intensivists who, throughout these 25 years, have developed their cardiac pacing activity and collaborated developing the registry.

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Innate cell response in severe SARS-CoV-2 infection in children: Expression analysis of CD64, CD18 and CD11a

Respuesta de celular innata en infeción pediátrica grave por SARS-CoV-2: análisis de expresión de CD64, CD18 y CD11a

Dear Editor,

In January 2020, a new coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was described in Wuhan, China. The virus, which produces coronavirus disease 2019 (COVID-19), has been declared a global health emergency and pandemic by the World Health Organization. Spain is one of the more severely affected countries.[1]

The immune response to SARS-CoV-2 infection appears to be a critical factor in the development and prognosis of COVID-19 patients.[2] In children, severe forms of the disease like the pediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 appears to be related with some immune dysregulation.[3] So, increase knowledge about the innate cellular immune response to SARS-CoV-2 is of great interest. To this, the study by flow cytometry (FC) may provide critical data and further understanding of this novel disease.[3]

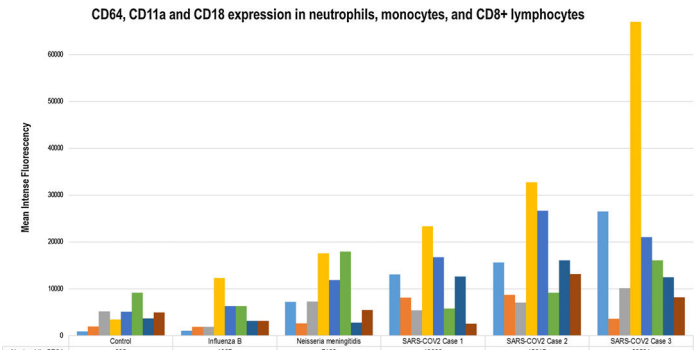
In this paper, we study three molecules which are part of the innate cellular response to infection: CD64, CD18 and CD11a. The CD64 is a type I high-affinity receptor for the Fc fraction of the immunoglobulin G. It is located on monocytes, macrophages, dendritic cells, and neutrophils. The CD64 density on the cell surface is related to the stimulation received by inflammatory cytokines[4]. The CD18, also known as integrin beta-2, participates in leukocyte adhesion and signaling. The CD11a associates with CD18 to form the lymphocyte function-associated antigen 1, or LFA-1. This LFA-1 on leukocytes plays a central role in leukocyte cell-cell interactions and lymphocyte stimulation.

We study in this report three children with severe SARS-CoV-2 infection. Also, we compare them with a healthy control, a case of severe influenza infection and a case of Neisseria meningitidis sepsis. All cases

Table 1 Epidemiologic characteristics, clinical features, radiologic findings, and management of children admitted for pediatric critical care due to *Influenza B*, *Neisseria meningitidis* and SARS-CoV-2 infection.

	Influenza B	Neisseria meningitidis	Case 1	Case 2	Case 3
Age in years	4	9	12	11	7
Sex	Male	Male	Male	Male	Female
Referring department	Emergency department, 1 day of symptoms	Emergency department, 1 day of symptoms	Emergency department, 3 days of symptoms	Emergency department, 2 days of symptoms	Pediatric ward, 4 days of symptoms (one day of admission)
Previous diseases	No	No	No	No	No
Signs and symptoms prior to PICU admission	Tachipnea, hypoxemia, fever	Tachycardia, hypotension, fever	Fever, nausea, vomiting, diarrhea	Fever, nausea, diarrea, adenopathy	Fever, abominal pain
Cause of PICU admission	Respiratory isntability	Hemodynamic instability	Hemodynamic instability	Hemodynamic instability	Hemodynamic instability
PRIMS III	0	3	4	4	7
Total leukocytes/μL	9640	5710	11,410	7880	3820
Neutrophils/μL	4360	3280	10,510	7150	3160
Lymphocytes/μL	4450	2110	320	430	410
PCR mg/dl (0.01–1)	8.2	13	15.88	16.67	11
PCT ng/ml (0.1–0.5)	0.33	3.75	4.28	10.29	1.78
Ferritin ng/mL (7–140)	ND	ND	888	1110	1349
D-dimer mg/L (0–0.5)	ND	ND	3.85	4.22	7.37
IL-6 pg/ml (≤7)	ND	ND	63.2	11	85
Chest X-ray on PICU admission	Bilateral peribronchial thickening	No pathological findings	Bilateral pneumonia	No pathological findings	Bilateral pneumonia
Bilateral pneumonia developed while PICU treatment	No	No	Yes	No	Yes
Echocardiogram	Not done	Normal heart function	Normal heart function	Normal heart function	Normal heart function
Maximal respiratory support	BiPAP	Nassal cannula	HFNC	HFNC	HFNC
Inotropic support	No	No	No	No	Yes
Other support	No	No	No	No	No
Broad-spectrum antibiotics because of suspected bacterial coinfection	Yes	Yes	No	No	Yes
Azithromycin	No	No	Yes	Yes	Yes
Lopinavir/ritonavir	No	No	Yes	Yes	Yes
Remdesivir	No	No	No	No	No
Hydroxychloroquine	No	No	Yes	Yes	Yes
Steroids	No	No	Methylprednisolone (1 mg/kg/day)	Methylprednisolone (1 mg/kg/day)	Methylprednisolone (1 mg/kg/day)
Immunoglobulins	No	No	No	Yes	No
Tocilizumab	No	No	No	No	Yes
Heparin	No	No	Yes, prophylactic	Yes, prophylactic	Yes, prophylactic
Confirmed coinfection	No	No	No	No	No
Days of PICU admission	5	7	5	6	9

PICU: pediatric intensive care unit; HFNC: high flow nasal cannula; BiPAP: Bilevel Positive Airway Pressure; pSOFA: Pediatric Sequential Organ Failure Assessment; PRISM III: Pediatric Risk of Mortality Score; ND: not done.



	Control	Influenza B	Neisseria meningitidis	SARS-COV2 Case 1	SARS-COV2 Case 2	SARS-COV2 Case 3
Neutrophils CD64	902	1007	7182	13069	15617	26501
Neutrophils CD11a	1953	1865	2607	8130	8700	3591
Neutrophils CD18	5156	1865	7258	5391	7075	10161
Monocytes CD64	3411	12341	17540	23326	32750	89009
Monocytes/CD11a	5061	6283	11855	16772	26696	21047
Monocytes/CD18	9148	6283	17970	5754	9137	16087
CD8/CD11a	3662	3160	2792	12643	16080	12450
CD8/CD18	4958	3160	5497	2528	13166	8196

Figure 1 (A) CD64 staining on granulocytes, monocytes, and lymphocytes in peripheral blood samples obtained on pediatric critical care unit (PICU) admission. From left to right, we can observe the CD64 expression. CD64 is expressed on monocytes and neutrophils but not lymphocytes (internal negative control). The positive CD64 region is located to the right of the dotted line. As can be seen, neutrophils gated in the control case are crossed by this line. (B) Mean fluorescence intensity (MFI) values for CD64, CD11a, and CD18 are given for each case in the form of a bar chart. As can be seen in all cases, CD64 and CD11a expression is higher in SARS-CoV-2 cases. This observation is clear for CD64. The CD11a expression on CD8+ lymphocytes is also upregulated compared to previous data.

included had SARS-CoV-2 infection confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) on nasopharyngeal swab samples. The cases trajectories, complementary tests, and therapy approaches are summarized in Table 1. The children were studied after informed consent was obtained. One 0.5 ml sample of peripheral blood was extracted on admission to the pediatric intensive care unit (PICU). The samples obtained were collected in sterile EDTA at room temperature or refrigerated at 4 °C, after which they were used for CD45+ cell-marker studies and analyzed by FC within 24 h. Cell surface expression of CD64, CD18, and CD11a was measured by BD FACS Canto II flow cytometer (Becton Dickinson, New York, USA). CD64 (clone 10.1), CD18 (clone CBR LFA-1/2), and CD11a (clone HI111) monoclonal antibodies were obtained from Biolegend® (San Diego, CA, USA). Expressions were measured in monocytes, neutrophils, and lymphocytes. Cell viability was confirmed by 7-AAD staining. At least 10,000 events were recorded for each sample. Flow-cytometric settings and samples were prepared according to manufacturer instructions. Neutrophils, monocytes and lymphocytes were identified on a dot-plot and gated (Fig. 1). The intensity of CD64, CD18, and CD11a surface expression was measured as mean fluorescence intensity in arbitrary units (MFI, Fig. 1B).[5] The FC was performed on PICU admission in all cases. All patients received methylprednisolone prior to FC.

As results, we provide the description of CD64, CD18, and CD11a expression on neutrophils, monocytes and lymphocytes in children with severe SARS-CoV-2 disease. This expression appears to be higher compared to other infections and may point to an exacerbated cellular innate response in these children.[3–6]

The cytopathic effects of SARS-CoV-2 combined with the host immune response may play a major role in disease severity. A dysregulated immune response may result in inflammation and clinical worsening in patients with COVID-19. Elevated CD64 expression have been previously described in infectious and noninfectious diseases.[7] Our group carried out CD64 expression studies in acute bronchiolitis and severe viral and bacterial infections.[5] As can be seen in Fig. 1 children with SARS-CoV-2 show levels of CD64 expression that are higher than in previous published reports of bacterial or viral infections or autoinflammatory diseases.[5] Regarding the CD11a and CD18 complex or LFA-1, it is known that plays a key role in migration. Through these, leukocytes are mobilized from the bloodstream into tissues. One of the main findings in COVID-19 patients is the presence of lymphopenia.[8] It can be seen in our cases. This may be linked to the migration of CD8+ lymphocytes to the infected tissues. As seen in Fig. 1, the CD11a upregulation in CD8+ is clear and could be linked to this process. The LFA-1 is also involved in the process of cytotoxic T cell-mediated killing as well as antibody-mediated killing by granulocytes and monocytes.[9] The upregulation of both leukocyte populations is also observed in our cases (Fig. 1).

Immunomodulatory treatment seems to have a great role in COVID19. Their use should be based on a risk-benefit analysis. In SARS-CoV-2 infections the cytokine storm theory coupled with analytical data are used to justify these approaches.[2,10] Our FC results introduce a new approach to analyzing the immune response to this new virus. We confirm the activation of the innate cellular response. Besides we observed that is different and maybe higher than in other infections. The description of this immune status using FC could individualize the diagnosis and optimize the therapies applied.

In summary, we describe the immunophenotype of three children with severe SARS-CoV-2 infection. We observed significant upregulation of CD64, CD18, and CD11a expression on leukocytes. Compare to previous papers and to other types of infection it appears to be higher. This could inform about immune dysregulation triggered by SARS-CoV-2. The use of FC may lead to a better understanding of this response and optimize the therapies applies. Prospective studies with a higher number of cases should be conducted to confirm this observation.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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Restrictive or liberal transfusion for cardiac surgery: Spanish results of a randomized multicenter international parallel open-label clinical trial

Transfusión Restrictiva o liberal en cirugía cardiaca: Resultados españoles de un ensayo clínico aleatorizado, multicéntrico, internacional, paralelo y abierto

Dear Editor,

In cardiac surgery, red blood cell (RBC) transfusion is very frequent. Although transfusion is useful to treat anemia and avoid its complications, it represents a potential risk of acute kidney damage at 72 h after surgery, prolonged mechanical ventilation, need for hemodynamic support, increase-hospital mortality, as well as longer hospital stays.[1] Finding the optimal threshold of hemoglobin for indicating the transfusion with maximal benefit and minimal risk is an aim of the clinical practice. However, a survey in 34 Spanish centers performed in 2007 showed that 70% centers did not have homeostasis protocols and 75% of patients undergoing cardiac surgery were transfused.[2]

Because of the wide variability in transfusion practices and high rates of transfusion in cardiac surgery in Spain, we participated in the Transfusion Requirements in Cardiac Surgery (TRICS) III trial. This is a randomized, multicentre, international, controlled, open-label clinical trial to assess whether a restrictive transfusion strategy, in which lower hemoglobin concentrations for RBC transfusion, applied throughout the perioperative period, would be non-inferior, in terms of major morbidities and mortality, to a liberal approach among 5243 patients undergoing cardiac surgery.[3,4] This study was funded by national peer-review organizations from Australia, Canada, Spain (ISCIII and European Social Fund, CP15/00116) and New Zealand.

We present the results of the Spanish included patients followed up to 6 months after surgery.

Ethics approval was provided by each institutional review boards. All patients consented to participate in the clinical trial. An independent data and safety monitoring board provided trial oversight.

The inclusion criteria were patients undergoing cardiac surgery with cardiopulmonary bypass at moderate to high predicted risk for death, as defined by the European System for Cardiac Operative Risk Evaluation