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Implications of long-term medication of oral steroids and antimalarial drugs in primary Sjögren's syndrome

Tove Ragna Reksten [a,*], Karl A. Brokstad [a], Roland Jonsson [a,c], Johan G. Brun [b,c], Malin V. Jonsson [a,b]

[a] Broegelmann Research Laboratory, the Gade Institute, University of Bergen, Bergen, Norway
[b] Institute of Medicine, Section for Rheumatology, University of Bergen, Bergen, Norway
[c] Department of Rheumatology, Haukeland University Hospital, Bergen, Norway

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ABSTRACT

Background: Immunomodulating drugs are commonly used in treating patients with autoimmune diseases but with very different outcomes. We aimed to investigate differences in cytokine and autoantibody levels with regard to patient characteristics in patients with primary Sjögren's syndrome (pSS) receiving oral steroids or antimalarial drugs (AM) after a longer period of time.
Methods: Serum samples from 141 patients fulfilling the revised EU–US criteria and 99 healthy controls were analysed for 25 cytokines and 8 autoantibodies.
Results: AM-patients had lowered levels of IL-5, IL-10, IL-13 and IFN-γ, though non-significantly. Use of prednisolone was associated with reduced levels of IL-15, IL-2, IL-4, IL-12p40, TNF-α, MIP-1α and MIP-1β ($p<0.05$), and a trend towards decreased levels of IL-1RA and IL-1β was observed. No associations were seen between AM and antibody levels. Significantly higher protein levels of anti-Ro-52 and anti-Ro-60 were observed in the patients taking prednisolone ($p<0.05$). The proportion of patients positive for anti-Ro-52 and anti-La-48 did not differ significantly in the groups taking and not taking prednisolone, but a difference was seen for anti-Ro-60 ($p<0.05$).
Conclusions: Prednisolone is a potent anti-inflammatory and immunosuppressive drug commonly used in autoimmune diseases. Our study shows that oral steroids are associated with reduced levels of several pro-inflammatory cytokines, but increased levels of pSS specific autoantibodies. The association between steroid use and increased antibody levels is not readily explained by known steroid effects, and should therefore be confirmed in further studies. Lower levels of pro-inflammatory cytokines indicate a beneficial effect of oral steroids in this patient group.

1. Introduction

Primary Sjögren's syndrome (pSS) is a complex autoimmune rheumatic disease characterised by the presence of autoantibodies against the ribonucleoprotein (RNP) particles Ro/SSA and La/SSB [1], and focal mononuclear cell infiltration of exocrine glands. The salivary and lacrimal glands are the principal targets of a proposed T and/or B cell-mediated chronic inflammation, with an associated glandular atrophy and deficient function. The clinical consequences are dry mouth (xerostomia) and dry eyes (keratoconjuctivitis sicca). Due to disease development in multiple organs, there may be a number of systemic features of SS [2]. Serological markers of inflammation are commonly found in pSS patients, including higher titres of cytokines and circulating antibodies [3, 4]. Several factors may influence cytokine and autoantibody levels, including age [5] and we have previously observed aberrant titres of cytokines with regard to lymphoid organisation in pSS minor salivary glands [6].

The management of patients with pSS is difficult; the aetiology of the disease is largely unknown precluding direct treatment of a specific target. Current therapies include non-pharmacologic measures like punctual occlusion to preserve tears as well as the symptomatic relief of xerostomia and keratoconjuctivitis sicca, and use of immunomodulatory drugs [7,8]. A range of alternative medications have been tried to prevent tissue destruction [9], provide long-term relief of symptoms [10] and reduce the fatigue often seen in pSS [11]. Anti-TNF-α treatment through Infliximab and Etanercept has been tried in pSS patients though not successfully [12–14], whereas depletion of memory B cells through anti-CD20/Rituximab has been promising in preventing tissue destruction in salivary glands [15]. Hydroxychloroquine and prednisolone have been shown to delay SLE onset [16]. Recent trials of belimumab (anti-BAFF) have shown promising results in phase III studies in SLE patients and might prove useful in pSS in the future (BLISS-study [ClinicalTrials.gov identifier: NCT00410384]).

Immunomodulatory drugs may exert different effects in different diseases, but are in general thought to be of great benefit in autoimmune

* Corresponding author at: Broegelmann Research Laboratory, The Gade Institute, University of Bergen, The Laboratory Building, 5th floor, N-5021 Bergen, Norway. Tel.: +47 559 74 623.
E-mail address: tove.reksten@gades.uib.no (T.R. Reksten).

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diseases. In this study, our purpose was to investigate circulating autoantibody and cytokine levels in healthy individuals and pSS patients treated with antimalarial drugs and prednisolone over a longer period of time to see whether levels normalise.

2. Materials and methods

2.1. Patients and controls

The pSS cohort comprised patients (n = 141) diagnosed according to the revised European-American criteria [17] at the Department of Rheumatology, Haukeland University Hospital, Bergen, Norway. The cohort contained seven men and 134 women, mean age was 56 years (range: 24–74 years).

Nineteen (13.5%) patients had discontinued the use of prednisolone, eleven (7.8%) still used prednisolone and 111 (78.7%) patients had never used prednisolone. The median dose of prednisolone was 5 mg/day (range: 2–50 mg/day). Patients had been taking prednisolone for an average of 4.8 years (range: 3 months–17 years); patients who had discontinued prednisolone had used it for a short period of time within the past 20 years. Twenty-five (17.7%) patients were using antimalarial drugs (hydroxychlorochine), 35 (24.8%) had discontinued the use and 81 (57.4%) had not used antimalarial drugs. Four patients (2.8%) were using both prednisolone and antimalarial drugs. In addition, the patients commonly used lipid-lowering drugs, anticoagulants and non-steroidal anti-inflammatory drugs (NSAID), but neither class of medication is known to affect antibody or cytokine levels, and would affect all patient groups equally.

Serum samples and clinical data were gathered from all patients, with focus on general markers of inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), levels of immunoglobulins IgG, IgA and IgM, and complement factor C3 and C4 levels (Table 1). Previous measures of Ro/SSA, La/SSB and ANA were obtained from the medical records; unstimulated whole salivary secretion was measured by sialometry, and a visual analogue scale (VAS) score was used to assess change in patients' disease perceptions. Furthermore, extraglandular manifestations such as dry skin, arthralgia, myalgia, swollen joints and vasculitis were recorded.

Sera from 99 healthy blood donors recruited at the Haukeland University Hospital were used as controls. The mean age was 45 years (range: 22–64 years); 39 were female and 60 were male. As the controls could not be age and gender matched to the pSS group, statistical analyses were performed to compare autoantibody and cytokine levels in male versus female controls, and in the younger (26–36 years, n = 19) and older (56–64 years, n = 21) population. No significant differences were found.

All patients gave informed consent, and the study was approved by the Committee of Ethics at the University of Bergen (145/96-44.96 and 242.06).

2.2. Serological analyses

Serum cytokine levels were analysed using the Human Cytokine 25-Plex (kit cat. # LHC0009, Invitrogen, Carlsbad, CA, USA) according to manufacturer's protocol, measuring Eotaxin, GM-CSF, IFN-α, IFN-γ, IL-1RA, IL-1β, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, RANTES and TNF-α. A selection of serum autoantibodies was measured using the QUANTA Plex™ SLE Profile 8 immunoassay (cat. #708910, San Diego, CA, USA) as recommended by the manufacturer. The cut-off values were calculated as suggested by the manufacturer using the supplied calibration sample, and patients considered positive for the autoantibody in question (Sm, RNP, Ro-52, Ro-60, La-48, Scl-70, Jo-1, Ribosome P and Chromatin) if serum protein levels exceeded the lowest positive cut-off value.

Table 1
Comparison of pSS patients taking and not taking prednisolone.

Clinical features	Prednisolone (n = 11)	Discontinued prednisolone (n = 19)	Non-prednisolone (n = 111)
Age	62 ± 2	58 ± 2	51 ± 1
Female:male ratio	9:2	18:1	107:4
Disease duration (years)	14 ± 3	19 ± 3	14 ± 1
CRP (mg/L)	29.3 ± 24.5	4.2 ± 0.8	4.5 ± 0.4
CRP ≥ 10 mg/L	1/10[a]	1/18	6/104
ESR (mm/h)	32.8 ± 8.5	25.7 ± 2.7	24.2 ± 1.6
ESR > normal[b]	4/7	8/11	34/77
IgG ≥ 15.3 g/L	0/11	3/16	7/104
IgA ≥ 4.1 g/L	0/11	2/17	15/96
IgM ≥ 2.5 g/L	0/11	3/16	7/104
Mean C3 level g/L	1.05 ± 0.04	1.14 ± 0.1	1.18 ± 0.18
C3 ≤ 0.83 g/L	3/4	1/7	13/45
Mean C4 level g/L	0.20 ± 0.01	0.24 ± 0.03	0.19 ± 0.04
C4 ≤ 0.18 g/L	5/2	2/6	26/32
UWS (mL/15 min)	2.19 ± 0.90	2.58 ± 0.55	2.04 ± 0.20
Hyposalivation [c]	3/5	6/7	52/42
Dry skin	5/6	4/15	78/21
Arthralgia	5/6	13/6	83/28
Myalgia	3/8	16/3	68/43
Swollen joints	2/9	5/14	10/101

Data are presented as mean ± SEM.
None of the serological features are significantly different in the three groups.
[a] Fractions indicate the number of positive and the number of negative patients (p/n). Some values are missing due to incomplete data collection/patients medical records.
[b] Erythrocyte sedimentation rate (ESR) is considered normal when less than 15 mm/h in men <50 years, less than 2 mm/h in men >50 years and women <50 years, and when less than 30 mm/h in women >50 years.
[c] Hyposalivation defined as UWS ≤1.5 mL/15 min.

2.3. Statistical analyses

All statistical analyses were performed with PASW Statistics 18.0 for Mac (SPSS Inc., Chicago, USA). One-way ANOVA and the student's *t* test with Welch's correction for unequal variances were performed comparing autoantibody and cytokine levels. Chi-square or Fisher's exact test was applied for the clinical patient data, as well as the student's *t*-test. P-values below 0.05 were considered statistically significant.

3. Results

3.1. Serum cytokines and autoantibodies

Serum cytokine and autoantibody protein levels were generally elevated in pSS patients compared with the healthy controls, both for growth factors, chemokines and pro- and anti-inflammatory cytokines (Fig. 1A). Of the 99 controls, six had unexpectedly high cytokine levels, indicative of current or recent infection or inflammation.

A two-fold increase was noticed in interleukin (IL) 1β, the IL-12 and IL-23 subunit IL-12p40, macrophage inflammatory protein (MIP) 1β, monokine induced by interferon-γ (MIG) and monocyte chemoattractant protein (MCP) 1 protein levels, whereas a four-fold increase was seen in levels of IL-4 and granulocyte–macrophage colony stimulating factor (GM-CSF) when comparing healthy individuals to the pSS cohort. IL-1RA and IL-2R levels were six and seven times increased in pSS patient samples as compared with healthy controls.

Five cytokines, namely IL-2, IL-6, MIP-1α, RANTES and Eotaxin were not significantly increased in pSS patients compared with the controls. However, when excluding the 6 high-cytokine individuals, only Eotaxin ($p = 0.0592$) remained insignificantly different. Autoantibodies have been found in healthy controls in earlier studies, and in our cohort seven individuals were positive for one or more autoantibody; anti-SM (n = 2), anti-RNP (n = 1), anti-Scl-70 (n = 3) and anti-Chromatin (n = 7).

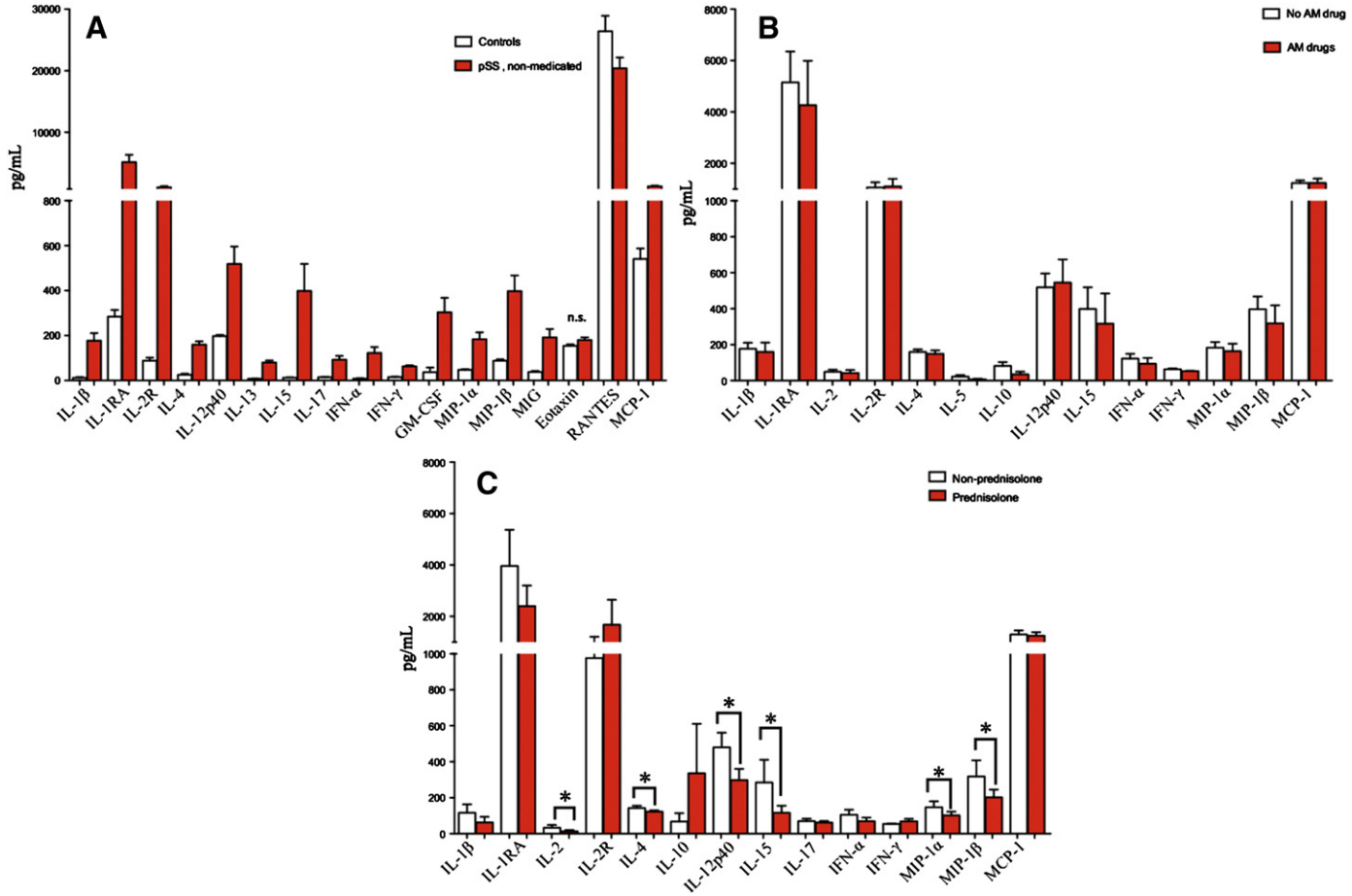

Fig. 1. (A) Serum cytokine levels were in general elevated when comparing patients with pSS (n = 141) to healthy controls (n = 99). (B) Minor differences were observed when comparing patients using (n = 25) and not using (n = 81) antimalarial (AM) drugs. (C) Significantly lower levels of cytokines were observed in patients using prednisolone (n = 11) compared to the patients never having used prednisolone (n = 111). Statistically significant differences ($p<0.05$) are marked with an asterisk (*).

Cytokine and autoantibody profiles were also studied in relation to medicinal treatment. Twenty-nine patients had not received any kind of anti-inflammatory treatment, 83 used or had used NSAIDs on a daily or weekly basis, six patients used or had discontinued use of DMARDs and one patient had previously received anti-TNF treatment. No differences were observed in cytokine or autoantibody levels in the NSAID or DMARD treated patients compared with untreated patients.

3.2. Antimalarial drugs

Based on findings in the medical records, patients typically received antimalarial drugs when they presented with symptoms of arthritis or vasculitis, or with high ESR/CRP values (data not shown). Comparing the levels of cytokines in patients taking antimalarial drugs alone (n = 18) and patients using no medication (n = 72) revealed a tendency toward lower levels of IL-5, IL-10, IL-13 and IFN-γ, though not statistically significantly ($0.10>p>0.05$) (Fig. 1B). With regard to antimalarial treatment, differences or trends in autoantibody levels were not observed; 70% of non-AM patients and 88% of AM patients had positive ANA ELISA results in the most recent test ($p = 0.06$ [Fisher χ^2]). There were no differences in unstimulated whole saliva, VAS score for mouth complaints or the number of *Candida albicans* colonies, but the change in whole saliva from previous measurement (2–10 years ago) was 0.49 mL/15 min in non-AM patients and −0.92 mL/15 min in patients receiving AM treatment ($p = 0.02$).

3.3. Prednisolone

3.3.1. Patient population comparisons

As no clear differences were found in patients without medication compared with those using AM drugs, the latter patients were included in the study cohort in order to increase power. Patient data were compared, revealing no statistically significant differences in the patients' age, disease duration or in a number of clinical and serological measures (Table 1).

Prednisolone had been administered to patients whose disease was "active" or patients who were "not well", as assessed by the treating rheumatologist. Prednisolone was discontinued if improvement was not achieved or if the patients suffered from adverse effects. Myalgia was more common in patients receiving prednisolone as compared with non-prednisolone patients ($p = 0.04$), though less common in previously treated patients compared with non-treated patients ($p = 0.03$). Manifestations such as dry skin, arthralgia and swollen joints were found to be slightly more prevalent in non-treated than in treated patients. The change in unstimulated whole saliva secretion in the prednisolone taking patients was −1.6857 mL/15 min and in non-prednisolone patients 0.2351 mL/15 min ($p = 0.24$). The mean levels of unstimulated whole saliva secretion were similar; 2.19 mL/15 min and 2.04 mL/15 min, prednisolone and non-prednisolone, respectively.

3.3.2. Cytokine measurements

Significantly lower cytokine levels were observed in the patients using prednisolone compared with those never having used prednisolone. There was a 50% decrease in IL-4 protein levels, a three-fold

decrease in IL-15 protein levels and a two-fold decrease in MIP-1α, MIP-1β and the IL-12 and IL-23 subunit IL-12p40 protein levels (Fig. 1C). Furthermore, a trend towards a two-fold decrease in IL-1RA protein levels ($p = 0.0508$) and a three-fold decrease in IL-1β protein levels ($p = 0.0532$) was noted. TNF-α and IL-2 protein levels were significantly decreased by a factor of three. However, the overall level of these cytokines was approaching the detection limit and should thus be considered with caution. In general, patients having discontinued prednisolone treatment had cytokine levels higher than the treated patients and lower than the untreated patients, though not significantly.

3.3.3. Effect on autoantibodies

All patients taking prednisolone ($n = 11$) were in the routine screening positive for Ro/SSA whereas only 48/111 (43%) of the non-prednisolone patients were. There was no significant difference in SSB/La positivity or other autoantibodies. All prednisolone patients were positive for ANA in the most recent test as compared to 79/111 (71%) of the non-prednisolone patients and 12/19 (63%) of previous prednisolone users, giving a likelihood ratio of 8.023 and $p = 0.02$. There was also a significant difference in the most recent ANA value (prednisolone: 5.20 g/L, non-prednisolone: 3.11 g/L [$p = 0.008$]).

Antibodies directed against Ro-52 and Ro-60 were found in twice as high protein levels in prednisolone treated patients compared with untreated ($p = 0.01$ and $p = 0.01$, respectively) and previously treated patients ($p = 0.02$ and $p = 0.01$, respectively). Anti-La-48 antibody levels were also elevated, though not significantly (Fig. 2). The proportion of patients positive or negative for Ro-60 and La-48 autoantibodies was not altered in the groups taking, previously taking and not taking prednisolone, however a higher risk of anti-Ro-52 antibodies was observed in the treated patients compared with never treated patients (relative risk: 6.71, $p = 0.0485$). Antibodies directed against Sm, Scl-70, Jo-1 and Ribosomal-P were not found in the prednisolone group, but detected in 3, 0, 1 and 1 of the patients in the non-prednisolone group, respectively. Anti-Chromatin was detected in one prednisolone-taking patient, in none previous users and in nine non-prednisolone patients; anti-RNP was detected in none prednisolone-taking patients, in one previous user and in four non-prednisolone patients.

4. Discussion

The effect of anti-inflammatory and immunomodulatory therapy on systemic cytokine and autoantibody levels in pSS has previously been poorly investigated. Though the drugs may be useful in relieving pain and reducing inflammation [18], as well as relieving ocular and oral symptoms [19], the pharmacological effect of antimalarial drugs in rheumatic diseases is not known. We were unable to detect signs of lower rate of autoantibody accumulation as previously reported for systemic lupus erythematosus (SLE) [16].

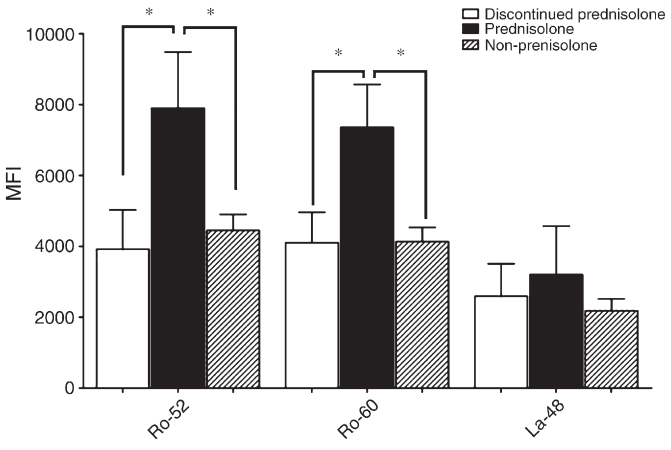

Fig. 2. The pSS specific autoantibodies directed against Ro-52, Ro-60 and La-48 were detected in patient serum samples. Anti-Ro-52 and anti-Ro-60 levels were significantly increased in patients receiving prednisolone treatment (black) as compared to patients previously using prednisolone (white) and never having used prednisolone (grey). Statistically significant differences ($p<0.05$) are marked with an asterisk (*).

Evidence that hydroxychloroquine influences the protein levels of T cell activation markers sIL-2R, sCD4 and sCD8 has previously been published along with reports of sustained reductions in serum levels of IL-6 [20,21], and that the drug inhibits the synthesis of TNF-α, IL-1β and IL-6 through different modes [22]. Our data did not reveal differences in protein levels in either of these cytokines, or of any other cytokine in the conducted analysis in patients taking antimalarial drugs over a longer period of time and those not taking them. As data on cytokine levels prior to administration of the drug are unavailable, we cannot exclude that the patients taking antimalarial drugs had higher levels of the cytokines in question at baseline. However, our data do not indicate any unfavourable long-term effects on cytokine and autoantibody levels in patients receiving antimalarial drugs over longer periods of time (5–10 years), and antimalarial drugs do not seem to restore normal cytokine values as seen in healthy individuals.

Although associated with significant adverse effects (reviewed in [23]), low-dose prednisolone has been used in the management of rheumatoid arthritis for decades, especially to relieve symptoms of early morning stiffness [24]. Important mechanisms underlying the anti-inflammatory effects of corticosteroids include the blocking of the effect of macrophage migration inhibitory factor and inhibition of neutrophil and monocyte–macrophage adherence to endothelial cells in the inflamed area (Summary of Product Characterisation, SPC [25]). Prednisolone-treated patients did indeed exhibit reduced levels of several pro-inflammatory cytokines, and especially macrophage associated cytokines, much in accordance with the mechanism of drug action. IL-15 is a cytokine in the IL-2 family capable of inducing B cell unresponsiveness towards glucocorticoids [26] and is also a potent activator of NK cells. IL-2 and IL-4 are cytokines known to be reduced by glucocorticoids, and their role in inducing glucocorticoid receptor (GCR) ligand resistance has recently been investigated [27]. Although prednisolone has no known effect on antibody producing B cells, one might hypothesise that reduced levels of IL-2 causes down regulation of the IL-2 receptor subunit CD25, which is also a subunit of the IL-15 receptor. In turn, this may affect the $CD4^+CD25^+Foxp3^+$ regulatory T cells, preventing regulation of B cell activity. Such an effect would, however, be in contrast to published reports on the positive effect of glucocorticoids on Tregs' suppressive functions in multiple sclerosis [28] and to findings indicating that prednisolone treated patients with myasthenia gravis display better suppressive potential of peripheral Tregs than untreated patients [29].

In an open, prospective pilot study, Miyawaki et al. [30] found that saliva production initially increased upon initiation of prednisolone treatment, however dropping again after 48 months of follow up. In our cohort, there is no difference in the volume of unstimulated whole saliva in patient groups, and Haldorsen et al. [31] concluded that neither antimalarial nor oral steroid treatment gave significant differences in 30% increase or decrease of unstimulated whole saliva.

Previous studies have shown prednisolone to decrease autoantibody levels in patients with anticardiolipin antibodies [32] or have no effect at all on antibody response to vaccines [33], and the SPC [25] state that glucocorticoids do not seem to have a significant effect on circulating antibodies at therapeutic dose levels. A small study, comprising only of 20 patients and no controls, found that low-dose prednisolone significantly reduced levels of Ro/SSA and La/SSB antibodies in primary Sjögren's syndrome [30], and that this effect was sustained after 48 months follow up of 5 patients. In our cohort, patients on long-term low-dose prednisolone treatment had significantly higher levels of Ro-52 and Ro-60 autoantibodies, and the proportion of anti-Ro-60 seropositive patients was significantly larger in

the prednisolone-using group. One might argue that this finding is a result of confounding by indication, since the patients received prednisolone because they have a more severe disease with higher levels of autoantibodies. However, if this were the case, one could argue that the patients who discontinued prednisolone treatment for various reasons would be expected to have the same levels. Oral steroids were administered to patients with extra-glandular manifestations, and a certain correlation between circulating autoantibodies and such manifestations in pSS have been found [34]. At the time of blood sampling, the treated patients presented with slightly fewer such manifestations than the untreated patients, and in the patients who discontinued prednisolone, autoantibody levels were as low as in the non-prednisolone group, though being in the same risk group with extra-glandular manifestations. Prednisolone treatment consequently had a positive effect on the extraglandular manifestations, but did not restore autoantibody levels to a lower level.

In conclusion, although this study was not designed to find treatment outcomes, the well-characterised patient cohort allows us to discuss potential long-term effects of medicinal treatment in pSS. Compliance cannot be accounted for; however, we do expect our cohort to reflect compliance in the general population. Common treatments such as NSAIDs did not seem to influence serological measures. The use of antimalarial drugs and oral steroids in the symptomatic treatment of pSS patients is regarded safe and well tolerated. To our knowledge, the patients on long-term treatment with hydroxychloroquin did not present with any negative effects of the medication. However, they did not present with any clear positive serological outcomes either. The effect of prednisolone on cytokines in patients with pSS has not previously been studied, and our findings are in concert with previous reports on steroid effects in other diseases. No differences were observed in typical T cell cytokines, and the treatment reduced levels of pro-inflammatory cytokine albeit without inducing anti-inflammatory cytokines. The discrepancy in autoantibody levels in patients taking and not taking prednisolone, warrant further investigations.

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References

[1] Garberg H, Jonsson R, Brokstad KA. The serological pattern of autoantibodies to the Ro52, Ro60, and La48 autoantigens in primary Sjögren's syndrome patients and healthy controls. Scand J Rheumatol 2005;34:49–55.
[2] Jonsson R, Bowman S, Gordon TP. Sjögren's syndrome. In: Koopman WJ, Moreland LW, editors. Arthritis and allied conditions — a textbook of rheumatology. 15th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 1681–705.
[3] Candon S, Gottenberg J, Bengoufa D, Chatenoud L, Mariette X. Quantitative assessment of antibodies to ribonucleoproteins in primary Sjögren's syndrome: correlation with B cell biomarkers and disease activity. Ann Rheum Dis 2008;68:1208–12.
[4] Moutsopoulos NM, Katsifis GE, Angelov N, Leakan RA, Sankar V, Pillemer S, et al. Lack of efficacy of etanercept in Sjögren's syndrome correlates with failed suppression of tumour necrosis factor α and systemic immune activation. Ann Rheum Dis 2008;67(10):1437–43.
[5] Ramos-Casals M, Garcia-Carrasco M, Brito MP, Lopez-Soto A, Font J. Autoimmunity and geriatrics: clinical significance of autoimmune manifestations in the elderly. Lupus 2003;12(5):341–55.
[6] Reksten TR, Jonsson MV, Szyszko EA, Brun JG, Jonsson R, Brokstad KA. Cytokine and autoantibody profiling related to histopathological features in primary Sjögren's syndrome. Rheumatology 2009;48(9):1102–6.
[7] Jonsson R, Haga H-J, Gordon T. Current concepts on diagnosis, autoantibodies and therapy in Sjögren's syndrome. Scand J Rheumatol 2000;29(6):341–8.
[8] Thanou-Stavraki A, James JA. Primary Sjögren's syndrome: current and prospective therapies. Semin Arthritis Rheum 2008;37(5):273–92.
[9] Seitsalo H, Niemelä RK, Marinescu-Gava M, Vuotila T, Tjäderhane L, Salo T. Effectiveness of low-dose doxycycline (LDD) on clinical symptoms of Sjögren's syndrome: a randomized, double-blind, placebo controlled cross-over study. J Negat Results Biomed 2007:11.
[10] Alpöz E, Güneri P, Önder G, Cankaya H, Kabasakal Y, Köse T. The efficacy of Xialine in patients with Sjögren's syndrome: a single-blind, cross-over study. Clin Oral Invest 2008;2:165–72.
[11] Hartkamp A, Geenen R, Godaert GL, Bootsma H, Kruize AA, Bijlsma JW, et al. Effect of dehydroepiandrosterone administration on fatigue, well-being, and functioning in women with primary Sjögren syndrome: a randomised controlled trial. Ann Rheum Dis 2008;1:91–7.
[12] Mariette X, Ravaud P, Steinfeld S, Baron G, Goetz J, Hachulla E, et al. Inefficacy of infliximab in primary Sjögren's syndrome: results of the randomized, controlled trial of remicade in primary Sjögren's syndrome (TRIPSS). Arthritis Rheum 2004;50(4):1270–6.
[13] Sankar V, Brennan MT, Kok MR, Leakan RA, Smith JA, Manny J, et al. Etanercept in Sjögren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. Arthritis Rheum 2004;50(7):2240–5.
[14] Steinfeld SD, Demols P, Appelboom T. Infliximab in primary Sjögren's syndrome: one-year followup. Arthritis Rheum 2002;46(12):3301–3.
[15] Pers JO, Devauchelle V, Daridon C, Bendaoud B, Le Berre R, Bordron A, et al. BAFF-modulated repopulation of B lymphocytes in the blood and salivary glands of rituximab-treated patients with Sjögren's syndrome. Arthritis Rheum 2007;5:1464–77.
[16] James J, Kim-Howard X, Bruner B, Jonsson M, McClain M, Arbuckle M, et al. Hydroxychloroquine sulfate treatment is associated with later onset of systemic lupus erythematosus. Lupus 2007;16(6):401–9.
[17] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61(6):554–8.
[18] Tishler M, Yaron I, Shirazi I, Yaron M. Hydroxychloroquine treatment for primary Sjögren's syndrome: its effect on salivary and serum inflammatory markers. Ann Rheum Dis 1999;58:253–6.
[19] Fox R, Dixon R, Guarrasi V, Krubel S. Treatment of primary Sjögren's syndrome with hydroxychloroquine: a retrospective, open-label study. Lupus 1996;5(1_suppl):S31–6.
[20] Wallace DJ. Antimalarials — the 'real' advance in lupus. Lupus 2001;10(6):385–7.
[21] Wallace D, Linker-Israeli M, Hyun S, Klinenberg J, Stecher V. The effect of hydroxychloroquine therapy on serum levels of immunoregulatory molecules in patients with systemic lupus erythematosus. J Rheumatol 1994;21(2):375–6.
[22] Jang C-H, Choi J-H, Byun M-S, Jue D-M. Chloroquine inhibits production of TNF-α, IL-1β and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. Rheumatology 2006;45(6):703–10.
[23] Gøtzsche P, Johansen HK. Short-term low-dose corticosteroids vs placebo and non-steroidal antiinflammatory drugs in rheumatoid arthritis. Cochrane Database of Systematic Reviews 2005, Issue 1. Art. No.: CD000189. doi:10.1002/14651858.CD000189.pub2.
[24] de Andrade JR, McCormick JN, Hill AGS. Small doses of prednisolone in the managment of rheumatoid arthritis. Ann Rheum Dis 1964;23:158–62.
[25] Prednisolone 1 mg Tablets - PL 29831/0177. Wockhardt UK Ltd 31/03/2008.
[26] Xu Q, Goleva E, Leung DYM. NK cells induce corticosteroid unresponsiveness in B-cells treated with IL-15. J Allergy Clin Immunol 2004;113(2, Supplement 1):S214–S.
[27] Goleva E, Li L-b, Leung DYM. IFN-γ reverses IL-2- and IL-4-mediated T-cell steroid resistance. Am J Respir Cell Mol Biol 2009;40(2):223–30.
[28] Xu L, Xu Z, Xu M. Glucocorticoid treatment restores the impaired suppressive function of regulatory T cells in patients with relapsing–remitting multiple sclerosis. Clin Exp Immunol 2009;158(1):26–30.
[29] Luther C, Adamopoulou E, Stoeckle C, Brucklacher-Waldert V, Rosenkranz D, Stoltze L, et al. Prednisolone treatment induces tolerogenic dendritic cells and a regulatory milieu in myasthenia gravis patients. J Immunol 2009;183(2):841–8.
[30] Miyawaki S, Nishiyama S, Matoba K. Efficacy of low-dose prednisolone maintenance for saliva production and serological abnormalities in patients with primary Sjögren's syndrome. Intern Med 1999;38(12):938–43.
[31] Haldorsen K, Moen K, Jacobsen H, Jonsson R, Brun JG. Exocrine function in primary Sjögren's syndrome: natural course and prognostic factors. Ann Rheum Dis 2008;67(7):949–54.
[32] Silveira LH, Hubble CL, Jara LJ, Saway S, Martìnez-Osuna P, Seleznick MJ, et al. Prevention of anticardiolipin antibody-related pregnancy losses with prednisone and aspirin. Am J Med 1992;93(4):403–11.
[33] Kapetanovic MC, Saxne T, Sjoholm A, Truedsson L, Jonsson G, Geborek P. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. Rheumatology 2006;45(1):106–11.
[34] Tsuzaka K, Ogasawara T, Tojo T, Fuji H, Tsukatani Y, Kubo A, et al. Relationship between autoantibodies and clinical parameters in Sjögren's syndrome. Scand J Rheumatol 1993;22(1):1–9.