



HAL
open science

Serum interferon-beta level determined by an ultrasensitive electrochemiluminescence immunoassay is increased in clinically active and inactive systemic lupus erythematosus

Karim Dorgham, Omaira da Mata Jardim, Clara Mellot, Suzanne Mouries-Martin, Maude Calixte, Ngoc Khanh Nguyen, Raphael Lhote, Julien Haroche, Fleur Cohen-Aubart, Micheline Pha, et al.

► To cite this version:

Karim Dorgham, Omaira da Mata Jardim, Clara Mellot, Suzanne Mouries-Martin, Maude Calixte, et al.. Serum interferon-beta level determined by an ultrasensitive electrochemiluminescence immunoassay is increased in clinically active and inactive systemic lupus erythematosus. *European Cytokine Network*, 2025, 36 (2), pp.15-23. <10.1684/ecn.2025.0502>. <hal-05116283v2>

HAL Id: hal-05116283

<https://hal.science/hal-05116283v2>

Submitted on 20 Jun 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

RESEARCH ARTICLE

Serum interferon-beta level determined by an ultrasensitive electrochemiluminescence immunoassay is increased in clinically active and inactive systemic lupus erythematosus

Karim Dorgham¹, Omaira Da Mata Jardin¹, Clara Mellot^{1,2}, Suzanne Mouries-Martin^{1,2}, Maude Calixte^{1,2}, Ngoc Khanh Nguyen¹, Raphael Lhote², Julien Haroche^{1,2}, Fleur Cohen-Aubart^{1,2}, Micheline Pha², Miguel Hié², Matthias Papo², François Chasset^{1,2,3}, Pascale Ghillani-Dalbin⁴, Hans Yssel¹, Zahir Amoura^{1,2}, Guy Gorochov^{1,4}, Alexis Mathian^{1,2}

¹ Sorbonne Université, Inserm, centre d'immunologie et des maladies infectieuses (Cimi-Paris), Paris, France

² Groupement hospitalier Pitié-Salpêtrière, AP-HP, centre de référence des maladies auto-immunes et auto-inflammatoires systémiques rares de l'adulte d'Ile-de-France, Centre et Martinique, service de médecine interne 2, Institut E3M, Paris, France

³ Sorbonne Université, service de dermatologie et allergologie, Hôpital Tenon, AP-HP, Paris, France

⁴ Département d'immunologie, groupement hospitalier Pitié-Salpêtrière, AP-HP, Paris, France

Correspondence: Karim Dorgham, Centre d'immunologie et des maladies infectieuses (Cimi-Paris), hôpital Pitié-Salpêtrière, département d'immunologie, bâtiment CERVI, 83, boulevard de l'Hôpital, 75013 Paris, France
<karim.dorgham@inserm.fr>

Article accepted on 01 May 2025

To cite this article: Dorgham K, Da Mata Jardin O, Mellot C, Mouries-Martin S, Calixte M, Khanh Nguyen N, Lhote R, Haroche J, Cohen-Aubart F, Pha M, Hié M, Papo M, Chasset F, Ghillani-Dalbin P, Yssel H, Amoura Z, Gorochov G, Mathian A. Serum interferon-beta level determined by an ultrasensitive electrochemiluminescence immunoassay is increased in clinically active and inactive systemic lupus erythematosus. Eur. Cytokine Netw. 2025; 36(2): 15-23. doi: 10.1684/ecn.2025.0502

ABSTRACT. *Background:* Type I interferons, which play an important role in the pathogenesis of various autoimmune diseases such as systemic lupus erythematosus (SLE), are expressed at very low levels under physiological conditions. In this study, we focused on IFN- β for its potential use as a biomarker of SLE activity and compared three different technologies for its quantification in the serum of healthy donors and patients with SLE. *Methods:* A total of 93 serum samples from healthy donors and 463 serum samples from lupus patients were tested using either ELISA, digital ELISA based on Single Molecule Array (Simoa[®]) technology, or a novel ultrasensitive immunoassay (S-Plex[®]) based on electrochemiluminescence. *Results:* Circulating IFN- β levels were detected in 1.3%, 6.7%, and 100% of healthy donors by Simoa, ELISA, and S-Plex technology, respectively. In patients with SLE, circulating IFN- β levels were detected in 7.5%, 18.8%, and 98.3% by Simoa, ELISA, and S-Plex technology, respectively, demonstrating the utility of the S-Plex technology for quantifying this cytokine in serum. S-Plex-determined serum IFN- β concentrations were higher in patients with SLE than in healthy donors (median, 0.297 pg/mL vs 0.205 pg/mL, $p=0.0004$, respectively), in patients with active SLE compared to those with inactive SLE (0.389 pg/mL vs 0.243 pg/mL, $p=0.0013$, respectively), in patients with severe flare compared to those without flare (0.462 pg/mL vs 0.244 pg/mL, $p=0.0009$, respectively), and in patients not in remission compared to those in remission (0.374 pg/mL vs 0.239 pg/mL, $p=0.0027$, respectively). However, according to ROC curve analyses, S-Plex-determined serum IFN- β levels demonstrated poor diagnostic performances for distinguishing disease clinical status in SLE. *Conclusion:* Using S-Plex technology, circulating IFN- β levels are quantifiable in the serum of healthy donors and are significantly higher in patients with SLE. They increase significantly in patients with clinically active disease. Although IFN- β is a biomarker of SLE activity, its serum levels in patient groups show considerable overlap, making it difficult to reliably distinguish between different states of disease activity.

Key words: interferon beta, systemic lupus erythematosus

Interferons (IFNs) are a family of cytokines identified by Isaacs and Lindenmann in 1957 through their antiviral properties [1]. IFNs are critical mediators in the immune system, providing a rapid response to viral infections and facilitating the development of a more targeted and sustained adaptive immune response. IFNs have also a role in antitumor and immunomodulatory responses. There are three distinct IFN families.

Type I IFN family is a multi-gene group encoding in humans 17 cytokines, including 13 partially homologous IFN- α subtypes, and single gene products for IFN- β , IFN- ω , IFN- ϵ , and IFN- κ . Most cell types produce IFN- β , whereas predominant producers of IFN- α are haematopoietic cells, particularly plasmacytoid dendritic cells. Type II IFN consists of a single gene product, IFN- γ , which is produced predominantly by T and

NK cells. The third class of IFNs is the type III IFN family consisting of four IFN- λ subtypes (IFN- λ 1, 2, 3, and the newly discovered IFN- λ 4). Type III IFNs exhibit functions akin to type I IFNs but demonstrate restricted mucosal activity due to predominantly epithelial cell receptor expression. Even though they bind to distinct receptors, all IFN cytokines signal through the Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway [2].

Physiological levels of circulating IFNs in biological fluids are dissimilar among the distinct subtypes. IFN- γ is reliably quantifiable in the serum of healthy donors by highly sensitive enzyme-linked immunosorbent assays (ELISA), while type I and III IFNs are predominantly undetectable due to their very low serum levels under standard conditions. As type I IFNs play a role in autoimmune diseases like systemic lupus erythematosus (SLE), their serum levels could serve as a biomarker of disease activity [3]. Therefore, ultra-sensitive technologies capable of precisely detecting low-abundance cytokines, like type I IFNs, are essential to address this challenge.

Recently, digital ELISA methods, such as Single Molecule Arrays (Simoa[®]), have achieved remarkable advancements in sensitivity, enabling the detection of proteins at subfemtomolar concentrations. The principle of this technology is based on the isolation of a single magnetic bead carrying enzyme-linked immunocomplexes into femtoliter-sized wells. Fluorescence is measured in hundreds of thousands of microwells simultaneously, and the counting of positive and negative wells generates a digital signal, reflecting the presence or absence of fluorescence, thereby enabling high sensitivity at extremely low concentration levels [4, 5]. A commercial Simoa[®] IFN- α assay developed by Quanterix[™] allows the detection of this cytokine in a proportion of healthy donors with a sensitivity as low as 5 femtogram per milliliter (fg/mL) [6]. A pan-IFN α digital ELISA has also been developed with human monoclonal antibodies (mAbs) isolated from patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) with relatively higher sensitivity [7, 8]. Serum IFN- α levels, measured by digital ELISA, correlated strongly with SLE disease activity [6] and type I IFN gene scores, offering a streamlined approach to assess IFN-I expression in clinical practice [7, 9].

Meanwhile, Meso Scale Discovery[®] (MSD[®]) has developed an ultrasensitive assay platform offering also detection limits in the low fg/mL range. The principle of the latter technology is based on electrochemiluminescence (ECL). The improved sensitivity of the S-Plex assays rely into the combination of a TURBO-TAG[™] with an antibody labeled with TURBO-BOOST[™] reagents, generating more signal than other ECL assays [10, 11].

Both digital Elisa and S-Plex assays report relatively similar performances for serum IFN- α quantification [12], with low limits of quantification (LLOQ), around 16 and 29 fg/mL, for the Simoa[®] IFN- α Advantage Kit from Quanterix[™], and the S-PLEX[®] Human IFN- α 2a Kit from MSD[®], respectively.

To date, mostly correlations between IFN- α and SLE disease activity have been reported [3] and it remains unclear whether IFN- β might also represent a useful

biomarker for SLE diagnosis and monitoring [13]. Moreover, quantification of IFN- β has remained a significant challenge to date.

Here, we compared the performance of three distinct technologies: (i) a high-sensitivity ELISA, (ii) a home-made digital ELISA based on Single Molecule Array (Simoa[®]), and (iii) a newly developed ultrasensitive assay based on ECL signals, for their ability to quantify IFN- β proteins in human serum. These methods were tested on serum samples from healthy donors and SLE patients.

METHODS

Study design and patients

This retrospective longitudinal study was conducted from February 2011 to October 2019 at the Reference Center for Rare Systemic Autoimmune and Autoinflammatory Diseases of Adults in Ile-de-France, Paris, France, regrouping out- or inpatients with active or quiescent, untreated or treated SLE. Blood samples were randomly obtained from patients diagnosed with SLE according to the 1997 American College of Rheumatology criteria for SLE classification or the 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for SLE [14, 15]. Known or suspected infection on day 0, or increased doses of hydroxychloroquine, prednisone and/or immunosuppressants within 4 weeks prior to day 0 were considered exclusion criteria. The Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) version of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), [16-18] the SLEDAI-2K, [19] and the therapeutic regimen were recorded on the day blood was drawn. The term “clinical” SLEDAI (cSLEDAI) refers to symptoms, signs and routine laboratory testing and disregards only scores contributed by the presence of anti-dsDNA antibodies and/or low complement [20]. We defined patient subgroups based on disease activity using different definitions. According to their cSLEDAI scores, patients were divided into groups with *inactive* (cSLEDAI=0) or *active SLE* (cSLEDAI \geq 1 or suffering from clinical manifestations related to SLE, but not recorded in the SLEDAI [e.g. acute transverse myelitis, autoimmune hemolytic anemia...]). The presence of a *severe* or *mild/moderate lupus flare* was recorded according to the SELENA-SLEDAI flare instrument [17, 18]. Alternatively, two disease activity statuses were defined, according to the definition of remission in SLE (DORIS) [21] without physician global assessment (PGA) [22], serum 50% hemolytic complement activity (CH50) and C4 analysis:

- *patients in remission*: cSELENA-SLEDAI and cSLEDAI-2K=0, no clinical manifestations related to SLE and not recorded in the SLEDAI score [e.g., myelitis, hepatitis, scleritis, autoimmune hemolytic anemia...], prednisone \leq 5 mg/day. Immunosuppressant and hydroxychloroquine were allowed;
- *patients not in remission*: cSELENA-SLEDAI or cSLEDAI-2K>0 or clinical manifestations related to SLE but not recorded in the SLEDAI score and/or prednisone >5mg/day.

Based on these definitions, serum IFN- β levels were analyzed in patients with SLE depending on (i) whether the disease was active or inactive, (ii) the presence or absence of a flare, and (iii) the classification of the remission status.

Blood samples from 93 age- and sex-matched healthy donors (Etablissement Français du Sang, Île-de-France, Hôpital Pitié-Salpêtrière) collected during the same period were included in the study.

Blood was centrifuged at 3,000 rpm for 10 minutes and serum was separated and conserved at -80°C until analysis. The study was approved by the hospital ethics committee (CPP 30052012) and the ethical committee of Sorbonne Université (CER2021-099), and informed consent was obtained from all participants. The research was carried out in compliance with the Helsinki Declaration.

Simoa IFN- β development

Serum IFN- β concentrations were quantified by digital ELISA using the Simoa[®] HD-1 Analyser and Quanterix[™] Homebrew kits, developed as previously reported [23], with minor modifications. The 710669-9 mouse monoclonal antibody (PBL Assay Science) was used as a capture antibody coated on paramagnetic beads at 0.2 mg/mL, and the 710323-9 mouse monoclonal antibody (PBL Assay Science) was biotinylated (biotin/antibody ratio = 40/1) and used as the detector antibody at 3 μ g/mL in a two steps assay, in combination with 50% Simoa Helper Beads and 50 pM of streptavidin- β -galactosidase (SBG). The human IFN- β 1a mammalian recombinant protein (PBL Assay Science) was used as calibrator, and 100 μ L of 1:4 dilution of serum in Simoa[®] Homebrew sample diluent B were used to quantify IFN- β concentrations. We determined that the limit of detection (LOD) of the assay, defined by the mean blank average enzyme per bead + 2.5 SD of all runs, was 157 fg/mL and that the lower limit of quantification (LLOQ), determining the concentration at which the coefficient of variation (CV) of the measurement is < 20%, was 304 fg/mL. Therefore, considering the 1:4 serum dilution, the analytical LOD and LLOQ were 0.628 and 1.216 pg/mL, respectively. The dynamic range of the assay was 1.2 – 200 pg/mL, and the concentration of each sample was calculated based on the four-parameter logistic fitting model generated with the standards. This test was subsequently referred to as “Simoa” throughout the manuscript. It should be noted that the antibody pair used in this assay is the same as that used in the IFN- β ELISA described below.

High sensitivity IFN- β ELISA

The VeriKine-HS Human Interferon Beta Serum ELISA Kit (PBL Assay Science) was used according to the manufacturer’s instructions. The absorbance was measured at 450 nm on a Multiskan EX plate reader (Thermo Scientific). The estimated LOD of the assay was 0.8 pg/mL, the LLOQ sensitivity 1.2 pg/mL, and the dynamic range 1.2 – 150 pg/mL. The concentration of each sample was calculated based on the four-parameter logistic fitting model generated with the calibrators.

This test was subsequently referred to as “ELISA” throughout the manuscript.

S-PLEX[®] Human IFN- β Kit

S-PLEX is an ultra-sensitive assay platform developed by Meso Scale Discovery[®]. The assay was performed according to manufacturer’s protocol. Briefly, streptavidin-coated S-PLEX plates were incubated with the biotinylated capture antibody before calibrators and samples addition in a blocking solution. In a third step, the TURBO-BOOST antibody solution was added into each well, before incubation with the enhance solution. Next the plate was incubated with the TURBO-TAG detection solution at 27 °C for 1 hour. Finally, MSD GOLD Read Buffer B was added and ECL signals were detected by MESO[™] QuickPlex SQ 120 plate reader (MSD[®]), kindly provided by MSD, and analyzed with Discovery Workbench Software (v4.0, MSD). According to the manufacturer’s data, the LOD of the assay was 17 fg/mL, the LLOQ sensitivity 64 fg/mL, and the dynamic range 0.064 – 81 pg/mL. The concentration of each sample was calculated based on the four-parameter logistic fitting model generated with the standards (concentration was determined according to the certificate of analysis provided by MSD). This test was subsequently referred to as “S-Plex” throughout the manuscript.

Statistical analysis

Quantitative parameters are expressed as the mean \pm SD or median (range), as appropriate. Statistical differences between groups were tested with the Mann-Whitney U test. Spearman’s correlation coefficients were computed for quantitative values. Values below the LOD were arbitrarily assigned as the LOD value for graphical representations and statistical analyses. The diagnostic performances of the S-Plex–determined serum IFN- β concentrations to detect SLE disease clinical status were investigated by analyzing receiver operating characteristic (ROC) curves, with clinical assessed disease activity as the gold standard for those analyses. The following activity status were successively analyzed: lupus in remission vs. lupus not in remission, clinically inactive lupus vs. active lupus, and lupus without flare vs. lupus with a flare. The areas under the ROC curves (AUCs) were calculated to differentiate SLE in remission vs. not in remission, inactive SLE vs. active SLE, SLE without flare vs. no flare, according to the S-Plex–determined serum IFN- β concentrations. All tests were 2-sided, and p values less than 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism software (v8.0.1).

RESULTS

Comparison of IFN- β detection by SIMOA, ELISA and S-Plex in healthy controls

Out of 93 healthy control (HC) samples, 80, 45, and 76 were assayed using Simoa, ELISA, or S-Plex assay, respectively. Serum IFN- β levels were tested

simultaneously with the three assays in 38 samples. Results are shown in *table 1* and *figure 1*.

Aberrant serum IFN- β concentrations (5.083 and 5.215 pg/mL) were measured by ELISA and S-Plex, respectively, in one healthy control sample that was considered an outlier and was discarded from subsequent analyses. Serum IFN- β levels were above limits of detection (LOD) of Simoa, ELISA or S-Plex in 1 (1.3%), 3 (6.7%) and 76 (100%) HC samples, respectively.

Comparison of IFN- β quantification by SIMOA, ELISA and S-Plex in patients with SLE

A total of 463 patients (416 women, 47 men, mean \pm SD age of 39.6 \pm 12.9 years) were included. Serum IFN- β levels were assessed in 454, 128, and 290 sera by using Simoa, ELISA, or the S-Plex assay, respectively. Results are shown in *table 2* and *figure 2*. IFN- β was detected above LOD in 7.5%, 18.8% and 98.3% of samples when measured by Simoa, ELISA and S-Plex, respectively. IFN- β was reliably quantified above LLOQ in 4.6%, 14.8% and 95.5% of samples, when assayed by Simoa, ELISA and S-Plex, respectively.

For samples above LLOQ, S-Plex-determined serum IFN- β concentrations were correlated with those measured by ELISA (n=15, r=0.69, p=0.005) but not with those measured by Simoa (n=14, r=0.32, p=0.260). Seventy-three sera were assessed simultaneously for IFN- β levels by the three assays. Cytokine concentrations above the LLOQ were detected concurrently by all three assays in only five samples (*figure 2C*).

Altogether, the S-Plex assay proved to be the most effective method for measuring IFN- β levels in the serum of healthy controls and patients with SLE, when compared to the Simoa and ELISA assays employed in this study.

Increased serum IFN- β levels in patients with SLE

S-Plex-determined IFN- β levels were then compared in patients with SLE (n=290) and healthy subjects (n=75). The baseline characteristics of the patients are described in *table 3*. Approximately 40 % of the patients had a clinically active SLE.

Table 1.

Descriptive statistics of IFN- β levels in the serum of healthy controls.

Assay	Simoa	ELISA	S-Plex
Limit of detection (LOD), pg/mL	0.628	0.800	0.017
Lower limits of quantification (LLOQ), pg/mL	1.216	1.200	0.064
Number of samples assessed, n	80	45	76
Detected, n (%)	1 (1.3)	3 (6.7)	76 (100)
Outlier, n	0	1	1
Median, pg/mL	<LOD	<LOD	0.204
Range, pg/mL	<LOD-0.733	<LOD-2.563	0.065-1.816

LOD, limit of detection

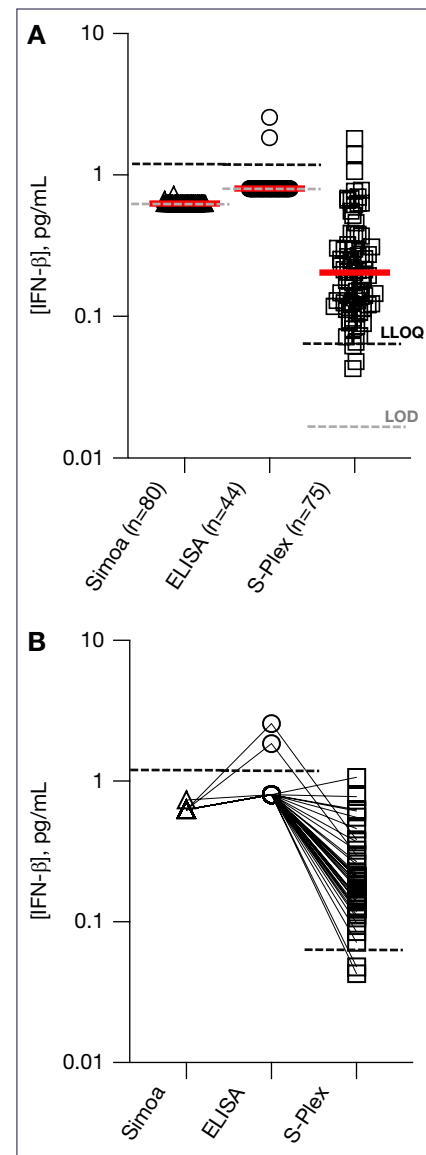


Figure 1

Detection of serum IFN- β in healthy controls. **A)** Black dotted lines indicate lower limits of quantification (LLOQ) using Simoa (1.216 pg/mL), ELISA (1.200 pg/mL) or S-Plex (0.064 pg/mL) assays, while grey dotted lines indicate limits of detection (LOD: 0.628, 0.800 and 0.017 pg/mL, respectively). Red bars indicate median values. LOD value was used when IFN- β was undetectable. **B)** Thirty-eight sera were tested simultaneously using the three assays. Each triangle, circle, or square represents a serum sample.

Table 2.

Descriptive statistics of IFN- β levels in the serum of patients with SLE.

Assay	Simoa	ELISA	S-Plex
Number of samples assessed, N	454	128	290
Detected (%)	34 (7.5)	24 (18.8)	285 (98.3)
Median (pg/mL)	<LOD	<LOD	0.297
Range (pg/mL)	<LOD-54.297	<LOD-54.400	<LOD-11.451

Serum IFN- β concentrations in patients with SLE (median 0.297 pg/mL, [IQR: 0.183–0.624]) were significantly higher than those in healthy controls (median 0.205 pg/mL [0.123–0.371]; p=0.0004) (*figure 3A*). Using the S-Plex assay, a positivity threshold was established

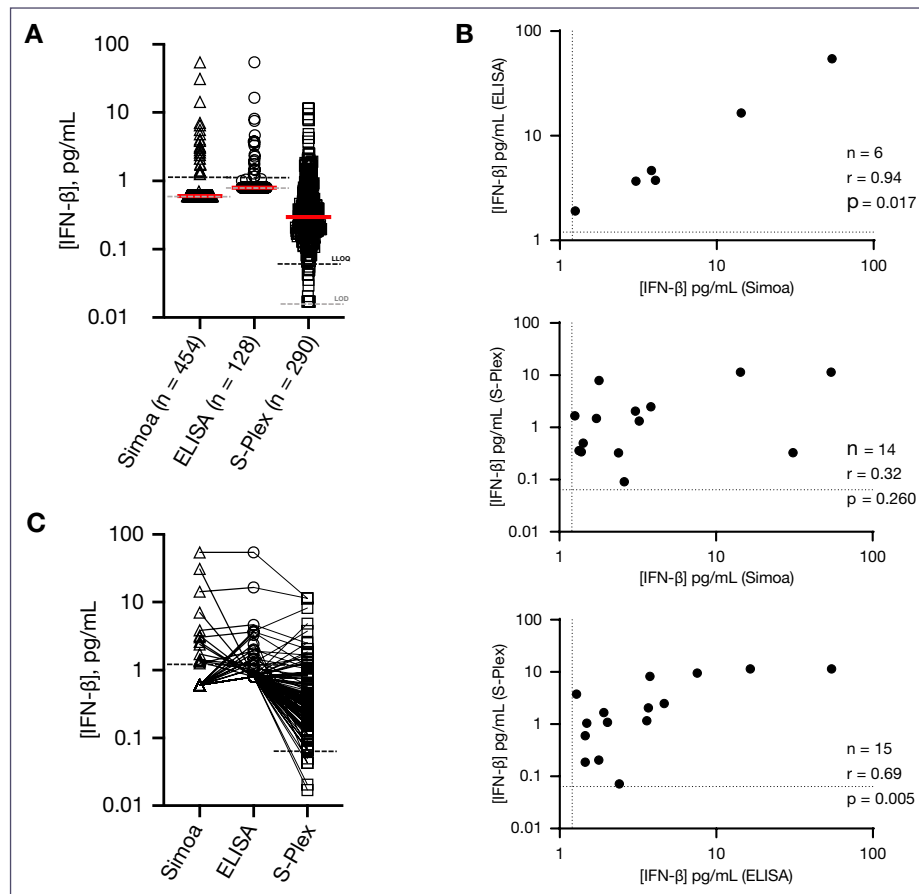


Figure 2

Detection of serum IFN- β in patients with SLE. **A)** Black dotted lines indicate lower limits of quantification (LLOQ) using Simoa (1.216 pg/mL), ELISA (1.200 pg/mL) or S-Plex (0.064 pg/mL) assays, while grey dotted lines indicate limits of detection (LOD: 0.628, 0.800 and 0.017 pg/mL, respectively). Red bars indicate median values. LOD value was used when IFN- β was undetectable. Each triangle, circle, or square represents a serum sample. **B)** Correlation of IFN- β levels in serum of patients with SLE when quantified by Simoa and ELISA (upper panel), by Simoa and S-Plex (middle panel), and by ELISA and S-Plex (lower panel). The dotted lines represent the limit of quantification of the assays. **C)** Seventy-three sera tested simultaneously using the Simoa, the ELISA and the S-Plex assays. Each triangle, circle, or square represents a serum sample. Black dotted lines indicate LLOQ.

based on the mean concentration in the serum of healthy controls plus 2.5 times the standard deviation, yielding a value of 1.069 pg/mL ($0.3044 + 2.5 \times 0.3058 = 1.069$ pg/mL). Using that cutoff value, the S-Plex assay was able to measure “elevated” serum IFN- β concentrations (i.e., >1.069 pg/mL) in 2.7% (2/75) of HCs, and 15.2% (44/290) of patients with SLE (figure 3A).

Serum IFN- β is a marker of disease activity.

S-Plex-determined serum IFN- β concentrations in patients with SLE not in remission ($n=142$, median 0.374 pg/mL [0.195–0.875]) were significantly higher than those in patients in remission ($n=148$, median 0.239 pg/mL [0.161–0.453]) ($p=0.0027$), and healthy controls ($p<0.0001$). Concentrations also differed significantly between SLE patients in remission and healthy controls ($p=0.038$). Increased IFN- β levels (i.e., above the positivity threshold value) were found in 8.8% (13/148) of patients in remission and 21.8% (31/142) of patients not in remission (figure 3B). The area under the receiver operating characteristic curve (ROC AUC) for the S-Plex-determined serum IFN- β levels to differentiate between SLE in remission and SLE not in remission was 0.60 (figure 4A).

S-Plex-determined serum IFN- β concentrations in patients with active SLE ($n=120$, median 0.389 pg/mL [0.196–1.029]) were significantly higher than those in patients with inactive SLE ($n=170$ median 0.243 pg/mL [0.163–0.485]) ($p=0.0013$) and healthy controls ($p<0.0001$). Concentrations also differed significantly between patients with inactive SLE and healthy controls ($p=0.026$). Increased IFN- β levels were found in 8.8% (15/170) of patients with inactive SLE and 24.2% (29/120) with active SLE (figure 3C). The ROC AUC for the S-Plex-determined serum IFN- β levels to differentiate between active and inactive SLE was 0.61 (figure 4B).

S-Plex-determined serum IFN- β concentrations in patients with SLE with severe flare ($n=73$, median 0.462 pg/mL [0.189–1.260]) were significantly higher than those in patients without flare ($n=180$, median 0.244 pg/mL [0.165–0.501]) ($p=0.0009$) and healthy controls ($p<0.0001$). S-Plex-determined serum IFN- β concentrations in SLE patients with mild or moderate flare ($n=37$, median 0.351 pg/mL [0.203–0.613]) were not significantly higher than those in patients without flare ($p=0.135$) but significantly higher than those in healthy controls ($p=0.0024$). Finally, elevated IFN- β levels were

Table 3.
Baseline characteristics and disease parameters in SLE patients.

Characteristic	Patients (n=290)
Women	261 (90.0)
Age, yr, mean±SD	38.2±11.9
Disease duration, yr, mean±SD	10.0±8.4
Disease in remission	148 (51.0)
SELENA–SLEDAI score, median (range)	2 (0–36)
SELENA–SLEDAI score ≥4	128 (44.1)
Clinically active SLE	120 (41.4)
Mild/moderate flare*	37 (12.8)
Severe flare*	73 (25.2)
Fever	30 (10.3)
Weight loss or anorexia	17 (5.9)
Lymphadenopathy	24 (8.3)
Active cutaneous lupus	51 (17.6)
Active lupus serositis	24 (8.3)
Active lupus arthritis	68 (23.4)
Active lupus nephropathy	34 (11.7)
Active neuropsychiatric lupus	6 (2.1)
Hydroxychloroquine use	248 (85.5)
Prednisone use	163 (56.2)
Prednisone ≥10 mg/day	49 (16.9)
Immunosuppressive agent use [†]	80 (27.6)
Positive Farr test	155/289 [‡] (53.6)
Positive anti-RNP Abs	99 (34.1)
Positive anti-Sm Abs	51 (17.6)
Positive anti-Ro/SSA 52 Abs	79 (27.2)
Positive anti-Ro/SSA 60 Abs	119 (41.0)
Positive anti-La/SSB Abs	32 (11.0)
Low C3	82/283 [‡] (29.0)

Values are expressed as n (%), unless stated otherwise.

* Defined using SELENA flare instrument.

[†] Excluding antimalarials and prednisone.

[‡] Positive assay/number of patients assessed.

SD, standard deviation; SELENA–SLEDAI, Safety of Estrogens in Lupus Erythematosus: National Assessment version of the Systemic Lupus Erythematosus Disease Activity Index.

found in 8.9% (16/180) of patients without flare, 13.5% (5/37) with mild or moderate flare, and 31.5% (23/73) with severe flare (*figure 3D*). The ROC AUC for the S-Plex–determined serum IFN- β levels to differentiate between an SLE flare from no flare was 0.61 (*figure 4C*).

DISCUSSION

It is now established that under homeostatic conditions, type I IFNs are constitutively secreted at a baseline level, and that lower or higher expression might have pathologic consequences. Patients with type I IFN deficiency are at risk for severe viral infection, and those with prolonged elevated levels exhibit overactivation of the immune response resulting in tissue damage and chronic inflammation [24, 25]. Moreover, type I IFNs are widely implicated in the pathogenesis of autoimmune diseases, foremost among which are SLE, Sjögren's disease, systemic sclerosis, autoimmune myositis, rheumatoid arthritis, and type 1 diabetes [26].

Type I IFN levels in biological fluids have been first assessed indirectly according to their antiviral potency, by quantifying the induced protection of virus-infected cells against death [27, 28]. Type I IFN family members can also be evaluated *ex vivo* by measuring mRNA expression by quantitative real-time PCR (qRT-PCR) in PBMCs or whole blood, thus reflecting expression of their respective proteins [29].

Another way to assess type I IFNs is to study interferon stimulated genes (ISGs) in blood. Several hundred gene transcripts define this IFN signature, and their expression levels are highly correlated. Thus, quantification of a small number of transcripts by qRT-PCR provides a good evaluation of the functional activity of type I IFNs *in vivo* [30]. However, although very sensitive, this technique is not specific of one subtype among type I IFNs [31].

Detection of low concentrations of biomarkers in healthy donors by non-invasive intervention is key to identify those implicated in pathological situation. Thereby, detection of low-expressed cytokines at the physiological state allows to define threshold in healthy controls allowing the identification of pathological or abnormal, disease-associated, levels. As an example, measurement of IFN- α by digital ELISA allowed the definition of its basal expression in a healthy population [6] and a prediction of a risk of relapse in lupus patients [32].

Thus, elevated circulating IFN- α levels are associated with increased clinical severity of viral infections and certain autoimmune diseases. Unfortunately, except for IFN- α , sensitive detection of other type I IFNs is not yet possible. Indeed, the current detection thresholds for IFN- κ , IFN- ω , and IFN- ϵ using standard ELISA exceed 10 pg/mL, which is significantly higher than the expected physiological levels. Therefore, it is essential to deploy ultra-sensitive technologies for the reliable detection of such weakly expressed biomarkers.

IFN- β was undetectable in serum of all healthy donors tested, using a homemade digital ELISA and in most of them using the highly sensitive ELISA distributed by *PBL Assay Science* (*figure 1A*), consistent with previous reports [23, 33, 34]. The lack of sensitivity of the digital ELISA might be relative to the low affinity of the antibodies used in this sandwich immunoassay. In this regard, Rules-Based Medicine (RBM) company developed an immunoassay based on the Simoa[®] bead technology to quantify IFN- β levels (RBM IFN beta - SIMOA) with an improved LLOQ as compared to the home-made digital ELISA described in this work (0.160 and 1.2 pg/mL, respectively). We did not test this new immunoassay under our experimental conditions.

In this study, by using the high-sensitive S-Plex assay from MSD[®] (LLOQ=0.064 pg/mL), IFN- β levels were measured in a cohort of 75 healthy donors, allowing the establishment of a threshold (1.069 pg/mL) above which circulating IFN- β levels can be considered above baseline secretion of this cytokine (*figure 3A*). In fact, the distribution of IFN- β concentrations in the serum of healthy controls is very broad, and its basal secretion [range: 0.043–1.816 pg/mL] is nearly 10 times higher than that of IFN- α previously determined by digital ELISA [range: 0–0.269 pg/mL] [6]. Conversely, the

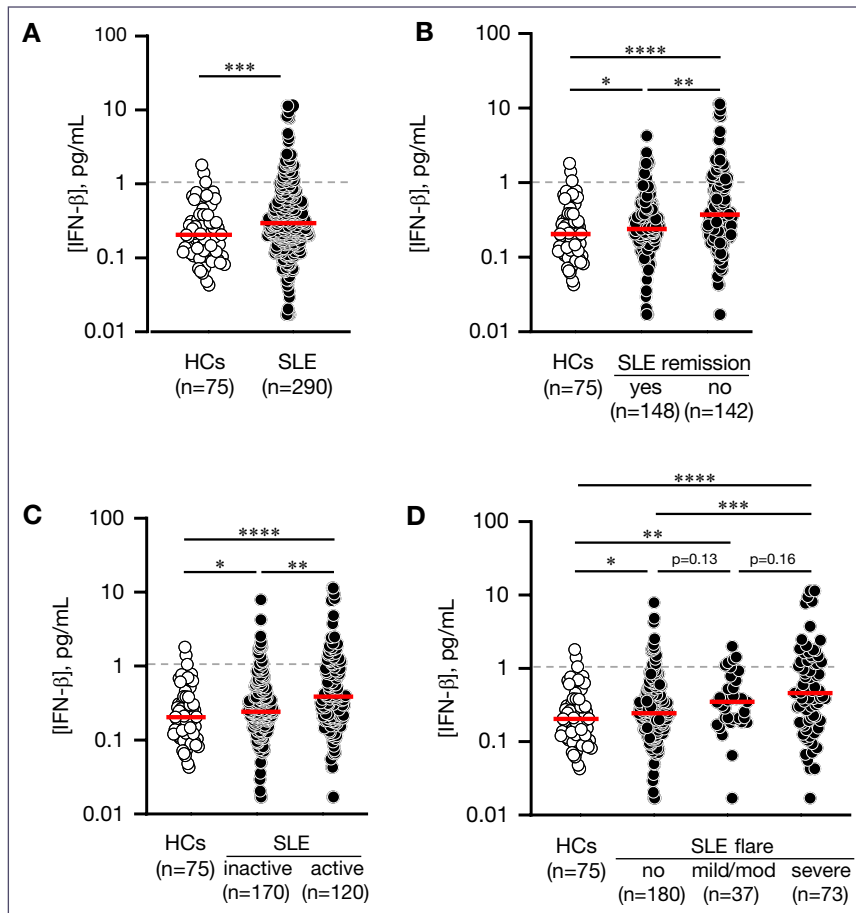


Figure 3

S-Plex-determined serum IFN- β levels in HCs and patients with SLE. **A)** Comparison of serum IFN- β levels in HCs and SLE patients, **B)** in SLE patients in remission or not in remission, **C)** in patients with inactive or active SLE, and **D)** in SLE patients without flare, with mild/moderate flare or with severe flare. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Red bars indicate median values. Each circle represents an individual. The grey dotted lines represent IFN- β positivity threshold calculated as $2.5 \times SD$ above the mean of serum IFN- β concentration from 75 healthy controls.

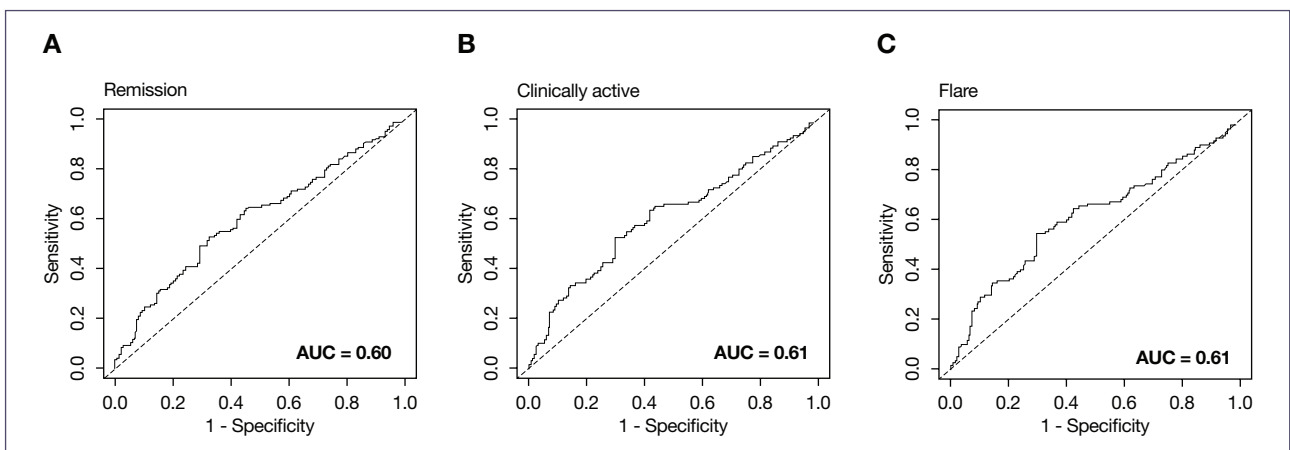


Figure 4

Receiver operating characteristic (ROC) curves of S-Plex-determined serum interferon- β (IFN- β) concentrations to discern active SLE. The diagnostic performances of the S-Plex-determined serum IFN- β levels to detect SLE **(A)** not in remission versus in remission, **(B)** clinically active versus inactive, and **(C)** with no flare or experiencing a flare were investigated by analyzing ROC curves. The area under each ROC curve (AUC) is provided.

distribution of serum IFN- β levels in patients with SLE [range: 0.017–11.450 pg/mL] is narrower compared to that of IFN- α [range: 0.005–>53 pg/mL] [6] (spanning 3 logs versus 4 logs, respectively). This difference may explain why using a cutoff value based on elevated serum IFN- β levels as a biomarker, to discriminate

patients with active SLE, is less effective compared to the measurement of IFN- α [6]. Indeed, IFN- β levels are readily detected in the sera of lupus patients who are in remission, inactive, or without flares. They rise significantly in SLE cases not in remission, with active disease, or in patients experiencing flares, indicating that IFN- β

is a biomarker of SLE activity. However, according to ROC curve analyses, where AUC values were low, S-Plex-determined serum IFN- β concentrations demonstrated poor diagnostic performances for distinguishing disease status in SLE. Thus, the levels of this cytokine in patients, whether in remission or not, active or not, in the absence or presence of flares are too overlapping to reliably discriminate the different states of SLE activity (figure 3). This expression pattern of IFN- β may be explained by the fact that IFN- β exhibits distinct gene expression patterns as compared to IFN- α [29]. IFN- β has also higher affinity than other type I IFNs for the binding to the type I IFN receptor [35] and may also regulate the internalization of the receptor in a different way [36].

To our knowledge, it is the first time that IFN- β concentrations can be measured using an ultrasensitive immunoassay in the serum of HCs and compared to those of patients with SLE, allowing determination of the baseline IFN- β secretion at the protein level. This work highlights the need of ultrasensitive techniques to the measurement of low expressed biomarkers to differentiate abnormal expression levels. Although serum IFN- β levels are elevated in patients with lupus, further studies are needed to refine associations of serum IFN- β levels with organ damage and other biomarkers, such as other IFNs, not only in SLE but also in other autoimmune diseases.

DISCLOSURE

Acknowledgments: The authors thank Fatima Lamraoui, Xavier Huet and Laurent Postolle from Meso Scale Discovery who allowed the use of, and the training on the MESO QuickPlex SQ 120MM instrument in our laboratory; Milena Milutinovic from Quanterix for the Simoa Home-Brew training; Darragh Duffy and Vincent Bondet from Pasteur Institute for their help in developing the digital assay; Dalila Es-Saleh and Juliette Bernardin for their technical assistance; the patients, the healthy donors, the nurses of the Department of Internal Medicine 2 staff who participated in this study.

Funding: This work was supported by Institut National de la Santé et de la Recherche Médicale (Inserm), Sorbonne Université, French Foundation for Medical Research (FRM) Paris, France (FRM EQU202203014622) “Investissement d’Avenir” launched by the French Government and implemented by the Agence Nationale de la Recherche (ANR) with the reference COVIFERON ANR-21-RHUS-08, EU Horizon HLTH-2021-DISEASE-04 UNDINE project, and by the Département Médico-Universitaire de Biologie et Génomique Médicales et Hygiène (DMU BioGeMH), APHP, Paris, France, by FAI²R (filière de santé des maladies auto-immunes et auto-inflammatoires rares), and by association lupus France. AM and CM thank the crowdfunding platform Thellie. MC received a research grant from Agence Régionale de Santé Hauts-de-France and Centre Hospitalier Universitaire Amiens-Picardie as part of a Master’s program.

Conflict of interest statement: AM has participated in advisory board related to lupus for AstraZeneca; received payment for expert testimony for GSK; received support for attending meetings and/or travel from AstraZeneca, GSK and Novartis; received consulting fees, speaking fees and honoraria from AstraZeneca, GSK, Novartis and Otsuka. ZA has received grant/research support from GSK, AstraZeneca, Roche, Novartis, Amgen; participated in advisory board related

to lupus for GSK, AstraZeneca, Kezar, Amgen, Otsuka; received consulting fees, speaking fees and honoraria from AstraZeneca and GSK.

Data availability statement: The data underlying this article will be shared on reasonable request to the corresponding author.

Ethical approval and patient consent statement: The study was approved by the ethical committee of Sorbonne Université (CER2021-099), and informed consent was obtained from all participants. The research was carried out in compliance with the Helsinki Declaration.

REFERENCES

1. Isaacs A, Lindenmann J. Virus Interference .1. The Interferon. *Proc R Soc B Biol Sci* 1957;147:258-67.
2. McNab F, Mayer-Barber K, Sher A, *et al.* Type I interferons in infectious disease. *Nat Rev Immunol* 2015;15:87-103.
3. Baechler EC, Gregersen PK, Behrens TW. The emerging role of interferon in human systemic lupus erythematosus. *Curr Opin Immunol* 2004;16:801-7.
4. Wilson DH, Rissin DM, Kan CW, *et al.* The Simoa HD-1 Analyzer: A Novel Fully Automated Digital Immunoassay Analyzer with Single-Molecule Sensitivity and Multiplexing. *JALA* 2016;21:533-47.
5. Rissin DM, Kan CW, Campbell TG, *et al.* Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol* 2010;28:595-9.
6. Mathian A, Mouries-Martin S, Dorgham K, *et al.* Monitoring Disease Activity in Systemic Lupus Erythematosus With Single-Molecule Array Digital Enzyme-Linked Immunosorbent Assay Quantification of Serum Interferon. *Arthritis Rheumatol* 2019;71:756-65.
7. Rodero MP, Decalf J, Bondet V, *et al.* Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *J Exp Med* 2017;214:1547-55.
8. Llibre A, Bondet V, Rodero MP, *et al.* Development and Validation of an Ultrasensitive Single Molecule Array Digital Enzyme-linked Immunosorbent Assay for Human Interferon-alpha. *J Vis Exp* 2018;(136).
9. Chasset F, Mathian A, Dorgham K, *et al.* Serum interferon-alpha levels and IFN type I-stimulated genes score perform equally to assess systemic lupus erythematosus disease activity. *Ann Rheum Dis* 2022;81:901-3.
10. Poorbaugh J, Samanta T, Bright SW, *et al.* Measurement of IL-21 in human serum and plasma using ultrasensitive MSD S-PLEX[®] and Quanterix SiMoA methodologies. *J Immunol Methods* 2019;466:9-16.
11. Diamandis EP, Stanczyk FZ, Wheeler S, *et al.* Serum complexed and free prostate-specific antigen (PSA) for the diagnosis of the polycystic ovarian syndrome (PCOS). *Clin Chem Lab Med* 2017;55:1789-97.
12. Dorgham K, Calixte M, Da Mata Jardin O, *et al.* Comment On: Association of serum interferon alpha-2a levels with disease severity and prognosis in systemic sclerosis. *Rheumatology* 2025.
13. Shao WH, Shu DH, Zhen Y, *et al.* Prion-like Aggregation of Mitochondrial Antiviral Signaling Protein in Lupus Patients Is Associated With Increased Levels of Type I Interferon. *Arthritis Rheumatol* 2016;68:2697-707.
14. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725-.
15. Aringer M, Costenbader K, Daikh D, *et al.* 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis* 2019;78:1151-9.

16. Bombardier C, Gladman DD, Urowitz MB, *et al.* Derivation Of The Sledai - A Disease-Activity Index For Lupus Patients. *Arthritis Rheum* 1992;35:630-40.
17. Buyon JP, Petri MA, Kim MY, *et al.* The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. *Ann Intern Med* 2005;142:953-62.
18. Petri M, Kim MY, Kalunian KC, *et al.* Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550-8.
19. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288-91.
20. van Vollenhoven R, Voskuyl A, Bertsias G, *et al.* A framework for remission in SLE: consensus findings from a large international task force on definitions of remission in SLE (DORIS). *Ann Rheum Dis* 2017;76:554-61.
21. van Vollenhoven RF, Bertsias G, Doria A, *et al.* 2021 DORIS definition of remission in SLE: final recommendations from an international task force. *Lupus Sci Med* 2021;8(1).
22. Ugarte-Gil MF, Wojdyla D, Pons-Estel GJ, *et al.* Remission and Low Disease Activity Status (LDAS) protect lupus patients from damage occurrence: data from a multiethnic, multinational Latin American Lupus Cohort (GLADEL). *Ann Rheum Dis* 2017;76:2071-4.
23. Llibre A, Bilek N, Bondet V, *et al.* Plasma Type I IFN Protein Concentrations in Human Tuberculosis. *Front Cell Infect Microbiol* 2019;9.
24. Gough DJ, Messina NL, Clarke CJP, *et al.* Constitutive Type I Interferon Modulates Homeostatic Balance through Tonic Signaling. *Immunity* 2012;36:166-74.
25. Davidson S, Maini MK, Wack A. Disease-Promoting Effects of Type I Interferons in Viral, Bacterial, and Coinfections. *J Interferon Cytokine Res* 2015;35:252-64.
26. Crow MK, Ronnblom L. Type I interferons in host defence and inflammatory diseases. *Lupus Sci Med* 2019;6(1).
27. Lebon P, Ponsot G, Aicardi J, *et al.* Early Intrathecal Synthesis Of Interferon In Herpes-Encephalitis. *Biomedicine* 1979;31:267-71.
28. Hooks JJ, Moutsopoulos HM, Geis SA, *et al.* Immune Interferon in the Circulation of Patients with Auto-Immune Disease. *N Engl J Med* 1979;301:5-8.
29. Yao Y, Higgs BW, Morehouse C, *et al.* Development of Potential Pharmacodynamic and Diagnostic Markers for Anti-IFN- α Monoclonal Antibody Trials in Systemic Lupus Erythematosus. *Hum Genomics Proteomics* 2009;2009.
30. Sen GC, Sarkar SN. The interferon-stimulated genes: targets of direct signaling by interferons, double-stranded RNA, and viruses. *Interferon* 2007;316:233-50.
31. Crow MK. Type I Interferon in the Pathogenesis of Lupus. *J Immunol* 2014;192:5459-68.
32. Mathian A, Mouries-Martin S, Dorgham K, *et al.* Ultrasensitive serum interferon-alpha quantification during SLE remission identifies patients at risk for relapse. *Ann Rheum Dis* 2019;78:1669-76.
33. Dorgham K, Quentric P, Gökkaya M, *et al.* Distinct cytokine profiles associated with COVID-19 severity and mortality. *J Allergy Clin Immunol* 2021;147:2098-107.
34. Bolko L, Anquetil C, Llibre A, *et al.* Ultrasensitive interferons quantification reveals different cytokine profile secretion in inflammatory myopathies and can serve as biomarkers of activity in dermatomyositis. *Front Immunol* 2025;16:1529582.
35. Wittling MC, Cahalan SR, Levenson EA, *et al.* Shared and Unique Features of Human Interferon-Beta and Interferon-Alpha Subtypes. *Front Immunol* 2021;11.
36. Marijanovic Z, Ragimbeau J, Van der Heyden J, *et al.* Comparable potency of IFN alpha 2 and IFN ss on immediate JAK/STAT activation but differential down-regulation of IFNAR2. *Biochem J* 2007;407:141-51.