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Reference:
Visser Annie, van Nimwegen Jolien F., Wilbrink Rick, Liefers Silvia C., van der Tuuk Karin, Mourits Marian J.E., Diercks Gilles F.H., Bart Joost, van der Vegt Bert, van Kempen Léon, ...- Increased interferon signalling in vaginal tissue of patients with primary Sjögren’s syndrome
Journal of rheumatology - ISSN 0315-162X - 51:7(2024), p. 687-695
Full text (Publisher's DOI): https://doi.org/10.3899/JRHEUM.2023-1068
To cite this reference: https://hdl.handle.net/10067/2050210151162165141

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ISG-expression pSS vaginal tissue

Increased interferon signalling in vaginal tissue of patients with primary Sjögren's syndrome

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Funding: This work was supported by grants from the Dutch Arthritis Society (ReumaNederland) [14-1-301] and the C&W de Boer Foundation.

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Conflict of interest: H. Bootsma has received unrestricted research grants, outside the submitted work, from Bristol-Myers Squibb and AstraZeneca and received consultancy fees from Argenx, Bristol-Myers Squibb, Roche, Novartis, Union Chimique Belge. F.G.M. Kroese and G.M. Verstappen are co-applicant on an unrestricted grant by AstraZeneca. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential competing interest.

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Key Indexing Terms: Sjögren's Syndrome, Autoimmunity, Interferons, Physiologic Angiogenesis, Vagina, Inflammation.

Statement of ethics and consent: This study complies with the Declaration of Helsinki and was approved by the Medical Ethics Committee of the University Medical Center Groningen (METC 2015/039). All participants gave written informed consent.

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ABSTRACT

Objectives: Vaginal dryness is an important factor influencing sexual function in women with primary Sjögren's syndrome (pSS). Previous studies showed a higher degree of inflammation in vaginal biopsies from pSS patients compared to non-pSS controls. However, the molecular pathways that drive this inflammation remain unclear. Therefore, the aim of this study was to investigate inflammatory pathway activity in pSS patients' vaginal tissue.

Methods: Vaginal biopsies of eight premenopausal pSS patients with vaginal dryness complaints and seven age-matched non-pSS controls were included. Expression of genes involved in inflammation and tissue homeostasis was measured using Nanostring technology and validated using TaqMan Real-Time PCR. Vaginal tissue sections were stained by immunohistochemistry for Myxovirus resistance protein 1 (MxA) and CD123 (plasmacytoid dendritic cells (pDCs)).

Results: The most enriched pathway in vaginal biopsies from pSS patients compared to non-pSS controls was the IFN signalling pathway ($p=0.01$). Pathway scores for JAK-STAT and Notch signalling were also higher ($p=0.01$, both pathways). Conversely, TGFβ-signalling and angiogenesis pathway scores were lower in pSS ($p=0.02$ and $p=0.04$, respectively). Differences in IFN signalling between pSS patients and non-pSS controls were confirmed by PCR and MxA tissue staining. No $CD123^{+}$ pDCs were detected in vaginal biopsies. Interferon-stimulated gene expression levels correlated positively with $CD45^{+}$ cell numbers in vaginal biopsies and serum anti-SSA/Ro positivity.

Conclusions: Upregulation of IFN signalling in vaginal tissue of women with pSS, along with its association with tissue pathology, suggests that IFNs contribute to inflammation of the vaginal wall and potentially also to clinical symptomatology i.e. vaginal dryness.

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INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease primarily affecting women, with a female:male ratio of 10:1 [1]. The pathophysiological process of the disease is marked by lymphocytic infiltrates in the lacrimal and salivary glands and presence of circulating autoantibodies (anti-SSA/Ro, anti-SSB/La and rheumatoid factor). Key elements in the pathogenesis are T-cell dependent B-cell hyperactivity and type-I interferon (IFN) activation [2–4]. The most reported clinical symptoms of pSS encompass dryness of the eyes (keratoconjunctivitis sicca) and mouth (xerostomia), pain and fatigue [1]. Less well-known, less spontaneously reported and less studied are complaints of vaginal dryness, experienced by the majority of female pSS patients [5–9]. Vaginal dryness is an important factor influencing sexual function in women with pSS. We reported that 56% of women with pSS experienced sexual dysfunction, measured by the Female Sexual Function Index, which includes six domains: desire, arousal, orgasm, lubrication, satisfaction and pain. Furthermore, pSS patients were more frequently sexually inactive compared to age-matched healthy controls, negatively affecting general wellbeing and quality of life [8].

Despite the considerable impact of vaginal dryness, the pathogenesis of this symptom in pSS patients is poorly understood. Normally, transudate from the venous and lymphatic networks in the lamina propria of the vaginal epithelium and mucus produced by endocervical glandular epithelial cells keep the vaginal surface humidified and lubricated [10]. In women with pSS, numbers of $CD45^+$ cells are increased in vaginal biopsies compared to non-pSS controls [11–14]. In addition, our previous study demonstrated a decrease in vascular smooth muscle cells in pSS patients' vaginal biopsies, indicating disturbed blood vessel homeostasis [11]. While these morphological changes may be caused by (chronic) inflammation of the vaginal wall in pSS, the underlying molecular pathways remain poorly understood.

We hypothesized that inflammatory pathways are activated in vaginal tissue of pSS patients, potentially contributing to reduced lubrication by disturbing blood vessel homeostasis. To test this hypothesis, we performed immune gene profiling on vaginal tissue biopsies from premenopausal pSS patients with vaginal dryness and non-pSS controls without an autoimmune disease. Differentially expressed genes and pathways were identified and expression levels were correlated to clinical parameters, including the Female Sexual Function Index (FSFI), Vaginal Health Index (VHI) and patient-reported vaginal dryness.

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MATERIALS AND METHODS

Patients and controls

For this study we used vaginal biopsies collected in a previous prospective exploratory case-control study. The study included ten premenopausal females with pSS who fulfilled 2016 ACR-EULAR criteria and reported vaginal dryness and ten premenopausal females without an autoimmune disease. Detailed in- and exclusion criteria have been previously described [11]. None of the participants used systemic corticosteroids or DMARDs ≤6 months before inclusion and participants with inflammatory or infectious gynaecological diseases were excluded. 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). All participants gave written informed consent.

Sample collection and processing

All participants underwent a mid-vaginal biopsy. Punch biopsies were collected and either snap-frozen (-80°C) or formalin-fixed and paraffin-embedded for immunohistochemistry. Immunohistochemical staining of tissue was performed for Myxovirus resistance protein 1 (MxA) and CD123 protein. For details see supplementary methods. Blinded scoring of slides was done by one researcher and three pathologists (BvdV, GD, JB). MxA expression was scored in epithelium, endothelium, fibroblasts and inflammatory infiltrates (when present). When staining was observed in all cell types, vaginal tissue was considered positive for MxA. From pSS patients, peripheral blood samples were collected in Paxgene tubes (Qiagen, Germantown, USA) and stored until use at -20 °C. Total RNA was extracted using the Paxgene Blood isolation Kit (Qiagen).

Patient-reported symptoms and clinical data collection

At the time of the biopsy, all participants completed a questionnaire concerning sexual function and vaginal dryness. Sexual function was measured by the 19-item FSFI. Higher scores indicate better sexual function [15]. Vaginal dryness was patient-reported using a numeric rating scale (NRS), ranging from 0 (no dryness) to 10 (the worst dryness imaginable) [16].

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Gynaecological examination was performed by an experienced gynaecologist by scoring the VHI. Higher scores indicate good vaginal health.

Immune gene profiling

Immune gene profiling was performed on RNA from frozen vaginal biopsies (RNeasy miniprep kit, Qiagen). Gene expression was measured using the nCounter® Nanostring PanCancer Immune Profiling Panel (Nanostring Technologies, Seattle, WA). For details, see supplementary methods. To validate Nanostrings' immune gene profiling results, RNA was reverse transcribed to cDNA and quantitatively analysed using an Applied Biosystems™ QuantStudio™ 6 Flex Real-Time (RT) PCR System (ThermoFisher Scientific, Waltham, USA). The relative expression (RE) was calculated based on the cycle threshold (Ct) value related to expression of the housekeeping gene GAPDH as follows: $RE = 2^{-(Ct\ Test\ gene\ -\ Ct\ GAPDH)}$.

To differentiate between type-I and type-II IFN dominant activation in pSS vaginal tissue, three IFN-related modules (M1.2, M3.4, M5.12) displaying a distinct inducibility by the different IFNs were analysed [17]. IFN-scores were calculated by summing up the individual RE per gene after normalization to the non-pSS control group as follows: $\sum(RE_{subject} - Mean_{control})/SD_{control}$. Two separate IFN-scores were calculated: the IFN12-score, based on the 12 ISGs of modules M1.2, M3.4 and M5.12 and the IFN3-score, based on three type-I IFN (IFNα) induced genes (*IFI44L*, *LY6E* and *MX1*) from module M1.2 [18]. An IFN-score was considered positive when it was higher than the mean+2SD of the control values.

Statistical analyses

Differential gene expression was analysed using nSolver™ Analysis 4.0 software provided by Nanostring Technologies. Because of the exploratory character of this study and the low number of samples, we did not use p-values, but genes were considered differentially expressed when their expression level was ≥1.5 times higher or lower than the expression level of non-pSS controls. Signalling pathway scores were calculated as the first principal component of the pathway genes' normalized expression. Pathway scores were expressed as median with interquartile range per pathway for patients and non-pSS controls. Pathway scores between groups were compared using the Mann-Whitney U test. A gene expression heatmap was generated by using the normalized expression data from nSolver™ into R statistical environment. Z-scores were calculated for each individual gene and subject.

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Hierarchical clustering was performed using Ward's method (Euclidean Distance). Spearman's correlation coefficient (ρ) was used to measure the strength and direction of association between two ranked variables.

Statistical analyses were performed in IBM SPSS statistics v28 and figures were created in GraphPad Prism 9.1.0 or R statistical environment (version 4.1.1). P-values <0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Clinical characteristics of the study participants are summarized in Table 1. We included eight out of ten pSS patients and seven out of ten non-pSS controls in our analysis. Reasons for exclusion were presence of Chlamydia trachomatis (pSS; n=1), endometriosis (control; n=2), or low RNA yield of the biopsy (pSS and control; n=2).

Dysregulated molecular pathways in vaginal tissue of pSS patients

Gene set enrichment analysis of nCounter® data showed divergent pathway scores in vaginal biopsies from pSS patients compared to non-pSS controls (Figure 1). Lower scores in pSS vaginal tissue were observed for several pathways, with a significant difference for TGFβ-signalling and angiogenesis (p=0.02 and p=0.04, respectively). Conversely, for several pathways higher pathway scores in pSS vaginal tissue compared to non-pSS controls were observed (Figure 1). The IFN signalling, JAK-STAT signalling and Notch signalling pathways showed the largest difference between groups, reaching statistical significance (p=0.01 for all). Since IFNs play a dominant role in the immunopathogenesis and inflammation of the salivary glands, the IFN signalling pathway was analysed in more detail. For this we looked at transcription levels of genes stimulated by IFNs, the IFN stimulated genes (ISGs), reflecting the activity of the IFN signalling pathway.

In total, 41 out of 58 (71%) genes involved in IFN signalling included in the probe panel were differentially expressed in vaginal tissue of pSS patients compared with non-pSS controls. For further analysis, the 20 ISGs with a fold change ≥1.5 in pSS versus non-pSS controls were selected (Supplementary Figure 1). Hierarchical clustering of all study participants based on these ISGs showed evident clustering of pSS patients with one independent cluster of four

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(50%) 'IFN-high' patients (pSS10, 13, 16 and 21) (Figure 2). The other four (50%) pSS patients (pSS9, 11, 19 and 25) showed moderate to low ISG expression levels. The seven non-pSS controls clustered together in a group with low ISG expression levels. All IFN-high patients were anti-SSA/Ro positive, whereas the two anti-SSA/Ro negative patients (pSS11 and pSS19) showed moderate to low ISG expression levels.

In addition to autoantibody status, we determined the relationship between increased ISG expression in vaginal tissue and the clinical parameters sexual function (FSFI), patient-reported vaginal dryness (NRS) and vaginal health (VHI). Patients and non-pSS controls were ranked based on their IFN signalling pathway score in vaginal tissue to facilitate a descriptive analysis of the three clinical parameters in relation to this score (Supplementary Table 1). Although pSS patients scored worse on nearly all FSFI domains, patient-reported vaginal dryness, and several VHI domains, as previously described [8,11], no clear differences were observed in clinical parameters between pSS patients with high or low IFN signalling pathway scores.

Validation of differential IFN stimulated gene expression

To validate nCounter® results, TaqMan RT-PCR analyses were performed to quantify expression of the 12 ISGs of the IFN12-score. At the individual gene level, a clear trend towards higher expression in pSS patients' vaginal biopsies was observed for the majority of measured ISGs (Supplementary Figure 2). Modules M1.2 (IFNα-induced) and M3.4 (IFNβ,γ-induced) were upregulated in four out of eight pSS patients (i.e., higher than the mean+2SD of the controls; Figure 3). Module M5.12 (IFNα,β,γ-induced) was upregulated in three out of eight pSS patients. The four pSS patients with elevated ISG transcript levels in M1.2 and M3.4, also showed an upregulated IFN3-score and IFN12-score. Thus, RT-PCR analysis shows increased IFN-scores in pSS patients' vaginal biopsies compared to non-pSS control biopsies, indicating more IFN activity confirming the nCounter® data.

Increased interferon scores and their correlation with the amount of $CD45^+$ cells in pSS vaginal tissue

Since the increased expression of ISGs in pSS patients' salivary glands has been associated with presence of lymphocytic infiltrates [19], we next investigated whether this was also the case in vaginal tissue. Immunohistochemical sections were previously stained for the pan-

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leucocyte marker CD45 [11], and were reanalysed by counting the number of positively stained cells per square millimetre of submucosa using QuPath [20]. At the group level there was an increase in the percentage (median 7.7 (IQR 7.1-9.0) vs. 6.1 (IQR 4.0-8.5) % positive cells, respectively) and number (median 340 (IQR 279-435) vs. 197 (IQR 155-256) of positive cells/mm^2, respectively) of $CD45^+$ cells in vaginal tissue of pSS patients compared to non-pSS controls. Even with a low number of samples, a strong correlation between the percentage of $CD45^+$ cells and the IFN-scores was observed, with the highest correlation for the IFN3-score (ρ=0.79). A moderate correlation was found between the number of $CD45^+$ cells and the IFN-scores, with the highest correlation for the IFN12-score (ρ=0.59).

Presence of MxA protein expression, but absence of plasmacytoid DCs, in pSS vaginal tissue

To explore whether elevated *MX1* transcript levels in vaginal tissue coincide with expression of this ISG at the protein level, we performed immunohistochemical staining on vaginal tissue sections (Figure 4). Tissue sections from six anti-SSA/Ro positive pSS patients, all with highly or moderately elevated *MX1* transcript levels as shown by Nanostring's immune profiling data, showed strong positive MxA protein staining in epithelial cells, endothelial cells, fibroblasts and infiltrates (when present). Previous work showed that these infiltrates mainly consist of T cells ($CD4^+$ and $CD8^+$) [11]. The two anti-SSA/Ro negative pSS patients (pSS11 and pSS19) and the non-pSS controls, all with moderate to low *MX1* transcript levels, showed only faint background staining in epithelial cells (and some endothelial cells) and were considered MxA negative. Thus, higher *MX1* gene expression corresponds to higher MxA protein expression in vaginal tissue, which is related to anti-SSA/Ro positivity.

To explore whether pDCs form a local source of type-I IFN in vaginal tissue, tissue sections were stained for the pDC cell membrane marker CD123. However, CD123 staining was not observed in vaginal biopsies from pSS patients or non-pSS controls. This observation was confirmed by nCounter® data, showing CD123-mRNA counts below the detection limit in all samples. Apparently, an alternative local source of type-I IFN (e.g., epithelium) or type-I IFN from the circulation is responsible for the observed increase in IFN signalling.

To investigate whether *MX1* expression in circulating immune cells and vaginal tissue was correlated, we compared transcript levels of the *MX1* gene in whole blood (RT-PCR) with the normalized expression of *MX1* in vaginal tissue (nCounter®). Despite differences in tissue type and molecular technique, we found a moderate correlation between blood and vaginal tissue

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MX1 expression at the group level (ρ=0.45, n.s.). The two anti-SSA/Ro negative pSS patients showed low *MX1* expression levels in both blood and vaginal tissue. These results indicate that there is some discordance in *MX1* expression levels between blood and vaginal tissue, although low levels in blood are in all cases accompanied by low levels in vaginal tissue (Supplementary Figure 3).

Interferon-stimulated gene expression and its relation to angiogenesis

Since elevated IFN levels may result in endothelial dysfunction [22–24], and that the angiogenesis pathway was downregulated in vaginal tissue of pSS patients, we hypothesized that higher ISG expression levels might be linked to endothelial dysfunction in vaginal tissue. We observed however no clear difference in angiogenesis pathway scores between vaginal tissue-based 'IFN-high' pSS patients and patients with moderate to low ISG expression levels (Supplementary Table 2). In addition, we analysed the expression of typical endothelial cell marker genes (i.e., *PECAM1*, *CDH5*, and *TIE1*) that were included in our probe panel, but not in the pre-defined angiogenesis pathway. While lower normalized expression values were observed for these genes in pSS patients compared to non-pSS controls, there was no clear difference between 'IFN-high' pSS patients and patients with moderate to low ISG expression levels (Figure 5).

Thus, while blood vessel formation in the vaginal wall seems to be impaired in the majority of pSS patients, our results do not indicate a direct relationship with enhanced IFN signalling.

DISCUSSION

Vaginal dryness and related sexual dysfunction are frequent complaints by women with pSS, affecting general well-being and quality of life. However, very little is known about the pathophysiology of vaginal dryness in pSS, while such knowledge may be beneficial for the treatment of this common symptom. Here, we used a probe-based technology to measure transcript levels of immune response genes in vaginal tissue of premenopausal female pSS patients with vaginal dryness complaints and non-pSS controls without an autoimmune disease. We demonstrate increased IFN activity in vaginal tissue of pSS patients. To our knowledge, this is the first study showing increased IFN activity in pSS outside the main target organs (e.g., salivary glands) and peripheral blood. We found a strong positive correlation

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between upregulation of ISGs and the frequency of $CD45^+$ cells in vaginal tissue, suggesting that these pathological features are linked.

IFN signalling is one of the major pathways that contributes to inflammation in salivary glands of pSS patients [2–4,19]. Increased IFN activity has been associated with more severe glandular pathology and dysfunction [25,26]. Whether IFN is involved in the pathophysiology of vaginal dryness in women with pSS remained unknown. By using immune gene profiling we found an IFN signature in vaginal tissue in 50% (4 out of 8) of the pSS patients. These 'IFN-high' patients showed elevated transcript levels of ISGs, reflecting the activity of the IFN signalling pathway. The other 50% of pSS patients, including two anti-SSA/Ro negative patients, showed moderate to low ISG transcript levels. All 'IFN-high' pSS patients were anti-SSA/Ro positive, confirming the strong link between activation of the IFN pathway and presence of anti-SSA/Ro autoantibodies in pSS [27]. The seven non-pSS controls clustered together in a group with low ISG expression levels.

IFNs are a large family of cytokines involved in anti-viral defence mechanisms, cell growth regulation and immune activation [28]. Type-I IFN (IFNα and IFNβ) and type-II IFN (IFNγ) are the most studied IFNs. To discriminate between more type-I or type-II-mediated ISG expression, we used IFN annotated modules, first described for SLE [17]. The four pSS patients who were assigned to the 'IFN-high' cluster based on nCounter® analysis, showed elevated ISG transcripts in the IFN modules M1.2 and M3.4, indicative for type-I IFN-mediated upregulation. Of these four pSS patients, three were also M5.12 positive, indicative of the concerted action of both type-I and type-II IFNs in vaginal tissue of these patients. As previously described in SLE and pSS patients' blood, ISGs in M5.12 were not upregulated without concomitant upregulation of ISGs in M1.2 and M3.4 [18,29]. The increased expression of both type-I and type-II-induced genes in the 'IFN-high' cluster was confirmed by higher IFN3-scores (type-I) and IFN12-scores (type-I and type-II). For the type-I induced gene *MX1*, gene expression results were validated at the protein level by immunohistochemistry. While MxA staining was considered negative in all non-pSS controls, six out of eight pSS patients (all anti-SSA/Ro positive) showed positive MxA staining while the two anti-SSA/Ro negative patients did not show MxA staining in vaginal tissue, in line with gene expression results. In MxA positive tissues, both non-lymphoid cells (epithelial cells, endothelial cells, fibroblasts) and lymphoid cells showed MxA expression, indicating responsiveness to type-I IFN.

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Next, we questioned whether upregulation of ISGs was induced by local production of type-I (and/or type-II) IFNs. Many different cell types can produce type-I IFN, upon activation of pattern recognition receptors. Plasmacytoid DCs are however specialized in type-I IFN production [30–32]. CD123-expressing pDCs have been observed in the salivary glands of pSS patients, but not in control glands [21]. In this study, we were not able to detect CD123-expressing pDCs in the vaginal wall. Apparently, other cells are responsible for the production of type-I IFN. Since there is some evidence that in the salivary glands of pSS patients epithelial cells produce type-I IFN [33–35], this could also be the case in vaginal tissue. The discordance between relative *MX1* gene expression in whole blood and normalized *MX1* gene expression in vaginal tissue supports the possibility that type-I IFN is produced locally in the vaginal tissue. For type-II IFNs, infiltrating T-cells may be a potential source. In a previous study [11], we have shown that the $CD45^+$ cell infiltrates largely consist of T-cells, including both $CD4^+$ and $CD8^+$ T-cells. The presence of (IFNγ-producing) T-cells could also explain the observed association between upregulation of ISGs and the percentage of $CD45^+$ infiltrating cells in the submucosa of the vaginal wall.

In addition to promoting inflammation, there is evidence that increased IFN activity may also result in endothelial dysfunction. In SLE patients, IFN alters the balance between endothelial cell apoptosis and vascular repair [22,23]. We therefore hypothesized that higher ISG expression levels might be linked to endothelial dysfunction in vaginal tissue, leading to vaginal dryness. Endothelial cells in the vaginal wall are critically involved in the formation of transudate, which humidifies the vagina, together with mucus produced by endocervical glands. Previously we observed a decrease in the amount of cells expressing caldesmon in the vaginal wall of pSS patients, an important protein for smooth muscle contraction. This decrease presumably reflects a lower amount of arterioles [11]. In line with these previous findings, the current study shows reduced transcript levels of endothelial cell marker genes (i.e., *PECAM1*, *CDH5*, and *TIE1*) in pSS patients compared to non-pSS controls. Apparently, the damage and/or loss of blood vessels cannot be repaired, as mirrored by a lower angiogenesis pathway score in pSS patients. Together, these results point towards a decreased number of arterioles in the vaginal wall of pSS patients that may well explain the vaginal dryness seen in these patients. We could however not demonstrate a direct link between increased IFN activity and reduced transcript levels of endothelial cell marker genes.

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A main limitation of our study is the small sample size, which results in low statistical power. This may also explain why we could not find a correlation between enhanced IFN activity in vaginal tissue (i.e., elevated expression of ISGs, higher IFN-scores) and relevant clinical parameters such as patient-reported vaginal dryness, vaginal health index (physician-reported), and sexual functioning (FSFI). Another complicating matter is that none of these parameters have been validated for pSS and the outcome of these clinical parameters is influenced by multiple factors (e.g., interpersonal and psychosocial factors). Also, patient reported outcomes such as NRS vaginal dryness may not adequately reflect objective vaginal dryness. Thus, absence of a correlation does not necessarily mean that IFN is not a contributing factor to clinical symptoms and further investigation is warranted.

In conclusion, the current study provides a unique insight into active immune response pathways in vaginal tissue of pSS patients compared to non-pSS controls. We demonstrated that IFN signalling is upregulated in vaginal tissue and potentially contributes to clinical vaginal symptomatology. Unravelling the pathophysiology of vaginal dryness in pSS is critical in developing treatment strategies to ameliorate this important, common but underreported and understudied symptom.

ACKNOWLEDGMENT

We thank Hannie Westra, Berber Doornbos and Mirjam Mastik for their technical support.

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FIGURE LEGENDS

Figure 1. Pathway scores in vaginal biopsies from pSS patients (black squares) compared to non-pSS controls (grey dots). Results are expressed as median with interquartile range per pathway for patients and controls. Signaling pathways scores that were significantly different ($p<0.05$) between pSS patients and non-pSS controls are marked in bold and with an asterix (*).

Figure 2. Heatmap showing gene expression in vaginal biopsies from pSS patients and non-pSS controls. Hierarchical clustering (Ward's method) was performed based on expression of 20 selected ISGs (≥1.5 fold linear fold change compared to controls including all subjects). Each column represents an individual study subject and each row represents a gene. Depicted on top of the heatmap: the group of the studied subject (pSS or non-pSS control), anti-SSA/B positivity of the pSS subjects and the calculated IFN-scores (IFN3, IFN12, IFN1.2, IFN3.4 and IFN5.12) of all subjects.

Figure 3. Interferon-scores calculated from TaqMan Real-Time PCR analysis of vaginal biopsies from pSS patients (black squares) compared to non-pSS controls (grey dots). IFN3-score (a). IFN12-score (b). IFNM1.2, IFNM3.4 and IFNM5.12 score (c-e). The dotted line represents mean plus 2xSD of controls. IFN3-score: *IFI44L*, *LY6E* and *MX1*. IFNM1.2-score: *CXCL10*, *IFI44L*, *IFIT3*, *LY6E*, *MX1* and *SERPING1*. IFNM3.4-score: *IFITM1*, *IRF7* and *STAT1*. IFNM5.12-score: *C1QA*, *IFI6* and *IRF9*. IFN12-score: M1.2 + M3.4 + M5.12.

Figure 4. MxA immunohistochemical staining on vaginal tissue sections of pSS patients and a non-pSS control. MxA staining of an 'IFN high' patient (pSS16) with positive MxA staining of epithelium, endothelium, fibroblasts and infiltrate (a), an 'IFN moderate to low' pSS patient (pSS9) with positive MxA staining of endothelium, endothelium and fibroblasts (b), an 'IFN moderate to low' pSS patient (pSS19) negative for MxA staining (c) and a non-pSS control negative for MxA staining. The patients pSS16 and pSS9 were both anti-SSA/Ro positive, patient pSS19 was tested anti-SSA/Ro negative.

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Figure 5. Angiogenesis pathway score and transcript levels of endothelial cell markers in vaginal tissue of pSS patients (black squares) compared to non-pSS controls (grey dots). The angiogenesis pathway score (a), and normalized expression (NE) values from Nanostring results for *PECAM1 (*b), *CDH5* (c), and *TIE1* (d), are displayed. Horizontal lines indicate the median. ANG: Angiogenesis, CDH: Cadherin, PECAM: Platelet and Endothelial Cell Adhesion Molecule, TIE: Tyrosine Kinase With Immunoglobulin Like And EGF Like Domains.

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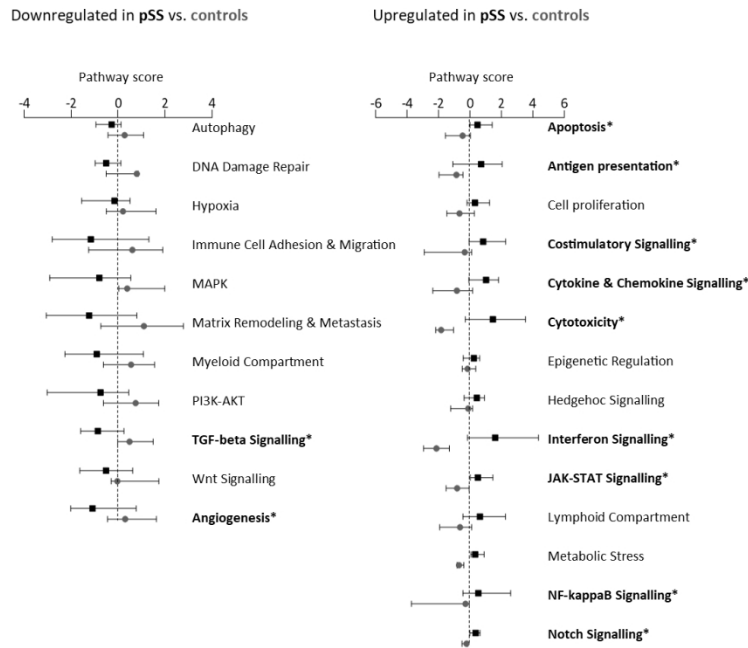

Figure 1.

Figure 1. Pathway scores in vaginal biopsies from pSS patients (black squares) compared to non-pSS controls (grey dots). Results are expressed as median with interquartile range per pathway for patients and controls. Signaling pathways scores that were significantly different ($p<0.05$) between pSS patients and non-pSS controls are marked in bold and with an asterix (*).

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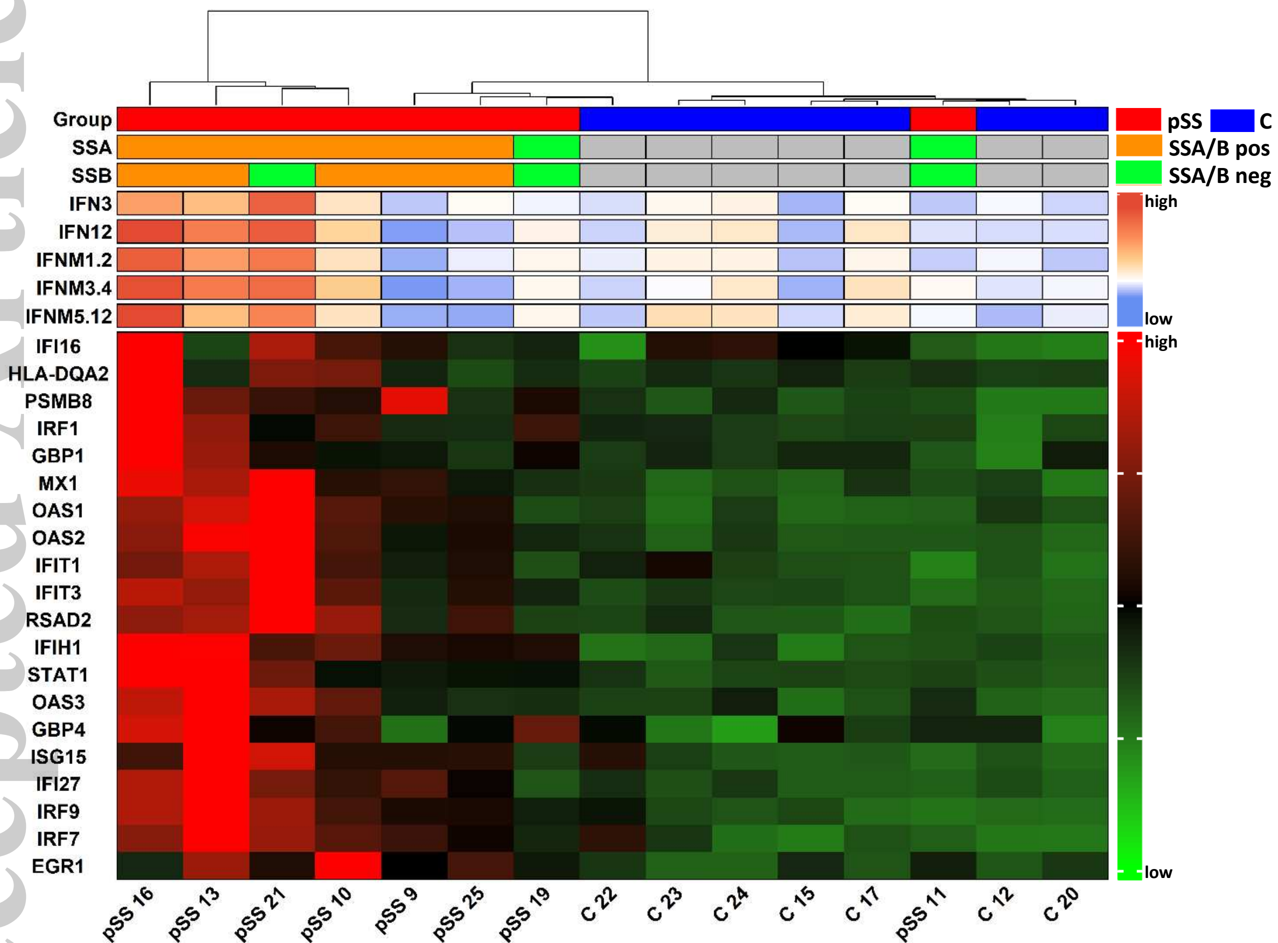

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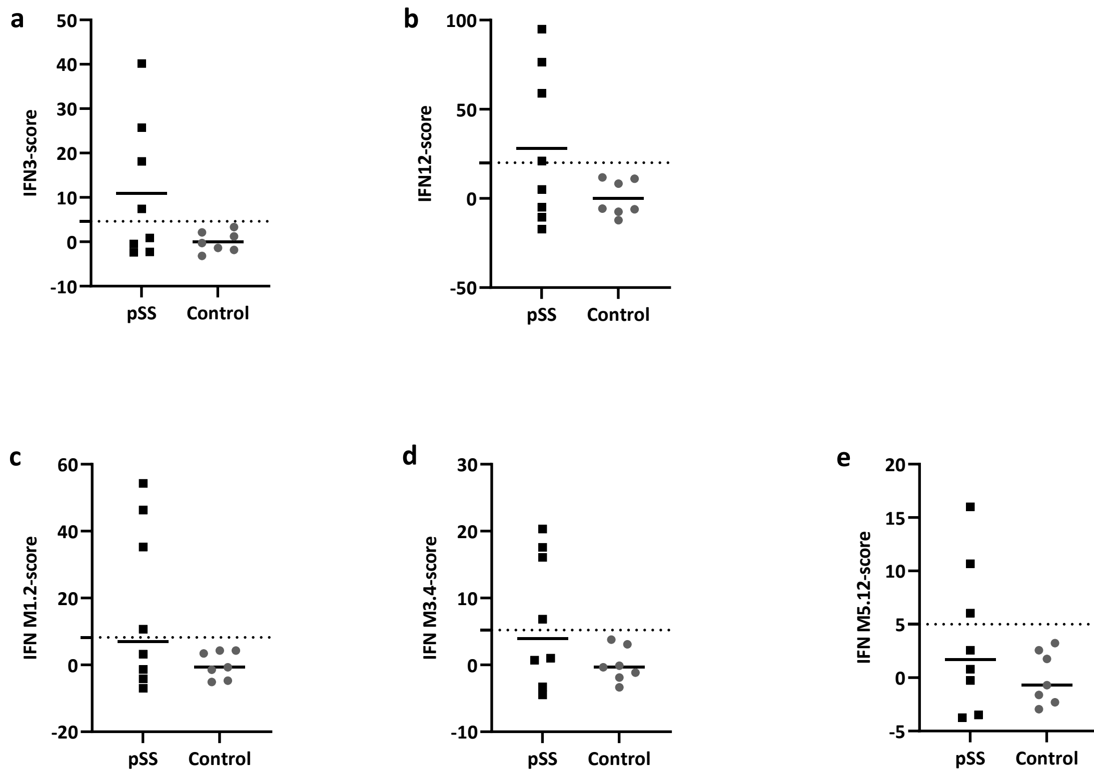

Figure 3.

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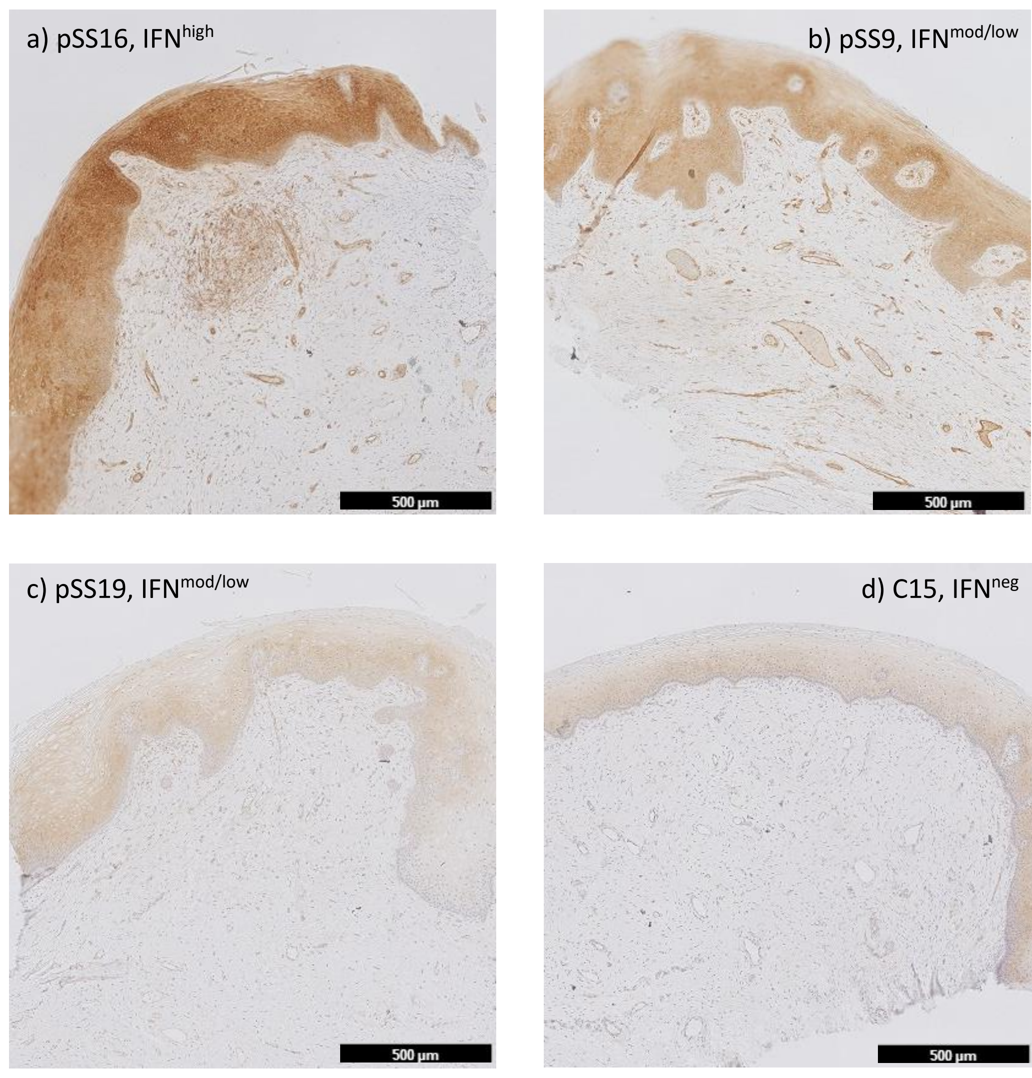

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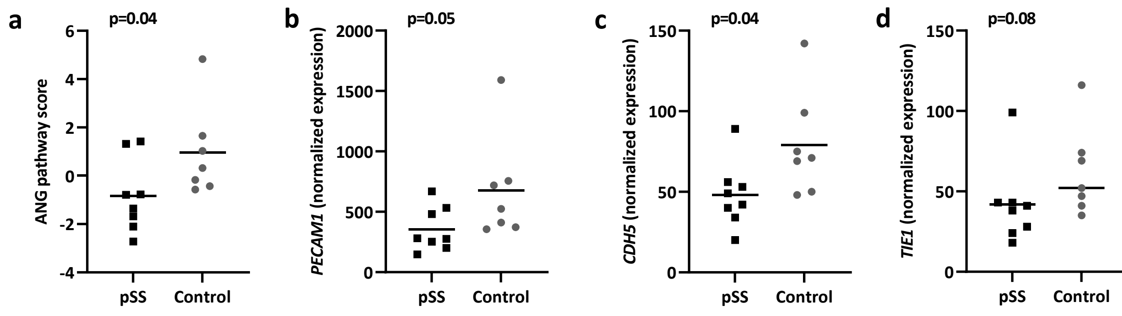

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TABLES

Table 1. Demographical and clinical characteristics of patients and non-pSS controls.

	pSS (n=8)	Controls (n=7)
Age, years	39 (35-46)	41 (36-42)
Anti-SSA positive, n (%)	6 (75)	
Anti-SSB positive, n (%)	5 (62)	
RF (U/ml)	46 (11-60)	
Positive salivary gland biopsy, n (%)	8 (100)	
ACR-EULAR score (range 0-9)[a]	8 (6-9)	
Time since diagnose, years	6 (3-10)	
Time since onset of symptoms, years	14 (9-20)	
ESSDAI – total (range 0-123), median (IQR)	6 (3-8)	
ESSPRI – total (range 0-10), median (IQR)	5 (5-6)	
ESSPRI subscale score (range 0-10), median (IQR)		
ESSPRI – dryness	7 (6-7)	
ESSPRI – fatigue	8 (4-8)	
ESSPRI – pain	2 (1-6)	
Previous use of DMARDs, n (%)	5 (63)	
Corticosteroids	2 (25)	
Hydroxychloroquine	2 (25)	
Abatacept	3 (38)	
Rituximab	1 (12)	

Data are presented as median (IQR), or number (%). RF: Rheumatoid factor, ACR: American College of Rheumatology, EULAR: European League Against Rheumatism, ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index, ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index, DMARD: Disease-Modifying AntiRheumatic Drugs.

[a] patients fulfil the 2016 ACR-EULAR criteria when the score is 4 or higher (Shiboski et al., DOI: 10.1002/art.39859).

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