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Ultrasensitive Interferons quantification in idiopathic inflammatory myopathies serve as biomarkers of activity in dermatomyositis and anti-synthetase syndrome

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Abstract

Objectives Inflammatory idiopathic myopathies (IIM) are a heterogeneous group of disorders, ranging from a muscle-specific autoimmune disease to a systemic one that are difficult to assess. Recent insights into IIM pathogenesis highlighted the role of interferon (IFN) in the pathophysiology. The aim of this study was to test if IFN serum levels can be used as a biomarker of disease activity in IIM.

Methods IFN type I and II were measured using an ultrasensitive detection technology and assess the potential of IFN.

Results One hundred and fifty-two patients (dermatomyositis (DM); n=50, anti-synthetase syndrome (ASyS); n=46, immune-mediated necrotizing myopathy (IMNM); n=32, inclusion body myositis (IBM); n=24) and 33 age-matched healthy donors were included. IFN- α levels were higher only in DM (0.07 pg/ml [0.03-0.23], $p<0.005$) and ASyS groups (0.07 [0.02-0.16], $p<0.05$) compared with controls (0.02 [0.01-0.05]). IFN- β was increased only in DM and IFN- γ among all IIM. IFN- α levels were correlated with disease activity in DM ($r=0.76$, $p<0.0001$). The predictive accuracy of IFN- α level to discriminate active and non-active disease was excellent as reflected by an area under the ROC-curve of 0.88. Using an IFN- α level cut-off above 0.11 pg/ml, the sensitivity was 75% and the specificity was 96% in DM patient. IFN- α and IFN- γ were correlated with disease activity in ASyS groups ($r=0.55$ and $r=0.46$ $p<0.05$)).

Conclusions IFNs are promising biomarker for DM and ASyS disease activity.

Key Messages

What is already known about this subject?

Idiopathic inflammatory myopathies are a heterogeneous group of diseases combining muscle and extra-muscular manifestations difficult to assess

Interferon signature correlates with dermatomyositis (DM) disease activity

What does this study add?

Serum levels of IFN- α and β (type I IFN) are increased in DM and IFN- α and γ in anti-synthetase syndrome (ASyS)

IFN- α is well correlated with disease activity and permit to discriminate active from inactive patients

How might this impact on clinical practice or future developments?

IFN- α can be considered as a biomarker of disease activity in DM and ASyS

IFNs increased levels in DM and in ASyS open new avenue for targeted treatment

Introduction

Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of autoimmune diseases including four main groups: dermatomyositis (DM), anti-synthetase syndrome (ASyS), immune-mediated necrotizing myopathies (IMNM), and inclusion body myositis (IBM) (1,2). IIM can either be a muscle-specific autoimmune disease (IBM and IMNM) or systemic involving mainly the skin, the joints and/or the lungs (DM and ASyS). The development of tools assessing disease activity is crucial in daily clinical practice to improve patients' care, as well as improving the design of randomized clinical trials. International Myositis Assessment and Clinical Studies Group developed a disease activity core set measures permitting to define an improvement using a total improvement score calculated with two timepoints measures (3). Nevertheless, reliable biomarkers are needed to assess the disease activity at one given timepoint. While creatine kinase (CK) level (one core set measure) is well correlated with IMNM disease activity (4), it may lack sensitivity in ASyS or DM patients (5). Interferons (IFN) are involved in the pathophysiology of IIM in which an overexpression of IFN-stimulated genes is observed (6,7). There are three main types of IFN. The type I IFN (IFN- α and β) blood levels are increased in DM (8,9) whereas the role of type I IFN in ASyS has not been yet clarified. In IBM, large amounts of type II IFN (IFN- γ) are produced by activated CD8 + T cells that play key role in muscle fibers damages (10,11). Serum level of type II IFN have not been evaluated in IIM yet. In addition, a new ultrasensitive technology has recently been developed and permits to detect very low level of proteins suitable for cytokine detection at femtomolar concentration such as IFN- α (12).

The aim of this study was to assess the potential of type I and II IFN, using an ultrasensitive digital ELISA technology, as a new blood biomarker of activity for IIM especially in DM and ASyS.

Methods

Patients And Sera

Patients were prospectively enrolled between 2011 and 2018 in a tertiary center of IIM (Pitié-Salpêtrière Hospital, Paris, France). They fulfilled the ACR/EULAR classification criteria for myositis (13). Patients were classified into four categories: IBM (Lloyd's criteria (14)), IMNM (ENMC 2017 (15)), ASyS in presence of anti-synthetase antibody and DM (1-14).

Sera were collected at diagnosis and/or during the follow-up and were rapidly (< 3 h) frozen after one centrifugation. All the sera were thawed only once to avoid potential freeze/thaw effects. Patients who had increased dose of corticosteroids (> 0.5 mg/kg and/or pulses) the week before the sampling were excluded as it may rapidly abrogate the IFN levels (16). Moreover, patients with active infectious diseases (e.g. flew or viral B hepatitis) were excluded. Thirty-three age-matched healthy donors (HD) from a French blood bank were used as negative controls.

The screening for Myositis-Specific Antibody (MSA) was performed with different line blot commercial assays as previously reported (1) including anti-melanoma differentiation-associated protein 5 (anti-MDA5), -transcription intermediary factor- γ (anti-Tif1 γ), -complex nucleosome remodeling histone deacetylase (anti-Mi2), -nuclear matrix protein 2 (anti-NXP2) and -SUMO-activating enzyme subunit 1 (anti-SAE1) for DM; -histidyl-ARNt synthetase (anti-Jo1), -threonine-ARNt synthetase (anti-PL7), -alanine-ARNt synthetase and -glycine-ARNt synthetase (anti-EJ) for ASyS; -signal recognition particle (anti-SRP) and – 3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) for IMNM. Myositis-associated autoantibodies (MAA) were screened by commercial assay including anti-Ro52, anti-Ro60, anti-La, anti-cyclic citrullinated peptide, and rheumatoid factor.

Disease Activity Assessment

Using International Myositis Assessment and Clinical Studies Group core set measures the following assessments were performed: Manual Muscle Testing 8 (MMT8), Creatine Kinase (CK) level, Myositis Disease Activity Assessment Tool (MDAAT), Physician Global Activity (PGA). Disease activity was assessed at the time of blood collection and the result was represented in a numeric scale (from 0 to 10; 0 corresponding to the remission without treatment and 10 the maximum disease activity).

IFN Serum Measurement By Single Molecule Array (Simoa®)

IFN- α and IFN- γ serum concentrations were measured using the high sensitivity Simoa® technology (Digital ELISA technology) (Quanterix Simoa™ IFN α Reagent Kit, Lexington, MA, USA and Quanterix Simoa™ IFN γ Reagent Kit, Lexington, MA, USA) according to the manufacturer protocols and as previously reported (9,17–19).

The limit of detection (LOD) was 0.0035 pg/ml for IFN- α and 0.026 pg/ml for IFN- γ . The positivity

threshold was defined as the mean plus three times the standard deviation of the 33 healthy donors (HD) and was 0.22 pg/ml for IFN- α and 1.97 pg/ml for IFN- γ .

For IFN- β serum quantification, we developed a homebrew assay using Simoa® reagents and mAbs from (PBL Assay Science, Piscataway, NJ, USA), as recently described (Llibre et al, In Press). The LOD was calculated by the mean value of the blank plus two times the standard deviation (positivity at 95% confidence) calculated on logarithmic values and was 1.24 pg/ml and the positivity threshold was defined by the mean plus three times the standard deviation of the HD and was 2.50 pg/ml.

Statistical analysis

Quantitative variables were expressed as median with inter-quartile range, and numbers with proportions for categorical variables. Multiple comparisons were performed using Kruskal-Wallis test then Dunn's post-hoc test for quantitative data. To analyse the correlation between IFN and disease activity assessed by the PGA, we performed Spearman's rank correlation tests using Graphpad prism 5. Positive threshold to discriminate active from inactive patient was assessed by ROC curve analysis. We used the point of the curve nearest the top of the left-hand corner to minimise both the number of false-positive and false-negative results. CK and IFN values were transformed through a base-10 logarithm for analysis. After verifying the absence of multicollinearity, we included IFN- α , IFN- γ and CK levels in binary multivariate logistic regression to determine the association with disease activity (binary outcome using PGA > 5 to define active patients). $P < 0.05$ was considered statistically significant. Multivariate statistical analyses were realized with R program version R-3.6.0.

Ethical

Written informed consent from each study patient and approval by local Ethics Committee (CPP Ile De France VI (2013-12-19), CCTIRS (N°14.323) and CNIL (AR158656)) were obtained.

Results

Patients' characteristics

One hundred fifty-two IIM patients (DM, $n = 50$; ASyS, $n = 46$; IMNM, $n = 32$ and IBM, $n = 24$) and 33 healthy donors were included. Main patients' characteristics are shown in Table 1.

Table I: Patients' characteristics at blood sampling timepoint

Diagnosis	DM	ASyS	IMNM	IBM	Total	p
n (%)	50 (33)	46 (30)	32 (21)	24 (16)	152 (100)	-
Age (year)	53.2 ± 15.4	48.6 ± 14.5	49.6 ± 19.1	69.3 ± 8.3	53.6 ± 16.6	p < 0.001
MSA, n (%)	35 (70)	46 (100)	32 (100)	12 (100)	125	-
MAA, n (%)	13 (26)	37 (80)	9 (28)	3 (13)	62	-
MMT8 (0-150)	142 [126-150]	150 [132-150]	132 [115-146]	120 [94-133]	138 [119-150]	p < 0.001
CK level (UI/ml)	112 [60-460]	550 [123-1500]	780 [249-1332]	586 [296-1123]	432 [109-974]	P < 0.001
MDAAT (0-60)	10 [3.5-17]	9 [2-21.5]	na	na	10 [2.5-17]	0.65
PGA (0-10)	5 [2-8]	5 [2-8]	5 [2-7]	na	5 [2-8]	0.85
Corticosteroids n (%)	33 (66)	25 (54)	22 (68)	0	80 (52)	0.36
Immunomodulator, n (%)	23 (46)	26 (57)	17 (53)	0	66 (43)	0.58

MMT8: manual muscle testing 8, CK: creatine phosphokinase, MDAAT: myositis disease activity

assessment tool, na: non available, PGA: physician global assessment. Immunomodulator:

methotrexate, azathioprine, ciclosporine, rituximab, cyclophosphamide, hydroxychloroquine. MSA:

myositis specific antibody (anti-Mi2, -Tif-1γ, -NXP2, -MDA5, -SAE1, -Jo1, -PL7, -PL12, -HMGCoA, -SRP, -

cn1a). MAA: myositis-associated antibody (anti-Ro52, -Ro60, RF, -CCP, -RNP, -DNA). DM:

Dermatomyositis, ASyS: Anti-synthetase syndrome, IMNM: Immune mediated necrotizing myopathie,

IBM: Inclusion body myositis.

As expected, IBM patients were older and displayed a lower MMT8 score compared to DM and ASyS.

MSA were detected in 70.6% of DM patients (anti-Mi2, n = 10; -TIF1γ, n = 12; -NXP2, n = 7; -MDA5, n = 5 and -SAE, n = 2), by definition all ASyS were antibody positive (anti-Jo1, n = 39; -PL7, n = 4; -PL12, n = 2 and -EJ, n = 1), and all IMNM patients were seropositive (anti-SRP, n = 13, -HMGCR, n = 19).

No difference was observed in the therapeutic profile, including the use of corticosteroids and immunosuppressors, between IMNM, DM, and ASyS while IBM patients did not receive any treatment.

Increased levels of type I and II IFNs depend on the myositis subgroups

Serum IFN-α level was significantly higher in DM (0.07 [0.03-0.23] pg/ml) and ASyS (0.07 [0.02-0.16] pg/ml) compared to HD (0.02 [0.01-0.05] pg/ml; p < 0.005 and p < 0.05 respectively) whereas it was not significantly different in IMNM (0.03 [0.01-0.09] pg/ml) or IBM (0.02 [0.02-0.03] pg/ml) compared to HD (Fig. 1a). One quarter (26%; n = 13/50) of DM and 20% ASyS patients (n = 9/46) had increased IFN-α level above the positivity threshold while only 3% of IMNM (n = 1/32) and 4% of IBM patients (n = 1/24) had increased levels.

Only DM patients had significantly higher IFN-β level (1.24 [1.24-6.31] pg/ml) compared to HD (1.24

[1.24–1.24] pg/ml, $p < 0.005$) (Fig. 1b). IFN- β was increased in 34% ($n = 17/50$) of DM patients, and 12% of IBM patients ($n = 3/24$) while no ASyS or IMNM patients presented an increased level. IFN- γ level was significantly increased in all IIM subgroups (ASyS (1.05 [0.47–2.46] pg/ml), DM (0.90 [0.55–2.09] pg/ml), IMNM (0.96 [0.42–1.29] pg/ml) and IBM (0.93 [0.42–2.09] pg/ml)) compared with HD (0.46 [0.29–0.59] pg/ml), $p < 0.05$) (Fig. 1c). One third of ASyS patients (37%; $n = 17/46$), one quarter of DM patients (26%; $n = 13/50$) and IBM patients (25%, $n = 6/24$) and 16% of IMNM patients ($n = 5/32$) had an increased level of IFN- γ .

IFN levels and disease activity

Type I and II IFN levels are correlated with disease activity of DM and ASyS

Correlation between IFN level and disease activity showed that disease activity was strongly correlated with type-I IFN, IFN- α ($r = 0.76$ [0.60–0.86], $p < 0.001$) (Fig. 2a) and IFN- β ($r = 0.58$ [0.35–0.74], $p < 0.01$) (Fig. 2c) in DM. A mild correlation with IFN- γ ($r = 0.36$ [0.05–0.56], $p = 0.02$) was observed.

ASyS also demonstrated that disease activity correlated with IFN- α ($r = 0.55$ [0.34–0.76], $p < 0.001$) (Fig. 2e) and IFN- γ levels ($r = 0.46$ [0.15–0.66], $p = 0.003$) (Fig. 2g). Of note, no ASyS patient presenting an active disease had increased IFN- β level.

In IMNM, only IFN- γ level was significantly correlated with disease activity ($r = 0.48$ [0.14–0.71], $p = 0.006$) whereas IFN- α ($r = 0.23$ [-0.14–0.55], $p = 0.2$) and IFN- β ($r = -0.07$ [-0.43–0.31], $p = 0.7$) were not. Of note, correlation between CK levels and disease activity was very high ($r = 0.87$ [0.73–0.94], $p < 0.001$) for IMNM patients.

Multivariate analysis including IFN- α , and IFN- γ showed that only IFN- α was associated with active disease in DM patients (OR = 9.5 [3.1–45.9], $p < 0.001$). Concerning ASyS patients, only IFN- α was statistically associated with disease activity (OR = 5 [1.9–17.9], $p = 0.004$), and there was a trend for IFN- γ ($p = 0.08$). No IFN subtype was associated with disease activity in the IMNM subgroup.

Longitudinal analysis

Focusing on DM and ASyS treatment-naïve patients at diagnosis, we performed a serial longitudinal analysis (DM, $n = 6/11$ and ASyS, $n = 4/11$) (supplementary Fig. 1 and Fig. 3). The majority of DM

patients had a high type I IFN level at diagnosis and a decrease parallel to the clinical improvement (Fig. 3a, Fig. 3b and supplementary Fig. 1). IFN- γ level wasn't associated with the disease activity in follow-up of DM (Fig. 3c). In most ASyS patients increased levels of IFN- α and IFN- γ but not IFN- β were observed at the diagnosis (Supplementary Fig. 2). Similarly, these levels decreased following the clinical improvement.

Sensitivity and specificity of IFNs to discriminate active and inactive DM and ASyS patients

Next, we aimed to define the threshold level of IFN corresponding to active disease. Active DM patients had higher level of IFN- α (0.26 [0.09–0.53] pg/ml) compared to inactive DM patients (0.03 [0.01–0.07] pg/ml, $p < 0.001$) (Supplementary Fig. 2a). ROC analysis showed that the area under the curve (AUC) was 0.88 (IC95 0.79–0.98; $p < 0.001$) (Fig. 2b). An IFN- α threshold above 0.11 pg/ml had a 75% sensitivity and 96.2% specificity, and 19.5 positive likelihood ratio to discriminate active from inactive DM patients (Table 2).

Table 2
Sensitivity and specificity of different IFNs as activity biomarkers

	IFN level (pg/ml)	Se (%)	Sp (%)	Likelihood ratio +
DM IFN- α	0.11	75	96	19.50
IFN- β	1.6	57	88	4.90
IFN- γ	1.06	71	77	3.07
ASyS IFN- α	0.06	82	81	4.42
IFN- γ	1.05	80	73	2.97

DM: Dermatomyositis, ASyS: Anti-synthetase syndrome, IFN: Interferon, Se : sensitivity, Sp :

Specificity.

Active DM patients had higher level of IFN- β (4.62 [1.24–27.96]) compared to inactive DM patients (1.24 [1.24–1.24] pg/ml, $p = 0.0001$) (Supplementary Fig. 2b) with a 0.75 AUC (IC95 0.61–0.89; $p = 0.0027$) (Fig. 2d). Of note, IFN- γ levels were higher in active DM patients (1.417 [0.81–2.74] pg/ml) compared to inactive ones (0.64 [0.38–1.20] pg/ml, $p = 0.007$).

Active ASyS patients had higher IFN- α level (0.16 [0.08–0.36] pg/ml) compared to inactive ASyS patients (0.03 [0.01–0.06] pg/ml, $p < 0.001$) (Supplementary Fig. 2c). The sensitivity was 82.3% and the specificity 80.8% for an IFN- α threshold above 0.06 pg/ml (AUC = 0.86, IC95(0.74–0.97); $p < 0.001$) (Fig. 2f). Active ASyS patients had higher level of IFN- γ (2.28 [1.18–3.26] pg/ml) compared to inactive ASyS patients (0.82 [0.34–1.33] pg/ml, $p = 0.004$) (Supplementary Fig. 2d) and sensitivity and

specificity were 80% and 73.1% respectively at a threshold above 1.05 pg/ml (Table 2) (AUC = 0.75, IC95(0.60–0.90); $p = 0.003$ (Fig. 2 h)).

Discussion

In this study, we showed that type I IFN is a reliable biomarker of disease activity in DM and ASyS patients. While IFN- α is increased in both conditions, IFN- β is only increased in DM patients. Type II IFN (i.e IFN- γ) is increased in all myositis subgroups but ASyS patients showed the best correlation with the disease activity.

Previous studies have described an IFN signature in muscle, skin and blood samples of DM patients (20–22). The IFN-stimulated genes levels can be assessed by an IFN score (6) combining a set of at least five genes, but it is not standardized and performed routinely in clinical practice, and requires RNA extraction. The IFN score in DM was only correlated with the cutaneous disease activity (9).

Digital ELISA for type I IFN is more sensitive than ELISA and was very well correlated with IFN-gene signature (9,17). For the first time, we were able to show a very good correlation with DM disease activity parameters and IFN-I levels. This technology was recently used in lupus with interesting data (19). Only one previous study used Digital ELISA to measure IFN- α blood level in adult DM (9). It did not show a significant correlation with disease activity but in this study, only the skin disease activity was assessed whereas we included all domains of disease activity (e.g skin, muscle and joints). In this study, IFN- β only was correlated with the skin disease activity (9) in line with a previous study showing that IFN- β was increased in DM (8). In our study, we included a large cohort of myositis patients and we confirmed that IFN- β is a DM specific IFN cytokine and showed for the first time that both IFN- α and β are reliable biomarkers. Nevertheless, we have to underline that the limit of detection of IFN- α was 300 times lower than for IFN- β it might be possible that interest of IFN- β level monitoring will increase when the limit of detection will be improved.

In addition, for the first time, we demonstrated that IFN- α is also associated with disease activity in ASyS. Along that line, it was previously shown that anti-Jo1 positive patients harbored an IFN signature (23). If both DM and ASyS have an IFN signature, we observed that only DM have increased level of IFN- β and that IFN- γ was a good biomarker in ASyS but not in DM. It was shown that only DM

patients expressed in the muscles IFN-related proteins (24,25) while muscle fibers of ASyS over-expressed MHC-II (26), a type II IFN inducible protein (27). Along that line, a recent study showed that different IFN signature is found in muscle biopsy of IIM (28). Altogether these results highlight that pathways in both conditions are different: namely IFN- α and - β in DM and IFN- α and - γ in ASyS. In IMNM, only IFN- γ was associated with disease activity but CK level was a better biomarker according to a previous study showing a strong correlation between the percentage of necrotic muscle fibers and CK level (4). Increased type II IFN levels may be due to Th-1 immune responses and CD8⁺ T cells or NK cells, two types of immune cells involved in pathophysiology of IBM and ASyS respectively (10,29), but also by macrophages (17), the most abundant cells infiltrating IMNM muscle (4).

DM and ASyS are two multisystemic conditions making disease activity assessment very complex. In this study, we demonstrated that IFNs are reliable biomarkers of disease activity. There is no gold standard to measure myositis disease activity and we cannot exclude that monitoring IFN levels is more sensitive to assess disease activity than current clinical tools (3). We acknowledge that we were unable to calculate the improvement score as recommended by the ACR/EULAR to assess patients' disease activity at two timepoints. Similarly, patients' reported outcomes such as health assessment questionnaire or patient visual analog scale were unavailable.

Conclusion

To conclude, this study showed that type I and II IFNs are reliable biomarkers for DM and ASyS disease activity and further independent prospective studies are required to confirm these results.

Abbreviations

Anti-synthetase syndrome: AsyS

Creatine kinase: CK

Dermatomyositis: DM

Immune-mediated necrotizing myopathy: IMNM

Inclusion body myositis: IBM

Inflammatory idiopathic myopathies: IIM

Interferon: IFN

Limit of detection: LOD

Manual Muscle Testing 8: MMT8

Myositis-Specific Antibody: MSA

Myositis Disease Activity Assessment Tool: MDAAT

Physician Global Activity: PGA

Declarations

Ethics approval and consent to participate

Written informed consent from each study patient and approval by local Ethics Committee (CPP Ile De France VI (2013-12-19), CCTIRS (N°14.323) and CNIL (AR158656)) were obtained. Patient were not involved in the design and conduct of our research

Consent for publication

Patient consent for publication were not required.

Availability of data and materials

All data relevant to the study are included in the article or uploaded as supplementary information.

Data are available upon reasonable request.

Competing interests

All the authors declare no disclosure.

Funding

Authors declare no funding.

Authors' contributions

LB, YA, OB and CA were involved in the study design. LB, DA, KD and GG realized IFN alpha and gamma measurement. LB, AL, VB and DD were involved in IFN beta measurement and validation of the test. LB, CA and KM realized statistical analysis. LB, ST, OLC, AM and BH realized clinical evaluation.

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Supplementary Figures

Supplementary figure 1: IFN level in naive patients at diagnosis. **a** IFN- α at diagnosis in IIM patients **b** IFN- β at diagnosis in IIM patients **c** IFN- γ at diagnosis in IIM patients. The dot line represents the mean + 3 standard deviations (positivity threshold).

Supplementary figure 2: Comparison between active (PGA>5) and inactive patients

(PGA $\leq$ 5). **a** IFN- α in active and inactive DM patients **b** IFN- β in active and inactive DM patients **c** IFN- α in active and inactive ASyS patients **d** IFN- γ in active and inactive ASyS patients. ** p<0.005 DM: Dermatomyositis, ASyS: Anti-synthetase syndrome,

Figures

Figure 1

IFN levels in IIM. DM: Dermatomyositis, ASyS: Anti-synthetase syndrome, IMNM: Immune-mediated necrotizing myopathies, IBM: Inclusion body myositis, HD: healthy donors - - mean $\pm$ 3 standard deviation or positivity threshold, *: $p < 0.05$, **: $p < 0.005$, ***: $p < 0.0005$, **** $p < 0.00005$

Figure 2

Correlation between IFNs and disease activity. Correlation between IFN-α and DM disease activity. b ROC curve concerning IFN-α level between active DM patients (PGA>5) and non-active DM patients (PGA≤5). c Correlation between IFN-β and DM disease activity. d ROC curve concerning IFN-β level between active DM patients (PGA>5) and non-active DM patients e Correlation between IFN-α and ASyS disease activity. f ROC curve concerning IFN-α level between active ASyS patients (PGA>5) and non-active ASyS patients (PGA≤5). g Correlation between IFN-γ and ASyS disease activity. h ROC curve concerning IFN-γ level between active ASyS patients (PGA>5) and non-active ASyS patients (PGA≤5) DM: Dermatomyositis, ASyS: Anti-synthetase syndrome, AUC: area under the curve, PGA: physician global activity.

Figure 3

Evolution of IFNs levels following treatment. a Evolution of IFN-α in DM patients. b Evolution of IFN-β in DM patients. c Evolution of IFN-γ in DM patients. d Evolution of IFN-α in ASyS patients. e Evolution of IFN-γ in ASyS patients. The dot line represents the mean + 3 standard deviations (positivity threshold). DM: Dermatomyositis, ASyS: Anti-synthetase syndrome,

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