



In the differential diagnosis of sepsis and hemophagocytic lymphohistiocytosis, procalcitonin and C-reactive protein (CRP) may be as determinant as ferritin

Arzu Oto ¹, Seher Erdogan ¹, Sinan Akbayram ², Mehmet Bosnak ¹

¹ Gaziantep University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Critical Care, Gaziantep, Turkey

² Gaziantep University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology and Oncology, Gaziantep, Turkey

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Abstract

Background: Hemophagocytic lymphohistiocytosis (HLH) and sepsis frequently appear as overlapping diagnoses in intensive care units. It is necessary to distinguish HLH, which has a very high mortality, from sepsis. In this study, we wanted to draw attention to the potential of procalcitonin (PCT) and C-Reactive Protein (CRP) as a marker like ferritin in differential diagnosis. Thus, HLH can be diagnosed as early as possible and the necessary aggressive immunosuppressive therapy can be added to the existing treatment.

Methods: All of the patients in the sepsis clinic who meet the HLH criteria Group HLH; patients not meeting the HLH criteria were defined as Group non-HLH. Files of all patients were reviewed in regard to HLH diagnosis criteria and H score.

Results: There were 16 patients in Group HLH and 15 in Group non-HLH. CRP and PCT levels were significantly lower ($p: 0.007$ and $p<0.001$, respectively) and ferritin levels were higher ($p<0.001$) in Group HLH. Hyperferritinemia was present in 15 (94%) and hemophagocytosis in bone marrow in 10 (63%) patients. In group HLH patients, the H score was 258 (230.7-281.5).

Conclusion: In critically ill children with suspected sepsis, if CRP and PCT values- two of acute phase reactants- do not explain the patient's poor clinical condition, HLH, which is an often overlooked diagnosis, must be excluded.

Key words: Children; ferritin; Hemophagocytic lymphohistiocytosis; procalcitonin; sepsis

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Correspondence / Yazışma Adresi: Arzu Oto, Gaziantep University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Critical Care, Gaziantep, Turkey e-mail: arzuhoto@gmail.com

Sepsis ve hemofagositik lenfohistiyositozun ayırıcı tanısında prokalsitonin ve C-reaktif protein (CRP) ferritin kadar belirleyici olabilir

Öz

Giriş-Amaç: Hemofagositik lenfohistiyositoz (HLH) ve sepsis, yoğun bakım ünitelerinde sıklıkla örtüşen tanılar olarak karşımıza çıkmaktadır. Mortalitesi çok yüksek olan HLH'yi sepsisten ayırmak gerekir. Bu çalışmada prokalsitonin (PCT) ve C-Reaktif Proteinin (CRP) ferritin gibi bir belirteç olarak ayırıcı tanıdaki potansiyeline dikkat çekmek istedik. Böylece HLH mümkün olduğunca erken teşhis edilebilir ve mevcut tedaviye gerekli agresif immünosupresif tedavi eklenebilir.

Yöntemler: Sepsis kliniğinde HLH kriterlerine uyan tüm hastalar Grup HLH; HLH kriterlerini karşılamayan hastalar Grup non-HLH olarak tanımlandı. Tüm hastaların dosyaları HLH tanı kriterleri ve H skoru açısından incelendi.

Bulgular: Grup HLH'de 16, Grup non-HLH'de 15 hasta vardı. Grup HLH'de CRP ve PCT düzeyleri anlamlı olarak daha düşük (sırasıyla p: 0,007 ve p<0,001) ve ferritin düzeyleri daha yüksekti (p<0,001). On beş (%94) hastada hiperferritinemi ve on (%63) hastada kemik iliğinde hemofagositoz mevcuttu. Grup HLH hastalarında H skoru 258 (230.7-281.5) idi.

Sonuç: Sepsis şüphesi olan kritik hastalarda akut faz reaktanlarından ikisi olan CRP ve PCT değerleri hastanın kötü klinik durumunu açıklıyorsa, sıklıkla gözden kaçan bir tanı olan HLH ekarte edilmelidir.

Anahtar kelimeler: Çocuklar; ferritin; Hemofagositik lenfohistiyositoz; prokalsitonin; sepsis.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is an aggressive pathological immune activation syndrome characterized by clinical signs and symptoms of excessive inflammation¹. Classified as primary or secondary, HLH creates a sepsis-like condition in a clinical and laboratory manner, due to general condition impairment, fever, cytopenia and high ferritin levels. The differential diagnosis of HLH and sepsis, which are life-threatening and necessitate very different treatment options, must be made by the clinician.

Sepsis is one of the main causes of morbidity and mortality in pediatric patients, it is defined as systemic inflammatory response syndrome associated with infection, and causes microcirculatory impairment and end-organ failure. The diagnosis of sepsis, which is a clinical diagnosis in critical pediatric patients, is made with the definitions made at the international consensus conference in 2005². Patients with sepsis are quickly transferred to intensive care units due to unstable clinical conditions.

Regardless of the cause, acute phase reactants such as C-reactive protein (CRP), procalcitonin (PCT), ferritin increase in response to inflammation. CRP is a pneumococcal C-polysaccharide reactant and is synthesized from the liver under the action of IL-6³. PCT is produced by thyroid C cells and also by the liver, intestines, monocytes and some neuroendocrine cells in cases of infection⁴. Serum PCT and CRP values have diagnostic and prognostic value for patients with sepsis⁵. Many studies have reported that high levels of acute phase proteins CRP, PCT and ferritin are detected in HLH with hyperinflammation⁶⁻⁹. HLH, which is extremely rare, may also develop secondary to sepsis. Unless suspected, the diagnosis is overlooked and the patient can only receive sepsis treatment. We recorded age, gender, Pediatric Index of Mortality (PIM), Pediatric Risk of Mortality (PRISM), Pediatric Logistic Organ Dysfunction (PELOD), Pediatric Multiple Organ Dysfunction scores (PMODS), outcomes, PCT, CRP and ferritin values by retrospective file scanning. In this study, we aimed to compare the measured PCT, CRP and

ferritin values of patients in our unit with HLH diagnosis accompanied by sepsis with the PCT, CRP and ferritin values of patients with sepsis. It is necessary to be careful to make the diagnosis of HLH, which should be added to the treatment with an aggressive immunosuppressant, as soon as possible. In addition to the HLH 2004 criteria, we anticipate that CRP and PCT, like ferritin, may be supportive at the time of diagnosis.

METHODS

This study was carried out retrospectively between March 2014 and March 2017 in the pediatric intensive care clinic of Gaziantep University Faculty of Medicine, which has third level 7 beds. The study was approved by Gaziantep University clinical research ethics committee (Decision No: 2017/145). Informed consent was waived due to its retrospective manner.

Sepsis was diagnosed according to pediatric age groups as defined in the International pediatric sepsis consensus conference guidelines². The HLH 2004 diagnostic criteria were used to diagnose HLH, which included a molecular diagnosis consistent with HLH or the presence of at least five of the following eight criteria: a) fever, b) splenomegaly, c) cytopenia affecting at least 2 series, d) high triglycerides and/or low fibrinogen, e) high serum ferritin, f) hemophagocytosis in bone marrow, spleen, liver or lymph node biopsies g) reduction or loss of natural killer (NK) cell activity, h) high level of free IL-2 receptor (CD25) in the blood¹. NK cell activity and IL-2 receptor level were not evaluated because we were not able to study these in our hospital. No difference in primary and secondary HLH treatments in the Histiocyte Society's current treatment protocol (HLH-2004). Therefore, patients were not classified as primary or secondary HLH, all patients who met the criteria were included in Group HLH. Secondary HLH is associated with all infectious microorganisms and malignant diseases¹. It is a

score evaluated according to the answers given to 9 variables for the H score used to distinguish secondary HLH. It is scored according to 3 clinical (high body temperature, organomegaly, immune suppression status), 5 laboratories (cytopenia, triglyceride, fibrinogen, ferritin, AST) and 1 cytological (hemophagocytosis) variable 10. The probability of having HLH is less than 1% if the H-Score is <90; and H-Score is above 99% with > 250¹⁰.

Sixteen patients with sepsis meeting the HLH criteria were defined as Group HLH. Fifteen patients hospitalized with the diagnosis of sepsis but excluded from the diagnosis of HLH were defined as Group non-HLH. A standard study form was filled out for each patient by a pediatric intensive care fellow. Age, gender, PIM, PRISM, PELOD, PMODS scores of the patients, and PCT, CRP and ferritin levels were recorded. Considering the diagnostic criteria of HLH, patient files were screened for vital signs, physical examination, family history, hemogram, biochemistry, coagulation, ferritin, bone marrow aspiration, and genetic results, if present, and these data were recorded.

Compliance of numerical data to normal distribution was tested by Shaphiro Wilk test. Student's T test was used to compare variables that fit normal distribution in two groups. Mann Whitney U test was used to compare variables that were not normally distributed in 2 groups. Relationships between categorical variables were tested with the Chi-square test. SPSS 22.0 package program was used in the analyses. P <0.05 was considered significant.

RESULTS

The study was conducted between March 2014 and March 2017 with a total of 31 pediatric patients, 11 (35%) male and 20 (64.5%) female, aged between 2 and 75 months. Patients who met at least five of the diagnostic criteria determined by the Histiocyte Association were considered as HLH, were evaluated, and these

patients were named Group HLH. Of the patients in group HLH, 11 were girls and 5 were boys, the median age was 10.5 (3.5-25) months, Six (37%) patients had a history of sibling death, 4 (25%) patients had relapsing HLH. One patient was followed for Griscelli syndrome. Molecular diagnosis could be studied in 9 patients, perforin gene mutation (homozygous W374X mutation in exon 3 region) was detected in 2 patients, one had also a history of sibling death. Eight (50%) patients died. In Table 1, demographic characteristics and scores were evaluated among the groups.

Table I: Evaluation of demographic characteristics and scores between groups

	Group HLH (n=16) mean± SD	Group Non-HLH (n=15) mean± SD	p ^a
Age (month)	10,5 (3,5-25)	12 (6,5-22,5)	0,98
Gender (M/F)	5/11	6 /9	0,44
PIM	42,43±30,68	47,72±35,81	0,06
PRISM	20,56±9,56	24,26±12,16	0,35
PELOD	23,62±7,12	26,86±13,36	0,40
P-MODS	7,68±3,13	7±3,46	0,56
Deaths (ex)	8/16	11/15	0,27

*a*Mann-Whitney U test; HLH; Hemophagocytic Lymphocytosis, PIM; Pediatric Index of Mortality; PRISM, Pediatric Risk of Mortality; PELOD, Pediatric Logistic Organ Dysfunction; P-MODS, Pediatric Multiple Organ Dysfunction Score

When Group HLH patients were evaluated in terms of HLH criteria, all patients had persistent fever, splenomegaly and cytopenia. When hemogram parameters were evaluated, anemia and thrombocytopenia were observed in all patients, neutropenia was observed in 8 (50%) patients. Hyperferritinemia was present in 15 (94%) patients and hemophagocytosis in the bone marrow in 10 (63%) patients. While hypofibrinogenemia was present in 12 (75%) and hypertriglyceridemia in 10 (63%) patients, it was observed that there were 15 (94%) patients meeting at least one of these two criteria. NK cell activity and interleukin-2

receptor level were not evaluated because they could not be studied in our hospital. In group HLH patients, the H score was calculated as 258 (230.7-281.5). In Table 2, patients were evaluated in terms of compliance with the HLH 2004 diagnostic criteria.

Table II: Percentage of cohort meeting the HLH 2004 diagnostic criteria.

HLH Criteria	Grup HLH (n=16) %(n)	Grup Non-HLH (n=15) %(n)	p ^a
Molecular Diagnosis	2/9		
Fever	100 (16)	100 (15)	Insignificant
Splenomegaly	100 (16)	7 (1)	<0,001
Cytopenia (in 2 or 3 series)	100 (16)	73 (11)	0,04
Hemoglobin (<9g/dL)	100 (16)	100 (15)	Insignificant
Trombocytopenia (<100×10 ⁹ /L)	100 (16)	67 (10)	0,01
Neutropenia (<1 × 10 ⁹ /L)	50(8)	13 (2)	0,05
High triglyceride or low fibrinogen	94 (15)	n.c.	
Hypertriglyceridemia(>265 mg/dL)	63 (10)	n.c.	
Hypofibrinogenemia (<150 mg/dL)	75 (12)	n.c.	
Hemophagocytosis in bone marrow	63 (10)	It was examined in only 1 patient and could not be detected.	
High ferritin >500 ng/mL	94 (15)	33 (5)	0,001

*a*Chi-square test; HLH; Hemophagocytic Lymphocytosis

Patients with sepsis, severe sepsis and septic shock who did not meet the HLH criteria were named Group non-HLH. Nine patients in Group non-HLH were female and 6 were male, their median age was 12 (6.5-22.5) months. Five patients had sepsis, 2 had severe sepsis, and 8 patients had septic shock. One patient had a history of sibling death due to Fanconi syndrome. All patients in the group had signs of fever, only 1 patient had splenomegaly, 11 patients had cytopenia. When hemogram parameters were evaluated, anemia was found in all patients, thrombocytopenia was observed in 10 (67%) and neutropenia was observed in 2

(13%) patients. Hyperferritinemia was observed in 5 (33%) patients. It was found that fibrinogen level was sent in 12 patients, but hypofibrinogenemia was not detected in any of them, triglyceride level was measured in 2 patients and it was found to be normal. There was no one meeting at least one of these two criteria in Group non-HLH. Hemophagocytosis was not observed in bone marrow aspiration, which was performed in 1 patient with fever, bicytopenia and high ferritin level. Spleen was not palpable in this patient. Secondary HLH was excluded because this patient's H score was 91. There was concomitant immune deficiency in 1 patient. Eleven (73%) patients died.

When the two groups were compared, no difference was found in terms of age and gender ($p > 0.05$). There was no difference between the two groups in terms of PIM, PRISM, PELOD and PMODS scores. However, CRP and procalcitonin levels of the patients in Group HLH were

significantly lower ($p: 0.007$, $p < 0.001$, respectively). Ferritin level in Group HLH was significantly higher than Group non-HLH ($p < 0.001$). There was no difference between the groups in terms of mortality ($p: 0.27$). Comparing patients who survived and those who died, there was no difference in PIM, PMODS scores and PCT, CRP and ferritin levels, while the PRISM and PELOD scores of the patients who were lost were higher ($p: 0.007$ and $p: 0.022$). The number of patients with severe sepsis and septic shock was higher among patients who were lost ($p: 0.021$). In Table 3, laboratory parameters were evaluated among the groups. Since fibrinogen, triglyceride, values of all patients in Group non-HLH were not evaluated and bone marrow aspiration was not performed in terms of hemophagocytosis, a comparison could not be made between the two groups in regard to these parameters. In Table 4, HLH 2004 criteria and H score at the time of diagnosis.

Table III: Evaluation of laboratory parameters between groups

	Group HLH (n=16)	Group Non-HLH (n=15)	p
PCT (ng/mL)	1,49 (0,53-12,6)	73,8(30,4-100)	<0,001 ^a
CRP (mg/L)	27,6 (5,62-67)	76,4(40,5-163)	0,007 ^a
Ferritin(mg/dL)	6815 (1497-8892)	264(49,8-564)	<0,001 ^a
Hemoglobin (g/dL)	5,97±1,86	7,39±1,03	Insignificant ^b
Trombocyte (10 ³ /L)	20500(15000-40500)	87000(71500-134000)	<0,001 ^a
ANC (10 ³ /L)	940(180-3050)	6550(1965-8450)	0,004 ^a
Bilirubin (mg/dL)	3,13±2,36	0,69±0,66	<0,001 ^a
AST (U/L)	404(135-1052)	62(30-182)	0,008 ^a
ALT (U/L)	136(67-274)	15(9-144)	0,024 ^a
Albumin(g/dL)	2,35±0,47	2,58±0,61	0,47
Sodium(mmol/L)	131±9,1	140(132-146)	0,28 ^b
LDH U/L	495(405-634)	na	-
INR	1,83±0,67	2,17±1,28	0,80 ^a
aPTT (sec)	55,6(37-95)	44(36,6-53,1)	0,07 ^a
Triglyceride (mg/dL)	283(199-359)	na	-
Fibrinogen (mg/dL)	115(96,5-162)	na	-

^a Mann-Whitney U test; ^b Student T test; HLH, Hemophagocytic Lymphocytosis; PCT, Procalcitonin; CRP, C-reactive protein; ANC, Absolute neutrophil count; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; LDH, Lactate dehydrogenase; INR, International Normalised Ratio; Aptt, activated partial thromboplastin time

Tablo IV: HLH 2004 criteria and H score at the time of diagnosis

Case no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Age (month)	3	75	56	5	26	2	6	24	3	18	2	8	13	25	8	25
Gender	F	M	F	F	M	M	F	F	M	F	M	F	F	M	F	F
Molecular Diagnosis	-	Griceli	-	perforin gene W374X mutation	-	-	-	-	-	-	-	perforin gene W374X mutation	-	-	-	-
Fever	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Splenomegaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cytopenia (in 2 or 3 series)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemoglobin, gr/dL	6,3	2,4	4,5	8,1	3,0	7,5	6,4	7,2	6,5	3,2	6,5	8,4	8,1	6,5	5,8	5,2
Platelets, 10 ⁹ /L	19	35	25	51	6	22	39	15	41	43	7	15	92	16	19	14
Neutrophils /mm ³	980	330	4500	40	2400	3200	900	30	3900	130	2600	1740	518	10	3200	340
High triglyceride or low fibrinogen	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Triglycerides	140	196	378	741	360	285	159	285	260	358	280	150	210	281	1246	301
Fibrinogen	110	142	98	101	140	107	74	120	253	180	168	80	96	629	72	145
Hemophagocytosis features on bone marrow aspirate	-	+	-	+	+	+	-	+	+	+	+	-	+	+	-	-
Ferritin ng/mL	1376	890	1098	57144	100000	5806	1861	8947	8346	8633	5182	19348	169	7824	8728	5543
Hepatomegaly	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-	-
SGOT >30 IU/l	94	45	363	529	742	3875	3090	123	446	1229	825	201	1128	210	111	172
SGPT IU/l	23	38	135	197	67	485	668	108	278	263	313	67	241	115	18	138
Known immunosuppression	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sibling death story	+	-	-	+	-	+	+	-	-	-	+	-	+	-	-	-
H score	214	267	204	319	274	289	214	284	244	299	274	228	249	269	239	249
Outcome	Alive	Death	Alive	Alive	Death	Death	Death	Alive	Alive	Death	Death	Death	Alive	Alive	Death	Alive

SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase

DISCUSSION

HLH, which makes organ infiltration and has dense cytokine release at the cellular level, shows similarities clinically to sepsis, causing impairment in microcirculation and end-organ failure^{2,11}. Classified as primary or secondary, HLH creates a sepsis-like picture clinically and laboratory, due to general condition impairment, fever, cytopenia, and high ferritin levels. It is usually very difficult to distinguish whether it is primary or secondary in patients. Primary HLH is an autosomal recessive disorder caused by perforin, syntaxin mutation and is often the first manifestation of another immunodeficiency syndrome^{12,13}. In group HLH, 1 patient had Griscelli syndrome and 2 patients had combined immunodeficiency diagnosis. Genetic diagnosis could be studied in 9 of 16 HLH patients, homozygous W374X mutation was detected in exon 3 region of perforin gene in 2 patients and 6 (37%) patients had a history of sibling death. No difference in primary and secondary HLH treatments in the Histiocyte Society's current treatment protocol (HLH-2004). Diagnosis of the microorganism, malignancy or autoimmune disease causing HLH does not change the treatment.

Secondary HLH is associated with all infectious microorganisms and malignant diseases¹. It is a score evaluated according to the answers given to 9 variables for the H score used to distinguish secondary HLH. It is scored according to 3 clinical (high body temperature, organomegaly, immune suppression status), 5 laboratories (cytopenia, triglyceride, fibrinogen, ferritin, AST) and 1 cytological (hemophagocytosis) variable¹⁰. The probability of having HLH is less than 1% if the H-Score is <90; and H-Score is above 99% with > 250 10. In a study in which both adult and pediatric patients with suspected HLH were evaluated, it showed that the H score in children was more sensitive than adults¹⁴. In our study, the H score in Group HLH patients was calculated as 258 (230.7-281.5).

Sepsis is the most common cause of mortality in pediatric intensive care units. Its treatment requires a multidisciplinary approach, including source control, effective antimicrobials, hemodynamic support and respiratory care. The HLH diagnostic criteria and the organ dysfunction associated with sepsis have common aspects. Due to the non-specific nature of the clinical picture, despite severe clinical conditions of patients the diagnosis of HLH is often overlooked¹⁵. HLH management mandates chemotherapy cycles, while sepsis management relies on the correct use of antimicrobials¹⁶. Aggressive immunosuppressive therapy, which is life-saving and found effective in HLH treatment, is not included in sepsis guidelines.

In Table 2, the HLH 2004 diagnostic criteria are reviewed for all patients. Fever is a criterion observed in both HLH and sepsis patients and cannot be used in differential diagnosis. Splenomegaly caused by lymphocytic and histiocytic infiltration is present in 90% of patients at the time of HLH diagnosis^{15,17}. The presence of splenomegaly without other known causes is beneficial for differentiating HLH from sepsis¹¹. In our study, when the two groups were compared, splenomegaly was significantly more common in Group HLH patients ($p < 0.001$). All patients in group HLH had cytopenia in at least 2 series, anemia and thrombocytopenia were observed in all patients, neutropenia was observed in 8 (50%) patients. In Group non-HLH, 11 (73%) patients had cytopenia, anemia was found in all patients, thrombocytopenia was observed in 10 (67%) patients and neutropenia in 2 (13%) patients. When the two groups were analyzed, the patients in Group HLH were significantly more cytopenic ($p = 0.004$).

Although ferritin is associated with sepsis as an acute phase reactant, extremely high levels should raise suspicion of HLH.

Hyperferritinemia is a very important marker for active HLH. It is an acute phase reactant and can be elevated at a lower level in several inflammatory or infectious processes. In children, however, ferritin level higher than 10000 mg/L suggests HLH with 90% sensitivity and 96% specificity¹⁸. Ferritin is a well known marker for HLH activity^{17,19} and prognosis²⁰. According to Bennett et al., higher ferritin levels (> 3,000 ng/mL) are associated with higher mortality²¹ and Garcia et al. reported 100% mortality in children with severe sepsis with high ferritin levels²². In our study, the median values of ferritin were 6815 ng/mL (1497-8892) in Group HLH and 264 ng/mL (49.8-564) in Group non-HLH. Hyperferritinemia (>500 ng/mL) was observed in 15 (94%) patients in Group HLH and 5 (33%) patients in Group non-HLH. When the two groups were evaluated for ferritin, ferritin levels in Group HLH were significantly higher than Group non-HLH ($p < 0.001$). Unfortunately, there is no single marker to differentiate HLH from sepsis. The most important diagnostic marker is hyperferritinemia. Ferritin levels should be checked to differentiate HLH in patients with suspected sepsis, and if it is high, HLH criteria should be reviewed one by one.

Hypofibrinogenemia or hypertriglyceridemia is one of the HLH diagnostic criteria. In Group HLH, it was observed that 12 (75%) patients had hypofibrinogenemia, 10 (63%) patients had hypertriglyceridemia, and 15 patients (94%) met at least one of these two criteria. Since all patients in the Group non-HLH did not have fibrinogen and triglyceride results, a comparison could not be made between groups. While the presence of hemophagocytosis in the bone marrow can help confirm the diagnosis of HLH, it is often absent, especially in the early stages^{23,24}. Although hemophagocytosis is present in the name of the disease, it is considered one of only 8 criteria and is not essential to diagnose HLH²⁵. In our study,

although 16 patients were diagnosed with HLH, hemophagocytosis was observed only in 10 (63%) patients. Since HLH was not considered in Group non-HLH patients, routine bone marrow aspiration was not performed, it was performed only in 1 patient with fever, cytopenia and high ferritin, and hemophagocytosis was not observed, splenomegaly was not found.

If CRP value induced by IL-6 is high, bacterial infection and autoimmune diseases are considered. Published data on CRP in HLH are limited, but there are only studies highlighting moderately high values, where mean median CRP values were 30⁶, 32⁷ and 71⁸ mg/L. However, in a review published for the purpose of differential diagnosis of sepsis and HLH, it is concluded that low CRP level can support HLH diagnosis¹¹. In our study, the CRP median levels in Group HLH were found to be 27.6 (5.62-67) mg/L, and in Group non-HLH 76.4 (40.5-163) mg/L. When high CRP was present in both groups, CRP levels in Group HLH were significantly lower than Group non-HLH ($p=0.007$). Low CRP may suggest HLH in a patient with suspected sepsis, but high CRP does not rule out this diagnosis because of probable infections secondary to neutropenia. In patients with sepsis clinic the infection should be treated effectively, but in HLH where hypercytokinemia needs to be treated with immunosuppressants, this is usually not enough.

PCT is normally produced by thyroid C cells⁴, but in case of an infection it is also produced by liver, intestines, monocytes and some neuroendocrine cells^{4,26}. PCT may be a more useful marker in patients with bacterial sepsis, since it has higher specificity and sensitivity than CRP²⁷. In a review of 5 adult HLH patients, increased PCT values were reported, but the highest PCT level within days was 7.1 ng/mL⁹. While the median values of PCT were 1.49

ng/mL (0.53-12.6) in Group HLH, it was 73.8 ng/mL (30.4-100) in Group non-HLH.

Although the PCT levels were high in both groups, PCT level in Group HLH was significantly lower than in Group non-HLH ($p < 0.001$). In this study, we aimed to present features that may help to differentiate HLH from sepsis in pediatric patients and wanted to emphasize that patients with treatment-resistant fever should definitely be reviewed in terms of HLH, especially if acute phase reactants such as CRP and PCT are not high enough to explain the clinical condition of sepsis. Although there were various publications (case presentations) stating that PCT increases, it was observed that PCT values were not as high as in patients with septic shock. To our knowledge, no case series comparing PCT and CRP values in patients with sepsis and HLH have been found in the literature. More studies are needed on this subject.

Covid-19 pandemic continues to claim lives nowadays and the cytokine storm, which has mortal consequences, has become one of the most frequently investigated topics. In an article compiling Covid-19 studies, it was reported that high serum procalcitonin and ferritin values should be considered as poor prognostic factors, but the cut-off PCT value was taken as ≥ 0.5 ng/mL²⁸. In a study comparing laboratory parameters in patients thought to have cytokine storm secondary to Covid 19, CRP and PCT levels were reported to be significantly increased in 65% and 5.7% of hospitalized patients, respectively, and even higher in patients with severe disease compared to the moderate group²⁹. However, careful evaluation of the study revealed that the optimal cut-off value of PCT was 0.07 ng/mL²⁹. In a study evaluating patients infected with the new type of coronavirus, most of the patients had normal PCT levels at admission, only 3 out of 12 patients requiring intensive care had high PCT,

but it should be noted that the cut-off value was reported as 0.5 ng/mL³⁰.

When the literature including secondary cytokine storm seen in HLH, MAS, Covid-19, which are diseases in which cytokine storm plays a major role, it was observed that the PCT level was high, but it was seen that the cut off value was taken as 0.5 ng/mL. The point we want to emphasize in this study is that even if the PCT values are high in intensive care admission of patients, it does not elevate to high values as in sepsis. Therefore, if the PCT and CRP values of the patient in a sepsis clinic do not explain the poor clinical condition of the patient at intensive care admission, HLH, which is an often overlooked diagnosis, must be excluded.

Machowicz et al. reviewed 62 studies, published on HLH and sepsis, to suggest criteria to help distinguish HLH from sepsis¹¹. Hyperferritinemia, especially when too high values are present, is a very useful marker in distinguishing HLH from sepsis, and in that case other HLH criteria should be evaluated¹¹. Deep cytopenias in sepsis are unlikely, and the likelihood of HLH increases in the absence of other explanations¹¹. In our study, cytopenia was observed significantly more frequently in Group HLH patients compared to Group non-HLH patients ($p < 0.004$), even thrombocytopenia was more common in Group HLH patients ($p = 0.01$).

More studies on HLH can provide important information regarding the optimal management of sepsis. This may thus lead to the identification of a subset of patients with sepsis in which tissue damage secondary to the hyperinflammatory state can be treated by immunosuppressive therapy.

This study has some limitations. First of all, it is a single center analysis and defines the approach of our unit. Retrospective study design, not studied sCd25 and NK cell activities,

and lacking genetic analysis in some patients are other limitations. However, we conclude that cytokine storms in these patients can be detected by using routine laboratory tests and mortality rate can be reduced by adding immunosuppressants to the treatment when necessary.

CONCLUSION

It should be remembered that hemophagocytic lymphohistiocytosis coincides with septic shock and can lead to refractory septic shock and death. Therefore, our aim in this study is to encourage the physician for further investigations, in order to exclude hemophagocytic lymphohistiocytosis, especially in patients with sepsis, where CRP and PCT values do not match the patient's severe clinical status. More importantly, early initiation of immunosuppressive therapy, which is the way to control cytokine storm, will be life-saving. However, larger prospective studies are needed to confirm this result.

Ethics Committee Approval: The study was approved by Gaziantep University clinical research ethics committee (Decision No: 2017/145). Informed consent was waived due to its retrospective manner.

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REFERENCES

1. Henter JI, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-31.
2. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med*. 2005;6(1):2-8.
3. Castell JV, Gómez-lechón MJ, David M, et al. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology*. 1990;12(5):1179-86.
4. Uzzan B, Cohen R, Nicolas P, et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. *Crit Care Med*. 2006;34(7):1996-2003.
5. Erenler AK, Yapar D, Terzi Ö. Comparison of procalcitonin and c-reactive protein in differential diagnosis of sepsis and severe sepsis in emergency department. *Dicle Med J*. 2017;44(2):175-82.
6. Lehmborg K, Pink I, Eulenburg C, et al. Differentiating macrophage activation syndrome in systemic juvenile idiopathic arthritis from other forms of hemophagocytic lymphohistiocytosis. *J Pediatr*. 2013;162(6):1245-51.
7. Kim J, Yoo SW, Kang S-R, et al. Clinical implication of F-18 FDG PET/CT in patients with secondary hemophagocytic lymphohistiocytosis. *Ann Hematol*. 2014;93(4):661-7.
8. Schram AM, Comstock P, Campo M, et al. Haemophagocytic lymphohistiocytosis in adults: a multicentre case series over 7 years. *Br J Haematol*. 2016;172(3):412-9.
9. Thomas D, Shah N, Patel H, et al. Hemophagocytic lymphohistiocytosis: A series of five clinical cases in adult patients at a single institution with a review of the literature. *N Am J Med Sci*. 2015;7(9):415.
10. Fardet L, Galicier L, Lambotte O, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheumatol*. 2014;66(9):2613-20.
11. Machowicz R, Janka G, Wiktor-Jedrzejczak W. Similar but not the same: differential diagnosis of HLH and sepsis. *Crit Rev Oncol Hematol*. 2017;114:1-12.
12. Henter JI. Biology and treatment of familial hemophagocytic lymphohistiocytosis: importance of perforin in lymphocyte-mediated cytotoxicity and triggering of apoptosis. *Med Pediatr Oncol*. 2002;38(5):305-9.

13. Janka G. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu Rev Med.* 2012;63:233-46.
14. Debaugnies F, Mahadeb B, Ferster A, et al. Performances of the H-score for diagnosis of hemophagocytic lymphohistiocytosis in adult and pediatric patients. *Am J Clin Pathol.* 2016;145(6):862-70.
15. Janka G. Hemophagocytic lymphohistiocytosis: when the immune system runs amok. *Klin Padiatr.* 2009;221(05):278-85.
16. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. *Pediatr Crit Care Med.* 2009;10(3):387-92.
17. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Eur J Pediatr.* 2007;166(2):95-109.
18. Allen CE, Yu X, Kozinetz CA, et al. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2008;50(6):1227-35.
19. Jordan MB, Allen CE, Weitzman S, et al. How I. treat hemophagocytic lymphohistiocytosis. *Blood.* 2011;118:4041-52.
20. Lin TF, Ferlic-Stark LL, Allen CE, et al. Rate of decline of ferritin in patients with hemophagocytic lymphohistiocytosis as a prognostic variable for mortality. *Pediatr Blood Cancer.* 2011;56(1):154-5.
21. Bennett TD, Hayward KN, Farris RW, et al. Very high serum ferritin levels are associated with increased mortality and critical care in pediatric patients. *Pediatr Crit Care Med.* 2011;12(6):e233-e6.
22. Garcia PCR, Longhi F, Branco RG, et al. Ferritin levels in children with severe sepsis and septic shock. *Acta paediatrica.* 2007;96(12):1829-31.
23. Bode SF, Lehmborg K, Maul-Pavicic A, et al. Recent advances in the diagnosis and treatment of hemophagocytic lymphohistiocytosis. *Arthritis research & therapy.* 2012;14(3):1-12.
24. Arico M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. *Leukemia.* 1996;10(2):197-203.
25. Tothova Z, Berliner N. Hemophagocytic syndrome and critical illness: new insights into diagnosis and management. *J Intensive Care Med.* 2015;30(7):401-12.
26. Oruç N, Ozütemiz O, Osmanoğlu N, et al. Diagnostic value of serum procalcitonin in determining the activity of inflammatory bowel disease. *Turk J Gastroenterol: the official journal of Turkish Society of Gastroenterology.* 2009;20(1):9-12.
27. Floriańczyk B, editor Structure and diagnostic value of procalcitonin. *Annales Universitatis Mariae Curie-Sklodowska Sectio D: Medicina;* 2003.
28. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. *Am J Hematol.* 2020.
29. 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.* 2020:104370.
30. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet.* 2020;395(10223):497-506.