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Original article

Systemic interferon type I and type II signatures in primary Sjögren's syndrome reveal differences in biological disease activity

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Abstract

Objective. To assess the relationships between systemic IFN type I (IFN-I) and II (IFN-II) activity and disease manifestations in primary SS (pSS).

Methods. RT-PCR of multiple IFN-induced genes followed by principal component analysis of whole blood RNA of 50 pSS patients was used to identify indicator genes of systemic IFN-I and IFN-II activities. Systemic IFN activation levels were analysed in two independent European cohorts ($n = 86$ and 55, respectively) and their relationships with clinical features were analysed.

Results. Three groups could be stratified according to systemic IFN activity: IFN inactive (19–47%), IFN-I (53–81%) and IFN-I + II (35–55%). No patient had isolated IFN-II activation. IgG levels were highest in patients with IFN-I + II, followed by IFN-I and IFN inactive patients. The prevalence of anti-SSA and anti-SSB was higher among those with IFN activation. There was no difference in total-EULAR SS Disease Activity Index (ESSDAI) or ClinESSDAI between the three subject groups. For individual ESSDAI domains, only the biological domain scores differed between the three groups (higher among the IFN active groups). For patient reported outcomes, there were no differences in EULAR Sjögren's syndrome patient reported index (ESSPRI), fatigue or dryness between groups, but pain scores were lower in the IFN active groups. Systemic IFN-I but not IFN-I + II activity appeared to be relatively stable over time.

Conclusions. Systemic IFN activation is associated with higher activity only in the ESSDAI biological domain but not in other domains or the total score. Our data raise the possibility that the ESSDAI biological domain score may be a more sensitive endpoint for trials targeting either IFN pathway.

Key words: Sjögren's syndrome, interferon type I, interferon type II, fatigue

Rheumatology key messages

- Sjögren's patients can be stratified in interferon negative, type I or type I + II positive subgroups.
- Interferon activation patterns could identify subgroups in SS most likely to benefit from targeted treatment.
- Total EULAR SS disease activity index (ESSDAI) score is no appropriate end point for trials targeting interferon pathways in SS.

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BASIC SCIENCE

Introduction

Primary SS (pSS) is characterized by lymphocytic infiltrations in salivary and lachrymal glands. This is accompanied by sicca symptoms and frequently also extraglandular manifestations [1–3]. Treatment is mainly symptomatic, and biologics so far have shown limited efficacy.

IFNs play a pivotal role in the pathogenesis of pSS. The presence of IFN-induced gene expression has been demonstrated in the salivary glands, peripheral blood mononuclear cells, isolated monocytes and B cells of pSS patients [4–8]. This so-called IFN type I signature was associated with higher disease activity and higher levels of autoantibodies [9]. Recent findings also show activation of IFN type II-induced gene expression in salivary glands of pSS patients [10, 11]. To our knowledge detailed analysis of modular IFN type I and II activation patterns in a large cohort of pSS patients has not been performed.

Type I and II IFN bind to different receptors, but induce partially overlapping gene expression patterns. Therefore, it is difficult to determine which type of IFN triggers the IFN-induced gene expression pattern observed in pSS. However, understanding the relative contribution of IFN type I and II may deepen our knowledge in pSS pathogenesis and promote a stratified approach to therapeutic development.

Systemic type I IFN activation has been extensively characterized in SLE. In clinical trials blocking of IFN type I had limited efficacy, possibly due to unopposed type II IFN activation [12–14]. In SLE, Chiche *et al.* have reported three strongly upregulated IFN-annotated modules (M1.2, M3.4 and M5.12) from peripheral blood transcriptomic data. Each of these modules has a distinct activation threshold [15]. The M1.2 transcriptional module was induced by IFNα, while both M1.2 and M3.4 transcripts were upregulated by IFNβ. M5.12 was poorly induced by IFNα and IFNβ alone. Transcripts belonging to M3.4 and M5.12 were only fully induced by a combination of type I and II IFNs and displayed a more dynamic pattern when studied over time in SLE. Interestingly, M5.12 was mainly upregulated in SLE patients with high disease activity and correlated with renal flares. These data indicate that detailed modular analysis for pSS can contribute to the discovery of better biomarkers and development of stratified therapeutic intervention.

Fatigue is a major complaint in pSS patients [16–20] and is associated with a poor quality of life [21]. Patients receiving IFNα treatment for viral hepatitis can develop severe fatigue [22] and in rare cases also develop pSS-like symptoms [23–25]. Here we investigate a possible correlation between IFN activation and fatigue.

In this study, we performed a detailed analysis, using the IFN annotated modules described for SLE, in two large clinically well-characterized pSS cohorts—the United Kingdom Primary Sjögren's Syndrome Registry (UKPSSR) and the Rotterdam (The Netherlands) cohort. Furthermore, we assessed the relationships between these IFN modules and fatigue as well as other clinical features.

Methods

Patient recruitment

PSS patients and healthy controls (HCs) from the UK cohort were from the UKPSSR collected in 30 centres [26]. PSS patients and HC from the Rotterdam cohort were recruited at the Erasmus Medical Centre, Rotterdam, The Netherlands. All pSS patients fulfilled the 2002 American–European Consensus Group classification criteria [27]. Disease activity was assessed using EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and Clinical ESSDAI (ClinESSDAI) [28, 29]. HCs did not suffer from autoimmune disease or use corticosteroid. Characteristics of patients are summarized in supplementary Table S1, available at *Rheumatology* online. Written informed consent was obtained from all participants in the study, in compliance with the Declaration of Helsinki. The Medical Ethical Review Committee of the Erasmus MC and North West Research Ethics Committee approved this study.

Blood collection, measurement of laboratory parameters and RT-PCR

Blood was collected in clotting tubes for serum preparation and in PAXgene RNA tubes (PreAnalytix, Hombrechtikon, Switzerland) for whole blood RNA analysis. RNA isolation, cDNA preparation and RT-PCR were performed according to the manufacturer's protocol. See supplementary methods, available at *Rheumatology* online, for extended protocols.

Calculation of IFN score for each module

To identify correlated groups of genes and reduce data complexity, the expression of IFN-inducible genes (from M1.2, M3.4 and M5.12) was added to a principle component analysis. Kaiser–Meyer–Olkin measure of sampling adequacy were respectively 0.882, 0.907 and 0.888 for M1.2, M3.4 and M5.12. In order to assess the amount of variance explained by each factor, eigenvalues were extracted.

The IFN score for each module was defined by the relative expression of five indicator genes. For M1.2 these genes were *IFI44*, *IFI44L*, *IFIT1*, *IFIT3* and *MxA*; for M3.4, *ZBP1*, *EIFAK2*, *IFIH1*, *PARP9* and *GBP4*; and for M5.12, *PSMB9*, *NCOA7*, *TAP1*, *ISG20* and *SP140*. Mean and S.D._{HC} of each gene in the HC group were used to standardize expression levels. IFN scores per subject represent the sum of these standardized scores, calculated as previously described [32, 33]. Patients were divided into groups that were positive or negative for M1.2, M3.4 or M5.12, using a threshold of mean HC + 2 × S.D. HC.

Assessment of fatigue and depressive symptoms

In the UK cohort, fatigue was assessed using the profile of fatigue and discomfort-Sicca symptoms inventory (PROFAD-SSI), visual analogue scale (VAS) for fatigue and the EULAR

Sjögren's syndrome patient reported index (ESSPRI) [28, 34, 35]. In the Rotterdam cohort fatigue was assessed using the Dutch version of the multidimensional fatigue inventory (MFI) [36]. Depressive symptoms were assessed using the hospital anxiety and depression scale (HADS) for the UK cohort and the Dutch-validated Center for Epidemiologic Studies Depression scale (CES-D) for the Rotterdam cohort [37, 38].

Statistics

An independent *t* test was used to compare means and the Mann–Whitney *U* test was used to compare medians. Categorical data were compared using Fisher's exact test and correlations were assessed using Spearman's rho (r_s). Multiple group comparisons were analysed using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test. For extended statistics see supplementary methods, available at *Rheumatology* online.

Results

Presence of IFN annotated modules in whole blood of pSS patients

To select five indicator genes for each of the previously described IFN annotated modules (M1.2, M3.4 and M5.12) [15], 11–16 genes were selected using micro-array data of differentially expressed genes in monocytes of pSS patients (supplementary Table S2, available at *Rheumatology* online; unpublished results, Versnel et al.) [4]. Expression levels of these genes were assessed in 50 pSS patients and 38 HCs (Rotterdam cohort) using RT-PCR and added into a principle component analysis to identify correlated groups of genes in order to reduce data complexity.

Five indicator genes for each module were selected and subsequently determined in a cohort of 86 pSS patients (UK cohort), followed by a replication cohort of 55 pSS patients (Rotterdam cohort). A flow chart summarizing this selection procedure is shown in supplementary Fig. S1, available at *Rheumatology* online. All IFN annotated modular scores were expressed significantly higher in pSS patients than in HCs (supplementary Fig. S2, available at *Rheumatology* online). Furthermore, the three IFN modules strongly correlated with each other as depicted for the UK and Rotterdam cohorts combined ($P < 0.001$) (Fig. 1A).

To study the modular expression over time, the modular IFN scores of 15 pSS patients of the Rotterdam cohort were determined at two different time points. The mean (S.D.) period between two time points was 1.8 (0.8) years. There were no significant differences in M1.2 and M3.4 score between the two time points. In the M5.12 module there was a significant difference in score between the two time points (Fig. 1B).

Of the M1.2 positive patients, 90–96% were also positive for M3.4 and 66–67% were also positive for M5.12 when both cohorts were combined. Only three patients were positive for M3.4 while negative for M1.2 and M5.12. There were no patients positive for M5.12 and negative for M1.2.

In the UK cohort 81, 78, 55, 53 and 19% of the patients were positive for M1.2, M3.4, M5.12, all the modules and none of the modules, respectively (Fig. 1C). In the Rotterdam cohort this was respectively 53, 51, 35, 33 and 47%. The percentage of patients positive for each module was lower in the Rotterdam cohort. Compared with the UK cohort, patients in the Rotterdam cohort used more HCQ (supplementary Table S1, available at *Rheumatology* online). However, there were no differences in IFN scores between patients treated or untreated with HCQ in both cohorts (supplementary Fig. S3A–F, available at *Rheumatology* online).

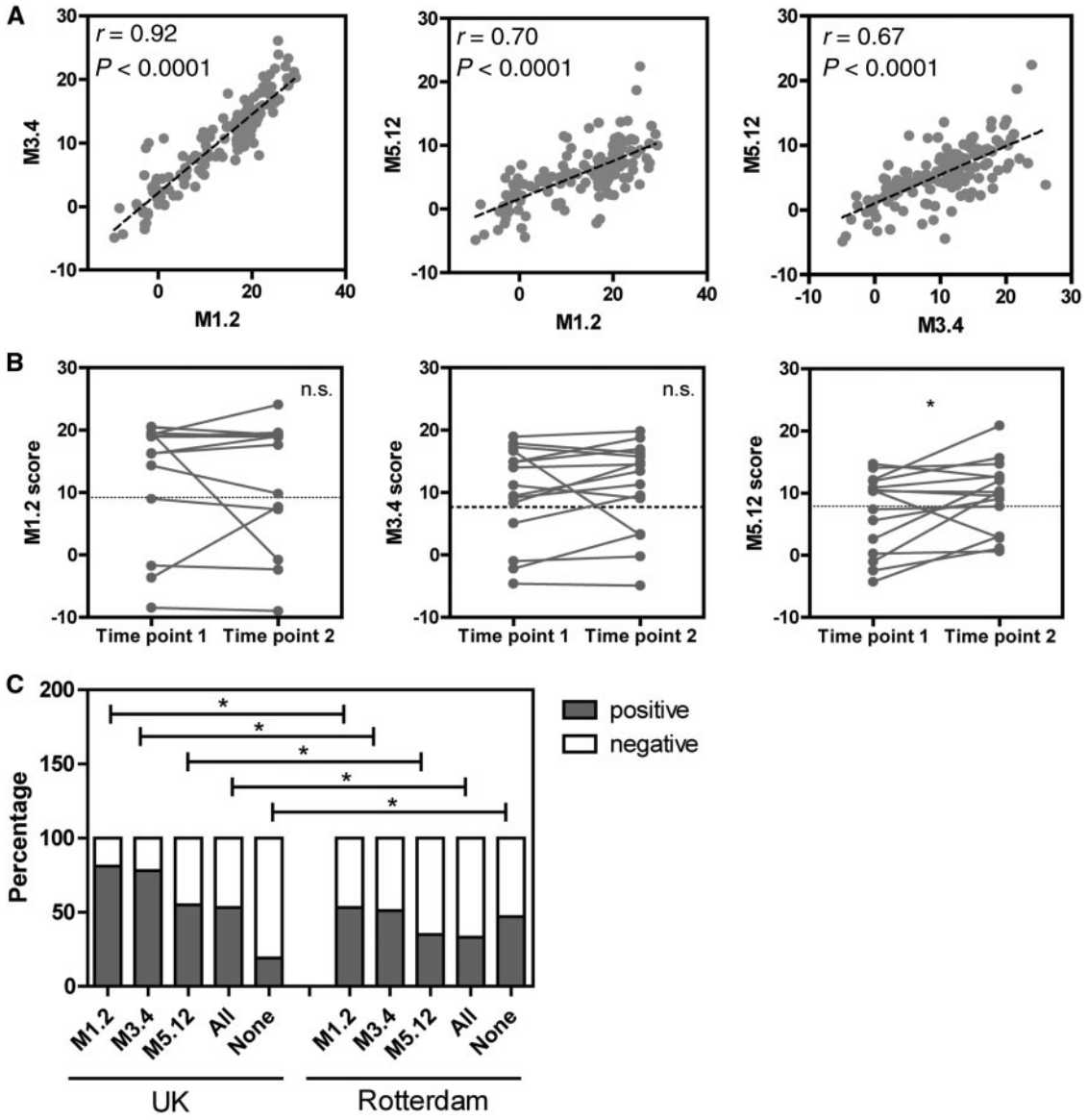
Systemic upregulation of IFN-inducible genes is associated with higher prevalence of autoantibodies

While M1.2 and M3.4 modular gene expression largely overlapped in pSS patients, there was a subgroup that was additionally positive for M5.12. Therefore, pSS patients were subgrouped into patients without (negative for modular IFN activation), IFN type I- (positive for M1.2 only) or IFN type I + II- (positive for M1.2 + M5.12) inducible gene expression. These three subgroups were subsequently investigated for associations with clinical data and functional tests. Functional tests were only available for the UK cohort. Patients with systemic IFN activation (I or I + II) were more often positive for anti-SSA and anti-SSB and had higher IgG levels compared with patients without systemic IFN activation in both cohorts (UK cohort: Table 1, Fig. 2A, D and E; Rotterdam cohort: supplementary Table S3, supplementary Fig. S4A–E, available at *Rheumatology* online). Furthermore, patients with IFN type I + II-inducible gene expression showed significantly higher IgG and ESR levels and lower lymphocyte counts and haemoglobin levels compared with patients with only IFN type I-inducible gene expression (UK cohort: Table 1, Fig. 2A–C). Schirmer's test scores were significantly lower in IFN type I + II positive patients compared with patients without IFN activation (UK cohort: Table 1 and Fig. 2F).

Systemic upregulation of IFN-inducible genes is associated with higher biological disease parameters but not clinical ESSDAI

To investigate differences in disease activity between patients without, with IFN type I- and with IFN type I + II-inducible gene expression, the ESSDAI and its subdomains were compared between the different subgroups. The frequency of pSS patients positive for the biological domain was higher in patients with IFN activation compared with patients without IFN activation (UK cohort: Table 2; Rotterdam cohort: supplementary Table S4, available at *Rheumatology* online). In fact, activity in the biological domain is largely confined to the IFN active groups. The frequency of pSS patients positive for the haematological domain was higher in patients with IFN type I + II-inducible gene expression compared with patients without IFN-inducible gene expression or with only IFN type I-inducible gene expression in the UK cohort. There were no

Fig. 1 Presence of IFN annotated modules in pSS patients from UK and Rotterdam cohort



(**A**) Correlation between modular scores of the UK and Rotterdam cohorts combined (n = 141). (**B**) Modular scores over time in pSS patients (Rotterdam cohort) (n = 15). (**C**) Comparison positivity for modules M1.2, M3.4, M5.12, all modules or none of the modules between the UK cohort and the Rotterdam cohort. Dotted lines indicate positivity threshold for each score. For correlations, Spearman's rho correlation test was used. Wilcoxon signed-rank test to compare dependent medians. Categorical data were compared using Fisher's exact test. * Represents P value of < 0.05, ** represents P value of < 0.005, *** represents P value of < 0.0005.

differences in total-ESSDAI or ClinESSDAI scores between the different subgroups.

Systemic upregulation of IFNs is not associated with fatigue or depression

To investigate if there was a difference in patient-reported symptoms between patients without, with IFN type I- and with IFN type I + II-inducible gene expression, validated questionnaires for fatigue, depression and anxiety were analysed. Patients without IFN activation and those with IFN type I-inducible gene expression had higher pain scores, compared with patients with IFN type I + II-inducible gene expression (Table 3). There were no differences in fatigue, depression or anxiety between the pSS subgroups.

Discussion

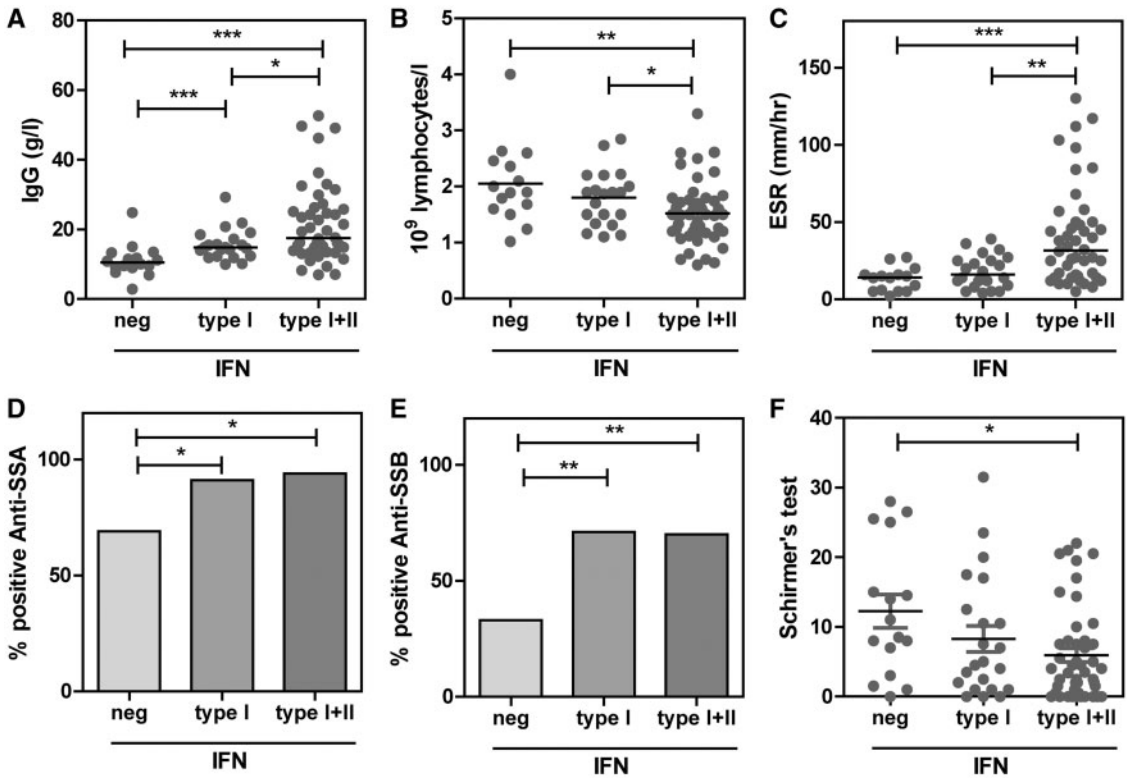
In this study, we show the presence of systemic upregulation of IFN type I and IFN type I + II signatures in two

Table 1 Comparison of laboratory parameters in the UK cohort after stratification on IFN activation

	Neg ($n = 16$)	IFN I ($n = 22$)	IFN I + II ($n = 47$)	*P*-value
Laboratory parameters				
Anti-SSA, *n* (%)	11/16 (69)	20/22 (91)	44/47 (94)	0.026
Anti-SSB, *n* (%)	5/15 (33)	15/21 (71)	32/46 (70)	0.007
IgG, median (IQR), g/l	10.9 (9.1–13.4)	14.9 (12.4–17.9)	18.6 (14.1–26.2)	<0.001
IgA, mean (S.D.), g/l	2.3 (1.8)	2.8 (1.0)	3.4 (1.8)	0.077
IgM, median (IQR), g/l	1.1 (0.9–1.7)	1.1 (0.8–1.5)	1.3 (1.0–1.7)	0.389
C3, mean (S.D.), g/l	1.3 (0.2)	1.2 (0.3)	1.3 (0.2)	0.403
C4, mean (S.D.), g/l	0.3 (0.04)	0.2 (0.06)	0.2 (0.1)	0.179
Hb, mean (S.D.), g/l	13.2 (1.0)	13.4 (1.1)	12.4 (1.1)	0.001
WCC, median (IQR), $\times 10^9$	6.5 (4.4–8.2)	5.7 (4.2–6.8)	5.7 (4.7–6.8)	0.217
Lymphocytes, mean (S.D.), $\times 10^9$	2.0 (0.7)	1.8 (0.5)	1.6 (0.5)	0.007
Neutrophils, median (IQR), $\times 10^9$	3.8 (2.3–5.1)	3.2 (2.3–4.4)	3.1 (2.7–4.3)	0.607
Plt, mean (S.D.), $\times 10^9$	305.8 (70.5)	276.5 (60.6)	264. (66.1)	0.087
CRP, median (IQR), mg/l	3 (2.0–5.0)	5 (2.9–5.0)	5 (2.6–5.0)	0.567
ESR, median (IQR), mm/h	14 (5.0–16.0)	19 (10.5–26.0)	31.5 (15.3–50.0)	<0.001
Functional tests				
Schirmer's test, mean of both eyes, median (IQR)	9.6 (4.0–22.5)	4.8 (1.0–13.6)	3.5 (0.5–8.0)	0.028
Unstimulated saliva flow, median (IQR), ml/5 min	0.7 (0.1–2.9)	0.4 (0.0–1.1)	0.2 (0.0–1.0)	0.274

Means or medians were compared using the one-way ANOVA or Kruskal–Wallis test. Categorical data were compared using Fisher's exact test. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Neg: IFN negative; IFN I: IFN type I; IFN I + II: IFN type I and II; Ig: immunoglobulin; C: complement; Hb: haemoglobin; Plt: platelets; WCC: white cell count.

Fig. 2 Relationship between modular IFN scores and laboratory and functional parameters



IgG levels (**A**), lymphocyte counts (**B**), ESR (**C**), percentage positive for anti-SSA (**D**), percentage positive for anti-SSB (**E**) and Schirmer's test (**F**) in IFN negative ($n = 16$), M1.2 positive (IFN type I inducible) ($n = 22$) or M5.12 positive (IFN type I + II inducible) ($n = 47$) pSS patients. Kruskal–Wallis (**A**, **C** and **F**), one-way ANOVA (**B**) and Fisher's exact test (**D** and **E**) were used to compare multiple groups. Asterisks represent *P*-values: $^*P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

Table 2 Comparison of the ESSDAI and its subdomains in the UK cohort after stratification on IFN activation

	Neg ($n = 16$)	IFN I ($n = 22$)	IFN I + II ($n = 47$)	*P*-value
ESSDAI, median (IQR)	3 (0.5–5.0)	2.5 (0.0–5.0)	4 (0.0–8.0)	0.472
ClinESSDAI, median (IQR)	4 (0.5–6.0)	2 (0.0–4.5)	4 (0.0–9.0)	0.929
ESSDAI domain, *n* (%)				
Constitutional	4/16 (25)	4/22 (18)	10/47 (21)	
Lymphadenopathy	0/16 (0)	2/22 (9)	3/47 (6)	0.879
Glandular	6/16 (38)	2/22 (9)	8/47 (17)	0.489
Articular	7/16 (44)	6/22 (27)	17/47 (36)	0.167
Cutaneous	1/16 (6)	0/22 (0)	2/47 (4)	0.664
Pulmonary	1/16 (6)	1/22 (5)	7/47 (15)	0.660
Renal	0/16 (0)	0/22 (0)	3/47 (6)	0.574
Muscular	0/16 (0)	0/22 (0)	0/47 (0)	0.867
PNS	0/16 (0)	0/22 (0)	2/47 (4)	–
CNS	0/16 (0)	0/22 (0)	0/47 (0)	0.437
Haematological	0/16 (0)	0/22 (0)	7/47 (15)	–
Biological	0/16 (0)	8/22 (36)	23/47 (49)	0.046

Medians were compared using Kruskal–Wallis test. Categorical data were compared using Fisher's exact test. Neg: IFN negative; IFN I: IFN type I; IFN I + II: IFN type I and II; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index.

Table 3 Comparison of fatigue, depression, symptom profile and disease damage index after stratification on IFN activation

	Neg	IFN I	IFN I + II	*P*-value
UK cohort	($n = 16$)	($n = 22$)	($n = 47$)	
SSDDI	7.3 (5.0–7.3)	7.3 (4.0–9.0)	7.0 (3.0–8.7)	0.791
Fatigue VAS	85.0 (75.5–93.5)	77.0 (20.5–87.8)	76 (15.0–84.0)	0.149
PROFAD-Physical	5.5 (4.5–6.0)	5.0 (2.6–6.0)	4.8 (1.8–5.5)	0.122
PROFAD-Mental	4.0 (2.5–5.8)	4.5 (1.0–5.4)	3.5 (0.5–5.0)	0.531
HADS anxiety	6.0 (4.5–7.0)	7.5 (5.0–11.0)	10.0 (5.0–12.0)	0.192
HADS depression	7.0 (3.0–11.0)	8.0 (2.5–10.0)	5.0 (1.0–10.0)	0.322
Total ESSPRI	7.0 (6.2–8.7)	6.7 (4.1–7.6)	5.8 (2.7–7.3)	0.047
ESSPRI sub-domains				
Pain	7.0 (5.0–9.0)	6.0 (2.3–8.0)	3.5 (1.0–7.0)	0.003
Fatigue	8.0 (7.0–9.0)	7.5 (3.5–9.0)	7.0 (2.0–8.0)	0.159
Dryness	7.0 (5.5–8.0)	7.0 (4.0–8.5)	6.0 (3.0–8.0)	0.938
Mental fatigue	7.0 (5.0–8.5)	5 (1.0–8.0)	3.0 (1.0–7.0)	0.058
Rotterdam cohort				
MFI sub-domains	($n = 25$)	($n = 11$)	($n = 19$)	
General fatigue	15.0 (12.0–17.8)	16.0 (13.0–18.0)	14 (2.75)	0.793
Physical fatigue	14.0 (12.0–16.0)	14.0 (10.0–15.0)	13.5 (9.0–20.0)	0.305
Mental fatigue	12.0 (8.0–15.0)	11.0 (5.0–12.0)	10.0 (8.0–15.0)	0.322
Reduced motivation	11.0 (8.0–14.0)	9.0 (5.0–13.0)	9.0 (5.9–11.0)	0.529
Reduced activity	11.0 (7.0–13.0)	11.0 (8.0–16.0)	11.0 (7.0–13.0)	0.941
CES-D	17.5 (8.0–23.5)	12.0 (8.0–20.0)	13.5 (10.0–20.0)	0.760

Data are presented as median (IQR). Medians were compared using Kruskal-Wallis. SSDDI, Sjögren's Syndrome Disease Damage Index; PROFAD, Profile of Fatigue and Discomfort; HADS, Hospital Anxiety and Depression; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; CES-D, Center for Epidemiologic Studies Depression Scale; MFI, Multiple Fatigue Inventory.

large clinically well-characterized European pSS cohorts, using five indicator genes of the previously described IFN annotated modules. IFN type I (M1.2) induced mainly by IFNα, was the most prevalent in both cohorts. IFN type I + II (M1.2 + M5.12), induced by IFNα, IFNβ and IFNγ, was present in ∼66% of the patients positive for IFN type I. Compared with patients without or with only IFN type I-inducible gene expression, pSS patients with IFN type

I + II-inducible gene expression were more often positive for the biological domain of the ESSDAI and had higher levels of IgG, higher ESR and lower lymphocyte counts in the UK cohort. In the Rotterdam cohort IgG levels of patients with IFN type I + II were also higher compared with HCs and there was a trend towards lower lymphocyte counts. There were no differences in patient-reported fatigue or depression between patients with and without systemic IFN activation.

We have previously shown systemic IFN activation in peripheral blood monocytes in a subset of pSS patients [9]. This type I IFN signature correlated with higher anti-SSA/anti-SSB autoantibody frequencies and hypergammaglobulinaemia. Comparison of these genes with the modules we tested in this study revealed that the IFN type I signature genes we used were all of the M1.2 module and thus type I induced. Indeed all patients positive for M1.2 were previously found to have a positive monocytic IFN signature [9].

Until now no detailed studies on the presence of a systemic IFN type II signature in pSS have been performed. A recent study in pSS has reported the presence of systemic type II IFN-induced gene expression, although using different genes from this study [39]. Similar percentages of types I and I + II positive patients were reported. However, 6.8% of the patients were exclusively positive for type II IFNs; in contrast, we did not find patients only positive for M5.12. This difference could be explained by the selection of *GBP1* as a gene mainly induced by IFN type II. According to the modular analysis, which our study was based upon, this gene belongs to the M3.4 module and therefore can also be induced by IFNβ.

The distribution of the modular IFN expression we detect in pSS is very similar to that earlier described for SLE. In pSS as well as SLE, M1.2 is the most prevalent module followed by M3.4 and M5.12 [15]. Additionally, similar to SLE, in pSS patients M5.12 was never upregulated without concomitant upregulation of M1.2 and M3.4. In SLE 87% of the patients showed upregulation of at least one of the modules. In our study 81% in the UK cohort was positive for at least one of the modules and in the Rotterdam cohort 53%. Longitudinal data indicated that in both diseases M5.12 is the module most susceptible to change over time, although our data are based upon a small sample number. A difference between SLE and pSS is that the M3.4 module largely overlaps with the M1.2 in pSS, while in SLE patients this was not observed [15]. The IFN modules correlated with auto-antibodies, anti-dsDNA titres in SLE and anti-SSA/anti-SSB in pSS. M5.12 in SLE correlated with SELENA-SLEDAI scores, flares and the cutaneous domain, and in pSS this module weakly correlated with the pulmonary and renal domain of the ESSDAI (data not shown) in the UK cohort, but not the total-ESSDAI scores. A reason why we did not detect significant differences in total-ESSDAI or most ESSDAI domain scores in pSS could be because extraglandular manifestations in pSS are less frequent than in SLE. Alternatively, IFN activity may be linked only to some but not all extraglandular manifestations.

Anti-inflammatory drugs can affect IFN signatures [40]. In this study, the frequency of patients positive for the modular IFN scores was lower in the Rotterdam cohort compared with the UK cohort. One possible explanation for this could be that patients in the Rotterdam cohort were treated more often with HCQ than patients in the UK cohort. We have shown before that patients treated with HCQ have lower IFN type I scores [40]. In addition, HCQ has been shown to impare IFNα production by plasmacytoid dendritic cells (pDCs) [41]. In this study, we also stratified patients based on HCQ use. Although there appeared to be a trend toward lower IFN type I (M1.2) scores among those taking HCQ, there were no significant differences detected in any of the modular scores. However, this is a cross-sectional study with no data on pre-treatment IFN scores. Moreover, because of the contribution of other IFNs in pSS the overall effect of HCQ may not result in a significant difference in IFN score. Consistently, in SLE it was shown that HCQ treatment only lowered expression of *MxA*, with other interferon-inducible genes such as *OAS1* and *IFI27* being unaffected [42].

Interestingly, there were no significant differences in total-ESSDAI or ClinESSDAI scores between patients without or with type I or type I + II IFN activation, except for the biological domain. This could have significant implications for selection of primary endpoints in clinical trials evaluating novel therapies. For instance, therapies targeting type I or II IFN may improve ESSDAI biological domain score, but have no impact on total-ESSDAI or ClinESSDAI scores. It is also of interest that systemic IFN activity is not associated with disease activity in other ESSDAI organ domains. One possible explanation is that the sample size in this study did not have the power to detect such differences. Another intriguing possibility is that mechanisms other than systemic IFN activation might be responsible for the clinical manifestations in these other organ domains.

Recently, salivary gland analysis of pSS patients revealed a predominant type II activation pattern [11, 39]. Comparing these data with our results on systemic IFN activation we conclude that systemically IFN type I expression dominated over IFN type II expression. This indicates that local and systemic IFN activation patterns within the same patient may differ. Future study of IFN activation patterns in paired samples from peripheral blood and salivary gland tissue of the same patient would be of interest and might help to define the role for systemic IFN activation as a biomarker for pSS.

Blocking systemic IFNα activation in SLE showed a reduction of SELENA-SLEDAI scores in a small subset of patients [13, 14, 43]. Interestingly, *post hoc* analysis revealed a possible effect in patients with low baseline IFN activity. This might be due to a contribution of IFN type II or IFNβ to the pathogenic process. A recent study targeting the Interferon type I receptor (IFNAR) in SLE patients with moderate-to-severe disease showed encouraging clinical effectivity in patients with a high IFN signature at baseline, while patients with low IFN signatures did not respond differently compared with the placebo group [44]. Our findings here in pSS and earlier finding in SLE

show distinct activation patterns (IFNα, IFNβ and/or IFN type II) which all lead to upregulation of IFN-inducible genes. Stratification of patients based on their IFN activation pattern will identify subgroups that are most likely to benefit from a specific targeted treatment. For instance, patients positive for M1.2 and M3.4 could benefit from blocking the IFNAR, while in patients additionally positive for M5.12, blocking the IFN type II pathway (as well as IFNAR blockade) might be necessary.

In contrast to our hypothesis, patients with IFN activation were not more fatigued than those without IFN signatures. This might be caused by the relatively low patient number or the subjective nature of fatigue. However, our data are in line with our previous study showing no correlation between IFN type I score and visual analogue scale fatigue score [45]. Additionally, we showed for the UK cohort lower levels of pro-inflammatory cytokines, among them IFN type II, in highly fatigued pSS patients [46]. These data indicate that fatigue is not directly related to activation of IFN-induced gene expression.

This study has several limitations. First is the study of gene expression levels in peripheral blood cells, instead of in a specific cell type. However, we previously investigated the IFN type I signature in monocytes of pSS patients and identified the same set of signature genes as here in whole blood cells. Also, all patients positive for the monocytic IFN signature were also positive for M1.2 when whole blood cells were collected simultaneously. Another limitation is that the Rotterdam cohort is collected in an academic reference centre and therefore may have a disproportionately higher percentage of atypical pSS patients while the UK cohort is a national biobank with 30 recruitment centres. This may also explain the differences in the prevalence of renal complications between the two cohorts.

Taken together, this study describes the prevalence of systemic (IFN types I and/or type II) activation in pSS. Stratification according to this activation pattern revealed differences in disease features. These data raise the possibility that the biological-ESSDAI rather than total-ESSDAI score may be a more sensitive end point for trials targeting either type I or type II IFN pathway.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Gabriel SE, Michaud K. Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. Arthritis Res Therapy 2009;11:1–16.

2 Fox RI, Howell FV, Bone RC, Michelson PE. Primary Sjogren syndrome: clinical and immunopathologic features. Semin Arthritis Rheum 1984;14:77–105.

3 Asmussen K, Andersen V, Bendixen G, Schiødt M, Oxholm P. A new model for classification of disease manifestations in primary Sjögren's syndrome: evaluation in a retrospective long-term study. J Int Med 1996;239:475–82.

4 Wildenberg ME, van Helden-Meeuwsen CG, van de Merwe JP, Drexhage HA, Versnel MA. Systemic increase in type I interferon activity in Sjögren's syndrome: a putative role for plasmacytoid dendritic cells. Eur J Immunol 2008;38:2024–33.

5 Gottenberg J-E, Cagnard N, Lucchesi C *et al*. Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjögren's syndrome. Proc Natl Acad Sci U S A 2006;103:2770–5.

6 Hjelmervik TOR, Petersen K, Jonassen I, Jonsson R, Bolstad AI. Gene expression profiling of minor salivary glands clearly distinguishes primary Sjögren's syndrome patients from healthy control subjects. Arthritis Rheum 2005;52:1534–44.

7 Emamian ES, Leon JM, Lessard CJ *et al*. Peripheral blood gene expression profiling in Sjogren's syndrome. Genes Immun 2009;10:285–96.

8 Imgenberg-Kreuz J, Sandling JK, Almlöf JC *et al*. Genome-wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. Ann Rheum Dis 2016;75:2029–36.

9 Brkic Z, Maria NI, van Helden-Meeuwsen CG *et al*. Prevalence of interferon type I signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. Ann Rheum Dis 2013;72:728–35.

10 Hall JC, Casciola-Rosen L, Berger AE *et al*. Precise probes of type II interferon activity define the origin of interferon signatures in target tissues in rheumatic diseases. Proc Natl Acad Sci U S A 2012;109:17609–14.

11 Hall JC, Baer AN, Shah AA *et al*. Molecular subsetting of interferon pathways in Sjögren's syndrome. Arthritis Rheumatol 2015;67:2437–46.

12 Bissonnette R, Papp K, Maari C *et al*. A randomized, double-blind, placebo-controlled, phase I study of MEDI-545, an anti–interferon-alfa monoclonal antibody, in subjects with chronic psoriasis. J Am Acad Dermatol 2010;62:427–36.

13 Kalunian KC, Merrill JT, Maciuca R *et al*. A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). Ann Rheum Dis 2016;75:196–202.

14 Petri M, Wallace DJ, Spindler A *et al*. Sifalimumab, a human anti–interferon-α monoclonal antibody, in systemic lupus erythematosus: a phase I randomized, controlled, dose-escalation study. Arthritis Rheum 2013;65:1011–21.

15 Chiche L, Jourde-Chiche N, Whalen E *et al*. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. Arthritis Rheumatol 2014;66:1583–95.

16 Segal B, Thomas W, Rogers T *et al*. Prevalence, severity and predictors of fatigue in primary Sjogren's syndrome. Arthritis Rheum 2008;59:1780–7.

17 Haldorsen K, Bjelland I, Bolstad AI, Jonsson R, Brun JG. A five-year prospective study of fatigue in primary Sjögren's syndrome. Arthritis Res Ther 2011;13:1–8.

18 Theander L, Strömbeck B, Mandl T, Theander E. Sleepiness or fatigue? Can we detect treatable causes of tiredness in primary Sjögren's syndrome? Rheumatology 2010;49:1177–83.

19 Karageorgas T, Fragioudaki S, Nezos A *et al*. Fatigue in primary Sjögren's syndrome: clinical, laboratory, psychometric, and biologic associations. Arthritis Care Res 2016;68:123–31.

20 Overman CL, Kool MB, Da Silva JAP, Geenen R. The prevalence of severe fatigue in rheumatic diseases: an international study. Clin Rheumatol 2016;35:409–15.

21 Champey J, Corruble E, Gottenberg J *et al*. Quality of life and psychological status in patients with primary Sjögren's syndrome and sicca symptoms without autoimmune features. Arthritis Care Res 2006;55:451–7.

22 Udina M, Castellvi P, Moreno EJ *et al*. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. J Clin Psychiatry 2012;73:1128–38.

23 Ojha JBI, Islam N, Cohen DM, Stewart CM, Katz J. Xerostomia and lichenoid reaction in a hepatitis C patient treated with interferon-alpha: a case report. Quintessence Int 2008;39:343–8.

24 Oishi A, Miyamoto K, Kashii S, Yoshimura N. Abducens palsy and Sjögren's syndrome induced by pegylated interferon therapy. Brit J Ophthalmol 2007;91:843–4.

25 Onishi S, Nagashima T, Kimura H *et al*. Systemic lupus erythematosus and Sjögren's syndrome induced in a case by interferon-α used for the treatment of hepatitis C. Lupus 2010;19:753–5.

26 Ng W-F, Bowman SJ, Griffiths B. United Kingdom primary Sjögren's Syndrome Registry—a united effort to tackle an orphan rheumatic disease. Rheumatology 2011;50:32–9.

27 Vitali C, Bombardieri S, Jonsson R *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;61:554–8.

28 Seror R, Ravaud P, Bowman SJ *et al*. EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. Ann Rheum Dis 2010;69:1103–9.

29 Seror R, Meiners P, Baron G *et al*. Development of the ClinESSDAI: a clinical score without biological domain. A tool for biological studies. Ann Rheum Dis 2016;75:1945–50.

30 Beillard E, Pallisgaard N, van der Velden VHJ *et al*. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – a Europe against cancer program. Leukemia 2003;17:2474–86.

31 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ method. Methods 2001;25:402–8.

32 Kirou KA, Lee C, George S *et al*. Coordinate overexpression of interferon-α–induced genes in systemic lupus erythematosus. Arthritis Rheum 2004;50:3958–67.

33 Feng X, Wu H, Grossman JM *et al*. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum 2006;54:2951–62.

34 Goodchild CE, Treharne GJ, Booth DA, Kitas GD, Bowman SJ. Measuring fatigue among women with Sjögren's syndrome or rheumatoid arthritis: a comparison of the Profile of Fatigue (ProF) and the Multidimensional Fatigue Inventory (MFI). Musculoskeletal Care 2008;6:31–48.

35 Shahid A, Wilkinson K, Marcu S, Shapiro CM. Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F). In: Shahid A, Wilkinson K, Marcu S, Shapiro CM, eds. STOP, THAT and One Hundred Other Sleep Scales. New York, NY: Springer New York; 2012: 399–402.

36 Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995;39:315–25.

37 Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 1983;67:361–70.

38 Schroevers MJ, Sanderman R, van Sonderen E, Ranchor AV. The evaluation of the Center for Epidemiologic Studies Depression (CES-D) scale: depressed and Positive Affect in cancer patients and healthy reference subjects. Qual Life Res 2000;9:1015–29.

39 Nezos A, Gravani F, Tassidou A *et al*. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: Contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. J Autoimmun 2015;63:47–58.

40 Maria NI, Brkic Z, Waris M *et al*. MxA as a clinically applicable biomarker for identifying systemic interferon type I in primary Sjögren's syndrome. Ann Rheum Dis 2014;73:1052–9.

41 Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic

cells in systemic lupus erythematosus. Arthritis Res Therapy 2012;14:R155.

42 Olsen NJ, McAloose C, Carter J *et al*. Clinical and immunologic profiles in incomplete lupus erythematosus and improvement with hydroxychloroquine treatment. Autoimmune Dis 2016;2016:9.

43 Khamashta M, Merrill JT, Werth VP *et al*. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. Ann Rheum Dis 2016;75:1909–16.

44 Furie R, Khamashta M, Merrill JT *et al*. Anifrolumab, an anti-interferon-α receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. Arthritis Rheumatol 2017;69:376–86.

45 James K, Al-Ali S, Tarn J *et al*. A transcriptional signature of fatigue derived from patients with primary Sjögren's Syndrome. PLoS One 2015;10:e0143970.

46 Howard Tripp N, Tarn J, Natasari A *et al*. Fatigue in primary Sjögren's syndrome is associated with lower levels of proinflammatory cytokines. RMD Open 2016;2:e000282.