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Keywords: Interferon-alpha; bacterial infection; emergency; febrile children; viral infection

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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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43 **Abstract**

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

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

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

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

65 **Clinical Trials Registration** clinicaltrials.gov (NCT03163628).

66 INTRODUCTION

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

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

85 METHODS

86 Participants

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

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

108

109 **Diagnostic process**

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

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

126

127 IFN- α and IFN- γ assay

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

135 IFN score assessment

136 RNA was extracted from whole blood contained in Paxgene® tubes (Kit PreAnalytix,
137 Qiagen©, SW) and quantified by spectrophotometry assay (Nanodrop 2000, Thermo
138 Scientific™, MA, USA). RNA integrity was then evaluated by Agilent RNA microarray
139 (Agilent Technologies©, Santa Clara, CA, USA). The mRNA quantification of 6 ISGs (IFI27,
140 IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1) and 3 housekeeping genes (ACTB, HPRT1,
141 POLR2A), was performed using nanostring technology (Nanostring Technologies©, WA,
142 USA). Data standardization was obtained using the geometric average of internal control and
143 housekeeping genes count number. Interferon score was calculated as previously described
144 (5)

145 Statistical analysis

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

148

149 **RESULTS**

150

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

160 Analysis of the Area Under the Curve [AUC - 95%CI] indicated that both IFN- α serum level
161 (0.930 [0.877;0.983]) and IFN score (0.908 [0.845;0.971]) robustly discriminated viral
162 infections from bacterial ones in febrile children (Fig1-D). To evaluate if IFN- α could
163 improve the diagnosis of febrile children attending PED, we assessed the performance of this
164 biomarker in combination with a clinically validated test that is the C-reactive protein (CRP)
165 quantification. The AUC shown in Figure 1D revealed that the ratio of the concentrations of
166 CRP and IFN- α strongly improves the classification of these patients compared to CRP alone
167 (0.936 [0.888; 0.985] versus 0.829 [0.747;0.910] respectively, Fig1-D).

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

174 DISCUSSION

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

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

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

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

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

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

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

220

221

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223

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228

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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 (%)		
<i>Escherichia coli</i>	35 (63,6%)	Respiratory syncytial virus 13 (28,9%)
<i>Streptococcus sp.</i>	6 (10,1%)	Influenza virus 9 [§] (19,6%)
<i>S. pneumoniae</i>	5 (9,1%)	[§] 1 co-infection influenza virus / norovirus
<i>S. pyogenes</i>	1 (1,8%)	Rotavirus 7 (15,6%)
<i>Staphylococcus aureus</i>	5 (9,1%)	Picornavirus 10 (21,7%)
<i>Salmonella sp.</i>	4 (7,3%)	7 Enterovirus (15.6%)
<i>S. enteridis</i>	2 (3,6%)	3 not specified (6.7%)
<i>S. typhimurium</i>	2 (3,6%)	Epstein-Barr virus 3 (6,7%)
<i>Mycoplasma sp.</i>	2 (3,6%)	Adenovirus 2 (4,4%)
<i>M. pneumoniae</i>	1 (1,8%)	Herspes Simplex virus 1 (2,2%)
<i>Fusobacterium necrophorum</i>	1 (1,8%)	Measles morbillivirus 1 (2,2%)
<i>Haemophilus influenzae</i>	1 (1,8%)	
<i>Proteus mirabilis</i>	1 (1,8%)	

0 Table 1 – Description of febrile patients

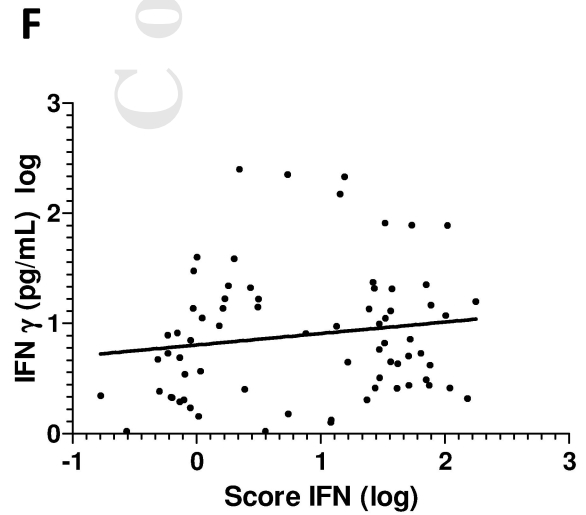
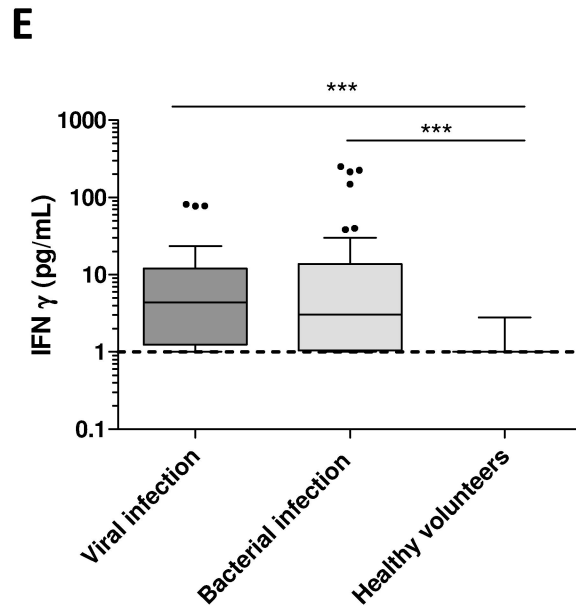
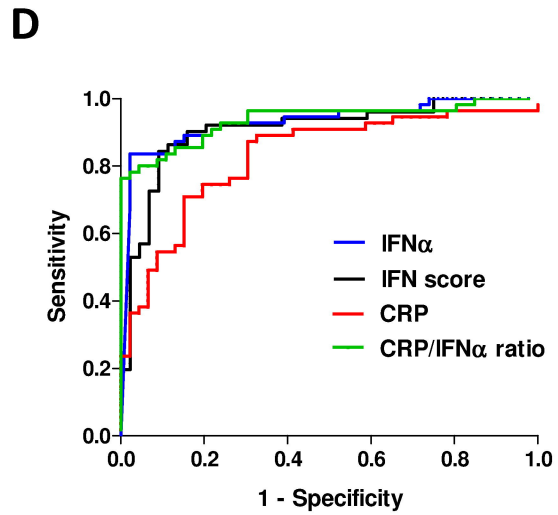
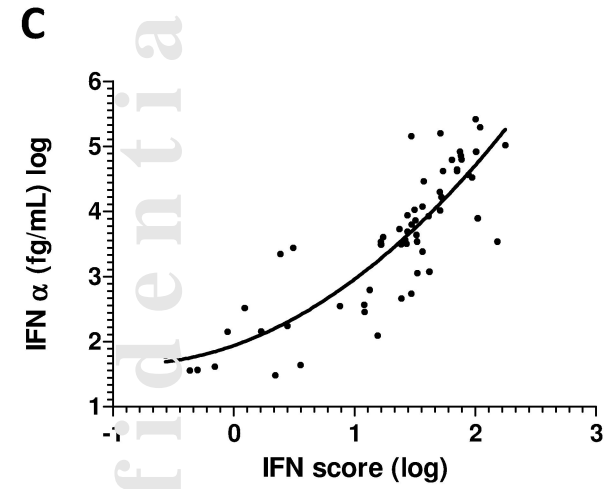
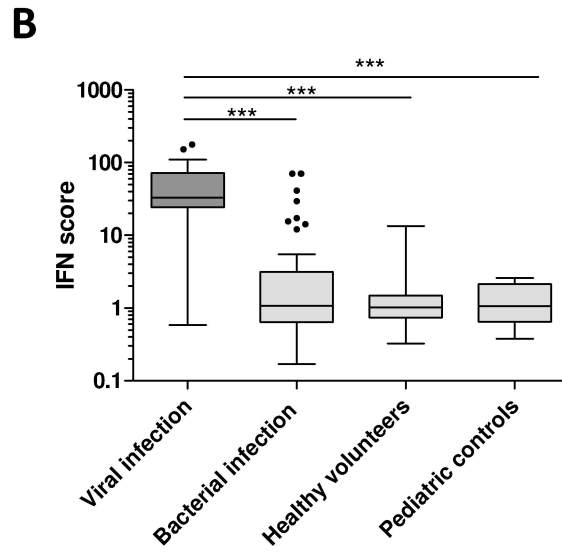
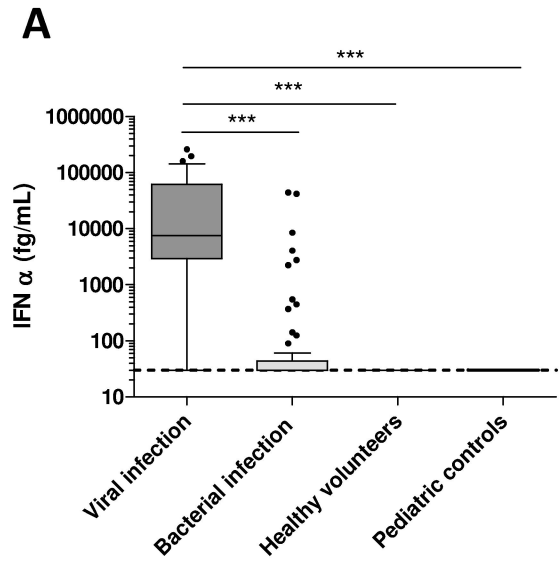
1 * t-test revealed a statistically significant difference between the two groups p<0.01. # Chi² test revealed a statistically significant difference between the two groups p<0.01

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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)