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1 **Article type**

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3

4 **Title**

5 2022 EULAR Points to Consider for the measurement, reporting and application of IFN-I
6 pathway activation assays in clinical research and practice

7

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19 syndrome; polymyositis; dermatomyositis; systemic sclerosis;

20

21 **Manuscript content:** 4319 words, 3 tables

22

1 **KEY MESSAGES**

2

3 **What is already known about this subject?**

- 4 • Type I interferons (IFN-I) play a role in a number of rheumatic and musculoskeletal
5 conditions (RMDs)
- 6 • The IFN-I pathway activation can be measured at different levels and using different
7 readouts
- 8 • Assays measuring IFN-I pathway activation have not progressed into clinical practice and
9 uncertainty exists pertaining clinical applications

10

11 **What does this study add?**

- 12 • These are the first EULAR endorsed Points to Consider (PtC) for the measurement and
13 reporting of IFN-I assays in clinical research and practice
- 14 • PtC concerned terminology and reporting practices to promote consistency and
15 harmonization, as well as delineate clinical applications in specific settings

16

17 **How might this impact on clinical practice or future developments?**

- 18 • Implementation of IFN-I pathway assays show a strong potential to improve clinical
19 management in rheumatology and other specialties
- 20 • This consensus document creates a framework for the future implementation of other
21 biomarkers

22

1 **ABSTRACT**

2

3 **Background:** Type I interferons (IFN-I) play a role in a broad range of rheumatic and
4 musculoskeletal diseases (RMDs), and compelling evidence suggests that their measurement
5 could have clinical value, although testing has not progressed into clinical settings.

6 **Objective:** To develop evidence-based Points to Consider (PtC) for the measurement and
7 reporting of IFN-I assays in clinical research and to determine their potential clinical utility.

8 **Methods:** European Alliance of Associations for Rheumatology (EULAR) standardised
9 operating procedures were followed. A taskforce including rheumatologists, immunologists,
10 translational scientists, and a patient partner was formed. Two systematic reviews were conducted
11 to address methodological and clinical questions. PtC were formulated based on the retrieved
12 evidence and expert opinion. Level of evidence and agreement was determined.

13 **Results:** Two overarching principles (OP) and eleven PtC were defined. The first set (PtC 1-4)
14 concerned terminology, assay characteristics and reporting practices to enable more consistent
15 reporting and facilitate translation and collaborations. The second set (PtC 5-11) addressed
16 clinical applications for diagnosis and outcome assessments, including disease activity, prognosis
17 and prediction of treatment response. The mean level of agreement was generally high, mainly in
18 the first PtC set and for clinical applications in systemic lupus erythematosus. Harmonization of
19 assay methodology and clinical validation were key points for the research agenda.

20 **Conclusions:** IFN-I assays have a high potential for implementation in the clinical management
21 of RMDs. Uptake of these PtC will facilitate the progress of IFN-I assays into clinical practice
22 and may be also of interest beyond rheumatology.

1 INTRODUCTION

2 Effects of Type I interferons (IFN-I) range from anti-viral defence to the crosstalk between innate
3 and adaptive immune responses [1]. Due to their immune stimulatory effects, IFN-I and their
4 signalling pathway have gained attention in the breakdown of tolerance and the development and
5 perpetuation of autoimmune and autoinflammatory phenomena. Thus, there is an extensive body
6 of evidence supporting the participation of IFN-I in the pathogenesis of rheumatic and
7 musculoskeletal diseases (RMDs). Compared to other cytokines, type I IFN have been implicated
8 in a wide range of different RMDs [2]. Moreover, this involvement covers the whole disease
9 process, from disease development (and diagnosis) to exacerbation (prognosis) and prediction of
10 therapeutic responses [2]. At the mechanistic level, the IFN pathway activation has been reported
11 to participate from genetic susceptibility to disease perpetuation and progression [2]. Finally,
12 consistent evidence supports the IFN-I pathway as a therapeutic target [3–5]. Taken together, all
13 this evidence asserts a particularly promising role of IFN-I as (multifaceted and multipurpose)
14 biomarkers in rheumatology.

15 The IFN pathway activation can be measured at different levels, including several targets (IFN
16 proteins, transcripts etc) and methods (immunoassays, qPCR etc) reported in the literature. A
17 number of studies have revealed associations between assays measuring IFN-I pathway activation
18 (or IFN-I assays) and clinical features in different RMDs, thereby suggesting potential roles in
19 several clinical applications such as diagnosis, prognosis, prediction of response to therapy and
20 patient stratification. However, results have been heterogeneous and IFN-I assays have largely
21 not progressed into routine clinical practice, with few exceptions mostly in infectious diseases
22 [6]. A key impediment has been the enormous diversity of approaches used for measuring IFN-I
23 pathway activation, which ranged from IFN-I proteins, IFN-stimulated protein scores, the
24 assessment of IFN-stimulated gene expression scores and signatures, to cell-based functional
25 assays. In addition to the intrinsic differences across assay methods, the use of different
26 biological samples, the lack of standardization within each approach as well as the lack of a
27 reference standard for all IFN-I assays have challenged the comparison and synthesis of the
28 results. Under these circumstances, the exact added value of IFN-I measurements and the need of
29 such assays for the clinical setting remains to be established.

30 For these reasons, a EULAR taskforce was convened to elaborate points to consider (PtC) to cover
31 this gap, in order to enable more consistent reporting and facilitate uptake into clinical practice as
32 well as to appraise the current evidence on the clinical value of IFN-I measurements in RMDs to
33 determine potential clinical utility.

1 **METHODS**

2 The EULAR Standardised Operating Procedures (SOP) were followed to produce these PtC [7].
3 After approval from the EULAR Executive Committee, the convenors (MV and EV) together
4 with the methodologist (PC) formed a multi-disciplinary taskforce of 17 members (from 8
5 EULAR countries and the United States of America), including rheumatologists, immunologists,
6 virologists, translational researchers and experts in interferonopathies. Two EMEUNET members
7 and one patient representative (member of PARE) were also involved. A first meeting was held
8 in July 2019 to introduce the project agenda and define the research questions (PICO structure).
9 Systematic literature reviews (SLR) were performed with all the literature published until
10 September 2019.

11 A second meeting (held remotely on two consecutive days in January 2021) was organized to
12 present the evidence collected and after an iterative process, the overarching principles (OP) and
13 PtC were derived.

14 The level of evidence (LoE) for each point was scored according to the Oxford Centre for
15 Evidence-Based Medicine. Furthermore, scorings on the level of agreement (LoA) for each
16 OP/PtC were retrieved by an online survey using a numeric scale (ranging from 0=“completely
17 disagree” to 10=“fully agree”). The final manuscript was reviewed and approved by all taskforce
18 participants.

1 RESULTS

2 Two OP and 11 PtC pertaining the IFN-I measuring and reporting in RMDs were produced (Table
3 1).

4 *A. The IFN pathway is a complex system with multiple subtypes of IFNs and diverse downstream
5 effects on gene and protein expression.*

6 The IFN pathway comprises multiple types of IFNs (IFN-I, IFN-II and IFN-III) and receptors. A
7 total of 16 subtypes can be distinguished within IFN-I proteins: 12 for IFN α , IFN β , IFN κ , IFN ω
8 and IFN ϵ . IFN-II (IFN γ) and IFN-III (IFN λ -1, IFN λ -2, IFN λ -3 and IFN λ -4) have different
9 proteins and receptors. Upon ligation with their shared surface receptor (IFNAR), IFN-Is regulate
10 the expression of hundreds of IFN-stimulated genes (including signalling proteins, transcription
11 factors, cytokines, etc), which have diverse functional effects on multiple cell types [8]. However,
12 there is a large overlap between the signalling pathways and IFN-stimulated genes induced by
13 ligation of IFNAR with the receptors for IFN-II and IFN-III. The composition and intensity of the
14 IFN-stimulated response are dynamic, variable, context-dependent, influenced by multiple other
15 stimuli, degree of activation, duration of the stimuli and negative regulation, and other factors,
16 including the distribution of the receptors. Because of this complexity, care must be taken when
17 planning and describing studies of this pathway.

18 *B. IFN-I pathway activation is a common hallmark in many RMDs. Although IFN-I pathway
19 activation is associated with some clinical manifestations, the utility of IFN-I pathway assays in
20 clinical practice requires further validation for most contexts.*

21 Sustained IFN-I pathway activation has been demonstrated in a wide range of RMDs, with
22 stronger evidence in SLE studies, followed by polymyositis/dermatomyositis (PM/DM),
23 rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), systemic sclerosis (SSc) and anti-
24 phospholipid syndrome (APS). This activation has been demonstrated using different approaches
25 and biological samples in most RMDs [9]. The level of activation differs across conditions. IFN-
26 I pathway activation has been related to several clinical features, but laboratory and clinical
27 methodological issues preclude translation to clinical practice for most contexts. The use of a
28 whole blood four-gene IFN-I gene signature to predict response to anifrolumab is a more strongly
29 validated application. Standardization and clinical validation for other applications are critical
30 clinical unmet needs for future biomarker research. Moreover, it must be noted that IFN-I pathway
31 activation also occurs in immune responses apart from RMDs, so measurements of IFN-I pathway
32 activation should be interpreted with caution and attention must be paid to clinical and biological
33 contexts.

1 *1. Taskforce consensus terminology should be considered for reporting IFN assays measurement.*
2 An important source of heterogeneity in reporting IFN research is the lack of a uniform
3 terminology [10–12]. The current taskforce has developed a consensus-based list of terms to cover
4 key aspects related to IFN measurement and reporting, to ensure comparability in future research
5 efforts (Table 2) [13]. It includes a clear definition of all the elements under the umbrella term of
6 “IFN-I pathway” that we found to be relevant from the biomarker literature (from IFN proteins
7 to IFN-stimulated mediators and effects), whose changes reflect IFN-I pathway activation and
8 thus represent targets of the different assays. This terminology can be applied beyond the field of
9 rheumatology.

10 *2. Existing assays measure different aspects of the IFN pathway; they do not reflect the entirety*
11 *of the pathway and some are not specific for IFN-I. The most appropriate assay will depend on*
12 *the research or clinical question and should be justified.*

13 The IFN-I pathway (Table 2) is a complex, dynamic biological entity encompassing a large
14 number of upstream and downstream processes [11,14,15]. Whether it is important to measure
15 the direct production of IFN-I or its downstream effects (and which ones) should be taken into
16 consideration, depending on the clinical or research question. For example, assays measuring
17 IFN-I proteins directly may not assess all relevant IFN subtypes, and cellular sources, and tissues,
18 nor the strength of downstream effect induced. Whereas on the other hand, assays measuring
19 downstream effects (certain chemokines, sets of IFN-stimulated genes, etc.) may not be specific
20 for IFN-I pathway activation [1] or may differ in their degree of specificity [10,15] and
21 responsiveness to change (see PtC11).

22 Hence, existing assays each only capture a limited aspect of the whole pathway [13]. As such,
23 their readouts and their added value may differ, should not be considered as interchangeable, and
24 must be interpreted in the context of the clinical application. In fact, different assays differ in their
25 associations with clinical outcomes even in the same cohorts [13] [16]. Even though technical
26 advances have allowed the development of highly sensitive and specific assays for some IFN
27 proteins, such as Simoa, such assays still only evaluate part of the pathway and depend on specific
28 antibodies, and their (clinical) superiority cannot currently be established. Therefore, there is not
29 a single gold-standard for IFN-I assays, and the most appropriate assay (or combination of assays)
30 must be chosen (and justified) by a combination of theoretical, experimental, feasibility and
31 clinical evidence requirements. The same applies to sample choice[10]. [1][10,15][10]

32 *3. Publications on novel IFN-I pathway assays should report whether they specifically reflect*
33 *IFN-I, and to the extent possible, which IFN-I is measured.*

1 Assays that evaluate downstream effects of IFN-I may be influenced by multiple IFNs, or other
2 inflammatory mediators [3,10,11] [13]. This is not consistently tested in the literature. For
3 reporting novel assays measuring IFN-I pathway activation, experimental demonstration to what
4 degree they specifically measure IFN-I pathway activation is recommended. An analysis of the
5 comparative effect of other IFN proteins (e.g., IFN-II or γ , and/or IFN-III or λ) as well as non-
6 IFN controls on assays results should be included.

7 *4. For assays that evaluate pathways downstream of the IFN-I receptor (e.g. IFN stimulated gene*
8 *expression or protein scores) the choice of components needs to be justified. For gene expression*
9 *scores, the known subsets of IFN-stimulated genes should be described separately.*

10 Despite the broad use of assays measuring the indirect effects of IFN-I through downstream
11 mediators (IFN-stimulated genes or proteins), a lack of consistency (and thus, replication and
12 validation of clinical associations) was observed for both the choice of gene or protein
13 components analyzed as well as for their combinations [13]. Reasons underlying these choices
14 were not frequently disclosed. Considering that not all downstream mediators are specific for
15 IFN-I, they may differ in their degree of specificity and responsiveness to change [9], results from
16 different IFN-I scores may yield to different results, which has been shown to influence clinical
17 associations [17–20].

18 Therefore, for assays measuring pathway changes downstream IFN-I receptor, the specificity for
19 IFN-I must be proven to the extent possible, and the choice of the actual components (including
20 number of components and their analyses) needs to be justified based on experimental evidence
21 of existing literature demonstrating their specificity and clinical associations. [17–20]

22 *5. IFN-I pathway is consistently activated in several RMDs, but assays measuring IFN-I pathway*
23 *activation cannot be currently recommended for diagnostic purposes.*

24 There is compelling evidence of IFN-I pathway activation in several RMDs compared to healthy
25 controls [14,15,21,22]. The strongest evidence in terms of numbers of studies and assays came
26 from SLE [19,23–26]. SSc [27–30] and pSS [31–34] were also evaluated by different assays,
27 followed by RA [35–38] and PM/DM [39–41], where more consistent evidence was observed for
28 DM compared to PM [9]. However, despite the considerable number of studies, these generally
29 test association in pre-selected groups. We found few well-designed diagnostic studies with
30 appropriate diagnostic statistics, pre-test/post-test probability assessment, the inclusion of disease
31 controls, and replication cohorts. Consequently, most of this evidence was overall judged as
32 having high risk of bias for this application [9]. Further limitations include: (i) IFN-I assays are
33 not specific for RMDs, since IFN-I pathway activation is also observed in viral infections,
34 monogenic interferonopathies and even cardiovascular disease; (ii) IFN-I pathway activation

1 seems to be present in several RMDs with different clinical presentation, so they may differentiate
2 RMDs from normal, but not between specific RMDs; (iii) IFN-I assays only capture a certain
3 aspect of the IFN-I pathway, so a negative IFN-I assay cannot fully rule out the possibility that a
4 patient had an IFN-I pathway activation, perhaps in non-circulating tissues, and variation among
5 assays make difficult the comparison among studies, and (iv) IFN-I activation seems to be present
6 in some patients but not always in a disease population as a whole (see PtC 6). These observations
7 suggest that IFN-I pathway activation assays may be used in combination with other features
8 (clinical signs or autoantibodies) to improve patient diagnosis , but this has received reduced
9 attention in the literature and studies suffered from the same methodological limitations as above.
10 Furthermore, this application may be of limited impact beyond SLE and PM/DM populations,
11 since the level of IFN-I pathway activation is much lower (see PtC6) and thus less likely to aid in
12 diagnosis. Taken together, the use of IFN-I pathway assays for RMDs diagnosis cannot currently
13 be recommended.

14 *6. IFN-I pathway assays define more severe subgroups within many RMDs, so they should be*
15 *considered for stratification studies.*

16 Although several RMDs are hallmarked by a sustained IFN-I pathway activation [14,15,21,22],
17 evidence suggests that the level of activation differs across the RMD spectrum [42,43]. A higher
18 activation in blood has been observed in SLE, followed in order by PM/DM (especially in DM
19 compared to PM), RA, pSS, SSc and APS [42], although methodological differences do not allow
20 firm group comparisons [9]. Overall, patients with IFN-I pathway activation are often associated
21 with more severe clinical features, such as disease activity [10,23,27,32,33,42,44,45], organ
22 involvement [20,24,26,27,46,47], damage [26,48] or glucocorticoid use [49–51], across several
23 RMDs [9]. IFN-I pathway activation was found to have a greater effect than other clinical features
24 in sub-analyses and multivariate analyses, hence confirming an incremental value [20,23,26,52].
25 Further evidence published after the accompanying SLR reconfirmed these findings in
26 observational longitudinal studies [16] as well as in clinical trials [53,54]. Taken together, IFN-I
27 pathway activation is indicated for patient stratification in RMDs.

28 *7. IFN-I pathway activation is associated with disease activity in some RMDs, especially SLE and*
29 *myositis, but its added value in clinical decision-making is uncertain.*

30 There is substantial evidence that activation of the IFN-I pathway is associated with disease
31 activity in some RMDs, especially in SLE [20,24,25,42,44,48,55,56] and PM/DM [57,58]. The
32 association in other diseases such as RA [35,59] or SSc [27,28] depends on clinical subsets or
33 disease duration [9]. It is less clear whether knowledge of IFN pathway activation status would
34 change a decision compared to the existing standard of using symptoms, signs, and existing

1 biomarkers such as acute phase markers. There were no studies that evaluated the clinical impact
2 of including IFN-I biomarkers in assessment of disease activity. Therefore, although the
3 associations with disease activity are solid and consistent, the actual added value for clinical
4 management is unknown.

5 In appraising the literature and in planning future research it must be noted that some disease
6 activity instruments include laboratory biomarkers (e.g., CRP, ESR, complement and anti-dsDNA
7 levels) that may be directly influenced by IFN-I. Indices that only assess symptoms and signs are
8 recommended for studies analysing IFN-I pathway activation. In addition, disease activity
9 instruments such as the SLEDAI weigh organ-related activity differently, which makes testing
10 association of assays with specific organ manifestations more complex.

11 Further, it must be considered that some IFN-I assays, and certain ISG, are more variable over
12 time than others or present differential associations with some clinical aspects than others, which
13 can affect conclusions about correlations with disease activity in cross-sectional or longitudinal
14 analyses.

15 *8. IFN-I pathway assays can predict disease exacerbations, in particular flare occurrence in SLE*
16 *patients, but further work should be performed to determine to what extent they outperform*
17 *current instruments.*

18 There is evidence from many longitudinal studies reporting that IFN-I pathway activation can
19 predict flare occurrence in SLE patients [20,55,56,60–63]. However, similar limitations as
20 described in point 7 apply; despite evidence being consistent among studies using different IFN-
21 I assays, the added value of such measurements over conventional clinical features and existing
22 laboratory markers has to be established [55,56,60,62,64], and therefore also whether an IFN-I
23 assay would affect decision making.

24 *9. IFN-I pathway assays might predict progression from pre-clinical autoimmunity to clinical*
25 *disease.*

26 There is good quality and consistent evidence, albeit from a smaller number of longitudinal
27 studies, associating IFN-I pathway activation in ‘at risk’ pre-clinical autoimmunity individuals
28 with progression to SLE/CTD or RA. In RA, two studies (micro-array and qPCR) both supported
29 association between an IFN gene expression signature and progression from arthralgia to RA
30 [65,66]. IFN-I pathway activation showed a predictive value equivalent to that of autoantibodies
31 (RF/ACPA) and improved the predictive power of the latter when combined [56]. Other classical
32 risk factors such as age, shared epitope or acute-phase reactants did not exhibit predictive power.
33 In antinuclear antibody (ANA)-positive individuals, a pre-defined set of ISGs predicted

1 progression to SLE or pSS in a prospective study [63]. This effect was independent of other
2 clinical characteristics and routine immunology features as demonstrated in a multivariate
3 analysis[63].

4 Taken together, IFN-I pathway activation has been demonstrated to have an independent and
5 incremental value in predicting progression to RMD. The field of pre-clinical disease is still
6 emerging, and therefore so is the role of novel biomarkers, but existing evidence suggests an
7 equivalent effect than some autoantibodies, a greater effect than other conventional risk factors
8 and a promising potential to improve prediction over traditional features.

9 *10. In SLE, IFN-I pathway assays may be useful in predicting response to IFN-I targeting*
10 *therapies.*

11 A qPCR IFN signature may be useful to predict treatment outcomes in SLE patients undergoing
12 IFN-I-targeting treatments, as differences in clinical response were observed depending on the
13 level of IFN-I pathway activation [50,51,67,68]. At the time of this SLR, the evidence is limited
14 to phase II trials. Since that time, an analysis of pooled phase III data has been published
15 validating the greater efficacy of anifrolumab in patients with high interferon gene signature, so
16 this clinical application is the most strongly supported by the literature [69]. The use of IFN-I
17 assays to predict treatment outcomes in other conditions (RA, PM/DM) and non-IFN targeted
18 therapies was inconclusive. In RA patients, a higher IFN pathway activation was associated with
19 worse outcomes upon some treatments (conventional synthetic disease-modifying anti-rheumatic
20 drugs (csDMARDs) [35,59], Tumour Necrosis Factor inhibitors (TNFi) [35,70–73], tocilizumab
21 [74] and rituximab [75–78]), using different approaches, but heterogeneity and lack of replication
22 prevented firm conclusions to be drawn.

23 *11. IFN-I pathway assay results may be affected by some treatments (e.g. IFN-targeted therapies*
24 *and high-dose glucocorticoids), and timing of sample collection should be taken into account and*
25 *reported.*

26 IFN-I pathway activation may be suppressed by some treatments such as IFN-targeted therapies
27 [48,79–83] and high-dose glucocorticoids [84,85], whereas the effect of other drugs (TNFi,
28 hydroxychloroquine or rituximab) may be weaker or absent. However, treatment duration,
29 dosages, existing RMD and the assay used (and the choice of ISG, if applicable) should be taken
30 into account. Overall, most of the studies with no group-level changes in treatments or disease
31 exacerbation reported little or no change over time across different RMD and techniques.

1 DISCUSSION

2 This is the first systematic approach to evaluate the use of IFN-I assays in clinical research and
3 practice in rheumatology. The taskforce agreed on the formulation of 2 OP and 11 PtC, which
4 represent the consensus of a multi-disciplinary, international group covering all the range of
5 professionals and stakeholders in this field. The level of agreement was overall high, thus
6 supporting the broad acceptability of the statements produced. These PtC are expected to facilitate
7 the validation and use of IFN-I assays in routine practice and clinical trials, to guide future steps
8 in IFN-I research (Table 3) where the evidence was lower, and to facilitate international
9 collaborations.

10 Current literature on IFN-I pathway activation in RMDs is characterized by a great heterogeneity,
11 which represents major pitfall to obtain clinical validation and establish clinical utility.
12 Heterogeneity on IFN-I research is a multi-level issue, related to the complexity of the pathway
13 biology itself, but also to the assay choice, clinical applications, clinical context, terminology,
14 study designs and diversity in analysis and reporting practices. Assay-specific issues, such as the
15 low reliability of direct IFN protein measurements due to sensitivity, the presence of multiple
16 subtypes of IFN-I, cross-reactivity and potential interferences, also add to this complexity [13]
17 [86,87]. This heterogeneity may account for the lack of transition of IFN-I assays into clinical
18 practice and represents a major limitation that may preclude IFN-I potential to be realised. Under
19 these circumstances, the taskforce aimed at providing uniform guidelines for terminology, assay
20 choice, analysis and reporting. Of note, this set of statements (PtC 1-4) showed the highest
21 agreement, thus reinforcing their urge/priority and appropriateness for the experts. The use of
22 these points to consider will also enable international collaborations to solve clinical unmet needs.
23 Moreover, these PtC create a framework for the implementation of biomarkers in the long-term,
24 especially for complex pathways.

25 A greater understanding is imperative to maximize the clinical applications of the IFN-I pathway
26 activation, especially with the advent of IFN-I-targeted therapies. Despite decades of research,
27 the complexity of the IFN-I pathway remains only partially understood. In fact, specific and
28 redundant functions of IFN-I subtypes are not firmly established, the sets of genes induced by
29 different IFN-I subtypes in different types of cells or tissues are often partially known and many
30 known ISGs remain functionally uncharacterised. The harmonizing procedures herein developed
31 are expected to foster the advancement towards the proposed research agenda (Table 3).

32 Based on the existing literature, the taskforce strengthens that currently there is not a single,
33 unique, universal assay for IFN-I pathway activation in RMDs. Consequently, none of the assays
34 can be currently considered as a gold-standard, and thus assay decisions must be made considering
35 both assay technical properties and the clinical question. The lack of harmonization and the

1 absence of universal gold standard(s) as well as comparative studies challenged the comparisons
2 among the multiplicity of assays described in the literature. Moreover, as different assays measure
3 distinct biological entities of the IFN-I pathway activation, they may likely capture distinct layers
4 of information which differ in terms of their clinical correlate(s). This may account, at least in
5 part, for the discrepancy among assay results within the same clinical purpose in a given disease,
6 as observed in the SLR. The fact that evidence across RMDs was skewed represents an additional
7 limitation in defining considerations across the whole spectrum of RMDs. Therefore, the potential
8 integration of these PtC into clinical management needs to be evaluated within each RMD
9 according to the detected clinical unmet needs and potential of IFN-I assays.

10 Evidence was however higher in SLE, not only in number of studies, but also in terms of quality
11 and coverage of clinical applications. Therefore, SLE-specific PtC were formulated, which also
12 received a high agreement. These clinical applications were mostly derived from qPCR,
13 immunoassays and flow cytometry methods, which the taskforce considered as the most
14 informative for the setting of SLE. More recent evidence on these assays is reassuring [88–90],
15 including phase III trials [12]. Of note, these methods differ in terms of assay methodology and
16 biosamples, which provides a reassuring message on the clinical value of the IFN-I pathway
17 activation itself, regardless of the method performed. Nevertheless, although certain parallelism
18 may exist with other RMDs, whether this inference could be generalizable cannot be established
19 at this point.

20 Clinical heterogeneity in some RMDs, especially SLE and RA, may also represent a substantial
21 obstacle for the development and validation of IFN-I assays for clinical management. However,
22 IFN-I pathway activation may be a powerful instrument to decipher the biological complexity of
23 these heterogeneous conditions. As distinct from application in disease diagnosis, evidence was
24 stronger and more consistent for a role in patient stratification, which may guide differences in
25 management and perhaps resolve the apparent heterogeneity. Hence, assays measuring IFN-I
26 pathway activation have high likelihood of instructing the molecular taxonomy of RMDs,
27 enabling patient stratification and allowing reclassification into ‘molecular hubs’ or
28 mechanistically distinct subsets [91].

29 Apart from RMDs, IFN-I has numerous roles in other autoimmune, infectious, cardiovascular and
30 oncological contexts. These guidelines may therefore also be of interest for other specialties. The
31 observation of these statements beyond rheumatology will help to gain understanding towards the
32 IFN-I pathway activation in other clinical scenarios compared to RMDs. The taskforce felt that
33 one of these areas are monogenic interferonopathies, where clinical heterogeneity may be linked
34 to differential tissue expression of the constitutive IFN-I production and/or signalling, which is
35 characteristic of these rare disorders [92]. Assessment of IFN-I pathway activation may be of help

1 in the screening of interferonopathies in some subsets of RMDs and may represent a strong tool
2 for diagnosis assessment in this scenario.

3 This study has some limitations that should be noted. These PtC were built upon SLRs covering
4 all IFN research until 2019, and further evidence has been published subsequently. However,
5 recent evidence by no means changes the current PtC but confirm the value of IFN-I pathway
6 activation to predict therapeutic responses in SLE (PtC10) [53], to measure disease activity in
7 SLE and DM (PtC7) [16,93], and to demonstrate stability in the absence of treatment
8 changes/disease exacerbations [94]. Additional evidence has demonstrated that IFN-I pathway
9 activation can be useful to segregate patients (PtC6) but different assays measure different
10 pathway aspects and thus are not fully interchangeable (PtC2) [95,96]. Of note, the latest evidence
11 consistently exhibits the same weaknesses raised in these PtC, such as heterogeneous
12 nomenclature, lack of clinical validation for some applications and assessment of added value,
13 hence reinforcing the need for uniform practices and a consistent research agenda. Moreover, the
14 lack of clinical instruments in certain areas, such as progression from at-risk phases, may
15 represent an additional limitation to realise the potential of IFN-I assays.

16 In conclusion, the assessment of the IFN-I pathway activation has a high potential for
17 implementation in the clinical management of several RMDs, although further research is needed.
18 We have developed a set of points to consider that creates a framework for harmonization,
19 validation and application of IFN-I assays in clinical research and practice with the ultimate goal
20 of translating these assays into clinical care. Uptake of these considerations along with gains in
21 understanding from the proposed research agenda will facilitate updating of these statements that
22 may eventually be considered in the category of recommendations. Finally, this work represents
23 a model for the translation of other biomarkers, beyond the field of IFNs and rheumatology.

1 **Author contributions**

2 JRC, AB, PC, EMV and MAV led the literature search, data extraction and formulated the draft
3 PtC versions. All authors participated in the definition of the final versions of the PtC and provide
4 feedback for their interpretation and discussion. JRC and AB drafted the manuscript. PC, EMV
5 and MAV edited the manuscript draft. All authors contributed and approved the final version of
6 the manuscript.

7

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16

17 **Competing interests**

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25 declare.

26

27 **Ethics approval**

28 Not applicable.

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1 **TABLES**

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3 **Table 1: Overarching principles and points to consider for the measurement and reporting**
 4 **of IFN-I pathway assays in clinical research and practice**

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	Level of Evidence	Level of Agreement (mean±SD), n(%) scorings ≥8/10
<i>Overarching Principles</i>		
A. The IFN pathway is a complex system with multiple subtypes of IFNs and diverse downstream effects on gene and protein expression.	N/A	9.76±0.66 17 (100)
B. IFN-I pathway activation is a common hallmark in many RMDs. Although IFN-I pathway activation is associated with some clinical manifestations, the utility of IFN-I pathway assays in clinical practice requires further validation for most contexts.	N/A	9.29±0.98 16 (94.1)
<i>Points to Consider</i>		
1. Taskforce consensus terminology should be considered for reporting IFN assays measurement.	5	9.58±0.79 17 (100)
2. Existing assays measure different aspects of the IFN-I pathway; they do not reflect the entirety of the pathway and some are not specific for IFN-I. The most appropriate assay will depend on the research or clinical question and should be justified.	4	9.76±0.56 17 (100)
3. Publications on novel IFN-I pathway assays should report whether they specifically reflect IFN-I, and to the extent possible, which IFN-I is measured.	5	9.58±0.61 17 (100)
4. For assays that evaluate pathways downstream of the IFN-I receptor (e.g. IFN-stimulated gene expression or protein scores) the choice of components needs to be justified. For gene expression scores, the known subsets of IFN-stimulated genes should be described separately.	5	9.41±0.87 16 (94.1)
5. IFN-I pathway is consistently activated in several RMDs, but assays measuring IFN-I pathway activation cannot be currently recommended for diagnostic purposes.	2b/3b	8.58±1.83 12 (70.5)

	Level of Evidence	Level of Agreement (mean±SD), n(%) scorings ≥8/10
6. IFN-I pathway assays define more severe subgroups within many RMDs, so they should be considered in stratification studies.	2b/3b	8.70±1.31 12 (70.5)
7. IFN-I pathway activation is associated with disease activity in some RMDs, especially SLE and myositis, but its added value in clinical decision-making is uncertain.	2b/3b	8.82±1.18 14 (82.3)
8. IFN-I pathway assays can predict disease exacerbations, in particular flare occurrence in SLE patients, but further work should be performed to determine to what extent they outperform current instruments.	2b	9.00±1.00 16 (94.1)
9. IFN-I pathway assays might predict progression from pre-clinical autoimmunity to clinical disease.	2b	8.00±1.69 11 (64.7)
10. In SLE, IFN-I pathway assays may be useful in predicting response to IFN-I targeting therapies.	2b	8.76±1.20 14 (82.3)
11. IFN-I pathway assay results may be affected by some treatments (e.g. IFN-targeted therapies and high-dose glucocorticoids), and timing of sample collection should be taken into account and reported.	2b/3b	9.70±0.46 17 (100)

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1 **Table 2: Consensus terminology**

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Term (abbreviation)	Definition
Interferon (IFN)	Proteins (cytokines) with anti-viral activity; IFNs are mediators of an anti-viral response. They belong to the Type I, Type II and Type III IFN families.
Type I interferon (IFN-I)	The IFNs alpha, beta, omega, kappa, epsilon, secreted by any nucleated cell, and binding to the IFNAR, which is expressed on any nucleated cell.
Type II interferon (IFN-II)	IFN gamma, mostly secreted by T cells, binding to the IFNGR, which is expressed on most leucocytes.
Type III interferon (IFN-III)	IFN lambda, which are structurally more similar to IL-10 but share downstream signalling and gene expression with IFN-I.
Interferon-stimulated genes (ISG)	Genes whose expression is known to be upregulated by any kind of IFN. Individual ISGs may not exclusively represent Type I IFN pathway activation.
Type I Interferon pathway	Type I IFN pathway is a dynamic, biological system that includes the secretion of Type I IFN protein, binding to the IFNAR, initiation of JAK/STAT signalling pathways, expression of IFN-stimulated genes, and the expression of IFN-stimulated proteins.
Type I Interferon pathway activation	Any evidence for changes in function or levels of the components of the Type I IFN pathway.
Type I interferon pathway assay	An assay measuring one or more components of the Type I IFN pathway at a molecular or functional level.
Interferon stimulated gene expression signature	A qualitative description of coordinated expression of a set of ISGs that is indicative of Type I IFN pathway activation.
Interferon stimulated gene expression score	A quantitative variable derived from expression of a defined set of ISGs that is indicative of Type I IFN pathway activation.
Interferon stimulated protein score	A variable derived from expression of a defined set of soluble biomarkers known to be upregulated by IFN, although not specific for Type I IFN.

Interferonopathy	Mendelian diseases in which there is constitutive type I IFN pathway activation with a causal role in pathology. The clinical picture may resemble RMDs. However, most diseases with IFN pathway activation are polygenic disorders and not mendelian Interferonopathies.
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1 **Table 3: Research agenda**

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Research agenda
<i>Fundamental/basic unmet needs</i>
<ul style="list-style-type: none"> • A better understanding of whether different IFN-Is, in particular IFNαs, have unique and/or redundant functions may help in the development of more precise tools for clinical use. • For IFN-stimulated genes: <ul style="list-style-type: none"> ○ Identify the sets of ISGs induced by different IFNs in relevant primary cell types. ○ Characterize differences in cell sensitivity to IFN-Is and tissue and cell-specific ISGs profiles ○ Characterise molecular, cellular and biochemical functions of ISGs. ○ Identify which of the hundreds of ISGs typically induced actually mediate pathology in RMDs. ○ Investigate IFN-repressed factors. • Development of assays that directly, sensitively and specifically measure subtypes of IFN-I.
<i>Methodological unmet needs</i>
<ul style="list-style-type: none"> • For downstream assays (IFN stimulated gene expression, IFN stimulated protein assays) the sensitivity and specificity for subtypes of IFNs, including appropriate positive and negative controls needs to be tested • For interferon-stimulated gene expression assays: <ul style="list-style-type: none"> ○ Confirmation of the most appropriate reference genes (across RMD spectrum) ○ Investigation of the mechanistic explanation for the subgroupings of ISGs to decide which should be included in assays ○ Minimum number of genes needed to capture the information in existing scores ○ To confirm whether whole blood assays represent associations reported in PBMC or cell subset literature • For soluble interferon-stimulated protein assays: <ul style="list-style-type: none"> ○ Most appropriate sample type (e.g. serum or plasma) ○ Appropriate selection of proteins to be analysed, how many to include and how to summarise results ○ To evaluate potential confounding factors such as neutralising antibodies and rheumatoid factors

- For high sensitivity interferon protein assays (e.g. SiMoA)
 - Investigation of the effects of non-circulating interferons and other interferon subtypes that may not be captured by a serum IFN- α SiMoA
 - Evaluation of the potential confounding effect of other pathogenic factors, such as neutralising antibodies and rheumatoid factors
 - Comparison of the results using a pan-IFN- α or an IFN- α subtype (e.g. IFN- α) antibody
- For cellular interferon-stimulated protein assays (i.e. flow cytometry)
 - Confirmation of sample stability and transportation when used in routine clinical laboratories
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Clinical unmet needs in RMDs

All of the following clinical studies must account for above technical validation

- Diagnosis
 - Well-designed and powered formal diagnostic studies, controlling for existing clinical and routine laboratory tests, and in patient populations that are representative of the intended clinical context
 - Evaluation of the added value of interferon assays in combination with other parameters (e.g. autoantibodies, or clinical features) for each specific RMD
- Patient stratification
 - Establish the role of patient stratification within each RMD context according to management unmet needs
- Disease Activity
 - Confirmation of the added value of an interferon assay in determining disease activity as compared to an endpoint of an objective gold standard (e.g. imaging or biopsy), or a subsequent clinical outcome
- Prediction of Flare
 - Well-designed and powered formal prognostic studies, controlling for existing clinical and routine laboratory tests, and in patient populations that are representative of the intended clinical context
- Progression in At-Risk Cohorts
 - Validation studies for existing results in cohorts at risk of RA or CTD, including evaluation of appropriate clinical covariates
 - Confirmation of the added value of an interferon assay compared to an established, validated clinical instrument

- Assessment of the added value of interferon over conventional risk factors for progression (e.g. autoantibody profiling) once established
- Response to treatment
 - Validation of data for prediction of response to anifrolumab in phase III trials
 - Replication of similar studies for other conventional and targeted therapies
- Responsiveness
 - For specific therapies: evaluation of IFN-I assays at multiple time-points from baseline in a population receiving similar therapy
 - For change in disease activity: evaluation of IFN-I assays at multiple time-points in patients who are experiencing a change in clinical status (e.g. flare or improvement), which may not depend on a specific therapy.

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