Immune thrombocytopenia in a child with COVID-19: Is it the calm after the (cytokine) storm?

To the Editor:

Immune thrombocytopenia (ITP) is an autoimmune disorder characterised by isolated thrombocytopenia.\(^1\) It is due to a loss of immunological tolerance to platelet membrane antigens, resulting into an increased platelet destruction, sometimes coupled with impaired/inadequate platelet production.

Several viral infections can be a potential trigger for ITP:\(^2\) Coronavirus disease (COVID-19)-associated ITP has been reported in literature both in adults\(^3\)–\(^5\) and children\(^6\)–\(^8\) with about 7% of patients being asymptomatic for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.\(^7\) We present the case of a child with ITP and SARS-CoV-2 infection extensively investigated for the circulating cytokine profile and bone marrow evaluation.

An 11-year-old boy was admitted to our hospital for the onset of diffuse petechiae and ecchymoses. Physical examination showed petechiae and ecchymosis on his body, face and oral mucosa with no active mucosal bleeding or organomegaly. The complete blood count showed thrombocytopenia (5 × 10\(^3\)/μl; other routine tests, including blood clotting tests and D-dimer, were normal. Fever and cough had occurred about 4 weeks before the onset of the petechial rash (no SARS-CoV-2 nasal swab test was performed at this time). Past medical history was notable for Kawasaki disease when he was 2 years old. According to the internal protocol, a nasopharyngeal swab for SARS-CoV-2 was performed (RT-PCR) and resulted positive. Also, specific IgG for SARS-CoV-2 tested positive.

Considering ITP as the most likely diagnosis, treatment with intravenous immunoglobulin (IvIg) was performed (800 mg/kg, single infusion), with initial partial response (platelet count 45 × 10\(^3\)/μl at 48 hours after the end of the infusion. Because of the partial response to the first IvIg administration, the fast drop in the platelet count (10 × 10\(^3\)/μl 72 hours after the IvIg administration) and the documented SARS-CoV-2 infection, a bone marrow examination was performed. An increased number of megakaryocytes was found, some of them dysplastic, consistent with a peripheral destruction of the platelets, confirming the diagnosis of ITP. Interestingly, the biopsy also showed numerous haemopagocytic macrophages without any other sign of secondary haemophagocytic lymphohistiocytosis (sHLH) such as fever, lymphadenopathy or high inflammatory markers. Also, the bone marrow tested negative for SARS-CoV-2. An extended microbiological evaluation on peripheral blood, bone marrow and nasopharyngeal swab did not detect co-infections. Four days after the first infusion and after the bone marrow evaluation, a second infusion of IvIg was administered with a complete response (platelet count 216 × 10\(^9\)/μl at 48 hours from the end of the infusion). The nasopharyngeal swab for SARS-CoV-2 resulted negative 1 week after the first one, therefore no further therapies were administered. After 3 weeks, the patient was discharged and followed up in the outpatient clinic. He is currently in optimal clinical condition at 9 months of follow-up; the complete blood count is normal.

Several possible pathogenetic mechanisms of COVID19-related thrombocytopenia have been proposed.\(^10\)–\(^11\) In our case, a direct viral infection of bone marrow cells could be ruled out by the absence of the virus in the bone marrow, and platelet consumption due to the formation of microthrombi could also be excluded, as we did not observe any thrombotic complications. The finding of numerous large megakaryocytes in the bone marrow and the response after two IvIg infusions were strongly suggestive of immune-mediated thrombocytopenia.

Haemophagocytosis is not a typical feature of ITP, but it has been described in the bone marrow of adult patients with COVID-19, also with ITP,\(^12\) either with or without other signs of sHLH. Haemophagocytosis is a very nonspecific finding seen in many inflammatory conditions, and in this case it could be explained as an epiphenomenon of the ongoing systemic inflammation and, possibly, of increased bone marrow turnover.

Recently, an inflammatory syndrome defined as ‘multisystem inflammatory syndrome in children’ (MIS-C) related to SARS-CoV-2 infection was described.\(^13\) The clinical features of MIS-C overlap with Kawasaki disease and sHLH, while the pathogenesis is not well established yet; a delayed immunological phenomenon associated with inflammation following either symptomatic or asymptomatic COVID-19 is advocated.\(^14\)

In light of these considerations, we hypothesised that the thrombocytopenia could be due to a delayed systemic inflammatory response to the previous SARS-CoV-2 infection. To obtain further insight, we performed a cytokine profiling on the patient’s serum (drawn before the administration of the IvIg bolus) including cytokines, chemokines and growth factors. The results were compared with data reported in the literature from paediatric healthy subjects.\(^15\)–\(^16\) We found a relevant increase in the concentration of several molecules (Table 1). We then compared our findings to the published cytokine profiles of three subgroup of patients: patients with MIS-C,\(^17\)–\(^18\) ITP\(^19\)–\(^20\) and sHLH\(^21\)–\(^22\) (Figure 1). The cytokine profile confirmed an inflammatory status that...
TABLE 1  Cytokine mapping using Luminex technology in our patient

| Conc (pg/ml) | COVID-19/ITP patient | Healthy subjects | LOD     |
|-------------|----------------------|------------------|---------|
| IL-1β       | 42                   | 2.04 (0.17–24)   | 0.25–2.529 |
| IL-1RA      | 1950                 | 169.2 (134.7–203.6) | 5.98–8366 |
| IL-6        | 144                  | 2.91 (0.16–37.7)  | 0.38–2357 |
| IL-8        | 1162                 | 32.6 (28.2–39)    | 0.36–1170 |
| MCP-1       | 157                  | 52 (26.5–77.9)    | 0.6–3770  |
| MIP-1β      | 1581                 | 40.5 (3.2–227.2)  | 18.4–28,445 |
| MIP-1α      | 1561                 | 7.4 (6.3–8.2)     | 1.58–4211 |
| TGF-α       | 15                   | 3.2 (0.93–26.8)   | 0.93–6400 |
| TNF-α       | 647                  | 3.21 (0.93–26.8)  | 0.62–3066 |

Note: Concentration of the analytes in our patient obtained using the Human XL Cytokine Luminex Performance plate (R&D Systems, Minneapolis, MN, USA) read on a MAGPIX detection platform (Luminex Corporation) compared with healthy subjects as reported in literature.15,16 To confirm the results obtained, the low and high control contained in the manufacturers’ kit were also tested with a high reproducibility, except for some analytes that were thus excluded from the analysis. Analytes studied: IL-1β, IL-12p70, IL-13, IL-15, IL-1RA, IL-2, IL-6, IL-7, IL-10 and IFN-α, TNF-α, CD40L, Granzyme B, PDL1-B7, Eotaxin (CCL11), Fractalkine (CX3CL1), GRO-α (CXC1L), GRO-β (CXC2L), MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, IL-8/CXCL8, MIP-3 alpha/CCL20, MIP-3-beta/CCL19, RANTES/CCL5, TRAIL and IP-10/CXCL10, FLT3 ligand, EGF, G-CSF, GM-CSF, PDGF-AA, PDGF-AB/BB, TGF-α and VEGF.

FIGURE 1  Visual representation of the overlapping profiles of these different clinical scenarios. The analytes found with a high concentration in our patient are underlined. In particular, a common pathway of specific Th1 cytokines (IL-1β, IL-6, TNF-alpha) and chemokines (IL-8, MCP-1) related to the pathogenesis of these diseases was found. MIS-C, multisystem inflammatory syndrome in children17,18; ITP, immune thrombocytopenia19,20; sHLH, secondary haemophagocytic lympho-histiocytosis21,22.

overlapped, but did not coincide, with virus-triggered ITP, MIS-C and sHLH.

In this context, haemophagocytosis could represent either a truncated sHLH-like syndrome or a phenomenon related to a SARS-CoV-2-related cytokine storm. Moreover, the history of Kawasaki disease may suggest a genetically determined immunological predisposition to a hyperinflammatory response to viral triggers.

The presence of IgG antibodies at the time of admission and the history of fever and cough 4 weeks before (presumably indicative of the primary infection), support the interpretation of ITP as a delayed immunological effect of SARS-CoV-2 infection. The absence of clinical and laboratory findings consistent with an active infection corroborates this hypothesis, along with the very rapid negativisation of the RT-PCR test. Notably, the initial positive swab for SARS-CoV-2 RNA by RT-PCR (4 weeks after initial symptoms) was most likely due to persistence of replication-incompetent virus in the nasopharynx of the patient.

In conclusion, we extensively analysed a case of ITP in a child with recent SARS-CoV-2 infection and found evidence for a delayed and truncated inflammatory response as possible mechanism of pathogenesis. Further studies on a larger cohort of patients are necessary to define the inflammatory clinical and biological profile of SARS-CoV-2 infection in children.

AUTHOR CONTRIBUTIONS
Giulia Ceglie, Maria Antonietta De Ioris and Stefania Mercadante conceived the idea, researched the literature and wrote the paper. Giulia Ceglie and Silvio Marchesani performed the cytokine-mapping experiment and analysed the data. Francesca Del Bufalo contributed essential reagents and tools and critically revised the manuscript. Nicole Olivini, Emanuela Monteferrario, Emilia Bocciari and Jolanda Pianese collected the patients’ data and provided critical feedback. Francesca Cocca provided critical feedback. Giuseppe Palumbo conceived the idea, provided critical feedback and supervision.
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