University of Groningen

The value of rituximab treatment in primary Sjögren's syndrome

Verstappen, Gwenny M.; van Nimwegen, Jolien; Vissink, Arjan; Kroese, Frans G.M.; Bootsma, Hendrika

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

[Link to publication in University of Groningen/UMCG research database](#)

The value of rituximab treatment in primary Sjögren's syndrome

Gwenny M. Verstappen [a],[*],[1], Jolien F. van Nimwegen [a],[1], Arjan Vissink [b], Frans G.M. Kroese [a], Hendrika Bootsma [a]

[a] Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[b] Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

ARTICLE INFO

ABSTRACT

The rationale for B cell depletion therapy with rituximab in primary Sjögren's syndrome relies upon the well-established role of B cell hyperactivity in immunopathogenesis. In line with this notion, several biomarkers of B cell activity are significantly affected by treatment, both in the target organs and periphery. In contrast to most biological outcomes, clinical outcomes are not consistent between studies. Although two large RCTs did not meet their primary endpoint, several beneficial clinical effects of treatment have been shown. As discussed in this review, differences in study design and patient characteristics could explain the variation in results. Interestingly, a newly developed composite endpoint of subjective and objective outcomes did show a significant effect of rituximab in one of the large RCTs. Response predictors need to be identified to define more targeted inclusion criteria and achieve precision medicine. The positive effects seen on biological and clinical parameters warrant future studies to investigate this promising treatment modality.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease with a heterogeneous clinical presentation. Predominant symptoms of pSS are dryness of mouth and eyes, but many patients also suffer from extraglandular symptoms, including chronic fatigue, arthralgia and involvement of lungs, skin, kidneys and the nervous system. Dysfunction of exocrine glands is accompanied by periductal mononuclear infiltration of these glands, mainly by CD4^{+} T cells and B cells. Involvement of B cells in pSS pathogenesis is further illustrated by the presence of autoantibodies directed against SS-A/Ro and/or SS-B/La, elevated levels of rheumatoid factor (RF), hypergammaglobulinemia, elevated levels of Bruton's tyrosine kinase in B cells and a significantly increased risk of Non-Hodgkin B cell lymphoma, predominantly mucosa-associated lymphoid tissue (MALT) lymphoma [1,2].

The prominent role of B cell hyperactivity in pSS pathogenesis provides a rationale for the use of rituximab, a humanized anti-CD20 monoclonal antibody, to treat this disease. Binding of rituximab to CD20-expressing B cells results in a significant depletion of these cells via antibody-dependent cellular cytotoxicity, complement mediated cytotoxicity and apoptosis [3]. Plasma cells are not directly depleted by rituximab, because expression of CD20 is downregulated when B cells

differentiate towards plasma cells, but formation of new plasma cells may be impaired by B cell depletion therapy. Although initial studies in pSS showed improvement of both subjective and objective parameters [4–7], two large placebo-controlled trials [8,9] did not confirm all promising results of the earlier studies. Possible explanations for this discrepancy are heterogeneity in patient characteristics, primary end points and background medication use, which will all be discussed in this review. Consensus on the efficacy of rituximab in pSS is currently lacking, but treatment results in several clinical, biological and histological improvements. Furthermore, treatment studies with rituximab in pSS provided insights in the pathogenic mechanisms of the disease and post-hoc analyses of biological parameters have identified possible biomarkers that can predict response. These biomarkers may characterize subgroups of pSS patients that benefit from rituximab before start of treatment. Future studies with B cell-targeting therapy can contribute to identification of new predictors of response, as well as development of sensitive and accurate outcome measures for future clinical trials in pSS.

2. Effects of rituximab on B cell hyperactivity

2.1. Systemic markers of B cell hyperactivity

Several biomarkers of B cell activation, including gammaglobulins, autoantibodies (RF, anti-SS-A/Ro, anti-SS-B/La), β2-microglobulin, free light chains and B cell activating factor (BAFF/Blys) have been studied in the context of rituximab treatment in pSS (Fig. 1). A small but significant gradual decrease in total serum IgG after 24 weeks of treatment is seen in larger studies (Table 1) [4,8,10]. At the same time, a decrease in RF levels (up to 50%) is observed (Table 1) [4,6,10–12]. Interestingly,

* Corresponding author at: Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, HPC AA21, PO-Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail address: g.m.p.j.verstappen@umcg.nl (G.M. Verstappen).
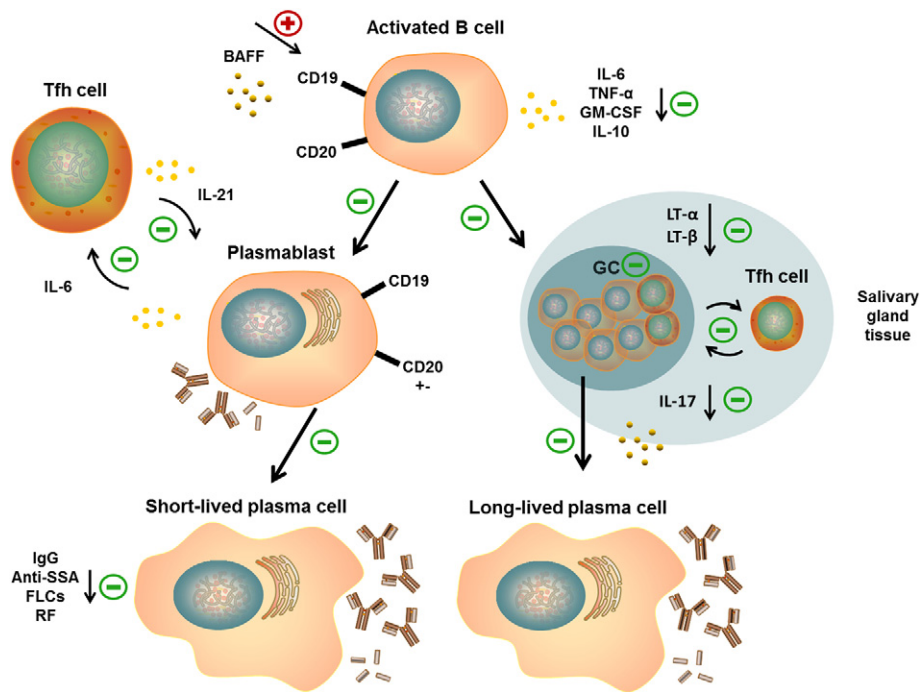[1] Authors contributed equally to this manuscript.

Fig. 1. Effects of rituximab treatment on immunopathogenesis of pSS. Cytokine production by B cells and (T cell-dependent) formation of plasmablasts and short-lived plasma cells are impaired by B cell depletion therapy with rituximab. B cell hyperactivity is reduced, as reflected by lowering of serum IgG, anti-SSA, free light chains and rheumatoid factor. Numbers of circulating Tfh cells and serum levels of IL-21 are also decreased. In salivary gland tissue, formation of ectopic lymphoid tissue and germinal centers is impaired. FLCs: free light chains, RF: rheumatoid factor.

Dass et al. found that a non-responder had less reduction in RF after treatment compared with responders [6]. Following B cell repopulation, RF levels rise again and this rise can predict relapse of clinical symptoms [5,12]. Similar to findings in rheumatoid arthritis (RA), combined presence of RF and disease-specific autoantibodies (anti-SS-A/Ro, anti-SS-B/La) may result in higher disease activity in pSS as well [13]. The mechanism behind this synergistic effect is unknown, but crosslinking and/or stabilization of immune complexes, consisting of autoantigens and autoantibodies, by RF is likely involved. In combination with the finding that higher RF levels seem to increase the risk of lymphoma [14], these data suggest that lowering RF levels by rituximab treatment is of clinical importance, as it may protect against disease progression and/or lymphoma development in pSS.

Several studies assessed the effect of rituximab on anti-SS-A/Ro or anti-SSB/La serum levels in pSS patients. While three studies did not find significant changes in anti-SS-A/Ro or anti-SS-B/La autoantibodies

after treatment [7,15,16], we found a significant reduction of ±25% in anti-SS-A/Ro and anti-SS-B/La titers at 16 weeks after treatment (Table 1) [17]. The discrepancy between studies may be explained by differences in study population size, baseline systemic disease activity, time point of measurements, or differences in reliability of the immunoassay, but methods for anti-SS-A/Ro and anti-SS-B/La measurement were not specified in most studies. The observed reduction in autoantibodies is likely a result of decreased generation of short-lived plasma cells, due to depletion of CD20+ precursor cells, and/or direct depletion of CD20-expressing (short-lived) plasma cells. There is evidence that anti-SSA/Ro60 antibody production depends –at least partially– on clonal turn-over of short-lived plasma cells and this may also be true for other autoantibodies [18]. B cell depletion therapy can therefore directly affect autoantibody production in pSS patients.

In addition to gammaglobulin and autoantibody levels, other indicators of B cell hyperactivity in pSS are also affected by rituximab

Table 1
Main biological effects of rituximab treatment analyzed in prospective clinical studies.

Study population	Patients treated (n)	IgG	RF	anti-SSA titer	Glandular B cells	Patients on concomitant immunomodulatory drugs (n (%))	
						DMARDS	Steroids
Pijpe et al. [11]	15	=	↓[a]	NA	↓	3 (20)	3 (20)
Devauchelle-Pensec et al. [15]	16	=	=	↓[b]	↓	0 (0)	0 (0)
Dass et al. [6,55]	8	↓[b]	↓	NA	NA	NA	NA
Meijer et al. [68]	5	NA	↓	NA	NA	0 (0)	0 (0)
Meijer et al. [4]	20	↓	↓	NA	↓	0 (0)	0 (0)
Gottenberg et al. [48]	78	NA	NA	NA	NA	29 (37)	17 (22)
Carubbi et al. [7]	19	=	=	=	↓	0 (0)	19 (100)
St. Clair et al. [16]	12	NA	↓[b]	=	NA	8 (67)	3 (25)
Devauchelle-Pensec et al. [8]	63	↓	NA	NA	↓	10 (19)	17 (32)
Meiners et al. [10]	28	↓	↓	↓	NA	0 (0)	0 (0)
Bowman et al. [9]	67	NA	NA	NA	NA	39 (58)	7 (10)

Arrows indicate a decrease. NA = not available.
[a] Only in patients with MALT/pSS.
[b] Not statistically significant.

treatment. β2-microglobulin levels show a 'delayed' drop at 16 weeks after treatment [8,12], which was not seen at 6 or 12 weeks after rituximab treatment [8,11]. Serum immunoglobulin free light chains (FLCs) are also affected by rituximab and decrease significantly from week 5 up to week 48 after treatment (unpublished data), in line with findings in RA [19]. Both β2-microglobulin and FLC baseline levels in serum of pSS patients are positively correlated to ESSDAI scores [20], suggesting that there is a link between the degree of B cell activation and systemic disease activity. Serum levels of several B cell-associated cytokines, including IL-6, GM-CSF, TNF-α and IL-10, are also lowered by rituximab [21]. Whether this decrease is the consequence of removal of cytokine-producing B cells, or is caused by indirect effects of B cell depletion on cytokine production by other cells is not yet known. In contrast to the B cell-associated cytokines mentioned above, serum BAFF levels increase after B cell depletion therapy, likely due to unavailability of BAFF receptors as a consequence of the absence of B cells [22]. This rise in BAFF levels may be unfavorable for the patient due to enhanced survival of autoreactive B cell clones and skewing of newly formed B cells towards an autoreactive phenotype [23,24]. Therefore, the efficacy of therapy combining B cell depletion and BAFF-blockade is currently under investigation (NCT02631538).

In summary, most biomarkers of B cell activation in the circulation are decreased by B cell depletion therapy (Fig. 1). Lowering of B cell activation likely contributes to amelioration of systemic disease activity in pSS patients, due to lower levels of autoantibodies and pro-inflammatory cytokines.

2.2. Histological markers of B cell hyperactivity

B cells infiltrate the glandular tissue of pSS patients, accumulate around the ductal epithelium and, together with stromal cells and follicular dendritic cells, orchestrate formation of ectopic lymphoid tissue. Importantly, rituximab clearly reduces the total number and proportion of infiltrating B cells in both minor and major salivary glands (Table 1) [7,25,26]. In addition, as shown in minor salivary gland tissue, rituximab decreases mRNA expression of lymphotoxin (LT)-α and -β, important for lymphoid organogenesis [7]. The reduction of lymphotoxin is likely a direct result of lower B cell numbers in the glands, as the heterodimer LTα1β2 is mainly produced by B cells [27]. Lowering of B cell numbers is further accompanied by a decline in germinal centers located within ectopic lymphoid tissue of the glands [28]. This decline is likely caused both by direct depletion of B cells, as well as reduced presence of Tfh cells (fig. 1) [17].

B cells often infiltrate the ductal epithelium of the salivary glands, resulting in the development of lymphoepithelial lesions. Most of these intra-epithelial B cells belong to a unique subset of cells expressing FcRL4 and these cells possibly function as precursor cells for MALT lymphoma [29]. We have found that intra-epithelial FcRL4+ B cells are almost completely depleted by rituximab [29]. Furthermore, rituximab treatment reduces the severity of lymphoepithelial lesions, and concomitantly leads to restoration of the epithelium [25]. It would be of value to study whether rituximab-treated patients develop MALT-lymphoma less frequently than untreated patients.

As expected, plasma cells can persist in parotid glands of pSS patients despite B cell depletion therapy, since they lack expression of CD20 [30]. However, it is not known if absolute numbers of plasma cells in salivary glands are affected by rituximab and whether this is associated with response to treatment. In synovial tissue of RA patients, a larger decrease in synovial plasma cells was observed in responders versus non-responders [31]. Therefore, it is of interest to study local plasma cell numbers in pSS patients after rituximab.

3. Effects of rituximab on the CD4+ T cell compartment

Depletion of B cells abrogates antigen presentation and cytokine production by these cells and rituximab treatment may therefore affect other cell types, in particular CD4+ T cells (Fig. 1) [32]. Patients with pSS have elevated proportions of circulating T follicular helper (cTfh) cells, defined as CXCR5+ PD-1+ CD45RA− CD4+ cells, compared with healthy controls [17,33,34]. The B cell hyperactivity that is present in pSS patients may favor differentiation of Tfh cells through secretion of IL-6 by activated B cells in conjunction with high expression of co-stimulatory molecules (e.g., CD40, ICOS-L) [35,36]. Tfh cells subsequently activate B cells and promote germinal center formation and plasma cell formation [36], providing a positive-feedback loop. We have recently shown that cTfh cells, and to a smaller extent also Th17 cells, are reduced by rituximab [17]. The decrease in cTfh cells correlates with lowering of ESSDAI scores, emphasizing their potential role in the disease process. Reduced frequencies and numbers of cTfh cells and Th17 cells during B cell depletion are accompanied by decreased serum levels of IL-21 and IL-17. Th17 cells in minor salivary glands are also reduced by rituximab, but the effect on local Tfh cells is not known yet [37,38]. Depletion of the small fraction of Th17 cells that co-expresses CD20 may contribute to the decrease in Th17 cells [39]. Thus, taking all the biological effects of rituximab on (T cell-mediated) B cell hyperactivity together, these findings may –at least in part– underlie beneficial clinical outcomes of rituximab in pSS patients.

4. Clinical efficacy of rituximab in primary Sjögren's syndrome

Several open-label and randomized controlled trials have been performed to date, including two larger RCTs: the TEARS and TRACTISS trials [8,9]. In Tables 2 and 3, population characteristics and clinical outcomes of all prospective clinical trials reported in literature are summarized. Despite the generally acknowledged beneficial effects of rituximab treatment on biological parameters, clinical outcomes vary between studies.

4.1. Effects on exocrine gland function and sicca symptoms

Objective measures of salivary gland function include unstimulated whole salivary flow (UWS) and stimulated whole salivary flow (SWS). UWS depends mainly on submandibular gland function, while SWS depends on both submandibular and parotid gland function. The ratio of parotid and submandibular saliva in SWS depends on the method of stimulation (mechanical vs. citric acid stimulation). UWS and SWS are both outcomes of interest. However, it is important to realize that patients show substantial intra-individual variability in salivary flow, resulting in a large standard deviation [40,41]. Therefore, adequate sample sizes are needed to show the effect of treatment on salivary gland function.

Meijer et al. and Carubbi et al. showed significant improvement in UWS after rituximab treatment [4,7]. In other trials, including the TEARS trial, no effect on UWS was observed [6,8,11,15]. Although the mean baseline UWS in the TEARS trial was comparable to the study of Meijer et al., the standard deviation was twice as high, which may influence the power of their analysis. St. Clair et al. did not find an effect on UWS, but included patients with low to absent UWS at baseline, who therefore may have had irreversible destruction of glandular parenchyma [16]. Recently, Bowman et al. showed that UWS of patients in the rituximab group of the TRACTISS trial remained stable, while the placebo group deteriorated [9].

Only few studies measured the effect of rituximab on SWS. Pijpe et al. showed that rituximab improved stimulated submandibular/sublingual salivary flow only in patients with residual salivary gland function at baseline (SWS > 0.10 ml/min) [11]. Similarly, in the RCT by Meijer et al. only patients with a SWS ≥ 0.15 ml/min were included, and SWS was significantly increased in the rituximab group, while it deteriorated in the placebo group [4]. Unfortunately, recent RCTs did not measure SWS.

Currently, there is a growing interest in salivary gland ultrasound for assessment of the salivary gland structure, as it is non-invasive and inexpensive. The first study using ultrasound showed a reduction in size of the parotid and submandibular glands after rituximab treatment [32].

Table 2
Study population characteristics.

	Study design	Patients (n)		Age (years)	Disease duration (years)	ESSDAI	Anti-SSA and/or -SSB positive (%)	IgG (g/L)	Unstimulated salivary flow (ml/min)	Stimulated salivary flow (ml/min)
		RTX	Control							
Pijpe et al. [11] (early pSS group)	Open label	8	0	46 ± 12	2 ± 1	NA	100	19 ± 5	0.04 (0–0.19)	0.38 (0.2–1.38)
Pijpe et al. [11] (MALT/pSS group)	Open label	7	0	54 ± 10	7 ± 4	NA	100	13 ± 6	0 (0–0.5)	0.01 (0–0.47)
Devauchelle-Pensec et al. [15]	Open label	16	0	55 ± 13	13 ± 10	NA	81	20 ± 13	0.1 ± 0.1	NA
Dass et al. [6,55]	RCT pilot	8	9	51 (22–64)	7 (1–18)	NA	100	19 (12–29)	NA	NA
Meijer et al. [68]	Open label *Re-treatment*	5	0	[a]	[a]	[a]	[a]	[a]	NA	0.09 ± 0.07[b]
Meijer et al. [4] and Moerman et al. [49]	RCT	20	10	43 ± 11	5 ± 4	8 (4–13)	100	23 ± 8	0.17 ± 0.19	0.70 ± 0.57
Gottenberg et al. [48]	Registry	78	0	60 (29–83)	12 (3−32)	11 (2−31)	69	NA	NA	NA
Meiners et al. [5]	Open label *Re-treatment*	28	0	43 ± 14	7 ± 4	8 ± 5	100	23 ± 7	0.16 ± 0.18	0.42 ± 0.37
Carubbi et al. [7]	Open label	19	22	40 (27–53)	1 (1–2)	20 (6–41)	NA	NA	0.08 ± 0.04	NA
St. Clair et al. [16]	Open label	12	0	51 (34–69)	8 (2–18)	NA	83	NA	0.03 (0.0–0.22)	0.05 (0.0–0.65)
Devauchelle-Pensec et al. [8]	RCT	63	57	53 ± 13	5 ± 5	10 ± 7	81	16 ± 6	0.2 (0.4)	NA
Bowman et al. [9]	RCT	67	66	54 ± 12	5 ± 5	5 ± 5	99	18 ± 7	0.08 (0.08)	NA

Results are presented as mean ± SD or median (range). For controlled studies, patient characteristics are presented for the rituximab-treated group.
[a] See Pijpe et al. [11].
[b] Stimulated submandibular/sublingual flow rate.

Table 3

Main clinical effects of rituximab treatment in prospective clinical studies.

Study	Study design	Patients (n) RTX	Control	Follow-up (weeks)	RTX dose	Salivary gland function	Tear gland function	Dryness VAS	Fatigue	Pain	ESSDAI	ESSPRI	SF-36
Pijpe et al. [11] Early pSS group	Open label	8	0	12	High	UWSF = Stim SM/SL ↑	RB ↓ Schirmer =	Oral ↓ Ocular =	MFI GF ↓	SF-36 BP =	NA	NA	Physical functioning ↑ Vitality ↑ Health change ↑ Other domains =
Pijpe et al. [11] MALT+ pSS group	Open label	7	0	12	High	UWSF = Stim SM/SL = [a]	RB ↓ Schirmer =	Oral = Ocular =	MFI GF =	SF-36 BP =	NA	NA	All domains =
Devauchelle-Pensec et al. [15,69]	Open label	16	0	36	Low	UWSF =	Schirmer =	↓	VAS ↓	VAS ↓	NA	NA	All domains except physical functioning ↑ MCS ↑ PCS ↑
Dass et al. [6,55]	RCT pilot	8	9	26	High	UWSF =	Schirmer =	NA	VAS ↓[d] PROFAD-SSI ↓[d]	VAS =	NA	NA	↑[f]
Meijer et al. [68]	Open label Re-treatment	5	0	48	High	SWSF ↑	NA	Oral ↓	MFI GF ↓	NA	NA	NA	Physical functioning ↑
Meijer et al. [4] and Moerman et al. [49]	RCT	20	10	48	High	UWSF ↑[d] SWSF ↑[e,f]	LG ↓[d] Schirmer = [e]	Oral ↓[d] Ocular ↓[e,f]	MFI GF ↓	NA	↓[f]	NA	Total score ↑[d] Vitality ↑[a]
Gottenberg et al. [48]	Registry	78	0	152[b]	High	NA	NA	NA	NA	NA	↓	NA	NA
Meiners et al. [5]	Open label Re-treatment	28	0	48	High	SWSF =	NA	Oral = [c] Ocular = [c]	MFI GF ↓[c]	NA	↓	↓	NA
Carubbi et al. [7]	Open label	19	22	120	High	UWSF ↑[d,f]	↑[f]	↓[f]	↓[f]	VAS ↓	↓[f]	NA	NA
St. Clair et al. [16]	Open label	12	0	52	High	UWSF = SWSF =	Schirmer =	Oral subscores ↓ Ocular =	VAS ↓	VAS =	NA	NA	Vitality ↑ MCS = PCS =
Devauchelle-Pensec, [8]	RCT	63	57	24	High	UWSF =	Schirmer =	↓[f]	↓[f]	VAS =	=	NA	MCS = PCS =
Bowman et al. [9]	RCT	67	66	48	High	UWSF = [e]	Schirmer =	=	VAS = PROFAD-SSI =	VAS =	=	=	All domains =

High-dose: 375 mg/m2/week (4×) or 1000 mg/m² /two weekly (2×). Low-dose: 375 mg/m2/week (2×).

Green arrows indicate significant improvements.

RTX: rituximab. NA: not available. VAS: visual analogue scale. ESSDAI: European League Against Rheumatism (EULAR) Sjögren's syndrome (SS) disease activity index. ESSPRI: EULAR SS patient reported index. UWSF: Unstimulated whole salivary flow. Stim SM/SL: Stimulated submandibular/sublingual salivary flow. SWSF: Stimulated whole salivary flow. RB: Rose Bengal. LG: Lissamin Green. MFI GF: Multidimensional Fatigue Index, General Fatigue domain. PROFAD: Profile of Fatigue and Discomfort—Sicca Symptoms Inventory (PROFAD-SSI). SF-36: Short-form 36-item Health Survey. SF-36 BP: SF-36 Bodily pain domain. MCS: mental component summary score. PCS: physical component summary score.

[a] Stim SM/SL improved in patients with baseline SWSF >0.1 ml/min (n = 2).

[b] Median follow up time.

[c] In 15/28 patients, reported in Meiners et al. [10].

[d] No significant change in control group, compared to baseline.

[e] Deterioration in control group, compared to baseline.

[f] Significant difference between RTX group and control group.

In a sub-analysis of the TEARS study, parotid parenchyma echostructure improved in 50% of the rituximab-treated patients versus 7% in the placebo group, visualizing histological changes induced by rituximab [33].

In summary, there seems to be a beneficial effect of rituximab on salivary gland function and structure, but the effect size is small and varies between studies. Echostructure of the gland seems to improve by rituximab, in line with the histological effects. The observed decrease in glandular B cells and (partial) restoration of the ductal epithelium in patients after treatment may contribute to the increase in salivary flow, but additional factors that affect salivary flow in pSS patients need to be identified. Lastly, it should be considered that severe destruction of parenchyma may not be reversed by immunomodulatory treatment, but such treatment could halt further damage in patients with residual gland function.

Tear gland function was assessed by Schirmer's test in most studies. Only one out of seven studies showed significant improvement in Schirmer's test after treatment (Table 3) [7]. The TEARS study showed a stable Schirmer's test result in the rituximab group, whereas the placebo group tended to deteriorate [8]. Of note, Schirmer's test may not be suitable to detect small changes over time, as it shows low to moderate reliability [42]. Measurement of the epithelial integrity of the ocular conjunctiva by rose Bengal or lissamin green, and cornea by fluorescein staining is more reliable to evaluate keratoconjunctivitis sicca [43]. Interestingly, studies using these ocular surface staining methods did show improvement after treatment [4,11]. This improvement may be caused by effects of rituximab on tear gland morphology and function, composition of tear fluid, as well as effects on the inflammatory micro-environment of the ocular surface. For example, B cell-derived IL-6 levels in tears correlate with the severity of ocular surface disease, reflected by a higher extent of ocular pain, irritation and staining [44]. More knowledge about the effect of rituximab on lacrimal gland inflammation would be valuable, and ocular surface staining should be evaluated in all clinical studies, instead of using Schirmer's test only.

Besides objective measures of dryness, patient-reported outcomes (PROs) with visual analogue scales (VAS) were used in most studies to assess subjective symptoms. Positive results on total dryness scores or subscores for oral and ocular dryness were seen in most studies (Table 3). Although no decrease in dryness VAS was seen in de TRACTISS study, improvement was seen in the TEARS study [8,9]. VAS dryness scores improved significantly among patients in the rituximab group, although <30 mm, which was set as minimum to achieve the primary end-point. Furthermore, in a post-hoc analysis, the SS Responder Index (SSRI) was developed, which includes VAS scores for fatigue, oral dryness and ocular dryness, as well as UWS and ESR. Using this composite endpoint, the proportion of patients with a 30% improvement was significantly higher in the rituximab group, compared to the placebo group [45].

Altogether, both subjective symptoms and objective measures of dryness seem to improve or at least stabilize during rituximab treatment in most studies. These findings are in accordance with histological improvements observed in the salivary glands. A lack of robust objective tests and the poor correlation between objective tests and symptoms in pSS may underlie the reported variation in study results [46].

4.2. Effects on extraglandular manifestations

Fatigue has a major impact on quality of life in pSS patients and is therefore an important target for treatment. However, fatigue is a complex and poorly understood feature of the disease and can only be measured subjectively [47]. Most studies measured fatigue by VAS, but more detailed instruments such as the multi-dimensional fatigue inventory (MFI) and the Profile of Fatigue and Discomfort (PROFAD) questionnaire were also used. Importantly, most studies show that fatigue is reduced in pSS patients. All studies, except for the TRACTISS study and a small group of patients with advanced disease and MALT lymphoma, showed a positive effect of treatment on fatigue (Table 3).

The largest decrease in fatigue is often seen at early time points (week 4 in Meijer et al. and week 6 in the TEARS study). This may explain why no effect was seen on fatigue in the TRACTISS study, as the first visit in this study was scheduled in week 16. Results at early time points may have been biased by initial prednisone treatment to prevent infusion reactions. However, fatigue also improved in the open-label study by Devauchelle-Pensec et al. where no initial prednisone treatment was given [15]. In summary, although the effect size is small, most studies did show improvements in fatigue. In contrast, symptoms of arthralgia and tendomyalgia do not seem to be ameliorated during rituximab treatment (Table 3).

Rituximab is often used off-label to treat severe systemic manifestations of pSS. The effect of rituximab on systemic disease activity was assessed by ESSDAI in several studies, including a prospective registry study of off-label treatment with rituximab (Table 3). Substantial heterogeneity exists within and between study populations regarding systemic disease activity (Table 2). A significant decrease in ESSDAI score following treatment was seen in the RCT of Meijer et al., as reported by Moerman et al., as well as two open label trials and the registry study [4,7,8,48,49]. Improvement was predominantly seen in the glandular, articular, hematological and biological domains [5], possibly because these ESSDAI domains are more likely to change [50]. The efficacy of rituximab on articular involvement was also confirmed using the 28-joint disease activity score (DAS-28) [51]. Results from the registry study and extrapolation of efficacy data from other autoimmune conditions further support the use of rituximab in pSS patients with vasculitis and pulmonary involvement [48,52]. Therefore, these specified clinical settings for rituximab treatment were recently included in the clinical practice guidelines of the Sjögren's Syndrome Foundation [52]. In contrast with earlier findings, no significant effect on ESSDAI score was seen in the TEARS and TRACTISS trials [8,9]. Whereas a lack of effect in the TRACTISS study can be explained by relatively low baseline ESSDAI scores (mean 5.3 ± 4.7 for the rituximab group), the mean baseline score in the TEARS study was 10 ± 7. Of note, in the TEARS study, the ESSDAI was determined retrospectively, which may influence the accuracy and reliability. Furthermore, in the TEARS study, the prevalence of baseline involvement in the domains that show the highest sensitivity to change, e.g. glandular, articular, hematological and biological domains, was 29%, 48%, 38% and 57%, respectively [8]. These percentages are relatively low in comparison to the study by Moerman et al. [49], in which these domains were active in 70%, 80%, 55% and 85% of patients, respectively (unpublished data). Meiners et al. and Carubbi et al. also reported a higher rate of involvement of most of these domains at baseline [5,7]. In conclusion, four prospective studies have shown beneficial effects of rituximab on systemic involvement [5,7,48,49]. The lack of effect in recent trials may be explained by low systemic involvement at baseline or heterogeneity in clinical systemic involvement.

4.3. Effects on quality of life

Several studies investigated the effect of rituximab treatment on quality-of-life using the 36-Item Short Form Health Survey (SF-36). Effects of rituximab treatment were seen in several studies in different domains of the SF-36, but with a large variability between studies (Table 3). Interestingly, vitality was often improved by treatment. However, the TEARS and TRACTISS trials did not observe a significant effect of rituximab treatment on SF-36 scores, compared with placebo. This is consistent with findings that subjective symptoms improved only slightly (TEARS) or not at all (TRACTISS) in the rituximab group, as subjective symptoms are strong predictors of health-related quality-of-life in pSS patients [53].

5. Predictors of response to rituximab

As described in the previous section, the efficacy of rituximab varies substantially between studies. Therefore, it is important to detect

possible predictors which enable selection of patients that are likely to respond to rituximab treatment. Several predictors of good clinical response to rituximab have, for example, already been identified in RA and SLE. In RA, these factors are RF or anti-CCP positivity, elevated serum IgG, low IFN activity, lower serum levels of BAFF and lower numbers of circulating plasmablasts [54]. Furthermore, the degree of B cell depletion was positively associated with clinical response in both RA and SLE [55,56]. SLE patients with a low-affinity FcγRIIIa genotype have less effective B cell depletion, as antibody-dependent cellular cytotoxicity, mediated by FcγRIIIa-positive effector cells (mostly NK cells), is impaired [57]. This genotype results in lower binding affinity of FcγRIIIa to anti-CD20 antibodies that are bound to the target B cells. Whether this FcγRIIIa genotype is also present in a subgroup of pSS patients is not known.

In pSS, some predictors of response to rituximab were evaluated. Baseline expression of B cell-related transcripts and presence of the IFN signature in blood or minor salivary glands were not associated with clinical response to rituximab in pSS [16,58]. Devauchelle-Pensec et al. did identify some candidate transcripts, but these need further validation [58]. Concerning response biomarkers in serum, lower serum BAFF levels at baseline were associated with clinical response to rituximab in pSS patients, as defined by a ≥ 30% improvement in at least two items of the SSRI [26]. As mentioned earlier, high BAFF levels may enhance the survival (and prevent the depletion) of autoreactive B cell clones, residing in glandular tissue and/or bone marrow. Besides lower BAFF levels, responders to rituximab –based on the SSRI– seemed to have lower baseline B cell activity, as reflected by a significantly lower B cell proportion within the glandular infiltrate in the labial salivary glands and lower levels of serum anti-SSA and FLCs [26]. Responders also had a lower focus score (median 0.3) and a lower salivary gland ultrasonography grade at baseline, compared with non-responders [26,59]. Based on these characteristics, responders may have less irreversible gland destruction and respond to rituximab based on SSRI improvement, since VAS dryness scores and UWS are two of the five measures that constitute the SSRI.

Using a different definition of clinical response, i.e., a decrease of ≥3 in the ESSDAI, we have shown that both baseline absolute numbers of B cells and the B cell proportion in parotid gland tissue are higher in responders versus non-responders [25,60]. Explanations for the apparent discrepancy between the study of Cornec et al. and our study have been extensively discussed elsewhere [60,61]. Our findings that high absolute numbers and proportions of B cells in the parotid gland are associated with ESSDAI response suggest that the number of B cells in the target tissue influences systemic disease activity. Likewise, the B cell proportion in the labial gland positively correlates with markers of systemic B cell hyperactivation [62]. Together, these data indicate that rituximab may be effective in either patients with low salivary gland inflammation, to prevent further glandular damage, or in patients with high numbers of infiltrated B cells and high systemic disease activity, to ameliorate activity in specified ESSDAI domains.

6. Why does the efficacy of rituximab vary between studies?

As discussed in the previous paragraphs, results from several trials of rituximab treatment for pSS vary. First, the use of different inclusion criteria, leading to differences in baseline patient characteristics, may explain part of this variation. Since rituximab has shown to –at least– halt further deterioration of glandular function, compared with placebo, treating patients early in the disease process may prevent progression of irreversible damage to the glands. Therefore, the majority of the studies incorporated a limited disease duration (range 2–10 years) as an inclusion criterion, but still there are large differences in disease duration between the study populations. Besides disease duration, patients characteristics such as mean age, IgG levels, and salivary flow also differ among study populations. For example, mean age is ±10 years lower in the studies by Meijer et al. and Carubbi et al., and mean IgG is higher in

the study by Meijer et al., compared to other studies [4,7]. In addition, there may be other unspecified patient characteristics that influence treatment response. For example, ±80% of pSS patients show poor correlation between reported ocular dryness symptoms and objective parameters of gland function, caused by either under- or over-reporting of symptoms [46]. The number of patients under- or over-reporting their symptoms included in a trial may influence the results. Moreover, a study by Lendrem et al. identified four phenotypic clusters using hierarchical clustering of patient-reported pain, fatigue, dryness, anxiety and depression, and found significant differences in IgG, lymphocytes, ESR, ESSDAI score, and UWS between groups [63]. Presumably, these groups may show different responses to rituximab treatment.

Another possible cause of discrepancies between studies is the use of (stable) background medication. In the TEARS and TRACTISS studies, respectively 51% and 68% of the patients used either concomitant DMARDs (mostly hydroxychloroquine) or prednisone (Table 1). Hydroxychloroquine and prednisone both have significant effects on the immune system, making it more difficult to show additional effects of rituximab.

Differences in statistical analysis may also contribute to the variation in reported outcomes. Several studies use paired tests between baseline and multiple time points, whereas specific methods for longitudinal data analysis are available that increase statistical power and reduce multiple testing problems. Generalized estimating equations (GEE), for example, take into account the fact that repeated measurements within one individual are correlated and GEE is therefore a more powerful tool to detect even small changes over time.

Finally, discrepancy between studies is also caused by the use of different outcome measures. No consensus has been reached about the ideal combination of outcome measures to measure treatment efficacy in pSS. The two large RCTs (TEARS and TRACTISS) have used change in subjective symptoms (VAS scores) as primary outcome measures [8,9]. Subjective symptoms such as fatigue and sicca symptoms account for a great loss in quality of life and are indeed an important target for treatment. However, the sensitivity to change of these outcome measurements has not been validated, and the response goals were set quite high (30 mm change in 2 out of 4 VAS scores in TEARS, 30% change of either oral dryness or fatigue VAS score in TRACTISS). These goals may have been too high, considering that the ESSPRI has a minimal clinically important improvement of 1 point (out of 10) or 15% change. Sensitivity to change may be improved by the use of more precise PROs, such as the Patient-Reported Outcomes Measurement Information System (PROMIS), developed by the National Institutes of Health [64]. Importantly, there is a poor correlation between subjective and objective measures of dryness in pSS [46]. Until we are able to understand these discrepancies, subjective and objective measurements of dryness should be equally weighted in the evaluation of treatment efficacy. In line with this notion, Cornec et al. proposed a new data-driven composite outcome which combines objective manifestations and subjective symptoms, the SSRI [45]. This outcome was established by combination of five outcome measures that were improved by rituximab in the TEARS trial. Although the combination of subjective and objective measures as primary outcome is of interest, the SSRI needs to be refined and validated in other clinical studies.

For objective measurement of systemic activity in pSS, introduction of the ESSDAI in 2010 has been a big step forward [65]. Before that, trials did not have a validated tool to assess the effect of rituximab treatment on systemic disease activity. In later trials, most improvement was seen in domains with the highest activity at baseline [5] and a minimal clinically important improvement in ESSDAI of at least three points was determined [66]. Recent trials in pSS have therefore focused on including patients with moderate-to-high ESSDAI scores (≥5), to be able to show an effect on extraglandular manifestations.

Although the ESSDAI has been validated and is now being used in most clinical trials, there are also disadvantages regarding the use of

ESSDAI as outcome measure. It is now recognized that not all ESSDAI domains show sensitivity to change [50]. Consequently, even in populations with comparable mean ESSDAI scores, differences in which ESSDAI domains are active at baseline may greatly influence response to rituximab. To prove efficacy of rituximab on systemic disease activity, future trials should therefore include patients with moderate-to-high ESSDAI scores and activity in at least one of the domains that is likely to change (biological, articular, hematological, pulmonary, and glandular domains). Prospective use of specific indices for separate domains, such as the DAS-28 for articular involvement, may provide more detailed information on efficacy. For example, it is difficult to detect moderate changes in patients with high baseline IgG levels, using the biological domain of the ESSDAI. Additionally, researchers should be aware of the complexity of ESSDAI, which needs to be completed by rheumatologists who are trained and experienced in doing so. In a multi-center setting, this may not always be the case. A more detailed user guide has been published, which may increase the accuracy of the ESSDAI [67]. Considering that rituximab shows effect in several domains of the ESSDAI, patients with high ESSDAI scores may be the target population that we should aim for. Future trials should explore composite endpoints, which include selected domains of the ESSDAI score, besides subjective symptoms and gland function.

7. Conclusions and future directions

Rituximab shows beneficial effects on B cell activity, glandular morphology, dryness, fatigue and several extraglandular manifestations in pSS patients. Although two large RCTs did not meet their primary endpoint, the sensitivity to change of their subjective endpoints may be limited. Future trials should evaluate clinical and biological predictors of response and explore the use of composite endpoints such as the SSRI. We believe that there is still room for new trials with anti-CD20 biologicals, as well as with other B cell-targeting therapies, such as anti-CD22 or anti-BAFF/Blys antibodies for the treatment of pSS, in well-defined populations with moderate to high ESSDAI scores. At the same time, data on long-term (>1 year) efficacy of rituximab and preventive effects on development of extraglandular manifestations and/or lymphoma are needed and may support the use of rituximab in pSS. The effectiveness of pSS has not been proven for all pSS patients, but in our opinion, rituximab is of great value to treat patients with systemic manifestations of pSS and we should not throw the baby out with the bath water.

Declaration of interest

Funding

References

[1] F.G. Kroese, W.H. Abdulahad, E. Haacke, N.A. Bos, A. Vissink, H. Bootsma, B cell hyperactivity in primary Sjogren's syndrome, Expert. Rev. Clin. Immunol. 10 (2014) 483–499, http://dx.doi.org/10.1586/1744666X.2014.891439.

[2] O.B.J. Corneth, G.M.P. Verstappen, S.M.J. Paulissen, M.J.W. de Bruijn, J. Rip, M. Lukkes, J.P. van Hamburg, E. Lubberts, H. Bootsma, F.G.M. Kroese, R.W. Hendriks, Enhanced Bruton's tyrosine kinase activity in peripheral blood B lymphocytes of autoimmune disease patients, Arthritis Rheum. (2017), http://dx.doi.org/10.1002/art.40059 (Epub ahead of print).

[3] T. Shaw, J. Quan, M.C. Totoritis, B cell therapy for rheumatoid arthritis: the rituximab (anti-CD20) experience, Ann. Rheum. Dis. 62 (Suppl. 2) (2003) ii55–ii59, http://dx.doi.org/10.1136/ARD.62.SUPPL_2.II55.

[4] J.M. Meijer, P.M. Meiners, A. Vissink, F.K.L. Spijkervet, W. Abdulahad, N. Kamminga, E. Brouwer, C.G.M. Kallenberg, H. Bootsma, Effectiveness of rituximab treatment in primary Sjögren's syndrome: a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 62 (2010) 960–968, http://dx.doi.org/10.1002/art.27314.

[5] P.M. Meiners, S. Arends, E. Brouwer, F.K. Spijkervet, A. Vissink, H. Bootsma, Responsiveness of disease activity indices ESSPRI and ESSDAI in patients with primary Sjogren's syndrome treated with rituximab, Ann. Rheum. Dis. 71 (2012) 1297–1302, http://dx.doi.org/10.1136/annrheumdis-2011-200460.

[6] S. Dass, S.J. Bowman, E.M. Vital, K. Ikeda, C.T. Pease, J. Hamburger, A. Richards, S. Rauz, P. Emery, Reduction of fatigue in Sjogren syndrome with rituximab: results of a randomised, double-blind, placebo-controlled pilot study, Ann. Rheum. Dis. 67 (2008) 1541–1544, http://dx.doi.org/10.1136/ard.2007.083865.

[7] F. Carubbi, P. Cipriani, A. Marrelli, P. Benedetto, P. Ruscitti, O. Berardicurti, I. Pantano, V. Liakouli, S. Alvaro, A. Alunno, A. Manzo, F. Ciccia, R. Gerli, G. Triolo, R. Giacomelli, Efficacy and safety of rituximab treatment in early primary Sjogren's syndrome: a prospective, multi-center, follow-up study, Arthritis Res. Ther. 15 (2013) R172, http://dx.doi.org/10.1186/ar4359; 10.1186/ar4359.

[8] V. Devauchelle-Pensec, X. Mariette, S. Jousse-Joulin, J.M. Berthelot, A. Perdriger, X. Puechal, V. Le Guern, J. Sibilia, J.E. Gottenberg, L. Chiche, E. Hachulla, P.Y. Hatron, V. Goeb, G. Hayem, J. Morel, C. Zarnitsky, J.J. Dubost, J.O. Pers, E. Nowak, A. Saraux, Treatment of primary Sjogren syndrome with rituximab: a randomized trial, Ann. Intern. Med. 160 (2014) 233–242, http://dx.doi.org/10.7326/M13-1085.

[9] S.J. Bowman, C.C. Everett, J.L. O'Dwyer, P. Emery, C. Pitzalis, W.F. Ng, C.T. Pease, E.J. Price, N. Sutcliffe, N. St Gendi, F.C. Hall, S.P. Ruddock, C. Fernandez, C. Reynolds, C.T. Hulme, K.A. Davies, C.J. Edwards, P.C. Lanyon, R.J. Moots, E. Roussou, I.P. Giles, L.D. Sharples, M. Bombardieri, Randomized controlled trial of rituximab and cost-effectiveness analysis in treating fatigue and oral dryness in primary Sjogren's Syndrome, Arthritis Rheumatol. (2017), http://dx.doi.org/10.1002/art.40093 (Epub ahead of print).

[10] P.M. Meiners, S. Arends, J.M. Meijer, R.V. Moerman, F.K. Spijkervet, A. Vissink, H. Bootsma, Efficacy of retreatment with rituximab in patients with primary Sjogren's syndrome, Clin. Exp. Rheumatol. 33 (2015) 443–444.

[11] J. Pijpe, G.W. van Imhoff, F.K. Spijkervet, J.L. Roodenburg, G.J. Wolbink, K. Mansour, A. Vissink, C.G. Kallenberg, H. Bootsma, Rituximab treatment in patients with primary Sjogren's syndrome: an open-label phase II study, Arthritis Rheum. 52 (2005) 2740–2750, http://dx.doi.org/10.1002/art.21260.

[12] R. Seror, C. Sordet, L. Guillevin, E. Hachulla, C. Masson, M. Ittah, S. Candon, V. Le Guern, A. Aouba, J. Sibilia, J.-E. Gottenberg, X. Mariette, Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome, Ann. Rheum. Dis. 66 (2007) 351–357, http://dx.doi.org/10.1136/ard.2006.057919.

[13] J. Sokolove, D.S. Johnson, L.J. Lahey, C.A. Wagner, D. Cheng, G.M. Thiele, K. Michaud, H. Sayles, A.M. Reimold, L. Caplan, G.W. Cannon, G. Kerr, T.R. Mikuls, W.H. Robinson, Rheumatoid factor as a potentiator of anti-citrullinated protein antibody-mediated inflammation in rheumatoid arthritis, Arthritis Rheumatol, 66, 2014, 813–821, http://dx.doi.org/10.1002/art.38307 (Hoboken, N.J.).

[14] G. Nocturne, A. Virone, W.-F. Ng, V. Le Guern, E. Hachulla, D. Cornec, C. Daien, O. Vittecoq, B. Bienvenu, C. Marcelli, D. Wendling, Z. Amoura, R. Dhote, C. Lavigne, R. Fior, J.E. Gottenberg, R. Seror, X. Mariette, Rheumatoid factor and disease activity are independent predictors of lymphoma in primary Sjögren's Syndrome, Arthritis Rheumatol. 68 (2016) 977–985, http://dx.doi.org/10.1002/art.39518 (Hoboken, N.J.).

[15] V. Devauchelle-Pensec, Y. Pennec, J. Morvan, J.-O. Pers, C. Daridon, S. Jousse-Joulin, A. Roudaut, C. Jamin, Y. Renaudineau, I.Q. Roué, B. Cochener, P. Youinou, A. Saraux, Improvement of Sjögren's syndrome after two infusions of rituximab (anti-CD20), Arthritis Rheum. 57 (2007) 310–317, http://dx.doi.org/10.1002/art.22536.

[16] E.W. St Clair, M.C. Levesque, E.T.L. Prak, F.B. Vivino, C.J. Alappatt, M.E. Spychala, J. Wedgwood, J. McNamara, K.L. Moser Sivils, L. Fisher, P. Cohen, Autoimmunity centers of excellence, rituximab therapy for primary Sjögren's syndrome: an open-label clinical trial and mechanistic analysis, Arthritis Rheum. 65 (2013) 1097–1106, http://dx.doi.org/10.1002/art.37850.

[17] G.M. Verstappen, F.G.M. Kroese, P.M. Meiners, O.B. Corneth, M.G. Huitema, E.A. Haacke, B. van der Vegt, S. Arends, A. Vissink, H. Bootsma, W.H. Abdulahad, B cell depletion therapy normalizes circulating follicular Th cells in primary Sjogren Syndrome, J. Rheumatol. 44 (2017) 49–58, http://dx.doi.org/10.3899/jrheum.160313 jrheum.160313.

[18] R. Lindop, G. Arentz, I. Bastian, A.F. Whyte, L.A. Thurgood, T.K. Chataway, M.W. Jackson, T.P. Gordon, Long-term Ro60 humoral autoimmunity in primary Sjögren's syndrome is maintained by rapid clonal turnover, Clin. Immunol. 148 (2013) 27–34, http://dx.doi.org/10.1016/j.clim.2013.03.015.

[19] T.G. Kormelink, J. Tekstra, R.M. Thurlings, M.H. Boumans, K. Vos, P.P. Tak, J.W. Bijlsma, F.P. Lafeber, F.A. Redegeld, J.A. van Roon, Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity, Ann. Rheum. Dis. 69 (2010) 2137–2144, http://dx.doi.org/10.1136/ard.2009.126441.

[20] J.E. Gottenberg, R. Seror, C. Miceli-Richard, J. Benessiano, V. Devauchelle-Pensec, P. Dieude, J.J. Dubost, A.L. Fauchais, V. Goeb, E. Hachulla, P.Y. Hatron, C. Larroche, V. Le Guern, J. Morel, A. Perdriger, X. Puechal, S. Rist, A. Saraux, D. Sene, J. Sibilia, O. Vittecoq, G. Nocturne, P. Ravaud, X. Mariette, Serum levels of beta2-microglobulin and free light chains of immunoglobulins are associated with systemic disease activity in primary Sjogren's syndrome. Data at enrollment in the prospective ASSESS cohort, PLoS One 8 (2013) e59868, http://dx.doi.org/10.1371/journal.pone.0059868.

[21] R.P. Pollard, W.H. Abdulahad, H. Bootsma, P.M. Meiners, F.K. Spijkervet, M.G. Huitema, J.G. Burgerhof, A. Vissink, F.G. Kroese, Predominantly proinflammatory cytokines decrease after B cell depletion therapy in patients with primary Sjogren's syndrome, Ann. Rheum. Dis. 72 (2013) 2048–2050, http://dx.doi.org/10.1136/annrheumdis-2013-203447.

[22] R.P. Pollard, W.H. Abdulahad, A. Vissink, N. Hamza, J.G. Burgerhof, J.M. Meijer, A. Visser, M.G. Huitema, F.K. Spijkervet, C.G. Kallenberg, H. Bootsma, F.G. Kroese, Serum levels of BAFF, but not APRIL, are increased after rituximab treatment in patients with primary Sjogren's syndrome: data from a placebo-controlled clinical trial, Ann. Rheum. Dis. 72 (2013) 146–148, http://dx.doi.org/10.1136/annrheumdis-2012-202071.

[23] M.R. Ehrenstein, C. Wing, The BAFFling effects of rituximab in lupus: danger ahead? Nat. Rev. Rheumatol. 12 (2016) 367–372, http://dx.doi.org/10.1038/nrrheum.2016.18.

[24] M. Mahévas, M. Michel, B. Vingert, J. Moroch, D. Boutboul, S. Audia, N. Cagnard, J. Ripa, C. Menard, K. Tarte, J. Mégret, S. Le Gallou, P. Patin, L. Thai, L. Galicier, B. Bonnotte, B. Godeau, F. Noizat-Pirenne, J.-C. Weill, C.-A. Reynaud, Emergence of long-lived autoreactive plasma cells in the spleen of primary warm auto-immune hemolytic anemia patients treated with rituximab, J. Autoimmun. 62 (2015) 22–30, http://dx.doi.org/10.1016/j.jaut.2015.05.006.

[25] K. Delli, E.A. Haacke, F.G.M. Kroese, R.P. Pollard, S. Ihrler, B. van der Vegt, A. Vissink, H. Bootsma, F.K.L. Spijkervet, Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment, Ann. Rheum. Dis. 75 (2016) 1933–1938, http://dx.doi.org/10.1136/annrheumdis-2015-208304.

[26] D. Cornec, S. Costa, V. Devauchelle-Pensec, S. Jousse-Joulin, P. Marcorelles, J.-M. Berthelot, L. Chiche, E. Hachulla, P.-Y. Hatron, V. Goeb, O. Vittecoq, A. Saraux, J.-O. Pers, Blood and salivary-gland BAFF-driven B cell hyperactivity is associated to rituximab inefficacy in primary Sjögren's syndrome, J. Autoimmun. 67 (2016) 102–110, http://dx.doi.org/10.1016/j.jaut.2015.11.002.

[27] P. Shen, S. Fillatreau, Antibody-independent functions of B cells: a focus on cytokines, Nat. Rev. 15 (2015) 441–451, http://dx.doi.org/10.1038/nri3857.

[28] E.A. Haacke, B. van der Vegt, P.M. Meiners, A. Vissink, F.K.L. Spijkervet, H. Bootsma, F.G.M. Kroese, Abatacept treatment of patients with primary Sjögren's syndrome results in a decrease of germinal centres in salivary gland tissue, Clin. Exp. Rheumatol. 35 (2017) 317–320.

[29] E.A. Haacke, H. Bootsma, F.K. Spijkervet, A. Visser, A. Vissink, P.M. Kluin, F.G. Kroese, FcRL4+ B cell in salivary glands of primary Sjögren's syndrome patients, J. Autoimmun. (2017), http://dx.doi.org/10.1016/j.jaut.2017.03.012 (Epub ahead of print).

[30] N. Hamza, H. Bootsma, S. Yuvaraj, F.K. Spijkervet, E.A. Haacke, R.P. Pollard, A. Visser, A. Vissink, C.G. Kallenberg, F.G. Kroese, N.A. Bos, Persistence of immunoglobulin-producing cells in parotid salivary glands of patients with primary Sjogren's syndrome after B cell depletion therapy, Ann. Rheum. Dis. 71 (2012) 1881–1887, http://dx.doi.org/10.1136/annrheumdis-2011-201189.

[31] R.M. Thurlings, K. Vos, C.A. Wijbrandts, A.H. Zwinderman, D.M. Gerlag, P.P. Tak, Synovial tissue response to rituximab: mechanism of action and identification of bio-markers of response, Ann. Rheum. Dis. 67 (2008) 917–925, http://dx.doi.org/10.1136/ard.2007.080960.

[32] F.E. Lund, T.D. Randall, Effector and regulatory B cells: modulators of CD4(+) T cell immunity, Nat. Rev. 10 (2010) 236–247, http://dx.doi.org/10.1038/nri2729.

[33] N. Simpson, P.A. Gatenby, A. Wilson, S. Malik, D.A. Fulcher, S.G. Tangye, H. Manku, T.J. Vyse, G. Roncador, G.A. Huttley, C.C. Goodnow, C.G. Vinuesa, M.C. Cook, Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus, Arthritis Rheum. 62 (2010) 234–244, http://dx.doi.org/10.1002/art.25032.

[34] Y. Zhao, P.M. Lutalo, J.E. Thomas, S. Sangle, L.M. Choong, J.R. Tyler, T. Tree, J. Spencer, D.P. D'Cruz, Circulating T follicular helper cell and regulatory T cell frequencies are influenced by B cell depletion in patients with granulomatosis with polyangiitis, Rheumatology (Oxford) 53 (2014) 621–630, http://dx.doi.org/10.1093/rheumatology/ket406.

[35] A. Awasthi, V.K. Kuchroo, Th17 cells: from precursors to players in inflammation and infection, Int. Immunol. 21 (2009) 489–498, http://dx.doi.org/10.1093/intimm/dxp021.

[36] C.S. Ma, E.K. Deenick, M. Batten, S.G. Tangye, The origins, function, and regulation of T follicular helper cells, J. Exp. Med. 209 (2012) 1241–1253, http://dx.doi.org/10.1084/jem.20120994.

[37] F. Ciccia, G. Guggino, A. Rizzo, R. Alessandro, F. Carubbi, A. Giardina, P. Cipriani, A. Ferrante, A. Cannizzaro, R. Giacomelli, G. Triolo, Rituximab modulates IL-17 expression in the salivary glands of patients with primary Sjögren's syndrome, Rheumatology (Oxford) 53 (2014) 1313–1320, http://dx.doi.org/10.1093/rheumatology/keu004.

[38] T. Koga, A. Mizokami, M. Nakashima, T. Shimizu, Y. Nakashima, H. Nakamura, M. Chiwata, N. Daisuke, A. Kawakami, Histological improvement in salivary gland along with effector memory Th17-1 cell reduction in a primary Sjögren's syndrome patient with dermatomyositis and diffuse large B cell lymphoma by R-CHOP therapy, Clin. Immunol. 165 (2016) 35–37, http://dx.doi.org/10.1016/j.clim.2016.03.005.

[39] A. Alunno, F. Carubbi, O. Bistoni, S. Caterbi, E. Bartoloni, P. Di Benedetto, P. Cipriani, R. Giacomelli, R. Gerli, IL-17 producing pathogenic T lymphocytes co-express CD20 and are depleted by rituximab in primary Sjögren's syndrome: a pilot study, Clin. Exp. Immunol. 184 (2016) 284–292, http://dx.doi.org/10.1111/cei.12771.

[40] F.R. Burlage, J. Pijpe, R.P. Coppes, M.E.W. Hemels, H. Meertens, A. Canrinus, A. Vissink, Variability of flow rate when collecting stimulated human parotid saliva, Eur. J. Oral Sci. 113 (2005) 386–390, http://dx.doi.org/10.1111/j.1600-0722.2005.00241.x.

[41] E.M. Ghezzi, L.A. Lange, J.A. Ship, Determination of variation of stimulated salivary flow rates, J. Dent. Res. 79 (2000) 1874–1878, http://dx.doi.org/10.1177/00220345000790111001.

[42] K.K. Nichols, G.L. Mitchell, K. Zadnik, The repeatability of clinical measurements of dry eye, Cornea 23 (2004) 272–285 http://www.ncbi.nlm.nih.gov/pubmed/15084861 (accessed December 29, 2016).

[43] J. Rose-Nussbaumer, T.M. Lietman, C.H. Shiboski, S.C. Shiboski, V.Y. Bunya, E.K. Akpek, M. Srinivasan, J. Mascarenhas, G. Massaro-Giordano, N.A. McNamara, J.P. Whitcher, B.D. Gaynor, Inter-grader agreement of the ocular staining score in the Sjögren's International Clinical Collaborative Alliance (SICCA) Registry, Am J. Ophthalmol. 160 (2015) 1150–1153.e3, http://dx.doi.org/10.1016/j.ajo.2015.08.021.

[44] S. Barabino, Y. Chen, S. Chauhan, R. Dana, Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease, Prog. Retin. Eye Res. 31 (2012) 271–285, http://dx.doi.org/10.1016/j.preteyeres.2012.02.003.

[45] D. Cornec, V. Devauchelle-Pensec, X. Mariette, S. Jousse-Joulin, J.-M. Berthelot, A. Perdriger, X. Puéchal, V. Le Guern, J. Sibilia, J.-E. Gottenberg, L. Chiche, E. Hachulla, P. Yves Hatron, V. Goeb, G. Hayem, J. Morel, C. Zarnitsky, J. Jacques Dubost, R. Seror, J.-O. Pers, P.M. Meiners, A. Vissink, H. Bootsma, E. Nowak, A. Saraux, Development of the Sjögren's Syndrome Responder Index, a data-driven composite endpoint for assessing treatment efficacy, Rheumatology 54 (2015) 1699–1708, http://dx.doi.org/10.1093/rheumatology/kev114.

[46] O.M. Bezzina, P. Gallagher, S. Mitchell, S.J. Bowman, B. Griffiths, V. Hindmarsh, B. Hargreaves, E.J. Price, C.T. Pease, P. Emery, P. Lanyon, M. Bombardieri, N. Sutcliffe, C. Pitzalis, J. Hunter, M. Gupta, J. McLaren, A.M. Cooper, M. Regan, I.P. Giles, D.A. Isenberg, S. Vadivelu, D. Coady, B. Dasgupta, N.J. McHugh, S.A. Young-Min, R.J. Moots, N. Gendi, M. Akil, K. MacKay, W.F. Ng, L.J. Robinson, L.J. Robinson, Subjective and objective measures of dryness symptoms in primary Sjögren's syndrome - capturing the discrepancy, Arthritis Care Res. (2016), http://dx.doi.org/10.1002/acr.23165 (Epub ahead of print).

[47] W.F. Ng, S.J. Bowman, Primary Sjogren's syndrome: too dry and too tired, Rheumatology 49 (2010) 844–853, http://dx.doi.org/10.1093/rheumatology/keq009.

[48] J.-E. Gottenberg, G. Cinquetti, C. Larroche, B. Combe, E. Hachulla, O. Meyer, E. Pertuiset, G. Kaplanski, L. Chiche, J.-M. Berthelot, B. Gombert, P. Goupille, C. Marcelli, S. Feuillet, J. Leone, J. Sibilia, C. Zarnitsky, P. Carli, S. Rist, P. Gaudin, C. Salliot, M. Piperno, A. Deplas, M. Breban, T. Lequerre, P. Richette, C. Ghiringhelli, M. Hamidou, P. Ravaud, X. Mariette, Club Rhumatismes et Inflammations and the French Society of Rheumatology, Efficacy of rituximab in systemic manifestations of primary Sjögren's syndrome: results in 78 patients of the AutoImmune and Rituximab registry, Ann. Rheum. Dis. 72 (2013) 1026–1031, http://dx.doi.org/10.1136/annrheumdis-2012-202293.

[49] R.V. Moerman, S. Arends, P.M. Meiners, E. Brouwer, F.K. Spijkervet, F.G. Kroese, A. Vissink, H. Bootsma, EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI) is sensitive to show efficacy of rituximab treatment in a randomised controlled trial, Ann. Rheum. Dis. 73 (2014) 472–474, http://dx.doi.org/10.1136/annrheumdis-2013-203736; 10.1136/annrheumdis-2013-203736.

[50] M.X. Gottenberg JE, R. Seror, A. Saraux, V. Devauchelle, E. Dernis Labous, P. Dieudé, J.J. Dubost, A.L. Fauchais, V. Goeb, C. Larroche, V. Le-Guern, E. Hachulla, P.Y. Hatron, J. Morel, A. Perdriger, S. Rist Bouillon, D. Sène, O. Vittecoq, J. Sibilia, P. Ravaud, Evolution of disease activity over a 5-year period in the 395 patients with primary Sjögren's syndrome of the assess prospective cohort - ACR meeting abstracts, Arthritis Rheum. (2016) 68 http://acrabstracts.org/abstract/evolution-of-disease-activity-over-a-5-year-period-in-the-395-patients-with-primary-sjogrens-syndrome-of-the-assess-prospective-cohort/ (accessed March 7, 2017).

[51] R.V. Moerman, S. Arends, P.M. Meiners, A. Vissink, F.K.L. Spijkervet, F.G.M. Kroese, E. Brouwer, H. Bootsma, Detailed analysis of the articular domain in patients with primary sjögren syndrome, J. Rheumatol. 44 (2017) 292–296, http://dx.doi.org/10.3899/jrheum.160459.

[52] S.E. Carsons, F.B. Vivino, A. Parke, N. Carteron, V. Sankar, R. Brasington, M.T. Brennan, W. Ehlers, R. Fox, H. Scofield, K.M. Hammitt, J. Birnbaum, S. Kassan, S. Mandel, Treatment guidelines for rheumatologic manifestations of Sjögren's syndrome: use of biologics, management of fatigue and inflammatory musculoskeletal pain, Arthritis Care Res. 69 (2017) 517–527, http://dx.doi.org/10.1002/acr.22968.

[53] D. Cornec, V. Devauchelle-Pensec, X. Mariette, S. Jousse-Joulin, J.-M. Berthelot, A. Perdriger, X. Puéchal, V. Le Guern, J. Sibilia, J.-E. Gottenberg, L. Chiche, E. Hachulla, P.Y. Hatron, V. Goeb, G. Hayem, J. Morel, C. Zarnitsky, J.J. Dubost, P. Saliou, J.O. Pers DDS, R. Seror, A. Saraux, Severe health-related quality-of-life impairment in active primary Sjögren's syndrome is driven by patient-reported outcomes: data from a large therapeutic trial, Arthritis Care Res. 69 (2017) 528–535, http://dx.doi.org/10.1002/acr.22974.

[54] M. Benucci, M. Manfredi, P.S. Puttini, F. Atzeni, Predictive factors of response to rituximab therapy in rheumatoid arthritis: what do we know today? Autoimmun. Rev. 9 (2010) 801–803, http://dx.doi.org/10.1016/j.autrev.2010.07.006.

[55] S. Dass, A.C. Rawstron, E.M. Vital, K. Henshaw, D. McGonagle, P. Emery, Highly sensitive B cell analysis predicts response to rituximab therapy in rheumatoid arthritis, Arthritis Rheum. 58 (2008) 2993–2999, http://dx.doi.org/10.1002/art.23902.

[56] E.M. Vital, S. Dass, M.H. Buch, K. Henshaw, C.T. Pease, M.F. Martin, F. Ponchel, A.C. Rawstron, P. Emery, B cell biomarkers of rituximab responses in systemic lupus erythematosus, Arthritis Rheum. 63 (2011) 3038–3047, http://dx.doi.org/10.1002/art.30466.

[57] J.H. Anolik, D. Campbell, R.E. Felgar, F. Young, I. Sanz, J. Rosenblatt, R.J. Looney, The relationship of Fc? RIIIa genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus, Arthritis Rheum. 48 (2003) 455–459, http://dx.doi.org/10.1002/art.10764.

[58] V. Devauchelle-Pensec, N. Cagnard, J.-O. Pers, P. Youinou, A. Saraux, G. Chiocchia, Gene expression profile in the salivary glands of primary Sjögren's syndrome patients before and after treatment with rituximab, Arthritis Rheum. 62 (2010) 2262–2271, http://dx.doi.org/10.1002/art.27509.

[59] D. Cornec, S. Jousse-Joulin, S. Costa, T. Marhadour, P. Marcorelles, J.-M. Berthelot, E. Hachulla, P.-Y. Hatron, V. Goeb, O. Vittecoq, E. Nowak, J.-O. Pers, V. Devauchelle-Pensec, A. Saraux, High-grade salivary-gland involvement, assessed by histology or ultrasonography, is associated with a poor response to a single rituximab course in primary Sjögren's syndrome: data from the TEARS randomized trial, PLoS One 11 (2016), e0162787. http://dx.doi.org/10.1371/journal.pone.0162787.

[60] K. Delli, E.A. Haacke, F.G. Kroese, R.P. Pollard, S. Ihrler, B. van der Vegt, A. Vissink, H. Bootsma, F.K. Spijkervet, 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, Ann. Rheum. Dis. 75 (2016) e34, http://dx.doi.org/10.1136/annrheumdis-2016-209317.

[61] D. Cornec, S. Costa, V. Devauchelle-Pensec, L. Chiche, A. Saraux, J.-O. Pers, 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. 75 (2016) e33, http://dx.doi.org/10.1136/annrheumdis-2016-209300.

[62] S. Costa, S. Schutz, D. Cornec, A. Uguen, I. Quintin-Roué, A. Lesourd, J.-M. Berthelot, E. Hachulla, P.-Y. Hatron, V. Goeb, O. Vittecoq, J.O. Pers, P. Marcorelles, A. Saraux, V. Devauchelle-Pensec, 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. 18 (2016) 21, http://dx.doi.org/10.1186/s13075-016-0924-2.

[63] D. Lendrem, N. Howard Tripp, X. Mariette, Johnsen SJA, J. Tarn, K. Hackett, B. Griffiths, S. Mitchell, A. Saraux, V. Devauchelle, K. Norheim, J.D. Isaacs, P. McMeekin, S. Bowman, R. Omdal, J.E. Gottenberg, Rethinking primary Sjögren's syndrome: stratification by clinical phenotypes to improve understanding of disease pathogenesis, trial design, clinical management and prospective health gains? - ACR meeting abstracts, Arthritis Rheumatol 2016, p. 68 Suppl 10 http://acrabstracts.org/abstract/rethinking-primary-sjogrens-syndrome-stratification-by-clinical-phenotypes-to-improve-understanding-of-disease-pathogenesis-trial-design-clinical-management-and-prospective-health-gains/ (accessed March 10, 2017).

[64] PROMIS, Clinical Outcomes Assessment, https://commonfund.nih.gov/promis/index (accessed March 8, 2017).

[65] R. Seror, P. Ravaud, S.J. Bowman, G. Baron, A. Tzioufas, E. Theander, J.E. Gottenberg, H. Bootsma, X. Mariette, C. Vitali, E.S.T. Force, EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome, Ann. Rheum. Dis. 69 (2010) 1103–1109, http://dx.doi.org/10.1136/ard.2009.110619.

[66] R. Seror, H. Bootsma, A. Saraux, S.J. Bowman, E. Theander, J.G. Brun, G. Baron, V. Le Guern, V. Devauchelle-Pensec, M. Ramos-Casals, V. Valim, T. Dorner, A. Tzioufas, J.E. Gottenberg, R.S. Laque, T. Mandl, E. Hachulla, K.L. Sivils, W.F. Ng, A.L. Fauchais, S. Bombardieri, R. Priori, E. Bartoloni, V. Goeb, S. Praprotnik, T. Sumida, S. Nishiyama, R. Caporali, A.A. Kruize, C. Vollenweider, P. Ravaud, P. Meiners, P. Brito-Zeron, C. Vitali, X. Mariette, On behalf of the EULAR Sjogren's Task Force, Defining disease activity states and clinically meaningful improvement in primary Sjogren's syndrome with EULAR primary Sjogren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI), Ann. Rheum. Dis. 75 (2016) 382–389, http://dx.doi.org/10.1136/annrheumdis-2014-206008.

[67] R. Seror, S.J. Bowman, P. Brito-Zeron, E. Theander, H. Bootsma, A. Tzioufas, J.-E. Gottenberg, M. Ramos-Casals, T. Dorner, P. Ravaud, C. Vitali, X. Mariette, K. Asmussen, S. Jacobsen, E. Bartoloni, R. Gerli, J.W. Bijlsma, A.A. Kruize, S. Bombardieri, A. Bookman, C. Kallenberg, P. Meiners, J.G. Brun, R. Jonsson, R. Caporali, S. Carsons, S. De Vita, N. Del Papa, V. Devauchelle, A. Saraux, A.-L. Fauchais, J. Sibilia, E. Hachulla, G. Illei, D. Isenberg, A. Jones, M. Manoussakis, T. Mandl, L. Jacobsson, F. Demoulins, C. Montecucco, W.-F. Ng, S. Nishiyama, R. Omdal, A. Parke, S. Praprotnik, M. Tomsic, E. Price, H. Scofield, K.L. Sivils, J. Smolen, R.S. Laque, S. Steinfeld, N. Sutcliffe, T. Sumida, G. Valesini, V. Valim, F.B. Vivino, C. Vollenweider, EULAR Sjogren's syndrome disease activity index (ESSDAI): a user guide, RMD Open 1 (2015) e000022, http://dx.doi.org/10.1136/rmdopen-2014-000022.

[68] J.M. Meijer, J. Pijpe, A. Vissink, C.G. Kallenberg, H. Bootsma, Treatment of primary Sjogren syndrome with rituximab: extended follow-up, safety and efficacy of retreatment, Ann. Rheum. Dis. 68 (2009) 284–285, http://dx.doi.org/10.1136/ard.2008.092601.

[69] V. Devauchelle-Pensec, J. Morvan, A.C. Rat, S. Jousse-Joulin, Y. Pennec, J.O. Pers, C. Jamin, Y. Renaudineau, I. Quintin-Roué, B. Cochener, P. Youinou, A. Saraux, Effects of rituximab therapy on quality of life in patients with primary Sjögren's syndrome, Clin. Exp. Rheumatol. 29 (2011) 6–12.