial salivary glands of pSS patients after RTX treatment [10]. However, in that study only a very low number of the pSS patients (6 out of 15) showed significant numbers of B-cells in the periductal infiltrates at baseline. This is remarkable, since B-cells usually make up to 20-60% of the lymphocytes in the infiltrates of the labial glands of pSS patients, depending upon the grade of the lesion [2].

In this study, at baseline the included patients had high systemic activity, as indicated by the relatively high ESSDAI scores, and the high numbers of GC/mm² [32]. We observed a strong reduction of GCs in the parotid tissue after RTX treatment; in several patients we even observed a complete absence of GCs. This is striking, since not all B-cells are depleted in the parotid glands, and GC B-cells may be relatively more resistant for anti-CD20 therapy compared to other B-cells, as shown by Gong et al. in a murine model for human CD20 expression [33]. A possible explanation for the strong depletion of GCs in the parotid tissue might be that RTX treatment also results in a significant reduction of follicular helper T cells (T_FH), as indicated by analysis of peripheral blood samples (Verstappen et al. 2015, manuscript in preparation). T_FH cells are essential for the development of GCs at local sites. These cells are present in the salivary gland tissue of pSS patients [34], where they may drive GC formation and generation of plasma cells. It is therefore possible that the absolute absence of T_FH in the salivary gland tissue after RTX treatment contributes to the loss of GC activity in these pSS patients.

LEL develop in striated ducts in pSS patients, particularly in the parotid glands. The epitheliotropic autoimmune inflammation of the intraepithelial lymphocytes results in the reaction of the epithelium and induction of these lesions [35]. RTX treatment not only results in a significant reduction of the number CD20⁺ B-cells in the periductal infiltrates, but also in a recovery of the LEL, as revealed by a considerable reduction of the severity of the lesions at all stages (Figure 1C). Such a restoration/redifferentiation of LEL was also observed in the small RTX treatment study (5 patients) described by Pijpe et al. [20]. Apparently, RTX treatment also results in depletion of B-cells within the basement membrane of striated ducts. To explain this, we have hypothesized that the trigger for LEL formation is diminished, and as a result less epithelial reaction takes place leading to reduced proliferation and finally anatomical restoration of the striated ducts. The trigger for LEL formation is unknown, but B-cell derived cytokines may possibly be responsible for this. This notion is in line with the finding of Pollard et al., who showed in the same cohort of RTX-treated patients that the serum levels of pro-inflammatory cytokines (e.g. IL-6) decreased significantly [36].

Patients with pSS have different genetic backgrounds, demographic features and prognosis and exhibit a wide variety of clinical manifestations, involving a number of pathophysiological pathways [37]. Personalized treatment, i.e. providing ‘the right patient with the right drug at the right dose at the right time’ [38], will therefore be the key to treating pSS. An important finding in our study was that clinical responders to RTX treatment had a higher number of CD20⁺ B-cells/mm² of parenchyma of parotid gland tissue at pre-treatment (baseline) compared to non-responders. Furthermore, we also observed a correlation between the change in the number of CD20⁺ cells/mm² of parenchyma and the change in ESSDAI. When higher numbers of B-cells are present in the parotid gland parenchyma, it is therefore possible that RTX treatment may result in depletion of more absolute numbers of B-cells responsible for the disease activity (measured by ESSDAI) than when lower numbers of B-cells are present in the tissue. The baseline number of B-cells/mm² of parenchyma of parotid gland may therefore determine patients’ response to treatment with RTX and may be considered as a biomarker for a more personalized treatment approach to pSS patients. The nature of these disease-associated B-cells that are reduced after RTX treatment needs to be elucidated. These cells are probably not antibody-producing cells, since antibody producing cells persist in the parotid salivary glands after RTX treatment [28]. Alternatively, these cells may represent cytokine-producing B-cells [36].

Although the change in number of B-cells in the infiltrates of the salivary glands correlated to the change in ESSDAI after RTX treatment, the absolute number of B-cells at baseline did not correlate to the ESSDAI. Furthermore, the focus score of the salivary glands did not correlate to the ESSDAI. However, Risselada et al. showed a significant correlation at baseline between the focus score and the cumulative ESSDAI in labial salivary glands of 174 pSS patients (p=0.166-0.284, ps=0.04) [39]. This discrepancy could be ascribed to the fact that the study by Risselada et al. was retrospective, where ESSDAI was assessed at any time point during disease (not necessarily at diagnosis and biopsy), that 21% of patients used immunomodulating medication at the time of the biopsy and correlations were considered to be sig-
nificant even if p was as low as 0.166-0.284. Moreover, the size of the focus (as we assessed by CD45 staining) is probably more relevant than the absolute number of present foci in the salivary gland tissue.

In conclusion, we demonstrated that in parotid gland tissue of pSS patients:

1. RTX treatment leads to major reduction of B-cells and a significant reduction in the number of GCs and LEL. This reduction in the LEL may be the consequence of a major decrease of local B-cell infiltration and may result in structural regeneration of the glands, especially the striated ducts.

2. The baseline number of CD20+ B-cells/mm² of parenchyma may serve as a prognostic biomarker to predict response to RTX treatment. As a result, baseline histopathological characteristics of a parotid biopsy may strongly contribute to a more personalized treatment approach to pSS patients.

Acknowledgements

The authors would like to thank Mr Klaas Sjolemma, UMCG Microscopy and Imaging Center (UMIC), University Medical Center Groningen, for his assistance during the immunohistochemical analysis of specimens with HistoQuest software and Dr Suzanne Arends, Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, for critical reading of the manuscript.

Funding

Supported by unconditional grants of Roche (Woerden, the Netherlands) and the Jan-Kornelis de Cock foundation (Groningen, the Netherlands).

References


18. Cornec D, Devauchelle-Pensec V, Mariette X, et al. Development of the Sjögren’s Syndrome Responder Index, a data-driven com-


**Supplementary table:** Number (%) of patients having any degree of activity per ESSDAI domain (score at least 1) before and after RTX therapy, stratified for clinical responders and non-responders.

<table>
<thead>
<tr>
<th></th>
<th>responders before</th>
<th>responders after</th>
<th>non-responders before</th>
<th>non-responders after</th>
</tr>
</thead>
<tbody>
<tr>
<td>cutaneous</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>pulmonary</td>
<td>5 (45)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>renal</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>articular</td>
<td>8 (73)</td>
<td>2 (18)</td>
<td>5 (100)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>muscular</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>peripheral nervous system</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>central nervous system</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>hematological</td>
<td>6 (55)</td>
<td>5 (45)</td>
<td>2 (40)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>glandular</td>
<td>9 (82)</td>
<td>4 (36)</td>
<td>4 (80)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>constitutional</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>lymphadenopathy</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>biological</td>
<td>11 (100)</td>
<td>8 (73)</td>
<td>2 (40)</td>
<td>2 (40)</td>
</tr>
</tbody>
</table>