Monocyte vacuoles in bone marrow aspiration are a sign of cytokine storm among children with Multisystem Inflammatory Syndrome, a case report

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Keywords: Monocyte; Macrophage activation syndrome; Multisystem Inflammatory Syndrome; COVID19; case report;

Abstract

Background: Children exposed to SARS-CoV-2 infection may develop a hyper-inflammatory response: the so-called Multisystem Inflammatory Syndrome associated with Coronavirus Disease 2019 (MIS-C). Clinical signs of SARS-CoV-2 infection hyperinflammatory manifestation can lead also to cytopenia.

Case presentation: We described three patients with Multisystem Inflammatory Syndrome associated with Coronavirus Disease 2019 (MIS-C) who presented with pancytopenia in addiction to thrombocytopenia that is typically described in MIS-C patients. To rule out malignancies or a secondary macrophage activation syndrome (MAS), they underwent bone marrow aspiration. In all three cases, bone marrow aspiration highlighted the prevalence of myeloid lines rich in granules and vacuoles that could be a sign of cytokines storm involving primarily myelomonocytic cells. Patients received a final diagnosis of MIS-C.

Discussion and conclusions: We suggest the presence of monocyte vacuoles in bone marrow as a peculiar finding in MIS-C.

Background

It is now well known that children exposed to SARS-CoV-2 infection may develop a hyper-inflammatory response: the so-called Multisystem Inflammatory Syndrome in Children (MIS-C) associated with Coronavirus Disease 2019[1]. Although MIS-C shares some clinical features with Kawasaki Disease, it is characterized by the older age of patients, the presence of gastrointestinal and respiratory symptoms, and peculiar cardiac involvement such as myocarditis and valvular involvement[2]. The majority of these patients shows elevation of inflammatory markers, coagulation abnormalities and in some patients, severe cytopenia is also present.

We describe three cases of MIS-c who underwent bone marrow aspiration for diffuse cytopenia resulting in the presence of immature myeloid cells enriched in vacuoles; this peculiar finding could be a sign of cytokines storm involving primarily myelomonocytic cells. However, a diagnosis of

Macrophage Activation Syndrome (MAS) among rheumatology disorders could also be done if appropriate criteria are applied.

Case presentation

Case 1.

In April 2020, a three-year-old girl of Caribbean ancestry was admitted to the Institute for Maternal and Child Health, IRCCS Burlo Garofolo of Trieste, Italy, for a 2-days history of high fever and abdominal pain with diarrhea. Clinical examination highlighted purpuric skin rash, labial cheilitis, palmar hands edema, and bulbar non-exudative conjunctivitis. A recent close contact with a relative with COVID-19 and a recent nasopharyngeal swab tested positive for SARS-CoV-2 was reported. Laboratory tests showed severe lymphopenia together with anemia, thrombocytopenia, increased C-reactive protein, liver enzymes, D-dimer levels, and hypofibrinogenemia (table 1). A thorax computed tomography (CT)-scan revealed multiple lung opacities as a consequence of previous primary covid pneumonia; echocardiography was normal except for a mild mitral valve insufficiency. Due to pancytopenia, bone marrow aspiration was done, highlighting a prevalence of myeloid cells with immature myeloid cells rich in vacuoles and granulations; reduced erythroblasts megakaryocytes and the lymphoid line was found; no histiocytosis or blasts were present. Cytokine study showed high levels of IL-1Ra, IL-6, IL-10. MIS-c diagnosis was made and treatment with intravenous (IV) methylprednisolone 2 mg/kg/day and intravenous immunoglobulin (IVIG) 2 g/kg was started. Non-invasive ventilation and noradrenaline infusion were needed as the patient developed the day after hypotension and dyspnea. Intravenous continuous infusion with anakinra 12 mg/kg/day was started with quick improvement of clinical conditions and discontinuation of ventilation and amine infusion. Anakinra was gradually tapered and stopped after eight days. The patient was discharged in good conditions with normal heart function on follow-up.

Patient 2.

In May 2020, a 10-year-old girl was admitted to the Institute for Maternal and Child Health, IRCCS Burlo Garofolo of Trieste, Italy, for a 5-days history of high-grade fever, abdominal pain with

vomiting, and headache. The patient appeared prostrated with cervical lymphadenopathy and papular skin rash. During the first day of hospitalization, the patient suddenly developed tachypnoea and hypotension. Laboratory exams showed lymphopenia, mild anemia and thrombocytopenia, together with the elevation of inflammatory markers, fibrinogen, ferritinemia, LDH, triglycerides, NT-pro BNP and D-dimer levels (table 1). Immunologic test including immunoglobulin level, immunophenotype, perforin expression and NK degranulation resulted normal. Cytokine levels of IL-1Ra, IL-6 and IP10 were markedly elevated. Heart ultrasonography revealed dilation of inferior vena cava without heart dysfunction or coronary abnormalities. Thorax CT scan showed a bilateral ground-glass pattern, and abdominal ultrasound was normal. Naso-pharyngeal swabs tested negative for SARS-CoV-2 as well as for other common viral infections. Antibodies for SARS-CoV-2 were not feasible at that time. Given to pancytopenia, bone marrow aspiration was done, highlighting a hypoplastic bone marrow and the prevalence of mature myeloid cells with granules and immature myeloid cells with vacuoles and high coexpression of CD16 on immunophenotype. No histiocytic macrophage or blast was found. MIS-c with macrophage activation pattern was diagnosed and treatment with IVIG and IV methylprednisolone 2 mg/kg and IVIG 2 g/kg was started with no significant clinical improvement; hence on day 3, subcutaneous anakinra 7 mg/kg/day was started together with antithrombotic prophylaxis with low-molecular-weight heparin observing fever defervescence in 24 hours and progressive laboratory test normalization.

Case 3.

A 17-year-old boy was admitted to the Department of Allergology, Rheumatology and Clinical Immunology, in Ljubljana Children's University Hospital, Slovenia, in November 2020 because of a 3-days history of low-grade fever (37.5^0C). One month earlier, he had a COVID-19 infection confirmed by a nasal swab, passed without complications. Vitals signs were unremarkable as was physical examination. Laboratory tests showed lymphopenia, neutropenia, thrombocytopenia and hyperferritinemia (Table 1). Heart ultrasound showed an edematous basal part of the left ventricle with preserved function of 68% and normal coronary arteries. Abdominal ultrasound highlighted

enlarged liver and spleen. Chest X-ray was normal. Bone marrow aspiration was done, showing hemophagocytosis and vacuoles in monocytes (figure 1) without malignant infiltration. Intravenous immunoglobulins (IVIG) at the dosage of 60 g and methylprednisolone was started in a dose of 1 mg/kg. Considering high levels of D-dimer, he received enoxaparin and acetylsalicylic acid in prophylactic doses. On follow-up, the disease course was uneventful, and no cardiologic complication was found on ultrasound and magnetic resonance.

Discussion and conclusions

The burden of COVID-19 consists not only in a direct viral infection but, especially in children, it may also trigger a hyperinflammatory response called MIS-C.

MIS-c is characterized by the presence of fever (>38°C for more than 24 hours) with signs and symptoms of at least two-organ involvement (cardiovascular, pulmonary, kidney, gastrointestinal, musculoskeletal or central nervous system), plus laboratory signs of systemic inflammation with or without lymphopenia. Moreover, infection other than SARS-CoV-2 must be excluded. Detection of SARS-CoV-2 infection may not be documented and it is not required for diagnosis[3].

National MIS-c case series highlighted that most patients presented with higher inflammatory markers compared with Kawasaki disease (KD); but they also present lymphopenia, lower platelet counts and anaemia[4,5]. These aspects, in particular, allowed to differentiated KD to MIS-c patients. However, patients with MIS-c who had cytopenia may also be confused as having a MAS or leukemia.

We described three patients with MIS-c who showed pancytopenia leading us to perform bone marrow aspiration to rule out malignancies, considering that at the time of the first two patients, MIS-c characteristics were not so well established. In all three cases, bone marrow aspiration highlighted the prevalence of myeloid line rich in granules and vacuoles that we suggest as a typical finding in MIS-C as an effect of the cytokine storm. Moreover, the presence of poorly cellulated bone marrow, and in the third patient the evidence of hemophagocytosis, could laso suggest the

presence of MAS given high levels of LDH and ferritin. Considering the inflammatory nature of MIS-c, we applied the criteria developed to define MAS in rheumatologic disorders[6].

Hyper-inflammatory immune response to SarsCOV-2 appears to be driven by the myelomonocytic response. In particular, it has been postulated that a delayed induction of interferons (IFNs) induces macrophages to produce high levels of proinflammatory cytokines like interleukin (IL-1, IL-6, tumor necrosis factor - TNF) and chemokines (C-C motif chemokine ligand (CCL-2, CCL-3, and CCL-5) with an autocrine action and excessive activation of monocytes. In particular, a significant expansion of populations of blood monocytes producing IL-6 was observed in patients with severe forms of COVID-19 requiring ICUs compared with those who did not require ICU hospitalization. The same chemokines involved in "COVID-19 related cytokine release storm" show similarities to those observed in macrophage activation syndrome[7-9].

Moreover, monocytes from peripheral blood of COVID-19 patients show an activated functional and morphologic phenotype, with an increased number of larger, atypical vacuoles[10]. What we found is the same morphologic changes in bone marrow smears in patients with MIS-C with a MAS-like phenotype. This finding could be further evidence of how hyperinflammatory state in MIS-C could be seen as a *continuum* with MAS through IL-1, IL-6 and TNF production by monocytes. This view could also consider the presence of thrombocytopenia in MIS-C without MAS phenotype[11]. In all of the three cases, a genetic susceptibility was ruled out through functional immunological studies.

Since the severity of the disease course, IVIG plus methylprednisolone was needed in one case and while high dose anakinra was added in the other 2 patients. As successfully proposed in an adult series by Cavalleri et al. IL-1 antagonists seem to confer a higher survival rate in adults with COVID-19 and a severe hyperinflammatory profile[12,13].

In conclusion, the COVID-19 outbreak pointed out the role of the host response to viral infection to engage a hyper-inflammatory state. Cytokine storm in MIS-c may mimick MAS and the treatment

for severe cases may involveI VIG plus corticosteroids, as well as anakinra for unresponsive cases. The central role played by myelomonocytic cells in MIS-c hyper inflammation syndrome should be considered to guide treatment.

Availability of data and materials All data generated or analyzed during this study are included in this published article.

Abbreviations

MISC: Multisystem inflammatory syndrome

MAS: Macrophage Activation Syndrome

CT: computed tomography

IV: intravenous

IVIG: intravenous immunoglobulin

Declarations:

Acknowledgements Not applicable

Ethics approval and consent to participate Not applicable

Consent for publication The parents/guardians, of all three patients, gave their written consent for their child's personal or clinical details to be published in this study.

Conflict of interest The authors have no conflict of interest to declare

Funding Data collection of this work was supported by CATTEDRA project funded by INTERREG Programme VA Italy - Slovenia 2014 - 2020 CUP C94I19008240001

Competing interests Not applicable

Contributions All authors have contributed to the manuscript. Conception and design: NT, AT. Acquisition of data: EC, JJ, SDP. Manuscript writing: EC, SDP, VM. Manuscript review: JJ, VK, NT, AT. The author(s) read and approved the final manuscript**.**

References:

1. Centre for Disease Control and Prevention. Emergency Preparedness and Response Multisystem Infammatory Syndrome in Children (MIS-C) Associated with Coronavirus Disease 2019.
2. Cattalini M, Della Paolera S, Zunica F, Bracaglia C, Giangreco M, Verdoni L et al. Defining Kawasaki disease and pediatric inflammatory multisystem syndrome-temporally associated to SARS-CoV-2 infection during SARS-CoV-2 epidemic in Italy: results from a national, multicenter survey. Pediatr Rheumatol Online J 2021; 16:19-29.
3. Cattalini M, Taddio A, Bracaglia C, Cimaz R, Paolera SD, Filocamo G et al. Childhood multisystem inflammatory syndrome associated with COVID-19 (MIS-C): a diagnostic and treatment guidance from the Rheumatology Study Group of the Italian Society of Pediatrics. Ital J Pediatr 2021; 8:47-24.
4. Feldstein LR, Rose EB, Horwitz SM, Collins JP, Newhams MM, Son MBF et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. N Engl J Med 2020; 23:334-46.
5. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P et al. Clinical Characteristics of 58 Children With a Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. JAMA 2020; 21:259-69.
6. Ravelli A, Minoia F, Davì S, Horne A, Bovis F, Pistorio A et al. 2016 Classification Criteria for Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis: A European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Arthritis Rheumatol 2016; 68:566-76.
7. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol 2020; 20:355-362.

8. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J Infect 2020; 80:607-13.
9. Lega S, Naviglio S, Volpi S, Tommasini A. Recent Insight into SARS-CoV2 Immunopathology and Rationale for Potential Treatment and Preventive Strategies in COVID-19. Vaccines 2020;8:224.
10. Kaur G, Sandeep F, Olayinka O, Gupta G. Morphologic Changes in Circulating Blood Cells of COVID-19 Patients. Cureus. 2021 Feb 18;13(2):e13416.
11. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. Ann Hematol 2020;99:1205-08.
12. Della Paolera S, Valencic E, Piscianz E, Moressa V, Tommasini A, Sagredini R, Kiren V, Comar M, Taddio A. Case Report: Use of Anakinra in Multisystem Inflammatory Syndrome During COVID-19 Pandemic. Front Pediatr 2021; 23;8:624248.
13. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D et al. Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol 2020;2:325-31.

Legend:

Figure 1: A. Vacuoled monocyte characterized by abundant blue-greyish cytoplasm with numerous small vacuoles and large bizarre shaped nucleus; B. Emophagocytated monocytes; C, D, E Erythrophagocytosis.