Type I Interferon in Children with Viral or Bacterial Infections

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Type I interferon in children with viral or bacterial infections

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Keywords: Interferon-α; febrile children; viral infection; bacterial infection; emergency department.

Running title: Type I interferon in febrile children

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Abstract

Background: Fever is one of the leading cause of consultation in pediatric emergency department (PED) under the age of 3 years old. Distinguishing between bacterial and viral infections etiologies in febrile patients remains challenging. We hypothesized that specific host biomarkers for viral infections, like type I-interferon (IFN), could help clinicians decisions and limit antibiotic overuse.

Methods: Paxgene tubes and serum were collected from febrile children (n=101) aged from 7 days to 36 months, attending PED in France with proven viral or bacterial infections. We have assessed the performance of IFN signature which is based on quantification of expression of IFN-stimulated genes using Nanostring® technology and plasma IFN-α quantified by digital ELISA technology (Quanterix ®).

Results: Serum levels of IFN-α were below the quantification threshold (30fg/mL) for 2% (1/46) of children with proven viral infections and for 71% (39/55) of children with bacterial infections ($p<0.001$). IFN-α levels and IFN score were significantly higher in viral compared to bacterial infection ($p<0.001$). We noticed a strong correlation between serum IFN-α concentrations and IFN score (p-pearson=0.83). Both serum level IFN-α and IFN score robustly discriminated (Area Under the Curve >0.91 for both) between viral and bacterial infection in febrile children, compared to C-reactive protein (0.83).

Conclusions: This study revealed for the first time, that IFN-α is increased in blood of febrile infants with viral infections. The discriminative performance of IFN-α femtomolar concentrations as well as blood transcriptional signatures could show a diagnostic benefit and potentially limit antibiotic overuse.

Clinical Trials Registration clinicaltrials.gov (NCT03163628).

INTRODUCTION

Type I interferons (IFNs) are a group of cytokines that are involved in the innate antiviral response. A chronic exposure to these cytokines is also linked to the pathophysiology of a group of autoimmune diseases called type I interferonopathies (1,2). As it is secreted at very low levels (femtomolar) during disease course, detection of type I IFN in patients remains challenging and led several groups to propose an alternative strategy for the monitoring of this group of cytokines. Based on the quantification of expression of IFN-stimulated genes (ISGs), blood transcriptional signatures (IFN signature) provide an indirect estimate of the exposure of cells to type I IFN and are currently proposed for the screening of autoimmune diseases (3). In addition, the recent development of digital ELISA (Single molecular array, Simoa) allows a very sensitive measurement of type I IFN molecules in the sera of patients.

In children, diagnosis of viral versus invasive bacterial infection is challenging since the main symptoms at onset are often similar and restricted to fever. Misdiagnosis is responsible for inappropriate antibiotic prescription contributing to the emergence of multi-drug resistant bacteria. Thus, we hypothesized that type I IFN, the key cytokine of antiviral response, may represent a new early biomarker of viral infection which could eventually help clinicians limit antibiotic overuse. The aim of this proof-of-concept study was to assess the performance of plasma IFN-α and IFN score in distinguishing documented viral and bacterial infections in a prospective cohort of children attending pediatric emergency departments (PED) for fever.

METHODS

Participants

This is an ancillary study of the prospective multicenter protocol ANTOINE (NCT03163628 - detailed in supplementary material and Fig-S1). The ANTOINE study was registered to the French National Data Protection Agency under the number 17-168 and has been approved by an ethics committee for biomedical research in May 2017 (Comité de Protection des Personnes Sud Méditerranée II) under the number 217-R18. For each participant, written informed consent was obtained from parents or legal guardians for the participation of the febrile children in the ANTOINE study. An additional specific written consent was required for their inclusion in this present ancillary study. Febrile children aged from 7 days to 36 months attending PED for a suspicion of infection were recruited prospectively in three different hospitals based in Lyon, Villefranche sur Saone and Colombes in France. Inclusion criteria were fever for more than 6 hours (temperature ≥38°C between 7 days and 3 months old and ≥38.5°C between 3 months and 36 months old) for which the physician prescribed venipuncture for suspected severe bacterial infection before any antibiotic treatment. For this study, Paxgene® tubes and serum were collected together with clinical blood tests.

Concomitantly, blood samples from healthy volunteers (HV, n=10) were obtained from the national blood service (Etablissement Français du Sang, Lyon, France, details in supplementary material). In addition, a pediatric disease control population (n=9) with no infection nor characterized type I Interferonopathies was also selected among patients attending consultations at the National Referee Centre for Rheumatic and AutoImmune and Systemic diseases in childrEn (details in supplementary material and patients characteristics detailed available in supplementary Table1).

Diagnostic process

The ANTOINE study was designed to evaluate the performance of several biomarkers in « real-life » condition. No other blood tests nor clinical investigation has been performed in addition to those requested by the emergency physician caring for febrile children. All patients underwent routine investigations as part of clinical care including blood count and differential, C-reactive protein, blood chemistry, blood, stools and urine cultures, and cerebrospinal fluid analysis where indicated. Chest radiographs were undertaken as clinically indicated. Bacteria culture and viral diagnostics using multiplex PCR were performed by routine microbiological laboratories of each hospital. For patients with suspicion of Respiratory Tract Infections (RTIs), specific PCRs targeting influenza virus or respiratory syncytial virus were performed systematically during seasonal peak.

According to the routine microbiology results and the clinical parameters recorded in a numerical clinical record folder (e-CRF), we retained patients with proven viral (n=46) or bacterial (n=55) infections for this study. The infections were proven on the basis of a definite identification of pathogens using routine microbiology testing. Assignment of patients to clinical groups was validated by consensus of an independent adjudication committee (cohort description in Table1).

IFN-α and IFN-γ assay

Serum IFN-α concentrations (in fg/ml) were determined by single molecule array (Simoa) using a commercial kit for IFN-α quantification (Quanterix™, Lexington, MA, USA). The assay is based on a 3- step protocol using an HD- 1 Analyzer (Quanterix; (4) see Supplementary Methods, available at http://onlinelibrary.wiley.com/doi/10.1002/art.40792/abstract)). IFN-γ concentrations were measured using the simple plex kit IFN-γ assay with the Ella platform (Protein simple©, CA, USA), according to manufacturers' instructions.

IFN score assessment

RNA was extracted from whole blood contained in Paxgene® tubes (Kit PreAnalytix, Qiagen©, SW) and quantified by spectrophotometry assay (Nanodrop 2000, Thermo Scientific™, MA, USA). RNA integrity was then evaluated by Agilent RNA microarray (Agilent Technologies©, Santa Clara, CA, USA). The mRNA quantification of 6 ISGs (IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1) and 3 housekeeping genes (ACTB, HPRT1, POLR2A), was performed using nanostring technology (Nanostring Technologies©, WA, USA). Data standardization was obtained using the geometric average of internal control and housekeeping genes count number. Interferon score was calculated as previously described (5)

Statistical analysis

Non-parametric Mann-Whitney tests and Spearman's correlation were calculated for all parameters using R software V3.6.1. A p-value <0.05 was considered statistically significant.

RESULTS

Serum levels of IFN-α were below the quantification threshold (30fg/mL) for 2% (1/46) of children with proven viral infections and for 71% (39/55) of children with bacterial infections ($p<0.001$). Median [IQR] IFN-α levels were significantly higher in viral (7856 [3096-62305] fg/mL) compared to bacterial infections (406 [68-3708] fg/mL, $p<0.001$; Fig1-A). Similarly, the IFN score was increased 30-fold in viral infections compared to bacterial ones ($p<0.001$; Fig1-B). Interestingly, no significant difference was observed between febrile children with bacterial infections and healthy volunteers or control pediatric population (Fig1-AB). There was a very strong positive correlation between serum IFN-α concentrations and the IFN score (r-spearman [95%CI] 0.85 [0.76;0.91] Fig1-C).

Analysis of the Area Under the Curve [AUC - 95%CI] indicated that both IFN-α serum level (0.930 [0.877;0.983]) and IFN score (0.908 [0.845;0.971]) robustly discriminated viral infections from bacterial ones in febrile children (Fig1-D). To evaluate if IFN-α could improve the diagnosis of febrile children attending PED, we assessed the performance of this biomarker in combination with a clinically validated test that is the C-reactive protein (CRP) quantification. The AUC shown in Figure 1D revealed that the ratio of the concentrations of CRP and IFN-α strongly improves the classification of these patients compared to CRP alone (0.936 [0.888; 0.985] versus 0.829 [0.747;0.910] respectively, Fig1-D).

In addition, knowing that ISG expression may also be driven by Type II interferon, we then evaluated whether serum IFN-γ concentrations could be correlated with the IFN score. No relation was observed between ISG expression and Type II interferon circulating levels (r-spearman 0.18 [-0.054;0.407]; Fig1-F). Of note, no significant difference was observed between IFN-γ serum levels of children with viral infections compared to those with bacterial ones (Fig1-E).

DISCUSSION

Biomarkers have become key tools during the clinical decision-making process for clinicians dealing with febrile children. In PED, CRP, procalcitonin (PCT), and white blood cell count are probably the most used markers despite their poor performance in guiding antibiotic prescription in such clinical settings (6). In combination with a marker specific for bacterial infections, a specific biomarker for viral infections could improve the management of febrile children. Recent clinical studies have shown that the combination of CRP or PCT with Myxovirus resistance protein 1 (MxA) coded by the ISG *mx1*, strongly improved both clinical sensitivity and specificity for differentiating infectious etiology (7,8). Nevertheless, MxA is

an intracellular protein which prevents its dosage in serum or plasma, the method currently used for dosage of CRP or PCT in routine labs (9). Here, we have assessed by digital ELISA the performance provided by the measurement of protein IFN-α itself, which is secreted by immune cells after recognition of viruses and detectable in serum or plasma. This preliminary study showed for the first time that IFN-α, at the protein level, is increased in blood of febrile infants with viral infections. The performance of this promising biomarker needs to be confirmed in a larger cohort of febrile children with suspected and proven infections.

Moreover, the biomarkers performance reported in this study have been evaluated in the ANTOINE clinical trial performed in «real-life» condition. In our cohort, only 15% of patients have a definite infection based on the routine microbiology results and the clinical parameters. This is in line with previous reports showing that laboratory tests failed to identify a causative pathogen in over half of children with severe illness or fever leaving an important place for biomarkers to improve the management of febrile children (10,11). In our study, the microbiological investigation was not exhaustive for each patient, explaining probably the low prevalence of rhinoviruses in our cohort. Consequently, viral co-infection was not investigated/reported in our cohort. This could explain the high level of interferon alpha observed for some patients with a documented bacterial infection.

The alternative to IFN-α protein quantification, represented by an IFN score measurement, was also evaluated in this study. Results suggest that this ISG-based signature has the same diagnostic performance than IFN-α protein quantification. Fast track RNA analyses using Nanostring® or FilmArray® platforms could thus be helpful to implement this marker in routine use. Of note, the circulating IFN-α concentrations as well as the IFN scores reported herein in case of viral infections were in the same order of magnitude than those observed in cases of autoimmune disease such as systemic lupus erythematosus (SLE) (12). Moreover,

several recent studies suggest that IFN-α could be a useful marker to monitor interferonopathie disease activity, to identify patients with high risk of relapse, and to select the best candidates for anti-IFNα treatment (13–15). Our results suggest that viral infections could interfere and possibly be responsible of false positive diagnoses when the IFN score or IFN-α protein is used for the screening and the monitoring of autoimmune diseases.

Of note, recent published data demonstrated that in specific clinical diseases, such as *Mycobacterium tuberculosis* infections, ISG expression was not mediated through plasma type I IFN (16). However, in febrile infants, the results herein showed that the expression of ISG was driven by IFN-α and not by IFN-γ.

According to the 68th World Health Assembly declaration, it is urgent to find effective diagnostic tools to guide optimal antibiotic use (17). Our study showed that measurement of IFN-α femtomolar concentrations as well as the use of an IFN score could offer new perspectives for improving diagnosis and limiting antibiotic overuse in febrile infants.

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Infection type	Bacterial		Viral	
Population. n (%)	55 (54.5%)		46 (45.5%)	
Male. n (%)	**20 (36.4%)#**		**27 (58.7%)#**	
Age (days). median [range]	**396 [10-1094]***		**121 [10-903]***	
Duration of fever (hours). n (%)#				
<12	**4 (7.2%)**		**11 (23.9%)**	
[12-24]	**12 (21.8%)**		**15 (32.6%)**	
>24	**39 (70.9%)**		**20 (43.5%)**	
CRP (µg/mL). median [range]	**99.0 [0.24-571.2]***		**14.6 [0.6-198.0]***	
Pathogens, n (%)	*Escherichia coli*	35 (63,6%)	Respiratory syncytial virus	13 (28,9%)
	Streptococcus sp.	6 (10,1%)	Influenza virus	9$ (19,6%)
	S. pneumoniae	*5 (9,1%)*	$ 1 co-infection influenza virus / norovirus	
	S. pyogenes	*1 (1,8%)*	Rotavirus	7 (15,6%)
	Staphylococcus aureus	5 (9,1%)	*Picornavirus*	10 (21.7%)
	Salmonella sp.	4 (7,3%)	7 Enterovirus (15.6%)	
	S. enteridis	*2 (3,6%)*	3 not specified (6.7%)	
	S. typhimurium	*2 (3,6%)*	Epstein-Barr virus	3 (6,7%)
	Mycoplasma sp.	2 (3,6%)	Adenovirus	2 (4,4%)
	M. pneumoniae	*1 (1,8%)*	Herspes Simplex virus	1 (2,2%)
	Fusobacterium necrophorum	1 (1,8%)	Measles morbillivirus	1 (2,2%)
	Haemophilus influenzae	1 (1,8%)		
	Proteus mirabilis	1 (1,8%)		

Table 1 – Description of febrile patients

* t-test revealed a statistically significant difference between the two groups p<0.01. # Chi² test revealed a statistically significant difference between the two groups p<0.01

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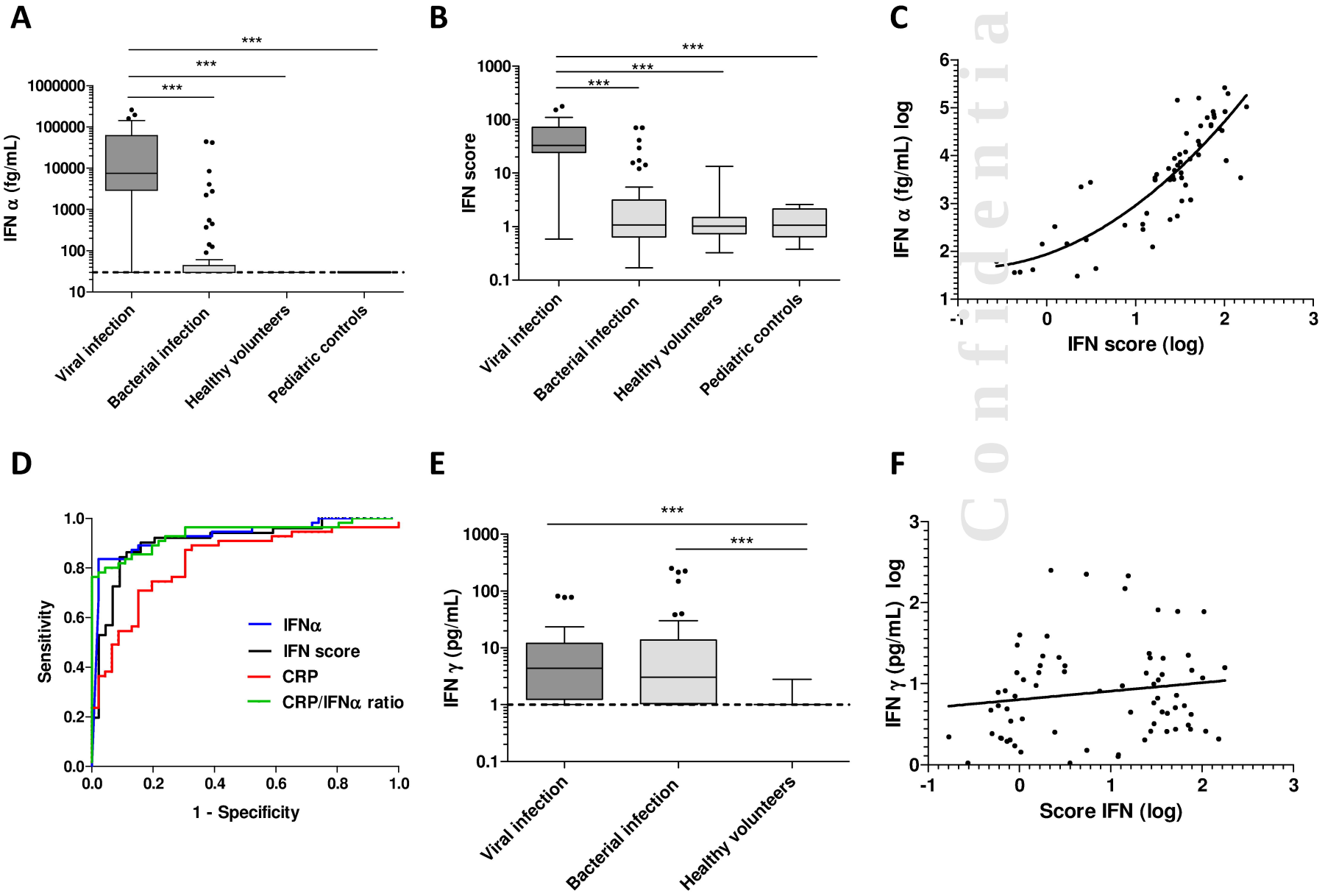

Figure 1 legend – Type I and II IFN quantification in febrile children with proven viral or bacterial infection

A. IFN-α (fg/mL) measured by digital ELISA (SIMOA®) **B.** IFN score as defined by a 6 Interferon Stimulated Genes transcriptional signature quantified using nanostring technology and obtained from Paxgene tubes of febrile infants with proven viral (n=46) or bacterial (n=55) infections attending pediatric emergency departments or from healthy controls (n=10) or from pediatric controls (n=9). Limit of quantification is indicated by the dotted lines. The IFN-α kit (Quanterix™, Lexington, MA, USA) used for this study detect IFN-α type 2. **C.** Spearman correlation between IFN score and serum IFN-α concentrations. **D.** Receiver operating characteristic curve of CRP, IFN score, serum IFN-α and CRP/ IFN-α ratio concentrations to discriminate between viral and bacterial infections. **E.** IFN-γ (pg/mL) measured in serum, one patient with bacterial infection had not sufficient blood volume and IFN-γ measurement have not been performed. **F.** Spearman correlation between IFN score and serum IFN-γ concentrations. **A-B-E.** Box-and-whiskers plots represent the median and inter-quartile range, whiskers are calculated by the Tukey's method. Mann-Whitney U test ***P<0,001 – IFN (interferon); CRP (C-reactive protein)