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To the Editor,

Since April 2020, an increasing number of authors have reported a new clinical syndrome related to SARS-COV2 infection in children, characterized by rapidly progressive systemic inflammation with multiorgan dysfunction. This syndrome has been addressed with many names since its description such as Multisystem Inflammatory Syndrome in children (MIS-C), Pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-ST), and SARS-CoV-2–Induced Kawasaki-Like Hyperinflammatory Syndrome (SCiKH).^{1–3} The pathogenesis of MIS-C is still unknown, even if the positivity of a nasopharyngeal RT-PCR assay for the SARS-COV2 and/or of specific antibody testing in most patients suggests that this syndrome develops while the immune system is activated against the SARS-COV2 virus.

Immunophenotype studies in COVID-19 patients have demonstrated a peculiar B cell response with increased plasmablast (PB) frequency.⁴ Interestingly, the increase in PB is not associated with an augmentation in spike protein-specific IgG titer. Recently Carter *et al.* showed that some MIS-C patients also have a progressive increase of PB proportion.⁵

The role of B lymphocytes in the acute immune response against coronavirus is still debated. Clinical observations are discordant; notably Quinti *et al.* have observed a milder disease course in two patients with X-Linked Agammaglobulinemia (who completely lack circulating B cells) compared to patients with Common Variable Immunodeficiency.⁶ On the other hand, Schulze-Koops *et al.* have reported a more severe SARS-COV2 infection in patients treated with Rituximab.⁷

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Between April and July 2020, we performed peripheral B lymphocyte immunophenotype in 5 patients with positive specific IgG against SARS-CoV2, who satisfied WHO preliminary case definition criteria for MIS-C,¹ and compared them with 8 children affected by COVID-19 and 15 age-matched healthy donors (HD). Ethical approval for this study was obtained from the local committee (protocol number: 00282/2020). In MIS-C and COVID-19 groups samples were collected within 7 days after disease onset, on the day of admission to our service (t0), and 4-6 weeks later, at post-discharge follow-up visit (t1).

B cells subsets were divided into Transitional B (TrB) cells (CD38+IgM+), naïve B cells (CD27-IgM+IgD+), IgM memory B cells (CD27+IgM+IgD+), switched memory B (SMB) cells (CD27+IgM-IgD-), and PB (IgM-CD38+++ cells). B cell subpopulations of the MIS-C and COVID19 patients were compared to one another at t0 and t1, and also with HD. Subsets were compared both as percentage of CD19+ cells and as absolute numbers (cell/mm³), expressed as median and Interquartile Ratio, using Mann-Whitney U-test. All tests were two tailed and the significance was set at $P \leq 0.05$.

Clinical and demographic features of MIS-C and COVID-19 groups, as well as lymphocyte count at t0 and t1, are described in Table1; disease severity was assessed according to Qiu *et al.*⁸

At t0, MIS-C patients had a considerably lower percentage of TrB cells than HD (7,2% vs 10,7%, $p=0.01$), the same difference was not present considering absolute TrB ($p=0.93$), demonstrating that the relative reduction is mainly due to the increase of non-TrB cells. As previously reported,⁴ the COVID-19 group had an increased percentage of PB (2.7%) compared to HD (0.8%, $p=0.02$); the same difference was present comparing COVID-19 and MIS-C groups (0.7%, $p=0.02$) (see figure 1).

When compared to HD, at t1 MIS-C patients showed a noticeable TrB percentage decrease (2,6% vs 10,7%, $p=0.01$). On the one hand, this could be explained by a slight absolute reduction of TrB (10,1/mm³ vs 22,7/mm³ $p=0,05$), and on the other hand by a marked increase in peripheral effector/memory B cells, including PB (14,4/mm³ vs 1,4/mm³ $p=0,004$), SMB cells (66,8/mm³ vs 16,8/mm³, $p=0.01$) and MZB (69,0/mm³ vs 34,9/mm³, $p=0.03$). MIS-C patients have an expansion of CD27+ B cells - specifically of SMB (66,8/mm³ vs 34,9/mm³, $p=0.03$) and MZB (69,0/mm³ vs 33,8/mm³, $p=0.04$) - even when compared with COVID-19 patients. Interestingly, there was no difference in PB count at t1 in COVID vs MIS-C, while both groups showed a significant increase, both in relative and absolute values, compared to HD (see figure 1).

In summary, MIS-C patients show a remarkable rise of circulating SMB and MZB cells over time; conversely, PB expansion seems to appear in all the patients with SARS-CoV2 infection, but it is delayed in the MIS-C group. Noticeably, the increase of SMB, together with the decrease of TrB, has been reported in adult patients admitted to ICU,⁹ possibly suggesting a similar immune activation in MIS-C and severe SARS-COV2 infection in adults.

Overall, the results suggest that B cell activation following SARS-COV2 infection has a different kinetic in MIS-C patients, as compared to children with acute infection. Indeed, a marked increase of CD27+ B lymphocytes overtime was observed in the former group. It is unknown why SARS-COV2 infection triggers PB expansion in all infected children, while SMB and MZB increase was seen exclusively in MIS-C patients. Nevertheless, the similarities between B cells response in MIS-C and in severe SARS-COV2 infection of adults support a common pathogenesis. This study presents some limitations, such as the small sample size and the use of a semi-quantitative method to assess specific SARS-CoV2 IgG, which prevented the comparison of IgG titers among groups.

Further studies in bigger cohorts are needed to clarify if the increased CD27+ B cells may produce the autoantibodies that have been detected in MIS-C patients¹⁰, and to investigate the potential utility of B immunophenotyping in differentiating MIS-C from other inflammatory conditions, such as Kawasaki disease.

Keywords: COVID-19; SARS-CoV-2; MIS-C; B cells; flow cytometry; plasmablasts; Pediatric;

The authors declare no conflict of interest.

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Table1: Demographic, clinical features and treatment of COVID-19 and MIS-C groups.
Continuous variables are expressed as median and IQR, categorical variables are expressed as relative frequency.

	MIS-C (n=5)		COVID-19 (n=8)	
Sex	80 % male		50 % male	
Age	8,3 (6,0-13,0)		11,0 (7,3-14,4)	
Disease severity:				
• moderate	• 0 %		• 75 %	
• severe	• 60 %		• 25 %	
• critical	• 40 %		• 0 %	
Clinical presentation:				
• Fever (>38°)	• 100 %		• 75 %	
• Cough	• 20 %		• 37,5 %	
• Pneumonia	• 0 %		• 25 %	
• Gastro-intestinal	• 80 %		• 12,5 %	
Increased pro-BNP	80 %		0 %	
Hypotension	80 %		0 %	
ICU admission	40 %		0 %	
Positive PCR SARS-COV2 at onset	60 %		100 %	
Seroconversion for SARS-CoV2	100 %		100 %	
IgG (mg/dL)	686 (612-836,5)		NA	
	T0	T1	T0	T1
Lymphocytes (*10 ³ /mmc)	1,61 (1,03-2,48)	2,15 (1,36-2,91)	2,58 (2,17-3,14)	2,97 (2,47-3,21)
Treatment:				
• Hydroxychloroquine	• 0 %		• 25,0 %	
• Azithromycin	• 0 %		• 37,5 %	
• IVIG	• 40 %		• 0 %	
• IV steroid	• 100 %		• 0 %	

