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Original article

Vaginal dryness in primary Sjögren's syndrome: a histopathological case–control study

Jolien F. van Nimwegen [1], Karin van der Tuuk[2], Silvia C. Liefers[1], Gwenny M. Verstappen[1], Annie Visser[1], Robin F. Wijnsma[1], Arjan Vissink[3], Harry Hollema[4], Marian J. E. Mourits[2], Hendrika Bootsma[1] and Frans G. M. Kroese[1]

Abstract

Objective. The aim was to study clinical, histopathological and immunological changes in the vagina and cervix of women with primary SS, which might explain vaginal dryness.

Methods. We included 10 pre-menopausal female primary SS patients with vaginal dryness and 10 pre-menopausal controls undergoing a laparoscopic procedure. The vaginal health index was recorded. Multiplex immunoassays and flow cytometry were performed on endocervical swab and cervicovaginal lavage samples to evaluate cellular and soluble immune markers. Mid-vaginal and endocervical biopsies were taken and stained for various leucocyte markers, caldesmon (smooth muscle cells), avian V-ets erythroblastosis virus E26 oncogene homologue (ERG; endothelial cells) and anti-podoplanin (lymphatic endothelium). The number of positive pixels per square micrometre was calculated.

Results. One patient was excluded because of *Clamydia trachomatis*, and two controls were excluded because of endometriosis observed during their laparoscopy. Vaginal health was impaired in primary SS. $CD45^+$ cells were increased in vaginal biopsies of women with primary SS compared with controls. Infiltrates were predominantly located in the peri-epithelial region, and mostly consisted of $CD3^+$ lymphocytes. In the endocervix, $CD45^+$ infiltrates were present in patients and in controls, but a higher number of B lymphocytes was seen in primary SS. Vascular smooth muscle cells were decreased in the vagina of primary SS patients. No differences were found in leucocyte subsets in the vaginal and endocervical lumen. CXCL10 was increased in endocervical swab samples of primary SS patients.

Conclusion. Women with primary SS show impaired vaginal health and increased lymphocytic infiltration in the vagina compared with controls. Vaginal dryness in primary SS might be caused by vascular dysfunction, possibly induced by IFN-mediated pathways.

Key words: Sjögren's syndrome, vaginal dryness, pathogenesis, vaginal health, gynaecology, interferon, CXCL10, smooth muscle cells, lymphocytes

CLINICAL SCIENCE

Rheumatology key messages

- Women with primary SS show impaired vaginal health and increased lymphocytic infiltration in the vagina.
- Numbers of vascular smooth muscle cells are decreased in the vagina of women with primary SS.
- Vaginal dryness in primary SS might be caused by vascular dysfunction, possibly induced by IFN-mediated pathways.

Introduction

Primary SS is a systemic autoimmune disease with a heterogeneous presentation, including sicca symptoms, systemic symptoms, such as fatigue, and extraglandular involvement [1]. A hallmark of primary SS is lymphocytic infiltration of the salivary and lacrimal glands. Besides sicca symptoms of the eyes and mouth, vaginal dryness is common in women with primary SS, which causes

[1]Department of Rheumatology and Clinical Immunology, [2]Department of Obstetrics and Gynaecology, University of Groningen , [3]Department of Oral and Maxillofacial Surgery and [4]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Correspondence to: Jolien F. van Nimwegen, Department Rheumatology and Clinical Immunology, University Medical Center Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail: j.f.van.nimwegen@umcg.nl

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dyspareunia and sexual dysfunction [2–6]. Although vaginal dryness usually occurs after menopause, in primary SS vaginal dryness often occurs at a younger age [7–9]. Two studies evaluating vaginal health in primary SS reported erythema of the vaginal epithelium [10, 11], whereas others did not find any macroscopic changes of the vagina and cervix [4, 8]. In a previous study, we did not observe changes in the vaginal microbiome in primary SS [12].

The pathophysiology of vaginal dryness in primary SS is still unknown. Normally, the vaginal surface is humidified and lubricated by transudate from the lamina propria, which contains rich venous and lymphatic networks, and by mucus produced by the endocervical glandular epithelium [13]. In pre-menopausal primary SS patients with dyspareunia, lymphocytic infiltrates were found in the stroma underlying the vaginal epithelium [2, 14]. Furthermore, chronic cervicitis was observed in biopsies of 42% of primary SS patients [11]. Local inflammation might influence the production of transudate from blood vessels in the vagina or compromise the function of the mucus-producing glandular epithelium of the endocervix.

In previous studies, few or no healthy controls were included, and no quantitative analyses were performed. Given that leucocytes are present physiologically in the vagina and cervix of healthy women [15–17], quantitative analysis and comparison with a control group are necessary to assess whether the lymphocytic infiltration observed in primary SS is indeed pathological. Furthermore, changes in the vascularization of the vagina were not taken into account as a possible cause of vaginal dryness.

To identify an appropriate treatment for vaginal dryness in primary SS, the pathogenesis of this symptom needs to be elucidated. The objective of this study was therefore to assess clinical and histopathological changes in the vagina and cervix of women with primary SS compared with controls, which might explain vaginal dryness. We also explored whether possible inflammatory changes in the vagina and cervix of primary SS patients were reflected by changes in immune cells and effector molecules in the vaginal lumen.

Methods

Study population

In a prospective exploratory case–control study, we included 10 women with primary SS who fulfilled ACR–EULAR criteria and reported vaginal dryness. We also included 10 age-matched controls without systemic autoimmune diseases who were scheduled for a laparoscopic procedure. To eliminate the influence of physiological hormonal changes to the vaginal mucosa, only pre-menopausal patients and controls were included. Other inclusion criteria were age $\geq$18 years and written informed consent. Exclusion criteria were pregnancy or breast-feeding, presence of inflammatory or infectious gynaecological disease, previous chemotherapy, current use of an intra-uterine contraceptive device, hormone replacement therapy or vaginal oestrogen supplementation, and use of systemic CSs or DMARDs $\leq$6 months before inclusion. The study complies with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the University Medical Center Groningen (METC 2015/039).

Study procedures

Participants were instructed not to have sexual intercourse or to use tampons, lubricants or any other vaginal products within 72 h before the study visit. On the day of examination, participants completed a questionnaire that included the female sexual function index and questions about co-morbidities, medication use, smoking status, vaginal symptoms and the presence of vaginal bacterial or fungal infections in the past year. In primary SS patients, the EULAR Sjögren's syndrome patient reported index and the EULAR Sjögren's syndrome disease activity index were recorded. Blood samples were obtained.

Gynaecological examination was performed by an experienced gynaecologist. The five domains of the vaginal health index (elasticity, fluid secretion, pH, epithelial mucosa and moisture) were scored on a scale from one to five, resulting in a total score of 5–25 (Supplementary Table S1, available at *Rheumatology* online) [18]. Cervicovaginal lavage (CVL) samples were collected by flushing 7 ml of PBS over the cervix and vagina, aspirating the PBS and then repeating the procedure [19]. Endocervical swab (ES) samples were collected by rotating eSwabs (Copan diagnostics, Murrieta, CA, USA) in the endocervical canal. The eSwabs were put in 5 ml of PBS. CVL and ES samples were immediately put on ice.

Another eSwab, suspended in eSwab transport medium, was used for PCR to detect *Chlamydia trachomatis* and *Neisseria gonorrhoea*. A vaginal secretion sample was collected for fungal culture. ThinPrep Papanicolaou tests (Hologic, Marlborough, MA, USA) were performed on cervical samples collected with a Cervex brush (Rovers Medical Devices, Oss, The Netherlands).

Finally, full-thickness mid-vaginal and endocervical punch biopsies were collected, after administration of local anaesthesia in primary SS patients or general anaesthesia in controls. Vaginal and cervical biopsies were fixed in 4% paraformaldehyde and embedded in paraffin.

Evaluation of vaginal and endocervical biopsies

Vaginal and endocervical tissue sections were stained with Haematoxylin and Eosin (HE), periodic acid–Schiff diastase (PAS-D) and various leucocyte markers (CD45, CD3, CD4, CD8 and CD20). Tissue sections were also stained for blood/lymphatic vessel-associated markers: avian V-ets erythroblastosis virus E26 oncogene homologue (ERG), which is a nuclear stain for endothelial

cells; anti-podoplanin (clone D2-40), which stains lymphatic endothelium; and caldesmon, which stains smooth muscle cells present in the tunica media of arterioles and larger venules. Endocervical tissue sections were additionally stained for CD138, because many plasma cells were seen in HE-stained tissue sections.

The HE- and PAS-D-stained sections were examined by a dedicated gynaecopathologist to check for gynaecological morbidity and fungal infections. Immunohistologically stained sections were analysed quantitatively by counting the number of diaminobenzinine-stained pixels per square micrometre of parenchyma, using the Positive Pixel Count algorithm (v.9.1) in ImageScope v12.1 (Leica biosystems, Wetzlar, Germany). For CD4, only strongly positive pixels were counted, to exclude non-specific staining. The epithelial layer was excluded from analysis of endothelial markers and CD138, because no blood or lymphatic vessels are present in the epithelium, and CD138 is expressed by stratified squamous epithelium. To quantify vaginal atrophy, the epithelial thickness and number of cell layers were counted at $\times 40$ magnification, in three areas of the biopsy in which the epithelium was thinnest and no dermal papillae were present. The mean epithelial thickness and number of cell layers were calculated.

Evaluation of cellular and soluble immune markers

Serum was frozen at -80°C. EDTA whole blood was lysed with ammonium chloride and centrifuged. The supernatant was discarded, and cells were washed and suspended in FACS buffer at a concentration of 10^6 cells/ml. To collect endocervical material, the swabs containing ES samples were gently scraped on the edge of the Falcon tubes in which they were kept after collection. The ES and CVL samples were then resuspended and centrifuged, after which the supernatant was frozen at -80°C, and cells were resuspended in FACS buffer at a concentration of 10^6 cells/ml.

Flow cytometry analysis of leucocyte subsets in cells from whole blood, ES and CVL was performed on the day of collection of the samples. Cells were washed and stained with antibodies directed against leucocyte markers (Supplementary Table S2, available at *Rheumatology* online), after which they were washed and resuspended in FACS buffer. Shortly before analysis, cells were stained with propidium iodide (eBioscience, San Diego, California, USA) and passed through a 35 μm nylon mesh. Antibody panel optimization and titrations were performed in cells from whole blood and confirmed in ES and CVL cells. Fluorescence-minus-one controls were included to determine the background fluorescence. Data were acquired using a LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Data were analysed using FlowJo (Tree Star, Ashland, Oregon, USA). The gating strategy is described in Supplementary Fig. S1, available at *Rheumatology* online.

Serum samples and supernatants of the CVL and ES samples were thawed and analysed for levels of APRIL (a proliferation-inducing ligand), BAFF (B-cell activation factor), IFN-γ, RANK-ligand, TNF-α, CCL2, CCL4, CX3CL, CXCL9, CXCL10, CXCL11, CXCL13, IL-6, IL-7, IL-8 and IL-17A, using a human magnetic Luminex premixed 16-plex assay (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol. Data were acquired on a Luminex 200 system.

Statistical analysis

Statistical analyses were executed using SPSS Statistics v.23 (SPSS, Chicago, IL, USA). The Mann–Whitney *U*-test, χ^2 test or Fisher's exact test was used as appropriate to compare differences between groups. Spearman's correlation coefficients were used to evaluate correlations. *P*-values of <0.05 were considered to indicate statistical significance.

Results

Clinical characteristics

One primary SS patient was excluded owing to the presence of *Chlamydia trachomatis*. Two controls were excluded owing to detection of endometriosis during laparoscopy, because the pathogenesis of endometriosis comprises immunological changes [20], and an association between endometriosis and primary SS has been described [21, 22]. Characteristics of remaining participants are shown in Supplementary Table S3, available at *Rheumatology* online. The median age was 36 [interquartile range (IQR) 33–46] years for primary SS patients ($n = 9$) and 41 (IQR 36–44) years for controls ($n = 8$). All primary SS patients had a positive salivary gland biopsy (focus score ≥ 1), and seven (78%) were anti-SSA antibody positive. Median ACR–EULAR score was 9 (interquartile range; IQR 5–9) and median EULAR Sjögren's syndrome disease activity index 6 (IQR 3–9).

Gynaecological symptoms and examination

Compared with controls, patients with primary SS showed lower female sexual function index scores (indicating sexual dysfunction), used lubricants more often and had an increased prevalence of superficial dyspareunia (Table 1). The vaginal health index score was significantly lower in primary SS patients, indicating impaired vaginal health (Table 1; Fig. 1). Of vaginal health index subdomains, the mucosa score was significantly decreased in primary SS, indicating frailty and a higher tendency of the epithelium to bleed. Upon inspection of the vulva, vagina and cervix, no major abnormalities were found. Some redness of the vulva was noted in three primary SS patients. One patient with active cutaneous vasculitis on her legs showed petechiae on the labia majora. Superficial vulvar rhagades were seen in three patients and one control. Vaginal pH did not differ significantly between groups,

Table 1 Patient-reported and gynaecological outcomes

Outcome	Primary SS (n = 9)	Controls (n = 8)	P-value
Patient-reported outcomes			
Sexual inactivity in past 4 weeks	3 (33)	2 (25)	1.000
Female sexual function index (range 2–36)[a]	22.2 (21.0–28.7)	30.6 (29.6–34.5)	**0.026**
Desire (range 1.2–6)	3.3 (2.6–3.6)	3.6 (3.0–5.0)	0.310
Arousal (range 0–6)	4.5 (2.9–5.7)	5.4 (4.7–5.7)	0.394
Lubrication (range 0–6)	4.4 (1.9–5.2)	5.9 (5.4–6.0)	**0.004**
Orgasm (range 0–6)	5.2 (4.1–5.7)	6.0 (5.4–6.0)	0.093
Satisfaction (range 0.8–6)	5.2 (4.2–5.6)	5.2 (4.8–6.0)	0.485
Pain (range 0–6)	3.2 (1.6–4.5)	6.0 (5.1–6.0)	**0.009**
Vaginal dryness (numerical rating scale, range 0–10)	5.0 (5.9–7.0)	1.0 (0.0–1.8)	**0.001**
Use of lubricants	5 (56)	0	**0.029**
Dyspareunia	9 (100)	2 (25)	**0.002**
Deep, during intercourse	4 (44)	1 (13)	0.294
Superficial, during intercourse	7 (78)	0	**0.002**
After intercourse	4 (44)	1 (13)	0.294
Vaginal or vulvar symptoms in past 2 weeks	8 (89)	3 (38)	0.050
Vaginal itching	3 (33)	1 (13)	0.576
Burning sensation in vagina/vulva	4 (44)	1 (13)	0.294
Reeking vaginal discharge	4 (44)	1 (13)	0.294
Abnormal vaginal discharge	3 (33)	1 (13)	0.576
Vaginal infections in past year	2 (22)	4 (50)	0.335
Gynaecological examination			
Vaginal health index (range 5–25)	19.0 (16.5–21.5)	23.0 (20.3–24.5)	**0.015**
Elasticity (range 1–5)	4.0 (3.5–4.5)	4.0 (4.0–5.0)	0.321
Fluid secretion (range 1–5)	4.0 (3.0–5.0)	5.0 (4.3–5.0)	0.074
Moisture (range 1–5)	4.0 (3.0–5.0)	5.0 (4.3–5.0)	0.139
pH (range 1–5)	5.0 (4.0–5.0)	4.5 (4.0–5.0)	0.673
Mucosa (range 1–5)	3.0 (3.0–3.0)	5.0 (3.5–5.0)	**0.008**
Vaginal pH	4.4 (4.1–4.9)	4.6 (4.4–4.7)	0.606

Data are presented as the median (interquartile range) or n (%), unless stated otherwise.
[a]For analysis of the female sexual function index, patients who were not sexually active in the past 4 weeks were excluded (three primary SS patients and two controls).

and none of the participants showed signs of vaginal atrophy.

Histological findings

No major abnormalities or fungal infections were found in vaginal or cervical HE- and PAS-D-stained tissue sections. One vaginal biopsy from a control was excluded from further analysis, because it was very superficial, consisting of 98% epithelium. Three primary SS patients and two controls were excluded from analysis of endocervical biopsies, because only ectocervical tissue or mucus was collected owing to difficulties in reaching the endocervical tissue through the external cervical ostium.

No significant differences were found in the number of cell layers (patients: median 25, IQR 21–33; controls: median 25, IQR 20–26) or thickness (patients: median 251 μm, IQR 197–271 μm; controls: median 243, IQR 142–252 μm) of the vaginal epithelium.

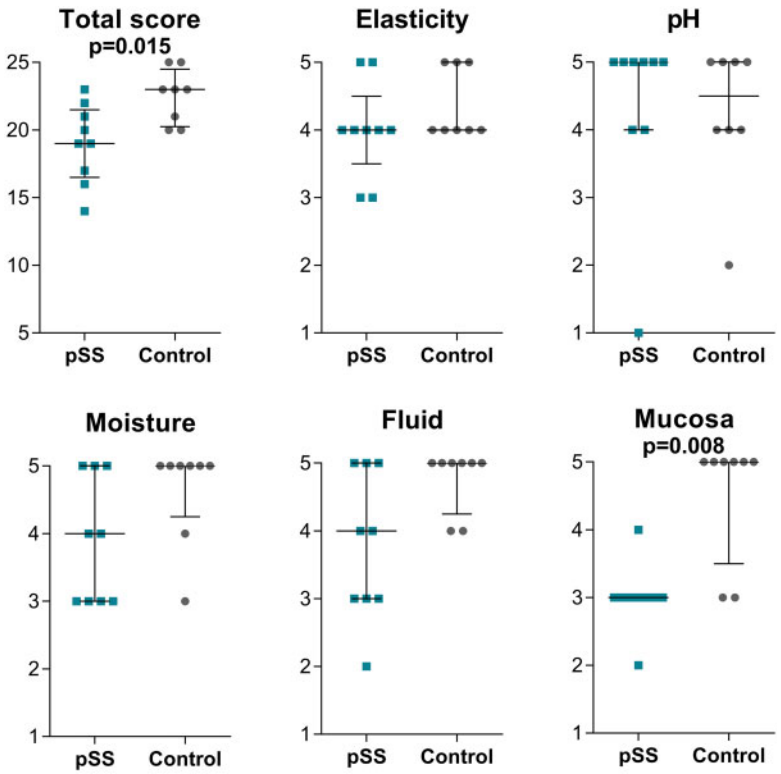
Lymphocytic infiltration in vagina and endocervix

Compared with controls, vaginal tissue from primary SS patients contained significantly higher numbers of $CD45^+$ cells (Table 2; Fig. 2). Lymphocytic infiltrates in primary SS patients were mainly located in the lamina propria immediately below the epithelium (peri-epithelial layer), with a peri-epithelial localization and aggregates in dermal papillae (Figs 3 and 4). Of all leucocyte subsets, only $CD3^+$ lymphocytes were significantly increased in the vagina. In endocervical tissue sections, there was no significant difference in the total number of $CD45^+$ cells, although the number of $CD20^+$ B lymphocytes was significantly higher in primary SS patients (Table 2; Fig. 2). Lymphocytic infiltration in the endocervix was also located mostly in the peri-epithelial layer (Figs 3 and 4).

Endothelial changes in vagina and cervix

To explore whether blood vessels and lymphatic vessels in the vagina and endocervix are affected in primary SS, we stained for endothelial markers (Supplementary Fig. S2, available at *Rheumatology* online). The number of $caldesmon^+$ cells was significantly lower in vaginal biopsies of women with primary SS, indicating a decrease in vascular smooth muscle cells (Table 2; Fig. 2). There

Fig. 1 Vaginal health index in patients and controls



Low scores correspond to low vaginal health. pSS: primary SS.

Table 2 Quantitative analysis of leucocyte and endothelial markers in the vagina and endocervix

Marker	Vagina			Endocervix		
	Primary SS (n = 9)	Control (n = 7)	P-value	Primary SS (n = 6)	Control (n = 6)	P-value
CD45	0.34 (0.26–0.53)	0.26 (0.12–0.27)	0.012*	1.12 (0.45–1.82)	0.60 (0.32–2.97)	1.000
CD3	0.49 (0.28–0.56)	0.19 (0.12–0.27)	0.008*	0.66 (0.38–1.28)	0.44 (0.20–1.57)	0.485
CD4	0.23 (0.14–0.34)	0.13 (0.12–0.32)	0.470	0.66 (0.25–1.21)	0.34 (0.24–1.25)	1.000
CD8	0.48 (0.32–0.99)	0.34 (0.22–0.51)	0.210	1.00 (0.73–1.49)	0.64 (0.28–2.05)	0.485
CD20	0.22 (0.17–0.47)	0.20 (0.14–0.40)	0.837	0.53 (0.44–2.45)	0.32 (0.25–0.55)	0.041*
ERG	0.23 (0.17–0.26)	0.26 (0.18–0.28)	0.470	0.50 (0.41–0.78)	0.67 (0.23–0.87)	0.818
Caldesmon	0.06 (0.03–0.07)	0.11 (0.07–0.21)	0.031*	0.15 (0.06–0.57)	0.14 (0.05–0.30)	0.818
D2-40	0.11 (0.06–0.26)	0.06 (0.04–0.09)	0.210	0.30 (0.12–0.41)	0.20 (0.09–0.27)	0.240
CD138[a]	ND	ND	ND	1.03 (0.17–2.01)	0.22 (0.11–2.87)	0.792

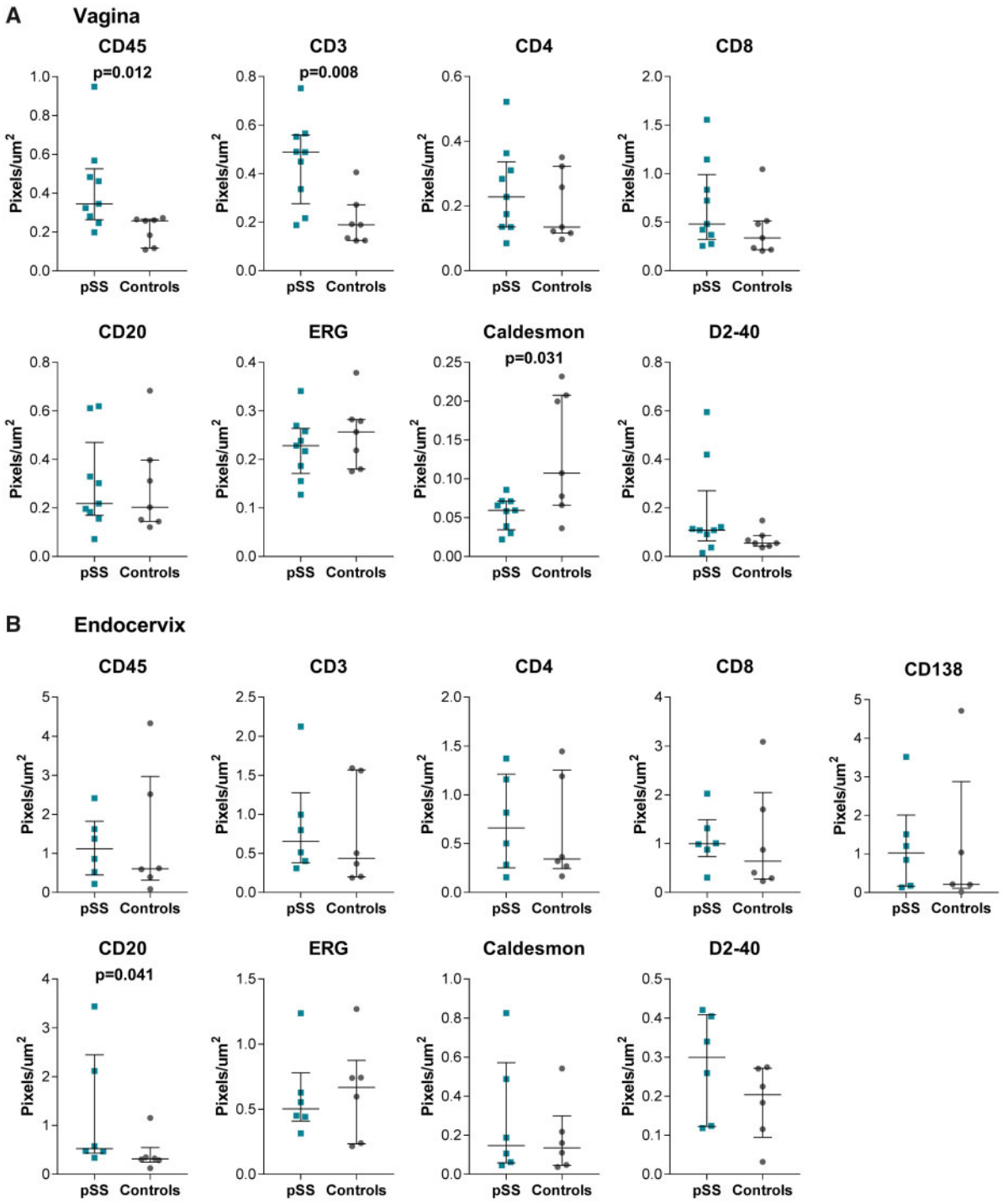
Values are the median (interquartile range) number of positive pixels per square micrometre.
[a]CD138 was analysed in six patients and five controls, because one control did not show representative endocervical tissue in the CD138-stained tissue section.
*$P<0.05$.
D2-40: anti-podoplanin (clone D2-40); ERG: avian V-ets erythroblastosis virus E26 oncogene homologue; ND: Not done.

seemed to be a tendency towards an increase in the number of lymphatic endothelial cells (D2-40) in primary SS. No significant differences were found in other endothelial markers in the vagina or endocervix.

Immune markers in blood, CVL and ES

Next, we explored whether the histological changes in the vagina and endocervix are reflected by cellular and soluble immune markers in the lumen. No differences

FIG. 2 Leucocyte subsets and markers for blood and lymphatic vessels in vaginal (**A**) and endocervical (**B**) tissue in patients with primary SS and controls

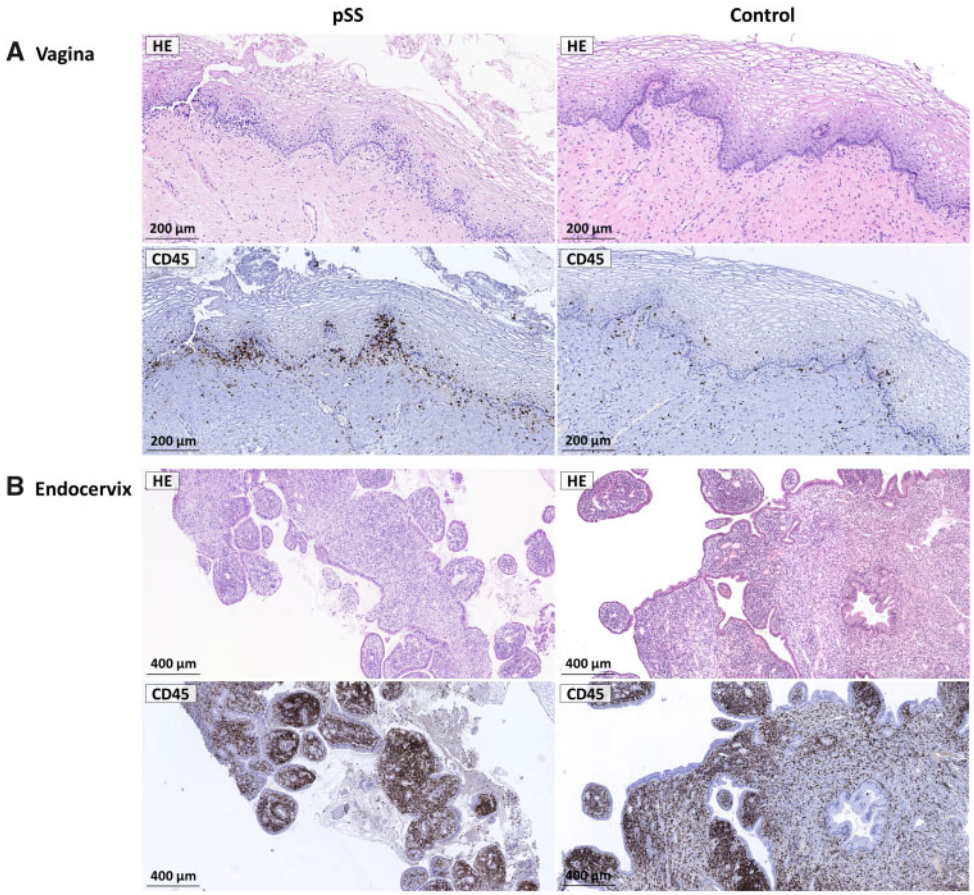


Markers are expressed as the number of positive pixels per square micrometre. D2-40: anti-podoplanin (clone D2-40); ERG: avian V-ets erythroblastosis virus E26 oncogene homologue; pSS: primary SS.

were found in the proportion of leucocyte subsets in CVL or ES (Supplementary Table S4, available at *Rheumatology* online).

A significantly higher level of CXCL10 was found in ES samples of patients with primary SS (Supplementary Table S5, available at *Rheumatology* online). No other

FIG. 3 Haematoxylin and Eosin and CD45 stains in the vagina and endocervix of a primary SS patient and a control



HE: Haematoxylin and eosin; pSS: primary SS.

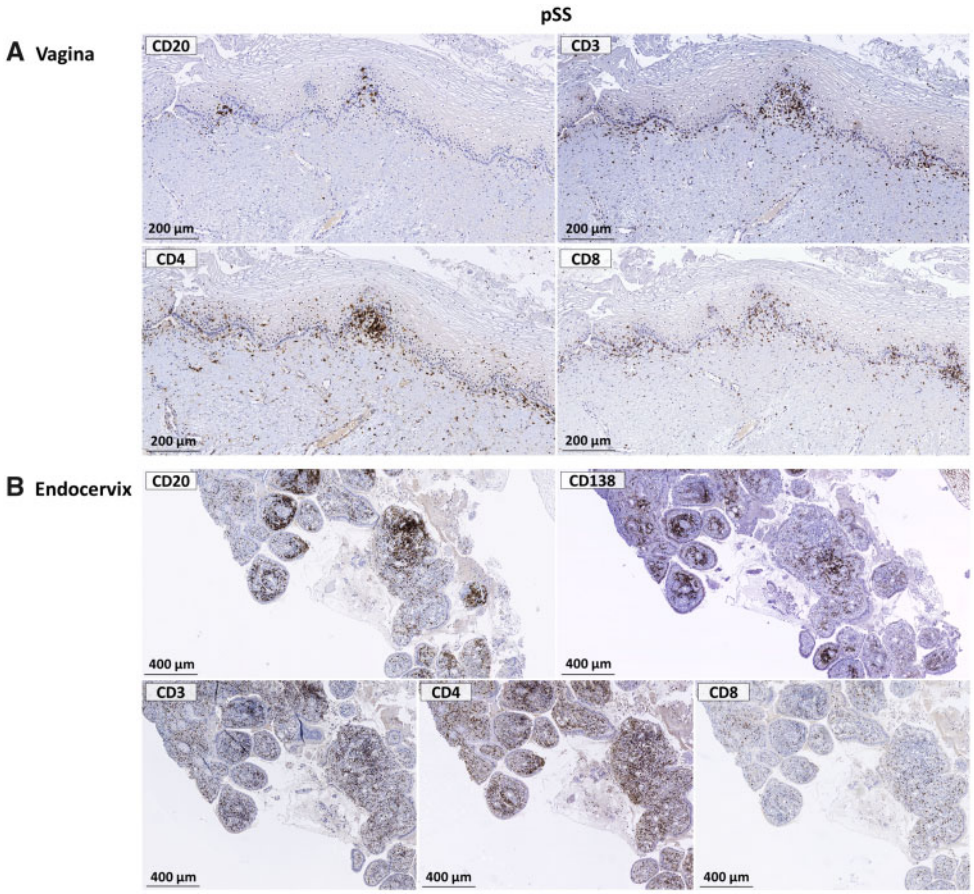
significant differences in chemokine or cytokine levels of patients and controls were found in ES or CVL samples. In serum, CXCL10 and CXCL11 were significantly increased in primary SS patients. Within the group of primary SS patients, a strong correlation was seen between CXCL10 in ES and CXCL10 in serum ($\rho = 0.717$, $P = 0.03$) and between CXCL10 in serum and the number of $CD45^+$ cells in the vagina ($\rho = 0.667$, $P = 0.05$). Levels of IFN-γ, IL-17A, CCL4, CX3CL and CXCL9 were below detection limits in serum, CVL and ES in most patients.

Discussion

Women with primary SS and vaginal sicca symptoms often experience sexual dysfunction and dyspareunia. We observed that women with primary SS have impaired vaginal health and an increased tendency of the vaginal epithelium to bleed. Furthermore, we found a peri-epithelial infiltration and decreased number of vascular smooth muscle cells in the vaginal wall of primary SS patients, which are likely to contribute to vaginal dryness. In contrast to post-menopausal women, the vaginal dryness in women with primary SS cannot be explained by atrophic vaginitis, because no signs of atrophy or increased pH were found.

Our study provides the first in-depth, quantitative evaluation of immunological and histopathological markers in the cervicovaginal mucosa of a well-defined group of primary SS patients, compared with healthy controls. By including only pre-menopausal patients, matching patients for age and screening for infections, we minimized the influence of confounders. We found higher numbers of infiltrating $CD45^+$ cells in vaginal biopsies of primary SS patients, with a peri-epithelial localization and aggregates in dermal papillae. This difference in $CD45^+$ cells seems to be largely attributable to $CD3^+$ T cells. Although both $CD4^+$ and $CD8^+$ T cells were present in peri-epithelial infiltrates, neither were significantly overrepresented in primary SS patients. The exact phenotype of the infiltrating $CD3^+$ T cells in the vagina of primary SS patients remains to be established. In the endocervix, $CD45^+$ infiltrates were present in patients and controls, but with a higher number of B lymphocytes in primary SS patients. The vaginal and endocervical epithelium remained intact in primary SS. Lymphocytes did not seem to migrate through the

FIG. 4 Lymphocyte subsets in the vagina and endocervix of the same primary SS patient shown in Fig. 3



epithelial layer, because no differences were found in the composition of leucocyte subsets in the vaginal and endocervical lumen using flow cytometry.

Our findings are in line with previous observations showing the presence of inflammatory infiltrates in the vagina and cervix of women with primary SS in HE-stained sections [2, 11, 14]. Why lymphocytes migrate to these sites is not yet known, but it is likely that CXCL10 is involved. This IFN-induced chemokine plays a dominant role in the pathogenesis of primary SS, and increased levels are reported in saliva, tear fluid, serum and now also in ES samples [23, 24]. The origin of CXCL10 in the ES samples is not yet known. Given the correlation with serum levels, a part of CXCL10 in the ES samples might be derived from serum by transudation, but it might also be produced locally. Salivary gland ductal epithelial cells produce CXCL10, which subsequently results in formation of periductal infiltrates [25]. Likewise, vaginal and endocervical epithelial cells might produce this chemokine, explaining the characteristic peri-epithelial vaginal infiltrate in the lamina propria.

The formation of transudate from the lamina propria, which is rich in capillaries and post-capillary venules, is important for humidification of the vagina. The lymphocytic infiltrate might either damage capillaries/post-capillary venules at these sites or otherwise interfere with generation of the transudate. Importantly, we observed that numbers of vascular smooth muscle cells are significantly decreased in the vagina of primary SS patients. Whether this decrease reflects destruction of vascular smooth muscle cells or a decrease in total number of arterioles remains to be elucidated. Either way, a decrease in smooth muscle cells might disturb the production of transudate, considering the important role of smooth muscle cells in the regulation of the blood flow in the vaginal vascular network during sexual arousal [26].

Although the reason for the decrease in smooth muscle cells is not clear, there are several studies showing that blood vessel homeostasis is disturbed in primary SS. First, numbers of circulating endothelial precursor cells are increased in primary SS, indicating endothelial damage [27]. Second, there is an increase in numbers of circulating angiogenic T cells, which contribute to endothelial repair but may also have cytotoxic and pro-inflammatory effects [28]. Third, soluble intracellular adhesion molecule 1 and soluble vascular cell adhesion molecule 1 are elevated in serum of primary SS

patients, which are associated with endothelial cell activation and dysfunction [29]. Fourth, functional impairment of the arterial wall and vascular smooth muscle cells has been described in primary SS [29, 30]. Taken together, we hypothesize that vaginal dryness is impaired in primary SS patients as a result of vascular dysfunction. Endothelial damage might also explain the increased bleeding tendency of the vaginal epithelium in primary SS patients. The development of vascular dysfunction might be mediated by the IFN pathway, in a similar manner to that in SLE, in which IFN alters the balance between endothelial cell apoptosis and vascular repair mediated by endothelial cell progenitors and myeloid angiogenic cells [31, 32].

This study focused on the vaginal and cervical epithelium, because these are the main sources of vaginal lubrication. Whether the vestibular glands (Bartholin's and Skene's glands) are affected by primary SS remains unknown. However, Bartholin's glands provide only a small contribution to lubrication of the vestibule of the vagina of healthy individuals [33] and whether the paraurethral glands (Skene's glands) contribute to lubrication of the vulva is still under debate [34]. Skene's glands most probably produce some fluid only during orgasm, if ever.

Limitations of our study are the small sample size and subjective measurement of vaginal dryness. Furthermore, given that we did not include primary SS patients without vaginal dryness or non-primary SS controls with vaginal dryness, it still has to be evaluated whether the cervicovaginal changes that we found in women with primary SS are the cause or a consequence of vaginal dryness, and whether they are specific for primary SS patients. Future studies should quantify vaginal lubrication objectively in a larger group of patients and evaluate the relationship of vaginal dryness to our findings. Lastly, although we aimed to include all patients during the follicular phase of the menstrual cycle, two controls were included in the luteal phase, because their laparoscopic procedures could not be planned in the follicular phase. Menstrual cycle phase might influence soluble immune markers in the vagina and cervix, but probably does not influence cellular markers [15, 19, 35].

In conclusion, our study shows that women with primary SS and vaginal dryness have sexual dysfunction, impaired vaginal health and increased lymphocytic infiltration in the vaginal lamina propria. We postulate that vaginal dryness in women with primary SS is caused by vascular dysfunction, possibly induced by IFN-mediated pathways.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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