ANDES PEDIATRICA
REVISTA CHILENA DE PEDIATRÍA
www.scielo.cl

Andes pediatr. 2021;92(3):382-388
DOI: 10.32641/andespediatr.v92i3.3316

ORIGINAL ARTICLE

Hematologic parameters and biomarkers predictors of severity in Multisystem Inflammatory Syndrome in children associated with SARS-CoV-2

Parámetros hematológicos y biomarcadores predictores de gravedad en Síndrome Inflamatorio Pediátrico Multisistémico asociado a SARS-CoV-2

Patricia Verdugo[a,c], Patricia Álvarez[b,c], Patricia Aroca[b], Vicente Montes[d], Cecilia Poli[e], Lorena Tapia[c], Fernanda Cofré[g], Verónica Soto[a,c], Jorge Mackenney[c], Cristian Carvajal[f], Valeria Acevedo[b,c]

[a]Servicio de Onco-Hematología. Hospital Roberto del Río. Santiago, Chile
[b]Servicio de Cardiología y Cardiocirugía, Hospital Roberto del Río. Santiago, Chile
[c]Departamento de Pediatría, Campus Norte, Facultad de Medicina, Universidad de Chile. Santiago, Chile
[d]Alumno de Medicina, Facultad de Medicina, Clínica Alemana, Universidad del Desarrollo. Santiago, Chile
[e]Unidad de Inmunología-Reumatología, Hospital Roberto del Río. Santiago, Chile
[f]Unidad de Infectología, Hospital Roberto del Río. Santiago, Chile
[g]Unidad de Paciente Crítico Pediátrico, Hospital Roberto del Río. Santiago, Chile

Received: September 13, 2020; Approved: December 9,2020

What do we know about the subject matter of this study?

PIMS-TS is a late immune response triggered by SARS-CoV2 virus interaction with the host. It manifests as a hyperinflammatory state secondary to a cytokine storm, evidenced by a marked increase of blood biomarkers.

What does this study contribute to what is already known?

A series of Chilean pediatric patients with PIMS-TS in which we studied the role of biomarkers in identifying those patients who will develop more severe clinical forms and require a timely referral and specific therapy.

Abstract

The multisystem inflammatory syndrome in children associated with SARS-CoV-2 (MIS-C) is characterized by a hyperinflammatory state resulting from a cytokine storm, evidenced by alterations in laboratory blood testing and acute-phase proteins. Objective: to describe the clinical and laboratory characteristics of patients hospitalized due to MIS-C and identify predictive markers of severity. **Patients and Method:** Retrospective study of 32 patients. The group was divided into critical and non-critical according to clinical presentation and therapy used. Clinical and laboratory aspects were studied, including complete blood count, coagulation tests, and biomarkers. **Results:** 18/32 were ma-

Keywords:
MIS-C;
SARS-COv-2;
Hematologic Parameters;
Pediatrics;
Inflammation;
Kawasaki Disease

Correspondence:
Dra. Patricia Verdugo
pverdugo33@hotmail.com

How to cite this article: Andes pediatr. 2021;92(3):382-388. DOI: 10.32641/andespediatr.v92i3.3316

les, with a median age of 6.8 years. The most frequent manifestations were cardiovascular (84.3%), digestive (84%), and mucocutaneous (59%). The group of critical patients included 15 patients, 12 were males with a median age of 8.9 years, and the non-critical group included 17 patients, 6 were males with a median age of 5.4 years. The laboratory parameters at the admission in the global group showed increased C-reactive protein, D-dimer, leukocytes, neutrophils, ferritin, and fibrinogen. In contrast, albumin and blood sodium levels were decreased. At admission, the critical group was characterized by presenting thrombocytopenia, hypoalbuminemia, prolonged prothrombin time, and elevated ferritin. At the time of deterioration, there was an intensification of thrombocytopenia, increased C-reactive protein together with increased neutrophils level. **Conclusion:** The blood count, C-reactive protein, and albuminemia at admission proved to be significantly important in the identification of patients at risk of clinical deterioration.

Introduction

By the end of 2019, COVID-19 infection emerges as a serious disease in the adult population, however, since April 2020 in the northern hemisphere, the first case series of a new clinical entity in children, currently called Pediatric Multisystem Inflammatory Syndrome temporally associated with SARS-CoV-2 (PIMS-TS)[1], have been published.

The diagnostic criteria for this syndrome are still under discussion, and the most widely used are those of the Royal College of Paediatrics and Child Health, Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO)[2-4]. All three include the presence of fever, with significant systemic inflammation evidenced by severely altered laboratory parameters and involvement of one or more organs.

Typically, this disease presents 4-6 weeks after contact with the virus, and its pathophysiology is still unclear, intermingling clinical elements of Kawasaki disease, suggesting a hyperinflammatory vascular and probably autoimmune origin[5]. In addition, this entity presents hematologic and coagulation compromise and alteration of acute-phase proteins[6].

The objective of this study is to analyze the laboratory parameters of a series of patients with PIMS-TS diagnosis who required hospitalization, in order to describe the findings observed in this disease and to identify those laboratory markers that are potential predictors of severe evolution and that can guide management and treatment.

Patients and Method

Retrospective cohort study of 32 patients admitted to the *Hospital Roberto del Río*, between May 11 and August 2, 2020, with diagnosis of PIMS-TS according to the CDC[3]. The following was considered as exposure to the virus: RT- PCR COVID-19 (+), IgM (+), or IgG (+) for SARS-CoV-2, and/or history of contact with patient COVID-19 (+). Parents signed informed consent.

Data were obtained from electronic medical records, including age, sex, symptoms, and signs on admission, duration of fever, admission to pediatric intensive care unit, and days of hospital stay.

The following laboratory tests at admission, in case of clinical deterioration (if any), and before discharge were analyzed: complete blood count, C-reactive protein, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, ferritin, albumin, creatinine, and natremia.

The patients were categorized as **critical** and **non-critical**, according to the Yale School of Medicine severity classification[7]. The first group, called **critical**, consisted of patients who presented shock requiring vasoactive drug support, or mechanical ventilation, or who presented organ failure, left ventricular dysfunction, or coronary compromise. The remaining patients formed the second group, called **non-critical**.

The forms of clinical presentation were analyzed in both groups. Cardiovascular involvement included shock, Kawasaki or Kawasaki-like disease, myocardial injury, myocarditis, pericarditis, and valvulitis. Non-cardiovascular clinical forms included mucocutaneous, digestive, respiratory, and neurological manifestations. Clinical and laboratory characteristics were analyzed between both groups and the results were compared. The type of therapy used was also studied as well as the response to treatment.

Statistical analysis: for each continuous variable, the Shapiro-Wilks test was used to determine whether it is parametrically or non-parametrically distributed. Then, these variables were described using absolute frequencies, percentages, and, depending on their distribution, mean and SD or median and IQR were used, respectively. To compare parametric variables, the Student's t-test was used for independent groups

and for the nonparametric ones, the Mann-Whitney U test.

Categorical variables were described as absolute frequency and percentages. The Chi-square test was used to compare these variables.

One-tailed tests were used in all statistical analyses since our working hypothesis is that one group is larger than another. A level $P \leq 0.05$ was considered statistically significant. All statistical analysis was done with SPSS software.

This study was authorized by the Hospital Management and approved by the Human Research Ethics Committee of the Faculty of Medicine of the Universidad de Chile.

Results

In the 32 patients admitted to the Pediatrics Service at the *Hospital Roberto del Río* with diagnosis of PIMS-TS, the median age was 6.8 years (IQR 2.73-11.07) and 18 were males (56.3 %). 15/32 patients were classified as **critical** whose median age was 8.9 years (IQR 4-13.5) and 12 were males (80%). The group of **non-critical** patients[17] presented a median age of 5.4 years (IQR 2.3-10.5) and 6 were males (35%) (table 1).

Fever was the first symptom in 84% of patients, above 39°C and with a median duration of 6 days.

Table 1. Clinical and demographic data of all patients diagnosed with PIMS-TS: total group, critical and non-critical group

	Total group	Critical	Non-critical
Total patients	32		
n	32	15	17
%		46.9	53.1
Males			
n	18	12	6
%	56.3	80	35.29
Females			
n	14	3	11
%	43.8	20	64.71
Days of hospitalization*	9	10	6
ICU	24	14	10
Days in ICU *	5	5	3
Days with fever*	6	6.5	6
Shock	12	12	0
%	37.50	100	0
Kawasaki	19	11	8
%	59.38	73.33	47.06

* Data are expressed as median. ICU: Intensive critical unit.

The clinical manifestations were digestive in 27 patients (84%) with diarrhea, vomiting, and abdominal pain as the most frequent symptoms, two patients had pancolitis, and one had hepatitis. 19 patients (59%) presented mucocutaneous manifestations. There were respiratory symptoms in 10 patients (31%), four of them had pneumonia. Headache was the most frequent neurological symptom (28%). Regarding cardiovascular symptoms, there were Kawasaki and/or Kawasaki-like disease in 19 (59%) patients, four with coronary involvement, shock in 12 (37.5%), myocardial injury in 14 (43%), pericardial effusion in 13 (40%), myocarditis in 8 (25%), and heart rhythm disorders in 7 (22%).

Cardiovascular clinical manifestations in the critical group were shock in 12 (80%) patients, 11 (73%) with Kawasaki and of them 4 with coronary involvement, 10 (66%) with myocardial injury, 8 (53%) with myocarditis, 9 (60%) with pericardial effusion, and 6 (40%) with arrhythmia (Table 1). The clinical manifestations for the non-critical group were Kawasaki disease in 8 (47%) patients, 5 (29%) with myocardial injury, and 4 (23%) with pericardial effusion.

Age was higher in the critical group as days of hospitalization were ($p = 0.006$) and males presented a more severe behavior of the disease ($p = 0.005$).

No deaths or patients were requiring extracorporeal circulation in this series.

From the study of laboratory parameters at admission, the overall group had elevated C-reactive protein, D-dimer, leukocytes, neutrophils, ferritin, and fibrinogen. In contrast, albumin and natremia were decreased and PT and aPTT were prolonged.

Comparing the critical group at admission, statistically significant differences were found in decreased albumin ($p = 0.001$), lower platelet count ($p = 0.038$), prolongation of PT ($p = 0.028$) and aPTT ($p = 0.049$), and elevated ferritin ($p = 0.002$) (figure 1). Blood sodium was also lower in this group ($p = 0.003$). Seven patients had elevated creatinine with an average value of 1.23 mg/dl (range 0.79 to 2.33), which is statistically significant compared with the non-critical group ($p = 0.019$).

From the analysis of tests of clinical deterioration in the critical group, there was a decrease in platelet count ($p = 0.013$), elevation of C-reactive protein ($p = 0.001$), and neutrophils ($p = 0.012$) (table 2).

The therapy used in the total group was intravenous immunoglobulin in 24 (75%) patients, of which three received two doses, methylprednisolone in 21 (65.6%), acetylsalicylic acid in 23 patients (72%), and enoxaparin in 24 (75%), indicated with an average D-dimer value of 2,445 ng/ml (range 980-5,000) for normal value < 250 ng/ml. Tocilizumab was also used in two patients and infliximab in one.

Discussion

This new PIMS-TS disease, although infrequent, is characterized by high morbidity since it presents shock secondary to myocardial compromise or vasoplegia due to systemic hyperinflammation, requiring intensive circulatory and ventilatory support. Children also present clinical manifestations of Kawasaki-like disease with coronary and myocardial compromise, as well as the presence of arrhythmias and even death[8-11].

This disease affects school-aged children with a higher incidence in males, with clinical polymorphism, frequently accompanied by gastrointestinal manifestations. The latter may raise an alarm in febrile children and should alert the physician to maintain a high diagnostic suspicion of PIMS-TS in the current epidemiological context, associated with the presence of hyperinflammation in laboratory tests, which we also observed in our series with gastrointestinal involvement in 84% of the children.

Elevated inflammatory markers are evidence of a hyperinflammatory state attributable so far to the cytokine storm that occurs as an individual and pathological response to the interaction with the virus[12-13]. The biomarkers that were most affected in our series were albumin, C-reactive protein, fibrinogen, ferritin, and D-dimer. C-reactive protein in our group was significantly elevated on admission and even more so on deterioration in critical patients. This constitutes a sensitive biomarker of inflammation and tissue da-

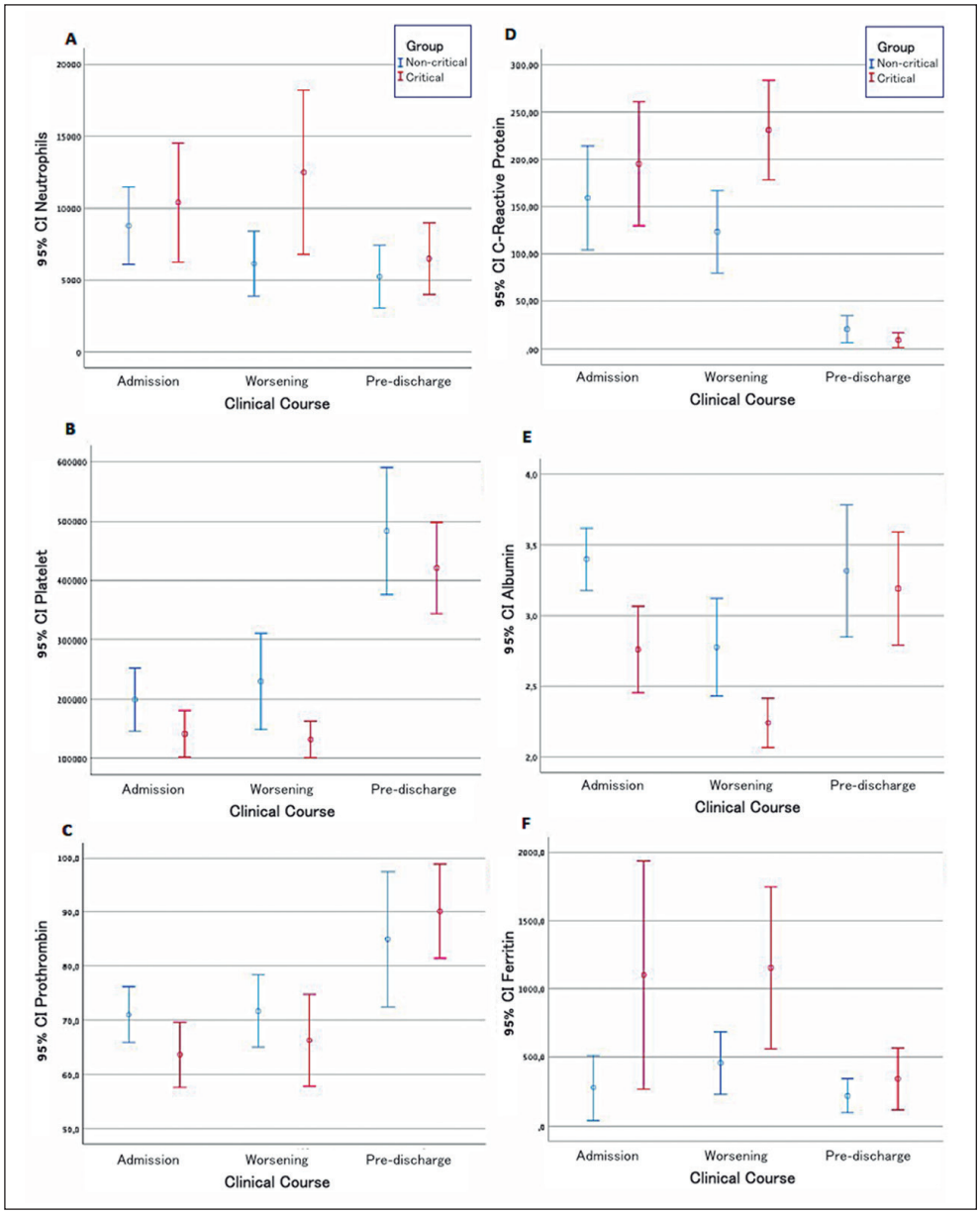

Figure 1. Changes in hematological parameters (**A** and **B**), prothrombin (**C**) and acute phase proteins (**D**, **E** and **F**), according to the moment of clinical course in each group studied, critical (red line) and non-critical (blue line).

Table 2. Laboratory findings in critical and non-critical patients at admission and clinical deterioration

Variable	Critical	Critical (SD or IQR)	Non-critical	Non-critical (SD or IQR)	P Value: Critical vs Non-critical
Eosinophils admission xmm^3	231	223	54.10	188.50	0.023
Eosinophils worsening xmm^3	123	174	94.60	389.50	0.370
Neutrophils admission xmm3	9 020	7 504	7 953.50	8 735.50	0.492
Neutrophils worsening xmm^3	9 603	9 493	4 978	7 452.50	0.012
Leukocytes admission xmm^3	11 600	7 800	10 800	11 455	0.500
Leukocytes worsening xmm^3	11 200	14 300	8 310	9 702.50	0.169
Lymphocytes admission xmm^3	1 076	1 782	1 431	2 016.25	0.293
Lymphocytes worsening xmm^3	1 307	1 284	2 069.50	2 105.50	0.128
Platelet admission mm^3	141 267	71 316	199 063	99 922	0.038
Platelet worsening mm^3	131 600	56 021	229 750	152 202	0.013
CRP admission mg/L	195.26	118.69	159.04	107.11	0.186
CRP worsening mg/L	231.03	91.47	123.29	82.11	0.001
D-dimer admission ng/ml	1 705	1 330	1 627	2 883	0.415
D-dimer worsening ng/ml	2 193	1 405	2 189	1 768	0.498
Ferritin admission ng/ml	673.75	1 215.50	266	228	0.002
Ferritin worsening ng/ml	812	1 612.50	383	558.25	0.013
Prothrombin admission %	63.67	10.93	71.08	9.30	0.028
Prothrombin worsening %	66.35	14.72	71.75	11.59	0.146
Natremia admission mmol/L	131.47	4.37	135.47	2.98	0.003
Natremia worsening mmol/L	135	7.50	135.50	4.50	0.170
Albumin admission g/dL	2.76	0.56	3.40	0.41	0.001
Albumin worsening g/dL	2.24	0.32	2.78	0.57	0.004
aPTTP admission sec	30	4.10	27.60	6.20	0.049
aPTTP worsening sec	30.20	9.20	28	6.55	0.117

*Reference values: eosinophils 50-500 x mm^3; neutrophils 1 500-10 000 x mm^3; leukocytes 6 000-17 000 x mm^3; lymphocytes 1.000-8.500 x mm^3; platelet 150 000-450 000 x mm^3; C-reactive protein (CRP) 0.3-5 mg/L; D-dimer 0-250 ng/mL; ferritin 6-67 ng/ml; prothrombin 75-113%; natremia 135-145 mmol/L; albumin 3.8-5.4 g/dL; aPTT 25-36.5 seconds.

mage which is induced by IL-6 in the liver, with rapid elevation and proportional to the severity of inflammation[14].

Within the analysis of biomarkers, a greater elevation of C-reactive protein and ferritin with a drop in albumin stood out in the group of critical patients at the time of deterioration, all of which are responses to the hypercytokinemia state of these patients, and it seems to us that this finding can help in therapeutic decisions.

Ferritin is a protein that regulates iron deposition, but it also increases because of interferon-gamma and IL-18, present in this cytokine storm, revealing macrophage hyperactivity that leads to erythrophagocytosis and secondary anemia, which are mechanisms involved in hemophagocytic syndrome[15]. In our series, no patient developed hemophagocytic syndrome.

Hypoalbuminemia is an early marker, present in the group of critically ill patients, a statistically significant fact in our series. This protein is an acute-phase protein, and its decrease is caused by an increase in vascular permeability secondary to endotheliitis and cytokines release[16].

In PIMS-TS, the hematologic involvement includes thrombocytopenia, lymphopenia, and neutrophilia. In our series, thrombocytopenia at admission was a statistically significant distinctive finding for the group of critically ill patients, decreasing even more with clinical deterioration. Thrombocytopenia is frequently observed in viral infections, and in the case of SARS-

CoV-2 virus, there are different theories about its cause such as platelet destruction by the appearance of IgG autoantibodies against platelet-specific receptors forming immune complexes, which are sequestered by the reticuloendothelial system, as well as depression of megakaryopoiesis by the direct action of the virus or of interleukins that inhibit growth factors in the bone marrow. In adults, there is an increased consumption due to thrombus formation[17-18].

In addition, several mechanisms have been proposed that cause lymphopenia, including direct lysis of the virus by expressing ACE-2 receptors and the action of interleukins and TNF-α that suppress lymphocyte proliferation. In our series, lymphopenia was an infrequent finding and no statistically significant difference was identified between critical and non-critical patients, a finding that is shown to be relevant in other publications[19].

The number of neutrophils was elevated in critical patients to their deterioration, which is an effect of the action of interleukins, mainly IL-1 and IL-6 which along with granulocyte colony-stimulating factor act in the bone marrow producing increased granulopoiesis. TNF-α also acts by recruiting neutrophils in the initial shock state[20].

Coagulopathy in PIMS-TS simulates disseminated intravascular coagulation but differs from it by having elevated fibrinogen, a higher increase in D-dimer, and mild thrombocytopenia, leaving unanswered questions as to whether it is an entity specific to this disease, combining elements of different types of coagulopathies. In our series, we had no thrombotic or hemorrhagic phenomena, leaving to discuss whether this was a product of an adequate and early anticoagulation management, or perhaps the elevated D-dimer seen in our patients could be a consequence of the increased macrophagic activity of fibrin generated by a hyperinflammatory state, rather than as a marker of thrombin generation and fibrinolysis with the consequent risk of thrombosis[21].

Therapy was based on that used for Kawasaki disease, including intravenous gamma globulin and acetylsalicylic acid. In case of no response or partial response, corticosteroid therapy was added. Series with a larger number of patients are needed to define the best therapeutic scheme for these patients.

Regarding the prevention of thromboembolic disease, enoxaparin was the most commonly used therapy, indicated when D-dimer increases more than 4 times the normal value, but more knowledge and observation time of this disease is needed to define its role.

In conclusion, a timely diagnosis based on clinical knowledge of this pathology together with serial evaluation of laboratory parameters allows early detection of patients with PIMS-TS and differentiation of those who could progress to a more serious condition. Blood count, C-reactive protein, and albuminemia are easily accessible tests and proved to be of high value in the identification of patients at risk of clinical worsening, which would help the treating physician to make the best therapeutic decision for these children, including their timely referral to hospital centers of greater complexity.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

References

1. Whittaker E, Bamford A, Kenny J, et al. Clinical Characteristics of 58 Children With a Pediatric Inflammatory Multisystem Syndrome Temporally Associated With SARS-CoV-2. JAMA 2020;e2010369.
2. Royal College of Paediatrics and Child Health. Guidance: paediatric multisystem inflammatory syndrome temporally associated with COVID-19. 2020. Disponible en: https://www.rcpch.ac.uk/resources/guidance-paediatric-multisystem-inflammatory-syndrome-temporally-associated-covid-19
3. Centers for Disease Control and Prevention. Emergency preparedness and response: health alert network. Publicado el 14 de mayo, 2020. Disponible en: https://emergency.cdc.gov/han/2020/han00432.asp
4. World Health Organization. Multisystem inflammatory syndrome in children and adolescents with COVID-19. Publicado el 15 de mayo, 2020. Disponible en: https://www.who.int/publications-detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19.
5. Hennon TR, Penque MD, Abdul-Aziz R, et al. COVID-19 associated Multisystem Inflammatory Syndrome in Children (MIS-C) guidelines; a Western New York approach. Prog Pediatr Cardiol. 2020; e101232.
6. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. Clin Chem Lab Med. 2020;58(7):1131-4.
7. Yale New Haven Children's Hospital. Guideline: Management Multisystem Inflammatory Syndrome in Children (MIS-C) Associated with COVID-19. 2020. Disponible en: https://app.lucidchart.com/documents/embeddedchart/e2fb545a-ac76-40b1-a11e-09bb6de8ec5b.
8. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020;395:1771-8.
9. Jones VG, Mills M, Suarez D, et al. COVID-19 and Kawasaki disease: novel virus and novel case. Hosp Pediatr. 2020;10:537-40.
10. Licciardi F, Pruccoli G, DeninaM, Parodi E. SARS-CoV-2 –induced Kawasaki-like hyperinflammatory syndrome: a novel COVID phenotype in children. Pediatrics. 2020;146(2):e20201711.
11. Davies P, Evans C, Kanthimathinathan H, et al. Intensive care admissions of children with paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS) in the UK: a multicentre observational study. The Lancet Child and Adolescent Health. 2020;4(9):669-77.
12. Riphagen S, Gómez X, González-Martínez C, et al. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet. 2020;395:1607-8.
13. Rodríguez Y, Novelli L, Rojas M, et al. Autoinflammatory and autoimmune conditions at the crossroad of COVID-19. J Autoimmun. 2020;16:e102506.
14. Liu F, Li L, Xu M, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. J Clin Virol. 2020;127:104370.
15. Mangalmurti N, Hunter CA. Cytokine Storms: Understanding COVID-19. Immunity. 2020;53(1):19-25.
16. Henry BM, Santos de Oliveira M, Benoit S, Lippi G, Plebani M. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58(7):1021-8.
17. Liao D, Zhou F, Luo L, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. Lancet Haematol. 2020;7(9):e671-8.
18. Panyang Xu, Qi Zhou, Jiancheng Xu. Mechanism of thrombocytopenia in COVID-19 patients. Annals of Hematology. 2020;99:1205-8.
19. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. Am J Hematol. 2020;95(7):834-47.
20. Fan BE, Lian Chong VC, Wei Chan SS, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020;95(6)e131-4.
21. Frazer JS, Tyrynis Everden AJ. Emerging patterns of hypercoagulability associated with critical COVID-19: A review. Trends in Anaesthesia and Critical Care. Publicado el 9 de julio 2020. Disponible en: https://doi.org/10.1016/j.tacc.2020.07.004.