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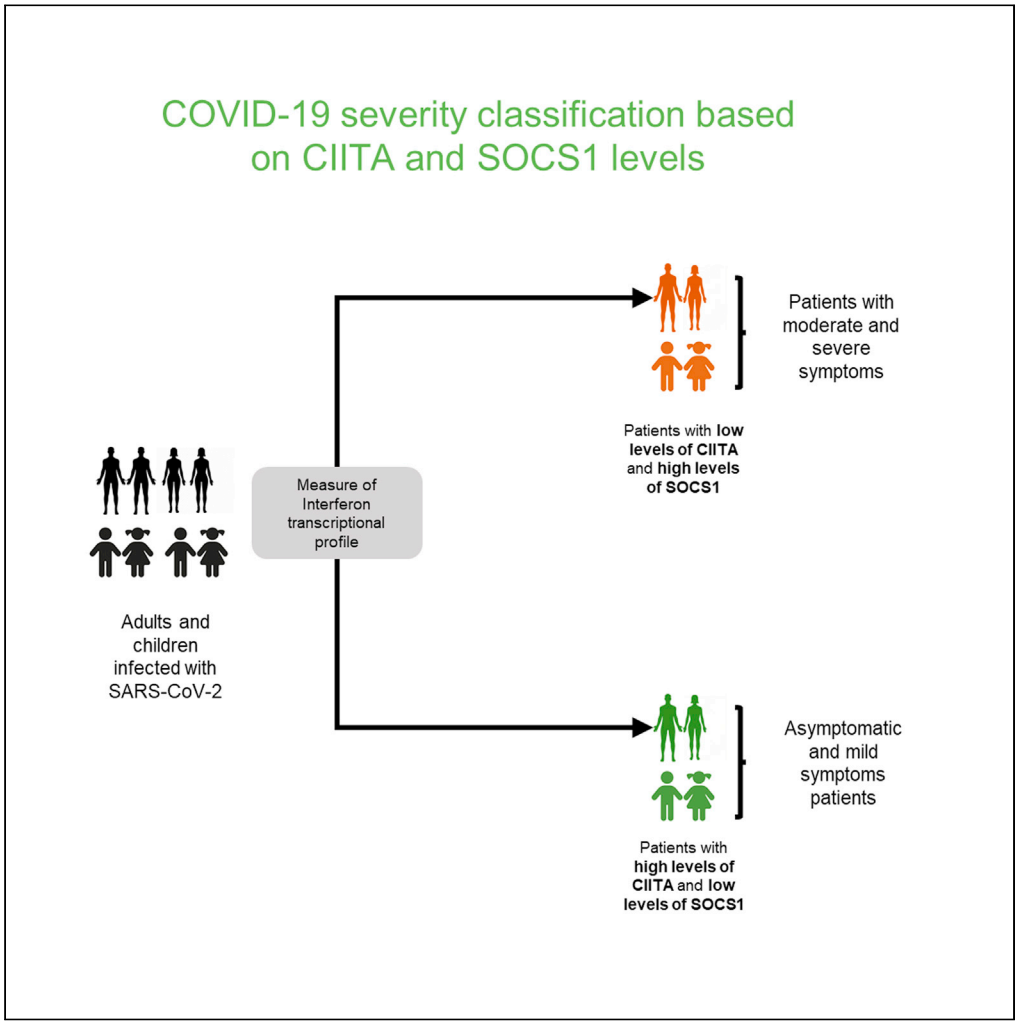

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Highlights

Interferon response seems to be pivotal for the severity of SARS-CoV-2 infection

When interferon response is deregulated, it leads to an overproduction of cytokines

Patients with severe COVID-19 had elevated cytokines, linked to interferon response

Low *CIITA* and high *SOCS1* values were indicative of severe disease

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Low levels of *CIITA* and high levels of *SOCS1* predict COVID-19 disease severity in children and adults

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SUMMARY

It is unclear why COVID-19 ranges from asymptomatic to severe. When SARS-CoV-2 is detected, interferon (IFN) response is activated. When it is insufficient or delayed, it might lead to overproduction of cytokines and severe COVID-19. The aim was to compare cytokine and IFN patterns in children and adults with differing severity with SARS-CoV-2.It was a prospective, observational study, including 84 patients. Patients with moderate/severe disease had higher cytokines' values than patients with mild disease (p< 0.001).Two IFN genes were selected to build a decision tree for severity classification: *SOCS1* (representative of the rest of the IFN genes) and *CIITA* (inverse correlation). Low values of *CIITA* and high values of *SOCS1* indicated severe disease. This method correctly classified 33/38(86.8%) of children and 27/34 (79.4%) of adults.

To conclude, patients with severe disease had an elevated cytokine pattern, which correlated with the IFN response, with low *CIITA* and high *SOCS1* values.

INTRODUCTION

In December 2019, a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated. It has caused a pandemic that has been declared a significant threat to international health by the World Health Organisation (WHO). To date, 193,657,725 cases have been reported, with 4,154,660 deaths (WHO, 2021).

In adults, the spectrum of clinical manifestations ranges from asymptomatic, or mild symptoms, to severe pneumonia with acute respiratory syndrome (ARDS) and death. Concretely, the mortality rate in hospitalized patients is around 20%–25% (Richardson et al., 2020). Secondarily to the severe outcomes of the disease, many researchers are struggling to detect risk factors or biomarkers to detect vulnerable patients in advance. On the one hand, some risk factors such as being male, older age, hypertension, obesity, and diabetes have been reported to be associated with more severe disease (Gao et al., 2021; Richardson et al., 2020). For this reason, some risk scores that include these clinical factors have been developed, in order to help to stratify the patients as soon as possible(Bello-Chavolla et al., 2020). On the other hand, immune factors have also been described as involved with a critical disease course, such as the ones related to the regulation of interferon (IFN). For instance, the presence of autoantibodies against IFN entails blocking IFN activity and, therefore, its capacity to control SARS-CoV-2 infection (Bastard et al., 2020). Furthermore, genetic inborn errors of IFN-related genes might be also detrimental for the adequate COVID-19 disease control (Zhang et al., 2020).

In children, the disease seems to be milder, with symptoms similar to other viral respiratory infections, with a low mortality rate of around 0%–0.2% (Ludvigsson, 2020; Sankar et al., 2020). There are some reasons that have been proposed to explain the milder disease in children. First, the higher levels of cross-neutralizing antibodies, lower levels of ACE-2 receptors in nasal epithelium, and immature B and T cells, might be responsible for a less specific but more immediate response. In addition, greater regulatory T cell responses, and lower IL-6 and TNF-alpha production may limit the inflammatory response (Wong et al., 2020).

However, in April 2020, an unexpected severe manifestation of COVID-19, named multisystem inflammatory syndrome (MIS-C), was reported in children. The diagnostic criteria are fever and elevated

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Continued

inflammatory biomarkers, evidence of COVID-19, as well as two of the following: rash, hypotension or shock, myocardial dysfunction, coagulopathy, and acute gastrointestinal problems (World Health Organization, 2020). It remains unclear why some children evolve to MIS-C while others manifest only a mild form of the disease (Ahmed et al., 2020). MIS-C is the main reason for requiring PICU in children with COVID-19 (Tagarro et al., 2021), and understanding the immune dysregulation underlying this disease will be fundamental to find other approaches for it.

Both in adults and children, long COVID is a concerning consequence still not well understood. It is defined by the persistence of symptoms beyond 4 weeks from the onset of the disease, and some mechanisms have been purposed, such as virus-specific pathophysiological changes, immunological alterations, and inflammatory damage, but it is still unclear (Asadi-Pooya et al., 2021; Naeije and Caravita, 2021).

Researchers are struggling to detect clinical or analytical biomarkers that could be of use in determining the risk of an individual patient to evolve to a more severe disease course, including MIS-C. In line with this, some genes have been reported to be linked to a worse prognosis (Bastard et al., 2020; Zhang et al., 2020), such as *TLR7*, whose loss-of-function variants have been associated with impaired IFN response (Van der Made et al., 2020), and other genes related to the binding of the ACE-2 cell surface receptor and also to entry at the initial stages, which could determine susceptibility to SARS-CoV-2 (Anastassopoulou et al., 2020). It thus seems that IFN is pivotal in controlling SARS-CoV-2 infection and avoiding a worse clinical course (Hadjadj et al., 2020).

Indeed, when a viral infection is detected by the innate immune sensors, IFN-I response is activated, and IFN-I response genes (IRG) are secreted by infected cells. They have three functions: (i) to limit the spread of the infectious agents, (ii) to modulate innate immune responses, and (iii) to activate the adaptive immune system (Ivashkiv and Donlin, 2014). IFN-I binds to the IFN receptor (IFNAR), and it activates an antiviral program of interferon-stimulated genes (ISGs) that can interfere with viral replication (Park and Iwasaki, 2020).

SARS-CoV-2 leads to a lower antiviral transcriptional response, with low IFN-I and IFN-III levels and elevated cytokine expression. Therefore, it seems that patients with severe COVID-19 might have a low or delayed IFN response, which could lead to high cytokine release, ending up in a hyperinflammatory state (Park and Iwasaki, 2020). That is to say, the infection has been proven to cause an immune dysregulation with overproduction of pro-inflammatory cytokines (IL-1, IL-6, IL-2R, IL-10, and TNF-alpha) in severe cases (Chen et al., 2020; Ong et al., 2020; Pedersen and Ho, 2020). What's more, in children with MIS-C, which is the severe manifestation of COVID-19, the cytokine response seems to be greater than in adults (Esteve-Sole et al., 2021).

We hypothesized that the IFN response and the cytokines released during acute SARS-CoV-2 infection differed depending on the severity of the disease. Moreover, they could be different in children and in adults. Therefore, we hypothesized that we would be able to stratify the risk of patients using these analytical values.

The main aim of the study, then, was to analyze the IFN response and pro-inflammatory cytokines in children and in adults with acute SARS-CoV-2. A secondary objective was to compare the immune response depending on the severity of COVID-19 disease using a machine learning model.

RESULTS

Eighty-four patients with SARS-CoV-2 were included in this study; 40 patients (47.6%) were children and 44 adults (52.4%). The distribution of patients in the different cohorts, genders, and age per study is presented in Table 1.

Patient demographics and characteristics

The main characteristics, risk factors, required support, and outcomes of patients with moderate and severe disease are summarized in Table 2. Patients with asymptomatic or mild disease were not included in the table because they did not have any previous relevant clinical background and they did not require any support.

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Table 1. Number of patients per cohort in interferon and cytokines studies

	Interferon transcriptional profile			Cytokine profile (14 cytokines)		
Total cohort population	Study set 72 (100)	Males	Age	Study set 77 (100)	Males	Age
Adults, 44 (52.38%)	**34 (47.22)**	**19 (26.39)**	**38.8 (29.6–46.7)**	**40 (51.95)**	**24 (31.17)**	**40.0 (32.4–46.2)**
Asymptomatic- 8 (9.52%)	8 (11.11)	4 (5.56)	49.8 (33.9–51.9)	6 (7.79)	4 (5.19)	50.9 (49.4–53.3)
Mild- 10 (11.90%)	8 (11.11)	6 (8.33)	42.5 (32.4–45.7)	10 (12.99)	6 (7.79)	41.4 (33.8–44.5)
Severe- 26 (30.95%)	18 (25)	9 (12.50)	36.0(28.3–40.2)	24 (31.17)	14 (18.1)	37.8 (31.0–41.8)
Children, 40 (47.6%)	**38 (52.78)**	**24 (33.33)**	**8.1 (2.7–13.3)**	**37 (48.05)**	**24 (31.17)**	**8.2 (3.6–13.4)**
Asymptomatic- 10 (11.90%)	10 (13.89)	7 (9.72)	5.8 (1.6–9.8)	10 (12.99)	7 (9.09)	5.8 (1.6–9.8)
Mild- 9 (10.71%)	9 (12.50)	6 (8.33)	8.9 (3.6–13.3)	8 (10.39)	5 (6.49)	9.7 (6.9–13.6)
Moderate- 10 (11.90%)	9 (12.50)	4 (5.56)	6.6 (2.4–8.3)	8 (10.39)	4 (5.19)	7.0 (2.5–9.3)
Severe- 11 (13.10%)	10 (13.89)	7 (9.72)	13.5 (6.5–15.5)	11 (14.29)	8 (10.39)	13.6 (6.7–15.4)

Values are expressed as frequency (percentage) for qualitative variables and as median (interquartile range) for quantitative variables. n: number of patients.

There were significant differences in some general blood test results between mild/asymptomatic patients and moderate/severe patients, both adults and children. Lymphocytes were lower in severe than in mild cases in adults (950/mm^3 vs. 1800/mm^3, $p< 0.001$) and also in children (600/mm^3 vs. 2450/mm^3, $p< 0.001$), while MR-proADM, procalcitonin, and C-reactive protein were also higher in severe cases ($p< 0.001$).

Circulating cytokine profile

The cytokine pattern was analyzed for 77 patients. All the different cytokines had positive correlations with each other, so they behaved in a similar manner (Figure 1). The differences in the circulating cytokine profile between different cohorts were analyzed.

Circulating cytokine profile in children–differences depending on severity

No significant differences were found in child circulating cytokine profile between severe and moderate patients; thus, they were grouped into a moderate/severe cohort (see Table S2).

Circulating cytokine profiles for severe/moderate infected patients showed significant differences when compared with those with mild/asymptomatic disease for all the analyzed cytokines, with higher levels in moderate/severe patients than in mild/asymptomatic ones (Figure 2, Table S3).

Circulating cytokine profile in adults–differences depending on severity

In adults, patients with severe disease showed higher values for all the studied cytokines than did patients with asymptomatic/mild disease (Figure 2, Table S3).

Circulating cytokine profile in children and adults–differences depending on age

Regarding the differences between the cytokine profile in adult severe and children moderate/severe patients, the latter with MIS-C had higher values of all the cytokines, except for IL-6 and MCP-3, which showed no significant differences between the two populations (Figure 2, Table S3).

The combination of IL-6 and lymphocyte counts correlates with disease severity in children and adults

IL-6 is the only cytokine that we are currently able to measure in a general routine blood test; as it behaves similarly to other cytokines, it was chosen to build a severity decision tree. The severity decision tree included IL-6 circulating levels and lymphocyte counts. Severe patients (adults and children) had low values of lymphocytes at admission and also high IL-6 values. In contrast, mild patients (adults and children) had higher lymphocyte count and lower IL-6 values (Figure 3).

Table 2. Patient characteristics, risk factors, biomarkers, and outcomes in moderate/severe COVID-19

	Adults	Children		Adult severe vs. Pediatric severe	Pediatric severe vs. Pediatric moderate
	n = 26	n = 21			
	Severe n = 26	Moderate n = 10	Severe n = 11	p values	p values
Risk factors, n (%)					
Obesity, n (%)	14 (53.8)	0 (0)	1 (9.1)	–	–
Asthma, n (%)	5 (19.2)	0 (0)	0 (0)	–	–
Arterial hypertension, n (%)	4 (15.4)	0 (0)	0 (0)	–	–
Diabetes, n (%)	1 (3.8)	0 (0)	0 (0)	–	–
Blood test					
Lymphocytes (/mm3)	950.0 (700.0–1100.0)	1150.0 (500.0–1975.0)	600.0 (400.0–700.0)	**0.007**	0.240
Lactate dehydrogenase, maximum (IU/L)	888.0 (683.5–1177.0)	621.0 (586.0–799.0)	689.0 (632.0–795.5)	0.077	0.462
Ferritin, maximum (ug/L)	945.0 (304.1–3614.5)	451.0 (235.5–619.7)	567.5 (470.0–1372.0)	0.724	0.257
D-dimer, maximum (mg/L)	2.0 (1.5–5.9)	2.8 (1.8–4.9)	6.4 (5.2–8.0)	**0.016**	**0.033**
MR-proADM (nmol/L)	0.7 (0.6–0.9)	0.9 (0.9–0.9)	1.7 (1.4–2.2)	**<0.001**	0.127
Procalcitonin (ng/mL)	0.2 (0.1–0.3)	2.4 (0.7–5.5)	11.6 (5.7–21.0)	**<0.001**	**0.017**
C-Reactive protein (mg/L)	122.6 (60.3–186.0)	186.1 (135.5–269.0)	299.0 (258.0–338.4)	**<0.001**	**0.041**
Respiratory support					
MV, n (%)	17 (65.4)	0 (0)	3 (27.3)	0.078	–
Length of MV (days)	15.0 (8.0–19.0)	–	4.0 (3.5–4.0)	**0.017**	–
NIV, n (%)	12 (46.2)	0 (0)	4 (36.4)	0.723	–
Length of NIV (days)	1.0 (0.0–3.2)	0	1.0 (0.2–1.8)	0.873	–
Haemodynamic support					
Inotropic treatment, n (%)	5 (19.2)	0 (0)	9 (81.8)	**0.001**	–
Inotropic length (days)	1.5 (1.0–2.0)	0 (0)	2.0 (2.0–3.5)	0.255	–
Maximum VIS	5.0 (5.0–10.0)	0 (0)	23.7 (12.0–30.0)	**0.034**	–
Outcomes					
Length of stay in PICU (days)	13.0 (10.0–18.8)	–	6.0 (4.0–6.5)	**<0.001**	–
Length of stay in hospital (days)	15.0 (11.0–22.8)	6.0 (5.0–7.8)	11.0 (10.5–12.0)	**0.030**	**0.002**

Values are expressed as frequency (percentage) for qualitative variables and as median (interquartile range) for quantitative variables. n: number of patients. MR-proADM: mid-regional pro-adrenomedullin. MV: mechanical ventilation. NIV: non-invasive ventilation. PICU: pediatric intensive care unit. VIS: vasoactive-inotropic score. p-values from Kruskal-Wallis test.

IFN transcriptional profile

The IFN transcriptional profile was analyzed for 72 patients. There were positive correlations for almost all the IFN genes, indicating that when any of the IFN genes was high the others also tended to be high. Only *CIITA* showed a negative correlation with all the other variables (Figure 1).

IFN transcriptional profile in children–differences depending on severity

No significant differences were found in child IFN transcriptional profile between severe and moderate patients; thus, they were grouped into a moderate/severe cohort (see Table S4).

There were differences in the IFN response between mild/asymptomatic vs. moderate/severe patients for the following genes: *GBP1*, *HERC6*, *IFIT3*, *OASL*, *SOCS1*, *and IFNA2*, which were higher in moderate/severe than in mild/asymptomatic patients, and *HERC6*, *OAS2*, *SIGLEC1*, *USP18*, *and CIITA*, which had lower levels in moderate/severe patients (Figure 4, Table S5).

After describing the values for the *Z* score for each IFN gene, a variable selection method was applied, and *CIITA* and *SOCS1* were found to be the most significant genes to build the decision tree (the correlation

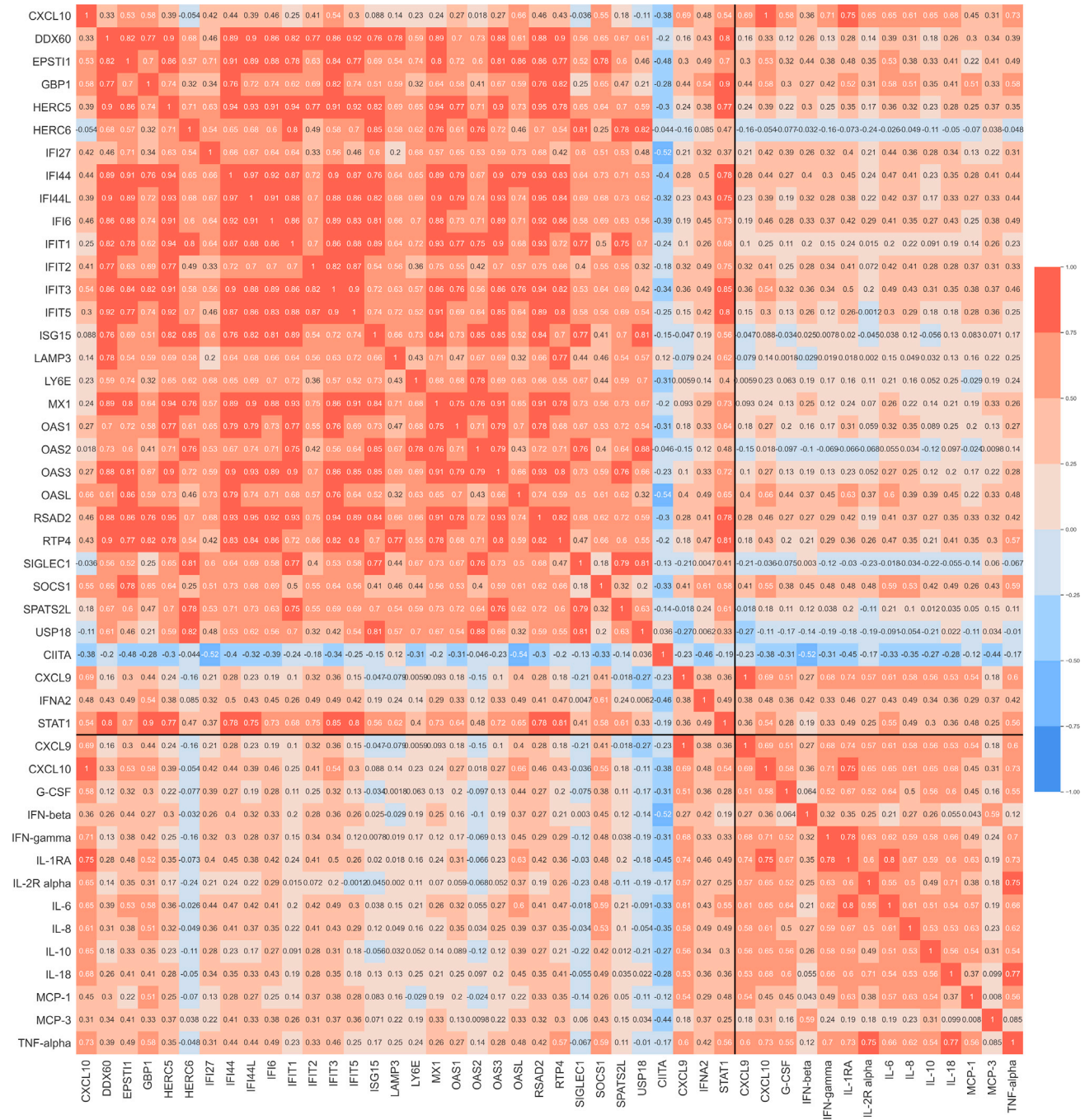

Figure 1. Correlation analysis for interferon genes and cytokines

The correlation coefficient was calculated using Spearman's rank correlation coefficient. From top to bottom and from left to right the variables plotted are *CXCL10, DDX60, EPSTI1, GBP1, HERC5, HERC6, IFI27, IFI44, IFI44L, IFI6, IFIT1, IFIT2, IFIT3, IFIT5, ISG15, LAMP3, LY6E, MX1, OAS1, OAS2, OAS3, OASL, RSAD2, RTP4, SIGLEC1, SOCS1, SPATS2L, USP18, CIITA, CXCL9, IFNA2, STAT1*, CXCL9, CXCL10, G-CSF, IFN-beta, IFN-gamma, IL-1RA, IL-2R alpha, IL-6, IL-8, IL-10, IL-18, MCP-1, MCP-3, and TNF-alpha. A color scale has been used to represent the correlations: red for positive correlations and blue for negative correlations.

coefficient between the two was −0.33), which is represented in Figure 5A. With *CITTA*≤ -1.205 and SOCS1>0.45, the patient was classified as moderate/severe; otherwise, the patient was classified as mild/asymptomatic. The decision tree correctly classified 33/38 observations (AUC 80%).

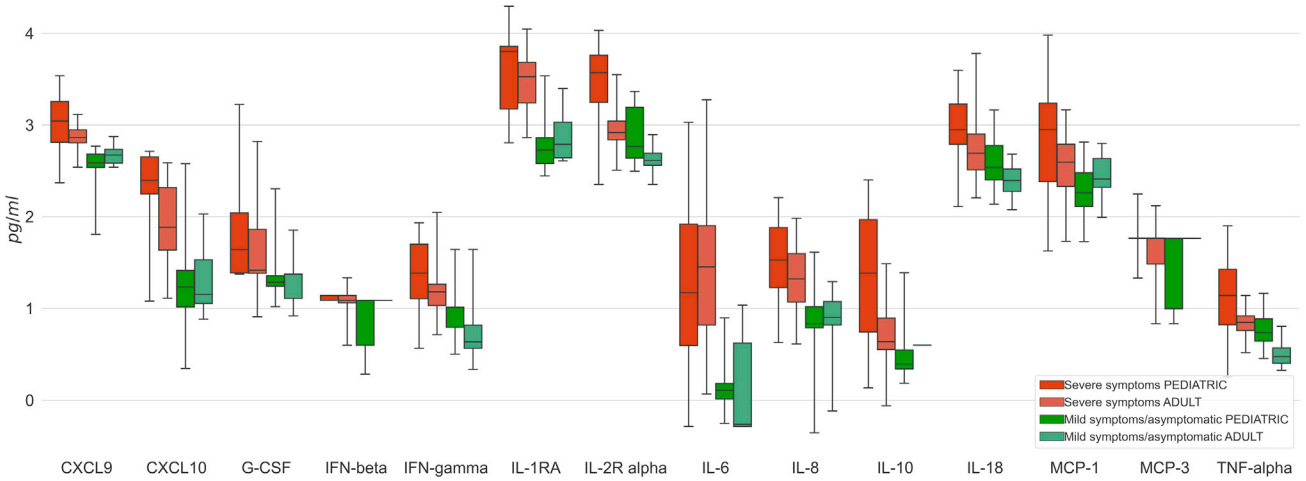

Figure 2. Cytokine profile in children and adults

Cytokines' values in patients with severe symptoms (red) and in patients with mild symptoms or asymptomatics (green). Children are represented in dark color and adults, in light color. From left to right the cytokines represented are CXCL9, CXCL10, G-CSF, IFN-beta, IFN-gamma, IL-1RA, IL-2R alpha, IL-6, IL-8, IL-10, IL-18, MCP-1, MCP-3, and TNF-alpha.

IFN response in adults–differences depending on severity

Differences were found in the following genes: *CXCL10, IFI27, IFI44L, SOCS1, CIITA, and IFNA2.* All of them presented higher values in severe patients than that in mild ones except for *CIITA,* which was lower in severe patients than in mild/asymptomatic patients (Figure 4, Table S5).

A decision tree with *CIITA* and *SOCS1* was also constructed for the adult population (Figure 5B). With *CITTA*≤ -2.441 and *SOCS1*>0.491, the patient was classified as moderate/severe; otherwise, the patient was classified as mild/asymptomatic. This model, built for the adult population, correctly classified 27/34 observations (AUC 70%).

IFN response in children and adults–differences depending on age

Regarding the significant differences between adults and children, moderate/severe children presented lower values of *IFI27, LY6E, OAS1, OASL, SIGLEC1, and SPATS2L* than did severe adults (Figure 4, Table S5).

Figure 4 shows the distributions of both variables used to train the models, *CIITA* and *SOCS1*, for the cohorts in adults and children. No significant differences were found in *CIITA* and *SOCS1* values when comparing moderate/severe adults vs. severe children ($p = 0.236$ and $p = 0.191$, respectively). However, children with mild disease had higher values for *CIITA* than did adults with mild disease (−0.3 vs. −1.4, $p = 0.009$). No differences were found in *SOCS1* values for mild/asymptomatic patients when comparing adults and children ($p = 0.202$).

IFN and cytokine correlation

Finally, the correlation between IFN genes and cytokines was analyzed (Figure 1, positive correlations in red and negative ones in blue). In general, the correlation between IFN genes and cytokines was not strong. However, some of the cytokines—*CXCL10, IFN-beta, IFN-gamma, IL-6, IL-8, IL-10, and TNF-alpha*—correlated in a strong positive way with *SOCS1* ($\rho = 0.55, 0.45, 0.48, 0.59, 0.53, 0.42, 0.59$, respectively) and in a negative way with *CIITA*. ($\rho = -0.38, -0.52, -0.31, -0.33, -0.35, -0.27, -0.17$, respectively).

DISCUSSION

This is the first study to analyze the IFN and circulating cytokine profiles in children and adults with COVID-19. The importance of the role of interferon has been pointed up in other studies (Bastard et al., 2020; Haddjadj et al., 2020; Zhang et al., 2020), and, related to its function, important data have come to light in this study. The main finding of the study is that the IFN response is different depending on the severity of the

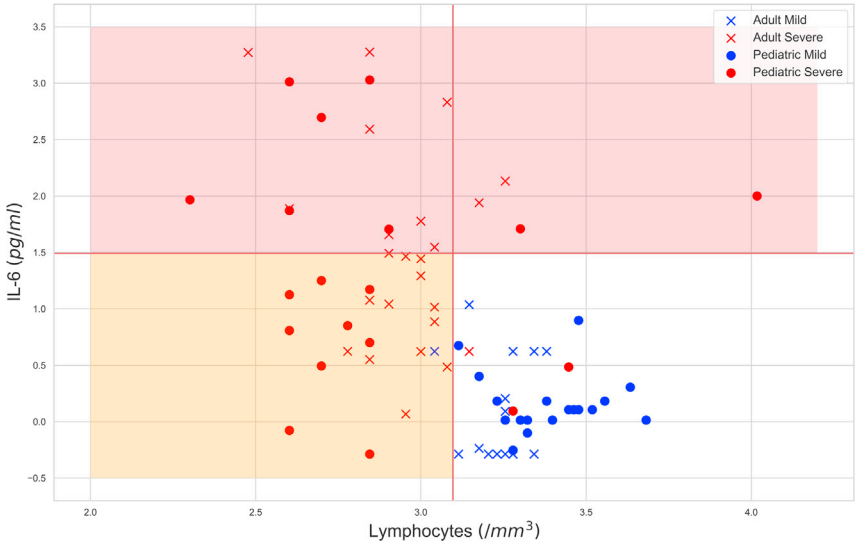

Figure 3. Severity decision tree classification for interleukin-6 and lymphocytes

Four sections were created. Each pediatric patient was represented with a colored circle, linked to the severity of the disease as follows: blue = mild, orange = moderate, red = severe. Each adult patient was represented with a colored times, linked to the severity of the disease as follows: blue = mild, red = severe. We can see that most patients with mild disease were located in the lower-right section, and most patients with moderate-severe disease in the upper-left section.

disease. Moreover, some circulating cytokine levels are tightly correlated with IFN gene expression, which could be useful as per future biomarkers and therapeutic targets.

First, from the general blood results, inflammatory biomarkers such as D-dimer, ferritin, LDH, and CRP were higher in severe cases than in milder disease, in line with findings in previous studies (Chen et al., 2020). Moreover, T lymphocytes seem to be generally decreased in COVID-19, but they were significantly lower in severe cases, which suggest a diminished and delayed adaptive immune response to the virus (Bolouri et al., 2021; Chen et al., 2020). Then, using the inflammatory biomarkers from the general blood test might be helpful to detect risky patients. For instance, *Gerotziafas et al.* have validated a risk score, which includes biomarkers (D-dimer, CRP, and lymphocytes count, among others) and clinical characteristics (obesity and male gender) to stratify patients into risk cohorts for severe disease (Gerotziafas et al., 2020). However, this study does not include pediatric population, or immune factors. Including cytokines and IFN response as biomarkers would probably increase the sensitivity and specificity for detecting those vulnerable patients.

In examining the cytokine pattern in the different cohorts, we saw that patients with severe disease (adults and children) had an increased inflammatory cytokine profile with respect to patients with mild disease, which is consistent with other studies (Esteve-Sole et al., 2021). Interestingly, even if the described hyperinflammation in MIS-C differs from the severe acute COVID-19 inflammation in adults (Consiglio et al., 2020), there was an increased inflammatory cytokine profile in both populations, although in children some inflammatory cytokines were higher than in adults. Higher values for IL-10 and TNF-alpha in children with MIS-C, in comparison to severe COVID-19 in adults, have previously been reported (Diorio et al., 2020). We have confirmed those results in our sample because both IL-10 and TNF-alpha were higher in children with severe disease than in adults.

After correlating the general blood test results with the circulating cytokine profile, interesting correlations were found. IL-6, which has been described as elevated in severe disease (Galván-Román et al., 2021) and which is available in the regular blood test, was chosen to build a decision tree together with the lymphocyte count at admission. This correctly classified most patients; thus, one of the main proposals emerging from the present study is to include them both in the regular screening of patients with COVID-19, because it allows physicians to discriminate severe from mild patients. Moreover, in cases with elevated IL-6, IL-6

Figure 4. Interferon transcriptional profile in children and adults

Interferon transcriptional profile in patients with severe symptoms (red) and in patients with mild symptoms or asymptomatics (green). Children are represented in dark color and adults, in light color. From left to right: *CXCL10, DDX60, EPSTI1, GBP1, HERC5, HERC6, IFI27, IFI44, IFI44L, IFI6, IFIT1, IFIT2, IFIT3, IFIT5, ISG15, LAMP3, LY6E, MX1, OAS1, OAS2, OAS3, OASL, RSAD2, RTP4, SIGLEC1, SOCS1, SPATS2L, USP18, CIITA, CXCL9, IFNA2, STAT1.*

blockers such as tocilizumab should be considered to diminish the inflammatory state and improve outcomes, as suggested in other studies (Gordon et al., 2021; Rubin et al., 2021).

Interestingly, some of the cytokines, including CXCL10, IL-6, IL-8, TNF-alpha, INF-beta, and INF-gamma, were correlated with the expression of IFN genes. The IFN pathway has been physiologically found to be a key point in the immune response (Calabrese et al., 2021; Lee et al., 2020). In the present study, all the genes included in the IFN signature correlated with each other, and all the variables behaved similarly. Higher values of expression of these genes were related to more severe disease in both adults and children. This is consistent with the data reported before, in which SARS-CoV-2 infection was proven to cause an immune dysregulation with overproduction of cytokines (IL-1, IL-6, IL-2R, IL-10, and TNF-alpha) and a reduction in IFN-γ expression in CD4 T cells, in severe cases (Chen et al., 2020; Ong et al., 2020; Pedersen and Ho, 2020). Therefore, anti-inflammatory treatments should be considered when it comes to severe diseases (Lee et al., 2020).

In contrast to the other IFN genes, which increase with the severity of the disease, low values of *CIITA* expression were linked to more severe disease and worse prognosis. *CIITA* is part of the IFN-stimulated immune response and it plays a role in the antiviral response as well as in antigen presentation. It can inhibit cathepsins, redirecting the virus into a degradation pathway in multivesicular bodies or lysosomes. Because it reduces viral transcription, assembly, and release, it plays a protective role (Bruchez et al.,

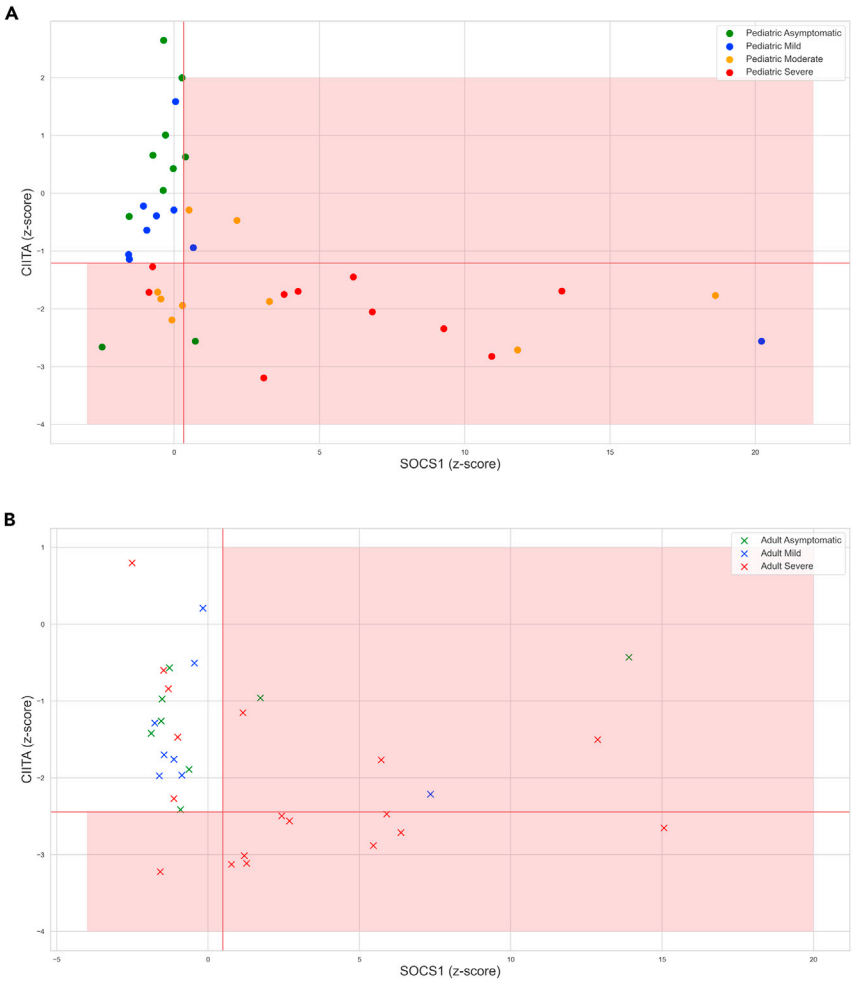

Figure 5. Decision tree for severity classification in children and adults

(A) Decision tree for severity classification in children. With the cut-off points of *CIITA* = −1.205 and *SOCS1* = 0.45, four sections were created. Each patient was represented with a colored circle, linked to the severity of the disease as follows: green = asymptomatic, blue = mild, orange = moderate, red = severe. We can see that most patients with moderate and severe disease were located in the lower-right section, and most patients with mild disease in the upper-left section.

(B) Decision tree for severity classification in adults. With the cut-off points of *CIITA* = −2.441 and *SOCS1* = 0.491, four sections were created. Each patient was represented with a colored circle, linked to the severity of the disease as follows: green = asymptomatic, blue = mild, orange = moderate, red = severe. We can see that most patients with moderate and severe disease were located in the lower-right section, and most patients with mild disease in the upper-left section.

2020; Butowt et al., 2020). This could explain why patients from this cohort with lower *CIITA* values had more severe disease.

It is true that there are already some biomarkers and risk scores that might help to detect patients at high risk of developing severe COVID-19 (Bello-Chavolla et al., 2020; Gerotziafas et al., 2020). However, this work adds valuable information, because it puts light on the physiopathology of the disease. Understanding the immune and inflammatory factors involved in SARS-CoV-2 might help to find other approaches and treatments. In concrete, *CIITA* might be a diagnostic tool, but it even might be a target in future research; because it seems a protective factor, increasing its activity might be useful for fighting against the disease. However, this is a preliminary data, thus these results need to be confirmed in further studies.

To sum up, there are different biomarkers that might be helpful to stratify patients with COVID-19 into risk cohorts. First, there is the lymphocyte count, because lymphopenia is related to severe disease. Secondly, a high cytokine response, including IL-6, may also be an indicator of severe disease, meaning that

anti-inflammatory treatment such as tocilizumab should be considered. Finally, IFN response seems to be extremely important in managing SARS-CoV-2-related infection. According to the findings of this study, the IFN pathway varies depending on the severity of the disease. Low *CIITA* values seem to be linked to more severe COVID-19; we propose as cut-off points for severe disease $CITTA \leq -2.441$ for adults and $CITTA \leq -1.205$ for children. In contrast, high values for *SOCS1*and for all the other IFN genes were found in severe disease, and the proposed cut-off points are $SOCS1 > 0.491$ for adults and $SOCS1 > 0.45$ for children.

We reckon that these results need to be confirmed in additional studies with larger samples, but meanwhile these IFN genes may be taken into consideration when looking for therapeutic targets against SARS-CoV-2.

Limitations of the study

We acknowledge several limitations in this study. It is a single-centre study with a relatively small sample size. Therefore, the results may not be extrapolable to other populations. Moreover, it would be extremely useful to examine the IFN expression and circulating cytokine profiles of the same patients at different time points of the disease, to better understand the overall immune response to it. Despite this, the results of the present study are promising, and further studies in line with this one should be performed so as to confirm them.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- METHOD DETAILS
  - Study design and participants
  - Ethics
  - Data collection variables
  - Sample analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Data analysis
  - Correlation analysis
  - Patient classification via machine learning models

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103595.

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AUTHOR CONTRIBUTIONS

Protocol design: M.G, A.E, S.B, M.H, L.A, I.J. Patient recruitment: M.G, A.E, S.B, M.H, L.A, I.J. Analytical analysis: A.M, A.C, C.J. Statistical analysis: G.A, XP.B, E.B. Results discussion and interpretation: All the

authors. Article writing: M.G, G.A, A.E, S.B, XP.B, E.B, M.H, L.A, I.J. Final manuscript revision: all the authors. The authors who verify these authors' contributions are L.A and I.J.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Tempus Blood RNA tube	Applied Biosystems™	Cat#4342792
Tempus™ Spin RNA Isolation Kit	Invitrogen™	Cat#4380204
nCounter XT Elements XT-ELE-036-P1TS	NanoString Technologies	Cat# 41121000003
XT Elements Master Kit 48 rnxs	NanoString Technologies	Cat# 041110064-K
NanoString Probes	Kim et al., 2018	NA
Human Magnetic Luminex Assay	BIO-TECHNE R&D SYSTEMS, SL	LXSAHM-16
Biological samples		
Whole blood, PBMC, serum and plasma from adults and children with SARS-CoV-2 infection	Hospital Sant Joan de Déu	NA
Pediatric Biobank for Research	https://www.irsjd.org/en/core-facilities/paediatric-biobank-for-research/	NA
Software and algorithms		
Python version 3.7	https://www.python.org/psf/	NA
nSolver software	NanoString Technologies	NA

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Iolanda Jordan (yolanda.jordan@sjd.es).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Data: Data reported in this study cannot be deposited in a public repository due to confidentiality reasons, which are mandatory according to the Ethical Committee. However, they might be available upon request to the Lead Contact. To request access, contact Iolanda Jordan (yolanda.jordan@sjd.es).

Code: This paper does not report original code.

Additional information: Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request. Clinical registry data: PIC-58-80.

Keywords: SARS-CoV-2; COVID-19; interferon; cytokines.

METHOD DETAILS

Study design and participants

This was a prospective and observational study, performed in the Hospital Sant Joan de Déu in Barcelona, which is a tertiary pediatric referral hospital with 326 beds.

Various cohorts of patients infected with SARS-CoV-2, from March 2020 to January 2021, for whom was possible to analyze the IFN transcriptional profile and the circulating cytokine profile, were included. They were classified into different groups depending on the age and the severity of the disease (see Table S1). These different populations were analyzed separately to compare their clinical characteristics and analytical patterns.

Ethics

The study followed the Declaration of Helsinki recommendations and all subjects signed the informed consent form. The study was approved by the hospital ethics committee and institutional review board with the registry number PIC58-20.

Data collection variables

The following demographic data were collected: age, gender, and comorbidities. Respiratory support used was also examined: oxygen, high flow nasal cannula, need for non-invasive ventilation (NIV), and mechanical ventilation (MV). Haemodynamic support requirement was analyzed. The analytical biomarkers recorded were C-reactive protein, procalcitonin, mid-regional pro-adrenomedullin (MR-proADM), ferritin, D-dimer, lactate dehydrogenase, and blood counts. The length of stay in the hospital and in the PICU, as well as the mortality rate, were also recorded.

Sample analysis

The sample analysis of IFN and cytokines was performed on admission, prior to any immunomodulatory treatment.

- IFN transcriptional profile: Whole blood samples were obtained in Tempus™ tubes (Applied Biosystems, California, USA) vigorously mixed and stored at −80°C. Total RNA was then isolated using the Tempus Spin RNA Isolation Kit (Applied Biosystems, California, USA) following the manufacturer's recommendations. RNA was quantified by spectrophotometry (Nanodrop, 2000; ThermoScientific, MA, USA) and assessed for quality using A260/A280 ratio. Gene expression of 32 genes was determined by Nanostring using the nCounter Elements Technology System according to the manufacturer's recommendations (NanoString Technologies, Seattle, WA). Twenty-eight of the 32 genes have been previously described as "type-I IFN response genes" (IRG) (Kim et al., 2018). In addition, we included 4 genes (*CIITA*, *CXCL9*, *IFNA2*, and *STAT1*) related to the IFN pathway but not strictly related with IFN-I response. Data were imported into nSolver analysis software (version 2.5) for quality checking and then were normalized, first to the geometric mean of six internal positive control sequences that are not native to any known organism, and second to the geometric mean of four housekeeping genes (*ALAS1*, *HPRT1*, *TBP*, and *TUBB*). A *Z* score for each gene was calculated using the mean and standard deviation of a cohort of 26 healthy controls, which included 18 healthy adults and 8 healthy children, using the following equation for each gene:

$$Z - scoreforeachgene = \frac{[genecount - meancount(HC)]}{[standarddeviation(HC)]}$$

The genes included were: *CXCL10, DDX60, EPSTI1, GBP1, HERC5, HERC6, IFI27, IFI44, IFI44L, IFI6, IFIT1, IFIT2, IFIT3, IFIT5, ISG15, LAMP3, LY6E, MX1, OAS1, OAS2, OAS3, OASL, RSAD2, RTP4, SIGLEC1, SOCS1, SPATS2L, USP18, CIITA, CXCL9, IFNA2, and STAT1*.

- Cytokine quantification was made by Luminex immunoassay (Bioscience) performed in serum or plasma of patients and healthy controls following the manufacturer's instructions. Cytokines detected included G-CSF, IFN-beta, IFN gamma, IL-1RA, IL-2R alpha, IL-6, IL-8/CXCL8, IL-10, IL-18, CXCL9, IP-10/CXCL10, MCP-1/CCL2, MCP-3, and TNF-alpha. Plates were read using a Luminex xMAP 100 analyzer.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis

Categorical variables were indicated as frequency (n) and percentage (%), whereas continuous variables were summarized as median and interquartile range (IQR) because they did not follow a normal distribution. Continuous variables were compared using the non-parametric Kruskal-Wallis test. Probability values < 0.05 were considered statistically significant.

Correlation analysis

Full correlation matrix between each pair of variables was computed. Since variables did not follow a normal distribution, correlations were analyzed with Spearman's rank correlation coefficient. Once computed, the correlation matrix was plotted using a heatmap where red indicates strong correlations (coefficient close to 1) and blue indicates negative correlations (coefficient close to −1).

Patient classification via machine learning models

With the objective of evaluating how well the patients could be classified based on the genetic variables collected, a machine learning supervised classifier was used. This type of method, when provided with a series of input variables and their known associated outcome class, automatically 'learns' the relationship between measured variables and outcome. Their output is then useful both to classify future cases and to quantify the relationships between inputs and clinical outcomes.

In this case, input variables were IFN gene variables values, and the class was the clinical outcome of the patients grouped in two classes: (1) asymptomatic and mild in one class and (2) moderate and severe in the other.

We used the classical CART algorithm as base classifier. This method maps the variables collected for each patient into their class in the shape of a decision tree. The tree is composed of nodes that branch to subsequent children nodes depending on the patient's variables. The tree is built in such a way that each branch separates the two classes as much as possible. This separation is measured as Shannon entropy, where a node with an entropy of zero means that the classification is perfect (either all or none of the patients developed a severe disease due to COVID-19) and an entropy of one is the worst possible mix (50%/50%). Once formed, each node contains a disease probability, calculated from the number of positive samples that reach the node, divided by the total number of samples. This approach has been widely used and validated in many applications and in other medical papers.

Due to the small sample size we wanted to avoid overfitting and sought to produce a simple, intuitive classifier that would explain the collected data in the simplest possible way. Therefore, instead of directly applying the classifier using all input variables, we first applied a variable selection process. Variable selection is a process for selecting those variables which contribute most to the target outcome. These techniques allowed us to reduce overfitting, improve accuracy, and reduce training time, because having redundant or irrelevant data as input in classification models increases the probability of making a decision based on noise. Variable importance is calculated as the decrease in node impurity weighted by the probability of reaching that node. The node probability can be calculated by the number of samples that reach the node, divided by the total number of samples. In this study we used the Extra Tree Classifier. This algorithm for a given numerical attribute selects its cut-point fully at random, i.e., independently of the target variable. At each tree node, this is combined with a random choice of a certain number of attributes from which the best one is determined. Applying the variable selection algorithm, we obtained the list of variables sorted by importance in explaining the target variable. From the output of the variable selection algorithm, we chose only the two most relevant variables that did not correlate strongly; variables are correlated when the correlation coefficient is greater than 0.4 (in absolute value).

Finally, using the variables selected, the CART algorithm was used to build a tree classifier. Since the goal of the study was to assess IFN response in children and evaluate the differences with respect to adults, different classifiers were built separately for children and adults, using the same variables. The classifiers were built using 10-fold cross-validation as recommended in TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis) guidance.

Once the tree classifiers were built, in order to make interpretability easier, in addition to the usual tree representation, we plotted the results in a bidimensional plot where each of the axes is one of the variables. The classification boundaries defined by the tree are shown with a red line and the patients are represented with a dot in different colors depending on their clinical outcome.

All statistical analysis was performed using Python version 3.7 (Python Foundation, USA).