Abatacept use for 24 weeks has a limited effect on salivary gland inflammation in Sjögren's disease patients

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Abstract

Objective

This study aimed to assess (1) effects of abatacept on salivary gland histology of Sjögren's disease (SjD) patients, (2) the predictive value of salivary gland histopathological characteristics at baseline for clinical response to abatacept treatment.

Methods

Patients (n=41) who participated in the Dutch ASAP-II and ASAP-III trials and international abatacept trial (IM101603) from whom a labial (n=13) or parotid (n=28) salivary gland biopsy was obtained at baseline and after 24 weeks of treatment with abatacept were included. Biopsies were analysed for SjD related histopathological features before and after abatacept (n=25) or placebo (n=16) treatment. Histopathological data at baseline were compared between clinical responders and non-responders to abatacept treatment.

Results

Comparison between abatacept- and placebo-treated patients revealed virtually no differences in histopathological parameters of parotid and labial salivary gland biopsies of SjD patients at baseline and 24 weeks after therapy. In labial glands, only the number of IgA plasma cells/mm^2 differed between the two groups over time (p=0.034). Correspondingly in parotid glands, the number of IgA plasma cells increased in the abatacept group (p=0.049) after 24 weeks. The number of CD20$^+$ B-cells/mm^2 in parotid glands of the placebo group increased compared to baseline (p=0.021). There were no evident differences in baseline histopathological parameters between CRESS or ClinESSDAI responders and non-responders treated with abatacept.

Conclusion

Abatacept has limited effects on salivary gland histology in SjD patients after 24 weeks of treatment. Besides possibly affecting numbers of IgA plasma cells and preventing increases in B-lymphocyte infiltration, salivary gland histopathology could not predict response to abatacept treatment in SjD patients.

Key words

Sjögren's disease, abatacept, salivary gland inflammation, histopathology

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Introduction

Sjögren's disease (SjD) is a systemic auto-immune disease, characterised by chronic inflammation of exocrine glands. In SjD, salivary and lacrimal glands are frequently affected causing typical sicca complaints such as xerostomia and dry eyes (1). In addition to exocrine glands, extra-glandular organ systems can be involved, underlining the systemic nature of the disease (2).

In SjD patients, minor and major salivary glands are typically infiltrated by lymphoid cells. These infiltrates are predominantly located around striated ducts and mainly consist of T- and B-lymphocytes, together with a variety of non-lymphoid cells (1, 3). The infiltrates may even be organised in ectopic lymphoid tissue as witnessed by evidently segregated B- and T-cell rich areas, presence of follicular dendritic cell (FDC) networks and development of germinal centres (GCs) (4). Another histopathological feature of salivary gland tissue of SjD patients is the presence of lymphoepithelial lesions (LELs) composed of lymphocyte-containing striated ducts together with hyperplasia of ductal epithelial cells (5). Finally, a marked increase in the number of IgG plasma cells can be seen, leading to a decline in the IgA/IgG plasma cell ratio (the so-called plasma cell shift) (6).

While much is still unknown about the pathogenesis of SjD, B-cell hyperactivity has been recognised to play an essential role (7). Activated $CD4^+$ T-cells enable and contribute to hyperactivity of B-cells. Therefore, inhibition of T-cell dependent B-cell hyperactivity may be a promising target for treatment of SjD. Abatacept, a fusion protein of CTLA-4 and IgG1-Fc, inhibits the interaction between CD80/CD86 on antigen-presenting cells, including B-cells, and CD28 on T-cells. Thereby the delivery of co-stimulatory signals essential for T-cell activation is prevented (8). Notwithstanding promising effects in an open-label phase II trial, clinical effects were not conclusive in two placebo-controlled randomised trials with a decrease in EULAR Sjogren's syndrome disease activity index (ESSDAI) as clinical endpoint (9, 10). The absence of a clear clinical response could partially be attributed to the large placebo response in ESSDAI. However, post-hoc analysis using the recently developed Composite of Relevant Endpoints in Sjögren's Syndrome (CRESS) showed discrimination between abatacept and placebo (11). Previous studies demonstrated that abatacept treatment induces multiple biological effects in SjD patients, including a reduction in the number of circulating follicular helper T-cells (Tfh), normalisation of the elevated levels of Bruton's tyrosine kinase (BtK) in B-cells, and decreased levels of disease-relevant biomarkers in serum, such as rheumatoid factor and CXCL13 (10, 12, 13). Despite these biological effects witnessed in peripheral blood, there were no significant histopathological changes when comparing parotid salivary gland biopsies prior to and after abatacept treatment in the open-label phase II study (14). However, major limitations of the study were the small number of patients and lack of a placebo group. Hence the aims of this study were (i) to assess the effect of abatacept on parotid and labial gland tissue of patients with SjD in comparison with baseline conditions and the placebo group and (ii) to assess the predictive value of histological characteristics present in labial and parotid salivary glands at baseline with regard to clinical response to abatacept treatment.

Materials and methods

Patients

For this study, patient samples from the open-label phase II Abatacept Sjögren Active Patients (ASAP) trial (ASAP-II) and the randomised placebo-controlled ASAP trial (ASAP-III) carried out at the University Medical Centre Groningen (UMCG; Groningen, the Netherlands), as well as the multi-centre international abatacept trial (IM101603) were combined. Abatacept was either intravenously (ASAP-II) or subcutaneously (ASAP-III and IM101603) applied. Intravenous abatacept infusions (10 mg/kg) administrated on days 1,15, and 29 and monthly thereafter for 24 weeks (9, 10, 15). For subcutaneous application, patients had received instructions to administer injections

at home, once a week, for 24 weeks. Injections contained 125 mg of abatacept or placebo. Informed consent was obtained from all patients according to Declaration of Helsinki principles. For trial information such as randomisation, follow-up time and clinical endpoints see previously published papers (9, 10, 15). All patients with a labial or parotid salivary gland biopsy at baseline and after 24 weeks of abatacept or placebo treatment, were included in this study. Exclusion criteria were insufficient biopsy material (<1mm^2), a focus score (FS) <1 at baseline and development of a MALT lymphoma (Supplementary Fig. S1A).

(Immuno-) histological staining and evaluation

Paraffin-embedded salivary gland biopsies (labial or parotid) of all three trials were similarly processed and analysed as previously described (16). Sections were stained with haematoxylin-eosin (H&E) and for cytokeratin (CK) 8/18, CD3, CD20, CD45, CD21, Bcl6, IgA, IgG and IgM. In addition, an immunohistochemical double staining technique was performed for IgA and IgG. Primary antibodies used are listed in Supplementary Table S1. Slides were digitised using a Philips UFS slide scanner (Philips, Best, the Netherlands). H&E-stained sections were used to determine the FS, defined as the number of periductal foci (clusters of ≥50 lymphocytes) per 4 mm^2. Staining for CK was used to visualise ductal epithelium in order to support the H&E and CD20 staining in the identification of the number of LELs/mm^2 and assessing the maximum severity of the LELs per section (5). All sections were evaluated for B/T cell segregation in one or more foci on the basis of staining adjacent sections for CD20 and CD3. Presence and number of CD21$^+$ FDC-networks and Bcl6$^+$ GCs per section were assessed as previously described (4). Dual staining for IgA and IgG was used to determine the presence of an IgA/IgG plasma cell shift (≥30% IgG plasma cells). Analyses were performed by a trained researcher (UN), an experienced lab-technician (SL) together with a senior head and neck pathologist (BvdV). Discrepancies between observers were resolved in a consensus meeting.

Table I. Demographic, clinical and serological characteristics of Sjögren's disease patients treated with placebo or abatacept.

	Placebo (n=18)	Abatacept (n=31)	*p*-value
Clinical parameters			
Age, years	46 [37-60]	46 [34-57]	0.71
Female, n (%)	18 (100)	30 (96.8)	0.63
Disease duration, years	1 [0-2]	1 [1-3]	0.13
Concomitant treatment, n (%)			
Oral corticosteroids	4 (22.2)	4 (12.9)	0.32
NSAIDs	4 (22.2)	15 (48.3)	0.06
Hydroxychloroquine	4 (22.2)	4 (12.9)	0.32
Schirmer, mm/5 min	4 [1-8]	7 [3-18]	0.14
SWS, ml/min	0.30 [0.15-1.05]	0.39 [0.15-0.57]	0.96
UWS, ml/min	0.10 [0.04-0.20]	0.11 [0.06-0.22]	0.75
ESSDAI, total	10 [8-17]	12 [8-16]	0.84
ESSDAI, glandular domain	1 [1-2]	1 [0-2]	0.95
ESSPRI	7 [6-8]	7 [6-8]	0.73
Serological parameters			
RF	22 [6-100]	43 [15-100]	0.22
Anti-SSA positive, n (%)	16 (88.9)	30 (96.8)	0.30
Anti-SSB positive, n (%)	8 (44.4)	21 (67.7)	0.07
IgG, g/L	16 [13-20]	18 [14-27]	0.17

NSAIDs: non-steroidal inflammatory drugs; ESSDAI: EULAR Sjögren's syndrome disease activity index; ESSPRI: EULAR Sjögren's syndrome patient reported index; RF: rheumatoid factor, SSA: Sjögren's syndrome-related antigen A; SSB: Sjögren's syndrome-related antigen B; SWS: stimulated whole saliva; UWS: unstimulated whole saliva.
Values are presented as median [IQR] unless otherwise specified.

- Digital image analysis

Tissue sections stained with H&E and for CD3, CD20, CD45, IgA, IgG and IgM were analysed using Digital Image Analysis (DIA) algorithms in QuPath (version 0.2.3) as previously described (16). Briefly, the total area of salivary gland parenchyma was digitally measured on the H&E-stained section. The number of CD3$^+$ T-cells, CD20$^+$ B-lymphocytes and IgA, IgG and IgM plasma cells per mm^2 was determined. The relative surface area of CD45$^+$ lymphocytic infiltrate was assessed in relation to the total surface area of salivary gland parenchyma.

Baseline histopathological characteristics in relation to clinical response

From all SjD patients included in the current study, a parotid or labial salivary gland biopsy was taken (within two years) before initiation of abatacept treatment. To evaluate the predictive value for response, salivary gland histopathology at baseline was compared between clinical responders and non-responders at 24 weeks of abatacept treatment. To define clinical treatment response, patients were categorised as responders or non-responders based on the CRESS, minimal clinically important improvement (MCII) in Clinical ESSDAI (ClinESSDAI) and ClinESSDAI low disease activity (LDA) (Suppl. Fig. S1B)(11, 17–19). Total CRESS response was defined as response on ≥3 out of the 5 items. MCII was defined as a decrease of ≥3 points. ClinESSDAI LDA was defined as a score <5.

Statistical analysis

Data were analysed using SPSS version 28 statistical software (SPSS Inc, Chicago, IL). Results were expressed as number of patients (%), mean±SD, or median (IQR) for categorical, normally distributed, and non-normally distributed data, respectively. Mann-Whitney U-test and Fisher's exact test were used to compare differences between the abatacept and placebo groups or between responders and non-responders. Wilcoxon signed-rank test and the McNemar test were used to compare differences over time within treatment groups. The difference between placebo and abatacept groups for change in histopathological parameters over time was evaluated using general-

Table II. Histopathological and immunohistochemical data of parotid and labial salivary gland biopsies of Sjögren's disease patients before and after placebo or abatacept therapy.

	Parotid salivary gland biopsies							Labial salivary gland biopsies						
	Placebo (n=11)			Abatacept (n=17)				Placebo (n=5)			Abatacept (n=8)			
	Baseline	Week 24	P-value	Baseline	Week 24	P-value	GEE P-value	Baseline	Week 24	P-value	Baseline	Week 24	P-value	GEE P-value
Focus score	1.8 (1.6-3.4)	1.5 (1.0-2.9)	0.53	4.3 (1.6-7.6)	3.3 (1.2-7.3)	0.62	0.55^	2.0 (1.4-2.3)	1.8 (1.5-5.5)	0.50	2.0 (1.7-4.6)	5.8 (1.4-9.3)	0.16	0.85^^
LELs/mm²	0 (0-0.13)	0.00 (0-0.18)	0.25	0.27 (0-0.53)	0.12 (0-0.74)	0.65	n/a[a]	0 (0-0.18)	0.12 (0-0.22)	0.29	0 (0-0.28)	0 (0-0.24)	0.72	n/a[a]
LEL⁺ patients, n(%)*	5 (45.5)	5 (45.5)	1.00	10 (58.8)	9 (52.9)	1.00	0.77	2 (40.0)	3 (60.0)	1.00	2 (25.0)	3 (37.5)	1.00	*
FDC-networks/mm²	0.23 (0-0.49)	0.31 (0.0-1.2)	0.09	0.41 (0-0.57)	0.16 (0-0.92)	0.64	*	0.22 (0.07-0.86)	0.23 (0.09-0.52)	0.23	0.38 (0.04-0.67)	0.28 (0-0.63)	0.75	0.84^
FDC-network⁺ patients, n(%)*	8 (72.7)	8 (72.7)	1.00	10 (58.8)	9 (52.9)	1.00	0.78	4 (80.0)	5 (100.0)	*	6 (75.0)	5 (62.5)	1.00	*
GCs/mm²	0.11 (0-0.33)	0 (0-0.20)	0.40	0 (0-0.29)	0 (0-0)	0.23	n/a[a]	0 (0-0.10)	0 (0-0.09)	0.32	0 (0-0.09)	0 (0-0)	1.00	n/a[a]
GC⁺ patients, n(%)*	6 (54.5)	4 (36.4)	0.69	5 (29.4)	3 (17.6)	0.50	*	1 (20.0)	1 (20.0)	1.00	2 (25.0)	1 (12.5)	1.00	*
CD3⁺cells/mm²	1212 (464-1904)	1547 (350-3053)	0.16	666 (277-1211)	711 (480-2144)	0.15	0.87^	528 (396-1054)	666 (485-1430)	0.35	1317 (404-2088)	1092 (495-1517)	0.33	0.16
CD20⁺cells/mm²	596 (232-1285)	873 (139-2350)	*0.021*	691 (193-1855)	449 (162-1379)	0.98	0.10^	327 (166-839)	393 (314-837)	0.50	1178 (249-1702)	573 (354-1564)	0.58	0.38^^
CD3/CD20 segregation, n(%)*	8 (72.7)	8 (72.7)	1.00	8 (47.1)	6 (35.3)	0.69	0.66	2 (40.0)	1.0 (20.0)	1.00	6 (75.0)	4 (50.0)	1.00	*
CD45⁺cells (%)	17.1 (14.0-25.8)	30.3 (5.6-42.7)	0.29	19.8 (5.5-36.5)	17.0 (8.7-29.1)	0.62	0.84^	17.0 (11.8-34.4)	26.9 (25.3-35.0)	0.14	35.7 (13.2-44.2)	28.8 (10.8-37.9)	0.21	0.10^^
IgA/IgG plasma cell shift, n(%)*	7 (63.6)	8 (72.7)	1.00	10 (58.8)	7 (41.2)	0.45	0.23	3 (60.0)	4 (80.0)	1.00	8 (100.0)	7 (87.5)	1.00	*
IgA plasma cells/mm²	326 (130-499)	296 (214-466)	0.48	110 (56-304)	252 (121-439)	*0.049*	0.43^	1655 (998-2168)	1378 (732-1695)	0.08	611 (316-1166)	656 (186-1907)	0.48	*0.034*^^
IgG plasma cells/mm²	205 (74-457)	446 (89-548)	0.25	97 (28-316)	121 (54-283)	0.29	0.47^	692 (362-898)	553 (290-866)	0.50	550 (259-1025)	804 (138-1172)	0.67	0.78^
IgM plasma cells/mm²	49 (12-106)	71 (32-134)	0.48	11 (3-43)	9 (2-45)	0.98	0.35^	566 (301-1445)	316 (210-457)	0.14	619 (242-804)	624 (213-1308)	1.00	0.13^^

[a] GEE analysis could not be performed due to non-normally distributed data
*GEE analysis could not be performed or was not reliable due to lack of power (groups consisting <5).
^ log transformation was applied to histopathological variables to obtain a normal distribution of residuals.
^^Square root transformation was applied to histopathological variables to obtain a normal distribution of residuals.

ised estimating equations (GEE). The GEE model included baseline values of the dependent variable, treatment, time and interaction of treatment and time. In order to reach normal distribution of residuals most histopathological parameters were transformed. The exchangeable correlation structure was used for all variables. Results of the GEE model were not given if it was not possible to obtain a normal distribution of residuals or if the power was too low. *p*-values <0.05 were considered statistically significant.

Results

For demographic, serological and clinical patient characteristics (Table I). At baseline, no differences in histopathological parameters between the placebo and abatacept groups were observed (Suppl. Table S2).

Effect of abatacept and placebo treatment over time on salivary gland biopsies of SjD patients

Comparison between the abatacept and placebo group by GEE analysis revealed that there were virtually no differences over time (baseline and week 24) in FS, amount of infiltrate, number and severity of LELs, CD21⁺ FDC-networks, Bcl6⁺ GCs, number of CD3⁺ T-cells, number of CD20⁺ B-cells, presence of B/T-cell segregation, presence of IgA/IgG shift and the number of IgG and IgM plasma cells/mm² neither in parotid nor labial salivary gland biopsies (Table II). Only the change in number of IgA plasma cells/mm² in labial salivary glands (but not parotid salivary glands) differed significantly between the abatacept and placebo group over time (p=0.034).

Analysis of the effect of abatacept after 24 weeks of treatment revealed that there were no clear changes in histopathological parameters compared to baseline in both types of glands (Table II). The only observed significant difference was an increase in the number of IgA plasma cells/mm² (p=0.049) in the parotid gland (but not the labial gland) (Fig. 1 A-B). Numbers of IgG and IgM plasma cells remained stable over time. The rise in IgA plasma cells was, however, not reflected by a concomitant altered IgA/IgG plasma cell ratio.

When analysing changes in the placebo group, a significant increase in infiltrating CD20⁺ B-cells in parotid glands (p=0.021) was observed (Fig. 1G). This increase was not associated by an increase in higher FS, percentage of infiltrated area, nor by other histopathological features. Furthermore, this rise in B-cell numbers was not seen in labial gland biopsies (Fig. 1H). In labial gland biopsies, placebo treatment revealed only a trend towards decrease in the number of IgA plasma cells/mm² (Fig. 1D) (p=0.08).

Minor or major salivary gland histopathology could not predict abatacept treatment response

Twenty-two out of 35 patients (63%) who were treated with abatacept were classified as CRESS responders because they reached response on ≥3 of 5 items. The remaining 13 patients were classified as CRESS non-responders. Parotid salivary gland biopsies were available from 13 responders and 8 non-responders and labial salivary gland biopsies were available from 9 responders and 5 non-responders. No significant differences in baseline histopathological parameters were observed between CRESS-responders and non-responders, neither in labial nor parotid salivary gland biopsies (Suppl. Table S3). When patients were classified as clinical responder based on ClinESSDAI MCII (67% responders), also no significant differences in histopathological parameters were observed on baseline between responders and non-responders. When response defined as maintenance or reaching ClinESSDAI low disease activity (62% responders), only the presence of IgA/IgG shift was higher in parotid (p=0.024) – but not labial gland biopsies – of responders at

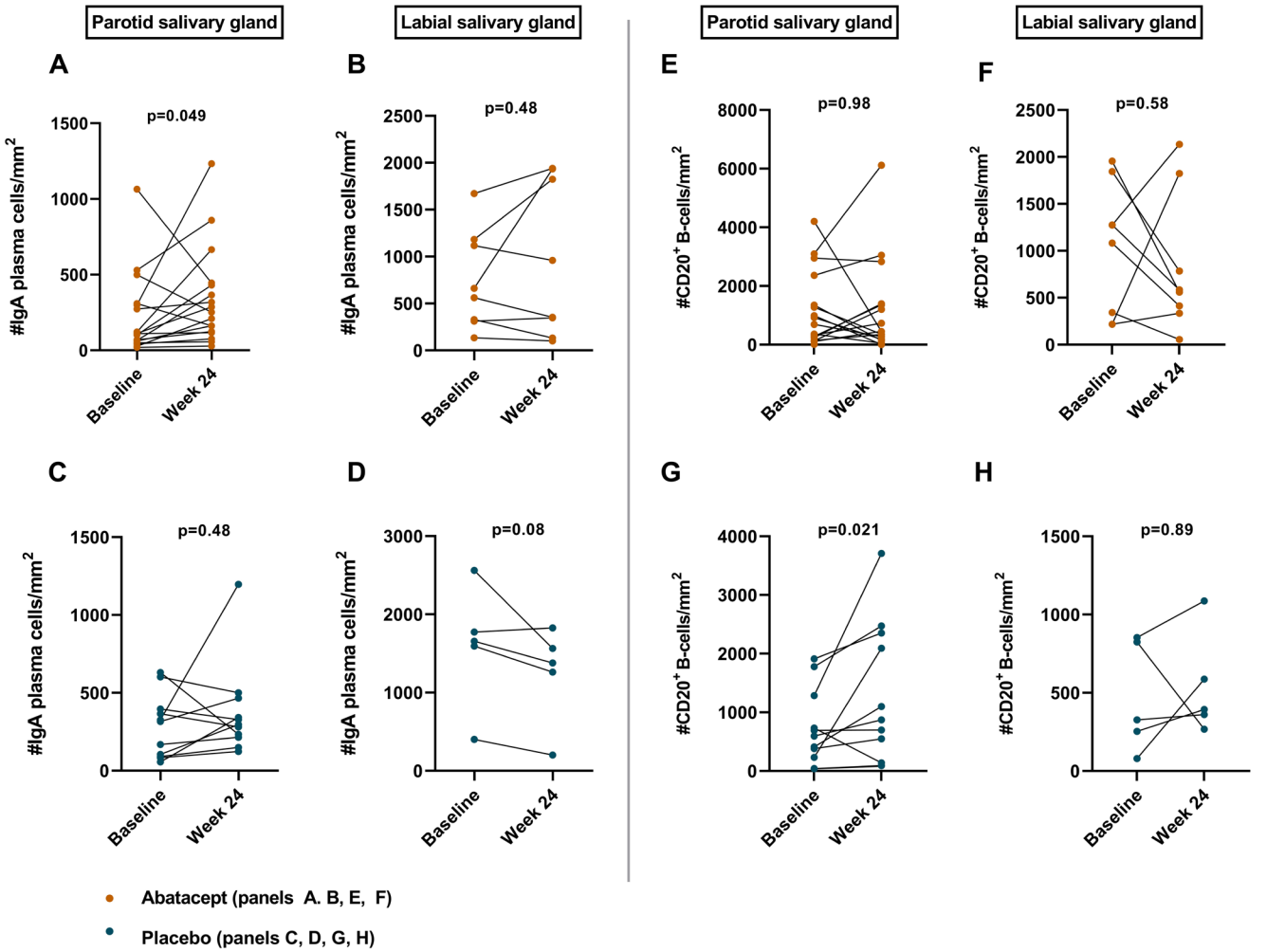

Fig. 1. Number of IgA$^+$ plasma cells/mm^2 (**A-D**) and CD20$^+$ B-cells/mm^2 (**E-H**) in parotid and labial salivary gland sections of Sjögren's disease patients at baseline and after 24 weeks of abatacept and placebo treatment.

baseline compared to non-responders (data not shown).

Discussion

In the work described here, we studied the effect of abatacept and placebo treatment over time on either parotid or labial gland biopsies of SjD patients. Overall, the effects of 24 weeks of abatacept treatment on parotid and labial gland tissue of SjD patients were limited, both when comparing differences over time between the abatacept and placebo groups as well as when the change within the abatacept group was compared to baseline. Furthermore, there were no differences in histopathological features at baseline between abatacept responders and non-responders based on the recently developed composite endpoint, the CRESS (11), nor on the ClinESSDAI (18, 19).

Although abatacept treatment showed in general no effect on histopathology, some subtle changes in absolute IgA plasma cell counts in salivary glands were observed after treatment. First, the change in number of IgA plasma cells in labial salivary glands differed in the abatacept treatment group compared to the placebo group. This seems to be largely attributed to a decrease in IgA plasma cell numbers in labial glands in patients receiving placebo. In this patient group there was a trend for a decline in IgA plasma cells, compared to baseline (p=0.08), while these numbers remained stable over time in the abatacept group. Second, a significant increase in the number of IgA plasma cells was seen in the parotid glands after abatacept treatment, compared to baseline, whereas IgA plasma cell numbers were stable in the placebo group. In both treatment arms and for both salivary gland types no effects were seen on IgG or IgM secreting plasma cells. Glandular IgA secreting plasma cells are part of the homeostatic mucosal immune system and IgA plasma cells are also normally present in healthy salivary glands (20). Although labial and parotid salivary glands appear to respond differently, abatacept treatment may contribute to a (slight) normalisation of the glandular microenvironment that supports differentiation towards IgA plasma cells. The exact role of abatacept in this process is not known, but a possible explanation is that blockade of costimulation may inhibit T-cell dependent (CD28-dependent) class switch recombination towards IgG, but not T-cell independent (CD28-independent) class switch recombination towards IgA. Important cytokines for T-cell independent

class switch recombination at mucosal sites towards IgA are BAFF and APRIL and these cytokines are abundantly expressed in serum, saliva and salivary glands of SjD patients. The absence of an effect on (T-cell dependent) IgG plasma cell numbers may be explained by the observation that plasma cells in salivary glands are long-lived cells, which form a relatively stable population of cells (15, 16). Moreover, according to Szysko *et al*. salivary glands of SjD patients provide niches rich in specific factors vital for survival of plasma cells (6). The long-lived plasma cells in these niches are possibly not affected by 24 weeks of abatacept treatment. Further research is needed to gain more knowledge regarding plasma cell populations residing in salivary glands of SjD patients, in particular on long-term effects of abatacept treatment on these cells.

Besides changes in numbers of IgA plasma cells, histopathological evaluation revealed an increase in the number of infiltrating B-cells in parotid salivary glands of patients in the placebo group after 24 weeks. Christodoulou et al. observed that in labial gland biopsies the number of B-cells are associated with the Tarpley biopsy score, and thus likely with the progression of the disease (21). In contrast to placebo, an increase in B-cell numbers was not seen in abatacept-treated patients, suggesting that further infiltration was halted by this immunomodulatory biological DMARD. Progression of B-cell infiltration was, however, not seen in labial glands of the placebo group. The reason for this is not clear, but untreated SjD patients harbour relatively more B-cells (but not T-cells) in parotid glands than in their labial glands, when paired glands are compared (16). An increase in the numbers of B-cells (in placebo treated patients) might therefore preferably be seen in parotid glands. In line with these findings, the open-label extension phase of the ASAP-III trial revealed glandular function improvement after 48 weeks while this was not observed after 24 weeks of abatacept treatment (22). Perhaps, glandular function and also histopathology, requires a longer treatment period to improve.

These findings are in line with our previous observations in parotid gland biopsies of abatacept treated patients in a smaller part of the study population (open label study; ASAP-II). In the previous ASAP-II open label study, GCs in parotid gland parenchyma were absent after abatacept treatment in those patients with GCs in their parotid glands on baseline (14). While in most of the SjD patients treated with abatacept, the number of GCs/mm^2 in salivary gland parenchyma decreased (5 out of 7 patients, *i.e*. 71%), a similar pattern was observed in the placebo group (6 out of 8, *i.e*. 75%). Therefore, we can conclude that abatacept does not affect the formation of GC significantly.

A widely used tool to measure clinically meaningful improvement in SjD is change in ESSDAI or ClinESSDAI score of ≥3 points (17). Despite the proven validity, reliability, and responsiveness of (clin)ESSDAI, several recent randomised controlled trials employing ESSDAI as the primary outcome measure were not able to discriminate in clinical response between the active treatment and placebo treatment groups. A main reason for this are the relatively large response rates in placebo groups (9, 23). The recently developed and validated composite endpoint CRESS was able to demonstrate superiority in clinical response of the active treatment compared to placebo treatment in *post-hoc* analysis of several clinical trials, including the abatacept trials (11). In the open-label study, presence of GCs at baseline predicted response to abatacept (14). Here, we did not observe histopathological differences between responders and non-responders. This discrepancy may be explained by the fact that in the study by Haacke *et al*. treatment response was not defined by CRESS, or reaching ClinESSDAI MCII or LDA, but as an increase or decrease of ESSDAI in the glandular domain.

In this study, we combined labial and parotid gland biopsies derived from patients from three abatacept trials and analysed a wide range of histological parameters in order to obtain valuable insights into the effect of abatacept versus placebo treatment on salivary gland histology. However, this was an explorative analysis with multiple testing, which was not pre-powered to find significant differences, and especially the number of labial biopsies was relatively low. Another limitation might be that biopsies were taken 24 weeks after treatment, which might not be sufficient in order to find significant differences over time. Future studies may be needed to confirm the effects of (non) treatment.

To conclude, in this study we showed that abatacept has a limited effect on salivary gland tissue in SjD, besides possibly affecting numbers of IgA plasma cells and preventing increases in B-lymphocyte infiltration. Additionally, we observed that salivary gland histopathology could not predict clinical response to abatacept in SjD patients.

References

1. MARIETTE X, CRISWELL LA: Primary Sjögren's syndrome. *N Engl J Med* 2018; 378(10): 931-39. https://doi.org/10.1056/nejmcp1702514
2. FOX RI: Extraglandular anifestations of Sjögren's syndrome (SS): dermatologic, arthritic, endocrine, pulmonary, cardiovascular, gastroenterology, renal, urology, and gynecologic manifestations. In: FOX RI, FOX CM (Eds.): Sjögren's syndrome. Springer, 2011; 285-316. https://doi.org/10.1007/978-1-60327-957-4_17
3. BRITO-ZERÓN P, BALDINI C, BOOTSMA H *et al*.: Sjögren syndrome. *Nat Rev Dis Primers* 2016; 2: 16047. https://doi.org/10.1038/nrdp.2016.47
4. NAKSHBANDI U, HAACKE EA, BOOTSMA H *et al*.: Bcl6 for identification of germinal centres in salivary gland biopsies in primary Sjögren's syndrome. *Oral Dis* 2020; 26(3): 707-10. https://doi.org/10.1111/odi.13276
5. VAN GINKEL MS, HAACKE EA, BOOTSMA H *et al*.: Presence of intraepithelial B-lymphocytes is associated with the formation of lymphoepithelial lesions in salivary glands of primary Sjögren's syndrome patients. *Clin Exp Rheumatol* 2019; 118(3): 42-48.
6. SZYSZKO EA, BROKSTAD KA, ØIJORDSBAKKEN G, JONSSON MV, JONSSON R, SKARSTEIN K: Salivary glands of primary Sjögren's syndrome patients express factors vital for plasma cell survival. *Arthritis Res Ther* 2011; 13(1): R2. https://doi.org/10.1186/ar3220
7. KROESE FGM, ABDULAHAD WH, HAACKE E, BOS NA, VISSINK A, BOOTSMA H: B-cell hyperactivity in primary Sjögren's syndrome. *Expert Rev Clin Immunol* 2014; 10(4): 483-99. https://doi.org/10.1586/1744666x.2014.891439
8. WESTHOVENS R: Abatacept: the first-in-class costimulation blocker for the treatment of rheumatoid arthritis. *Fut Rheumatol* 2006; 15-

22. https://doi.org/0.2217/17460816.1.1.15
9. VAN NIMWEGEN JF, MOSSEL E, VAN ZUIDEN GS *et al*.: Abatacept treatment for patients with early active primary Sjögren's syndrome: a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial (ASAP-III study). *Lancet Rheumatol* 2020; 3(2): E153-E163. https://doi.org/10.1016/S2665-9913(19)30160-2
10. BAER AN, GOTTENBERG JE, ST CLAIR EW *et al*.: Efficacy and safety of abatacept in active primary Sjögren's syndrome: Results of a phase III, randomised, placebo-controlled trial. *Ann Rheum Dis* 2021; 80(3): 339-48. https://doi.org/10.1136/annrheumdis-2020-218599
11. ARENDS S, DE WOLFF L, VAN NIMWEGEN JF *et al*.: Composite of Relevant Endpoints for Sjögren's syndrome (CRESS): development and validation of a novel outcome measure. *Lancet Rheumatol* 2021; 3(8): E553-E562. https://doi.org/10.1016/S2665-9913(21)00122-3
12. VERSTAPPEN GM, MEINERS PM, CORNETH OBJ *et al*.: Attenuation of follicular helper T cell–dependent B cell hyperactivity by abatacept treatment in primary Sjögren's syndrome. *Arthritis Rheumatol* 2017; 69(9): 1850-61. https://doi.org/10.1002/art.40165
13. CORNETH OBJ, VERSTAPPEN GMP, PAULISSEN SMJ *et al*.: Enhanced Bruton's tyrosine kinase activity in peripheral blood B lymphocytes from patients with autoimmune disease. *Arthritis Rheumatol* 2017; 69(6): 1313-24. https://doi.org/10.1002/art.40059
14. HAACKE EA, VAN DER VEGT B, MEINERS PM *et al*.: Abatacept treatment of patients with primary Sjögren's syndrome results in a decrease of germinal centres in salivary gland tissue. *Clin Exp Rheumatol* 2017; 35(2): 317-20.
15. MEINERS PM, VISSINK A, KROESE FGM *et al*.: Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). *Ann Rheum Dis* 2014; 73(7): 1393-96. https://doi.org/10.1136/annrheumdis-2013-204653
16. NAKSHBANDI U, VAN GINKEL MS, ARENDS S *et al*.: Histopathological comparison of Sjögren-related features between paired labial and parotid salivary gland biopsies of sicca patients. *Rheumatology* (Oxford) 2024 Mar 27. https://doi.org/10.1093/rheumatology/keae154
17. SEROR R, BOOTSMA H, SARAUX A *et al*.: Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). *Ann Rheum Dis* 2016; 75(2): 382-89. https://doi.org/10.1136/annrheumdis-2014-206008
18. SEROR R, MEINERS P, BARON G *et al*.: Development of the ClinESSDAI: a clinical score without biological domain. A tool for biological studies. *Ann Rheum Dis* 2016; 75(11): 1945-50. https://doi.org/10.1136/annrheumdis-2015-208504
19. DE WOLFF L, ARENDS S, PONTARINI E, BOMBARDIERI M, BOWMAN SJ, BOOTSMA H: Development and performance of the Clinical Trials ESSDAI (ClinTrialsESSDAI), consisting of frequently active clinical domains, in two randomised controlled trials in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2021; 39 (Suppl. 133): 100-6. https://doi.org/10.55563/clinexprheumatol/i8g5nd
20. BRANDTZAEG P: Mucosal immunity: Induction, dissemination, and effector functions. *Scand J Immunol* 2009; 70(6): 505-15. https://doi.org/10.1111/j.1365-3083.2009.02319.x
21. CHRISTODOULOU MI, KAPSOGEORGOU EK, MOUTSOPOULOS HM: Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010; 34(4): 400-7. https://doi.org/10.1016/j.jaut.2009.10.004
22. DE WOLFF L, VAN NIMWEGEN JF, MOSSEL E *et al*.: Long-term abatacept treatment for 48 weeks in patients with primary Sjögren's syndrome: The open-label extension phase of the ASAP-III trial. *Semin Arthritis Rheum* 2022; 53: 151955. https://doi.org/10.1016/j.semarthrit.2022.151955
23. TSUBOI H, MATSUMOTO I, HAGIWARA S *et al*.: Effectiveness of abatacept for patients with Sjögren's syndrome associated with rheumatoid arthritis. An open label, multicenter, one-year, prospective study: ROSE (Rheumatoid Arthritis with Orencia Trial toward Sjögren's syndrome Endocrinopathy) trial. *Mod Rheumatol* 2016; 26(6): 891-9. https://doi.org/10.3109/14397595.2016.1158773