Assessment of type I interferon response in routine practice in France in 2022

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ABSTRACT
An European Alliance of Associations for Rheumatology task force recently recommended specific points to consider for exploring type I interferon pathway in patients, highlighting the lack of analytical assays validated for clinical routine. We report here the French experience on a type I interferon pathway assay that has been set up and used routinely since 2018 in Lyon, France.

Rodríguez-Carrio et al recently recommended specific points to consider for exploring type I interferon (IFN) pathway activation in patients.1 They highlighted the lack of analytical assays validated for clinical routine mentioning the great heterogeneity of the assays with no standardisation, reported in another review from the same group,2 and the lack of clinical validation of these assays.1 We report here the French experience on a type I IFN pathway assay that has been set up and used routinely since 2018 in Lyon, France.3 In 2021, we have been certified as a French Reference Medical Biology Laboratory for this activity. The so-called interferon stimulated genes (ISGs) expression score is based on the measurement of the expression of six ISGs in whole blood; it is reproducible and repeatable demonstrating high diagnostic testing properties.5 Here, we describe our experience in the standardisation process, give a laboratory standpoint and provide an overview of the main clinical indications for which this ISG expression score has been performed in our laboratory last year (2022). We also report the demographic features of the patients tested.

In 2022, our medical laboratory performed 204 ISG expression scores for patients coming from Lyon (n=784), Paris (n=526) and Lille (n=197) university hospitals. These three hospitals host national referee centres for autoimmune/inflammatory disorders, explaining the overrepresentation of patients from these cities. However, it should be noted that samples were sent for testing from most big cities in France (figure 1A) and were all performed in the immunology laboratory of Lyon hospital. As pointed out by Burska et al2 before performing the analysis, the first step is to check that the samples met our preanalytical eligibility criteria. As such, we only processed samples when they arrived within 48 hours after sampling when they were collected on EDTA tubes. When peripheral blood was collected on Paxgene RNA tubes (Qiagen), this delay could be extended to 3 days if samples were kept at room temperature, 5 days at 4°C and weeks at −20°C, in accordance with the manufacturer recommendations. Consequently, 159 (7.3%) samples were not sent for analysis in 2022, mainly due to a delivery time longer than the recommended 48 hours for EDTA tubes. Then, cell lysis is performed the day the samples arrived (8 samples per day on average) and lysed samples are frozen at −80°C until RNA extraction. RNA extraction is carried out by series of 16 samples (representing 2 or 3 extraction runs per week) using maxwell 16 LEV simply RNA blood kit (Promega). After extraction, RNA quality (260/280 and 260/230 OD Ratios) and quantity are checked by spectrophotometry on a NanoVue (Thermofisher). Samples with low quality (260/280 ratio <1.8 and 260/230 ratio <0.8) or low quantity (<20µg/mL) are refused which was quite rare and for some reasons mainly concerned frozen Paxgene tubes. Extracted RNAs were then stored at −80°C until analysis. Our Nanostring-based ISG expression score starts with RNA hybridisation using fluorescent probes targeting the ISGs of interest and housekeeping (HK)
genes. Our assay is composed of six ISGs: SIGLEC1 (sialic acid binding Ig like lectin 1), IFI27 (interferon alpha inductive protein 27), IFI44L (Interferon induced protein 44 like), IFIT1 (interferon induced protein with tetratricopeptide repeats 1), ISG15 (interferon stimulated gene 15) and RSAD2 (radical S-adenosyl methionine domain containing 2). β-Actin, HPRT1 (hypoxanthine phosphoribosyltransferase 1) and POLR2A (RNA polymerase II subunit A) are the three HK genes used. The sequence of all the probes used was published previously.3 The hybridisation step is usually performed overnight during 16–21 hours at 67°C. Finally, the samples can be processed on the nCounter prep station during about 3 hours either fresh or stored at −80°C (depending on the availability of the instrument after the hybridisation step). Samples were analysed 12 at a time, and a home-made internal quality control (IQC) that was prepared by pooling RNA previously analysed with an ISG expression score close to our positivity threshold is included in each run. Immediately after, samples are transferred into the nCounter analyzer for 5 hours. Normalised counts are obtained dividing each ISG absolute count with the geometric mean of the HK genes counts. Then the relative expression is determined for each normalised ISG dividing by the median normalised expression of each ISG from a control group of 34 healthy volunteers. Finally, the median of these 6 ISGs’ relative expression is used to calculate the ISG expression score.

In 2022, 154 extraction runs and 209 Nanostring runs were performed. The whole process involved seven technicians, all empowered to pretreat samples (lysis step) and three of them qualified to perform the following steps (RNA extraction, hybridisation and analysis) and three MD/PharmD. The test is charged €189 at our hospital. As a comparison, the only transcript measurement assay that is reimbursed in France to our knowledge that is bcr-abl quantification, corresponding to the amplification of a single transcript, is charged €124. In average in 2022, it took 11 days from blood collection to the availability of the result. Next strategies for routine practice should try to reduce the number of ISG transcripts reflecting IFN-I to decrease the cost and consider a shorter time to process and validate the results.

Back to the present study, most of the patients were female (F/M ratio=1.8) and under the age of 20 (figure 1B). For each ISG expression score prescribed over this period, we collected the medical context, and we calculated the frequency of positive results (ISG expression score >2.3 corresponding to the average of the scores obtained in healthy volunteers +2 Standard Deviation (SD) in each group. As depicted in figure 1C, the frequency of positive results ranged from 86% for monogenic type 1 interferonopathies to 40% in patients who presented a neuroinflammatory syndrome and/or cerebral calcifications. Reasons for prescribing included help for diagnosis and monitoring of disease activity; but as we did not have access to the complete clinical and biological data for each patient nor the definitive diagnosis, we acknowledge that we cannot use the results to evaluate the sensitivity/specificity of our test but rather to describe the different medical contexts. In line with patients’ demographics, ISG expression scores mainly came from paediatrics departments (46%). Other units included internal medicine (28%), rheumatology (4%), dermatology and neurology (both children and adult, 4.5% and 3.7%, respectively). Intensive care units’ prescriptions (mainly in the context of severe COVID-19) represented 4% for adults and 3% for children (figure 1D).

Reproducibility and repeatability are critical for biological tests used in a clinical setting. To assess the robustness of our assay which is now accredited (ISO15189), we calculated the reproducibility Coefficient of Variation (CV) (repeated measurement of the same IQC over time) which was 7.5% and 7.4% on two different IQCs (figure 1E). The repeatability (calculated for two different ISG expression scores using the same sample measured six times) was also less than 10% even for ‘extreme’ interferon ISG expression scores (0.3 and 160) (figure 1F) making our assay compatible with a routine clinical practice.

Another comment from the European Alliance of Associations for Rheumatology (EULAR) task force was that novel IFN-I pathway assays should specifically monitor IFN-I activity. The selected ISGs in our ISG expression score are derived from the studies of a group of monogenic IFN-I-mediated diseases, Aicardi-Goutières syndromes, among 110 genes previously found over-expressed in the blood of patients with systemic lupus erythematosus (SLE).4 5 This reduce selection of six ISG is, therefore, representative of a significant number of ISGs and the use of the smallest combination of genes by the Nanostring multiplex technology has the advantage to minimise the cost per test. We and others previously showed that the ISG expression score monitored with our assay correlates with the level of plasmatic IFN-α2 (the most studied IFN-α among the 13 IFN-α) as measured using an ultrasensitive technique in a context of acute respiratory infections or various type 1 Interferonopathies including SLE.6 8 However, we found that IFN-γ can also increase the expression of these genes in vitro but to a lesser extent compared with IFN-α stimulation. Some discrepancies were also observed in our lab between weakly positive ISG expression scores in the absence of detectable IFN-α2 suggesting that our ISG expression score might not be as specific of IFN-I activation as originally thought. However, in our assay, IFN-I scores above 20 reflect IFN-I detection and cannot be explained by IFN-γ exposure. We believe that the selected ISGs are suitable for different type 1 IFN-related diseases since they are known to be the most upregulated in both monogenic and non-monogenic type I interferonopathies.5 9 The quantification of all 13 IFN-α at the protein level would overcome this issue. But this technique currently suffers from a limited availability of reagents, which make it incompatible with a routine practice.
Figure 1  (A) Geographical distribution of ISG expression score prescriptions in France in 2022. (B) Demographic features of patients for whom we performed an ISG expression score. (C) ISG expression score according to the indication for which the ISG expression score has been performed. Red line: median ISG expression score for each indication. Black dot line: positive threshold (2.3). (D) Overview of the prescribers of ISG expression score in 2022. (E) Reproducibility of our type I interferon pathway assay. Reproducibility Coefficient of Variation (CV) calculated from repeated measurement of the same IQC over time (two different IQC used, IQC#1 and IQC#2). (F) Repeatability of our type I interferon pathway assay. arb. unit: arbitrary unit. CAPS, cryopyrin-associated periodic syndrome; CTD, connective tissue disease; DM, dermatomyositis; FMF, familial Mediterranean fever; ICU, intensive care unit; IFN, interferon; IQC, internal quality control; JIA, juvenile idiopathic arthritis; PFAPA, periodic fever with aphthosis, pharyngitis and adenitis; PID, primary immunodeficiency; PIMS, paediatric inflammatory multisystem syndrome; RF, recurrent fever; SLE, systemic lupus erythematosus.
In addition, in order to improve the monitoring of type I IFN-related diseases, we believe that the EULAR task force should also consider the measurement of anti-IFN antibodies since they are critical for both severe COVID-19 and autoimmune conditions and they influence the ISG expression score and may reduce the severity of the autoimmune phenotype while predisposing to severe viral infections when they have neutralising properties.10 11 As such, it has been suggested that abnormally elevated IFN-I levels elicit an autoantibodies response that can eventually switch from non-neutralising to neutralising properties in some patients with SLE. Whether this observation can be extended to other non-monogenic or monogenic type I interferonopathies remains to be explored.

Association between type I interferon pathway activation and clinical outcomes in rheumatic and musculoskeletal diseases has already been reviewed.12 We are aware that a standardisation of assays measuring IFN-I pathway activation is essential for their use in clinical practice to facilitate the follow-up of a patient from one centre care to another and between physicians. In the case of the transcriptomic approaches, standardisation first requires that we harmonise the choice of ISGs to use. The extension of the Nanostring technology, not available in all medical centres, to more labs would also facilitate the standardisation through interlaboratory comparisons. In the meantime, the measurement of ISG expression by Real-Time quantitative PCR (RT-qPCR) might be a good alternative as we have shown previously that the two techniques have comparable performance.5 Joint efforts to compare and share protocols are essential to implement the IFN-I monitoring in chronic rheumatic diseases.