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Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

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Soluble interleukin-2 receptor in pediatric patients investigated for hemophagocytic lymphohistiocytosis – a single-center, ten-year-long experience

Солубилни рецептор за интерлеукин-2 код педијатријских пацијената испитиваних на хемофагоцитну лимфохистиоцитозу – десетогодишње искуство једног центра

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Received: October 28, 2022
Accepted: December 20, 2022
Online First: December 28, 2022
DOI: https://doi.org/10.2298/SARH221028119J

*__Accepted papers__ are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

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When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

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Soluble interleukin-2 receptor in pediatric patients investigated for hemophagocytic lymphohistiocytosis – a single-center, ten-year-long experience

Солубилни рецептор за интерлеукин-2 код педијатријских пацијената испитиваних на хемофагоцитну лимфохистиоцитозу – десетогодишње искуство једног центра

SUMMARY

Introduction/Objective Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition characterized by fever, splenomegaly and cytopenias. Diagnosis of HLH requires at least five of the eight criteria set by the Histiocyte Society and poses a significant challenge to physicians. HLH-2004 criteria include measurement of plasma levels of soluble receptor for interleukin-2 (sIL-2R), an invaluable tool in the diagnosis of HLH, particularly because it can be measured swiftly and inexpensively.

Methods We retrospectively analyzed medical records of 45 pediatric patients (28 boys and 17 girls, median age 8.1 years) who were investigated for suspected HLH in University Children's Hospital, Belgrade, in the period 2012-2022.

Results Ten children were diagnosed with HLH, while 35 did not have HLH. All ten HLH patients had secondary HLH: eight suffered from infection or inflammatory condition, one from an autoimmune disease, and one from malignancy. Level of sIL-2R was above the HLH-2004 cutoff value of 2400 IU/ml in 9/10 patients with HLH (sensitivity 90%) and 9/35 of patients who did not have HLH (specificity 74.2%).

Conclusion Soluble IL-2 receptor measurement is valuable in children suspected to have HLH. Sensitivity and specificity of this analysis can be further improved by strict patient selection and a comprehensive diagnostic approach.

Keywords: hemophagocytic lymphohistiocytosis; soluble IL-2 receptor; children

САЖЕТАК

Увод/Циљ Хемофагоцитна лимфохистиоцитоза (ХЛХ) је тешко хиперинфламаторно стање које се одликује грозницом, спленомегалијом и цитопенијама. Дијагноза ХЛХ изискује најмање пет од осам критеријума које је поставило Хистиоцитно друштво и значајан је изазов лекарима. Критеријуми ХЛХ-2004 обухватају мерење нивоа солубилног рецептора за интерлеукин-2 у плазми (сИЛ-2Р), драгоцено оруђе у дијагностици ХЛХ, посебно јер га је могуће брзо и економично мерити.

Методе Ретроспективно смо анализирали медицинску документацију 45 педијатријских пацијената (28 дечака и 17 девојчица, медијана узраста 8.1 година) који су испитивани због сумње на ХЛХ у Универзитетској дечјој клиници у Београду у периоду 2012-2022.

Резултати Код десеторо деце је постављена дијагноза ХЛХ, док 35 није имало ХЛХ. Код свих десеторо деце је ХЛХ била секундарна: осморо је патило од инфекције или запаљењског стања и по једно од аутоимунске и малигне болести. Ниво сИЛ-2Р је био изнад граничне вредности од 2400 IU/ml прописане критеријумима ХЛХ-2004 код 9/10 деце са ХЛХ (сензитивност 90%) и 9/35 деце без ХЛХ (специфичност 74,2%).

Закључак Мерење сИЛ-2Р је драгоцено код деце са сумњом на ХЛХ. Сензитивност и специфичност ове анализе је могуће додатно побољшати строгом селекцијом пацијената и свеобухватним дијагностичким приступом.

Кључне речи: хемофагоцитна лимфохистиоцитоза; солубилни ИЛ-2 рецептор; деца

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a severe and life-threatening hyperinflammatory condition characterized by fever, splenomegaly and cytopenias, as well as elevated serum ferritin levels, hypofibrinogenemia and hypertriglyceridemia [1]. The

cornerstone of HLH pathogenesis is abnormal macrophage activity causing overabundance of proinflammatory cytokines, thought to be a consequence of insufficient function of cytotoxic lymphocytes ($CD8^+$ T cells and natural killer [NK] cells), hampering the physiological termination of immune response and initiating a vicious circle of immune activation. Hemophagocytic lymphohistiocytosis can be primary or secondary. The former may occur as familial HLH or as part of certain primary immune deficiencies, while the latter can be triggered by a wide variety of infectious, autoimmune, malignant and other conditions [2].

Diagnosis of HLH requires at least five of the eight criteria set by the Histiocyte Society (HLH-2004) [3] and poses a significant challenge to physicians, since one or more criteria may be absent in some patients, particularly early in the course of the disease, and timely treatment is life-saving. HLH-2004 criteria include measurement of plasma levels of soluble receptor for interleukin-2 (sIL-2R), a molecule shed in great quantities by activated T cells, and thus a marker of T-cell activation [4]. With a cutoff value of 2400 IU/ml, sIL-2R measurement is an invaluable tool in the diagnostic workup of patients suspected to have HLH, particularly because it can be measured swiftly and inexpensively. However, the real-life impact of sIL-2R on the diagnosis of HLH may vary. We will review plasma sIL-2R levels found in pediatric patients investigated for HLH in the past ten years and assess their diagnostic significance.

METHODS

We retrospectively analyzed medical records of 45 pediatric patients (28 boys and 17 girls, median age 8.1 years) who were investigated for suspected HLH in University Children's Hospital, Belgrade, in the period 2012-2022. Data on disease course and outcome, initial and final diagnosis, treatment modalities, status of HLH-2004 criteria (fever, splenomegaly, bi/pancytopenia, hemophagocytosis in bone marrow, serum levels of fibrinogen, ferritin and tryglicerides), other relevant laboratory parameters (cerebrospinal fluid findings, C-reactive

protein [CRP], procalcitonin, transaminases and activated partial thromboplastin time [APTT]) were retreived from the patients' histories, as were the results of immunological tests (cytotoxic lymphocyte function) and genetic analyses, if performed.

Serum levels of sIL-2R were measured as part of the diagnostic workup. For this purpose, 2 ml of peripheral blood was drawn in a tube containing 0.38% Na-citrate as anticoagulant. Upon separating the serum by centrifugating the samples at 1600 x g, the analysis was either performed on the same day or the serum was kept frozen at -20 °C for a maximum of two months until the time of analysis. The level of sIL-2R was measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, USA) according to the manufacturer's instructions. The results were expressed in standardized international units (IU/ml).

The values of appropriate parameters were expressed as median, range and standard deviation. Statistical significance of differences between patient groups was determined by two-tailed Mann-Whitney's U test.

All procedures on human subjects were performed in accord with the Helsinki Declaration and were approved by the Ethical Committee of UCH Belgrade.

RESULTS

We classified all patients (N=45) in four groups according to the type of underlying condition: 1) detected or suspected infection or an inflammatory state of unclear origin (N=18); 2) known or suspected autoimmune disease (N=10); 3) pre-existing malignant disease (N=11); and 4) a transplanted organ (N=6).

Patients Diagnosed with HLH

The diagnosis of HLH was established in 10 patients (6 boys and 4 girls, median age 4.5 years). Of these, eight were in the infection/inflammation group and one in the autoimmune

and malignant groups, respectively. All HLH patients except one (9/10, 90.0%) had sIL-2R levels above 2400 IU/ml. All patients (10/10) were febrile, 6 (60.0%) had an enlarged spleen, while 7 (70.0%) displayed bi- or pancytopenia. Hemophagocytosis was found in bone marrow aspirate in 4 patients (40%). Hypofibrinogenemia was present in 6 (60%), hypertriglyceridemia in 5 (50%), and hyperferritinemia in 8 patients (80%). Functional capacity of cytotoxic T lymphocytes and NK cells was investigated in three patients, with results in the physiological range. Genetic investigations for HLH (clinical exome sequencing) were performed in one patient, who exhibited two heterozygous variants of undetermined significance (VUS) in the UNC13D gene. Of the ten HLH patients, one satisfied six HLH-2004 criteria, while the remaining nine satisfied five criteria. Median time from the onset of HLH to diagnosis was 2½ weeks (range 1-8 weeks).

In all ten children, HLH was classified as secondary. In three children, the underlying disorder was a clinically diagnosed pneumonia (without identification of the causative agent, although one child had a positive serologic test for West Nile virus). In two children, clinical and laboratory findings indicated a viral upper respiratory infection (with negative results of specific virological tests). One child had septicemia/systemic inflammatory syndrome (with sterile blood culture). This child also had a positive serological test for *Leischmania,* but without the finding of this organism on bone marrow examination. One child had an ANCA-positive systemic vasculitis accompanied by respiratory problems, with a positive serological test for *Chlamydia pneumoniae*. One child had a positive serological test for Epstein-Barr virus and clinical signs of Kawasaki disease. One child developed secondary HLH during maintenance therapy for acute lymphoblastic leukemia, while another was eventually diagnosed with lymphoma.

Five children were treated according to HLH-2004 protocol for secondary HLH (dexamethasone and cyclosporine without etoposide), two of whom also received intravenous

immunoglobulins enriched for IgM fraction. The remaining four children were treated by glucocorticoids with or without intravenous immunoglobulins. In total, disease had a fatal outcome in four children (40.0%), while six (60.0%) recovered. Among the fatalities, one child (age 2 years 11 months) received glucocorticoids and died of systemic vasculitis complicated by pneumonia five days after the diagnosis of HLH. One child (age 15 years 1 month) suffered from acute lymphoblastic leukemia, also received glucocorticoids, and died two months after the onset of secondary HLH. One child had unexplained septicemia/SIRS (age 12 years 11 months), was treated by dexamethasone, cyclosporine and immunoglobulins, and died two weeks after the diagnosis of HLH. One child (age 13 years 6 months) was treated with dexamethasone and cyclosporine and died after being transferred to another center; the underlying disorder was later determined to be lymphoma.

Patients Who Were Not Diagnosed with HLH

Among the 35 children who did not have HLH, 6 (17.1%) fulfilled only one HLH-2004 criterion, 11 (31.4%) fulfilled two criteria, 12 (34.3%) three, 3 (8.6%) four, 2 (5.7%) five, and 1 (2.9%) six criteria. The three children with 5-6 criteria were not deemed by their psysician to suffer from HLH, and were given other diagnoses (febrile neutropenia complicated by sepsis in two children with leukemia and a poorly defined viral infection in the remaining child).

In this group, six children died: four with malignant disease (three with acute leukemia and one with Langerhans cell histiocytosis), one as a consequence of systemic CMV infection complicated by acute respiratory distress, and one child with transplanted kidney who was killed in a traffic accident unrelated to medical issues. Only two of these six patients (both with leukemia) had an sIL-2R level above 2400 IU/ml. One of these two did fulfill five HLH-2004 criteria, although his condition was attributed to disease progression and not HLH. The other child had three criteria.

Levels of Soluble IL-2 Receptor

Median level of sIL-2R in patients with HLH was 3489.3 (range 2101.0-5536.0 IU/ml, SD 1664.9 IU/ml). This was significantly higher ($p<0.01$) compared to patients without HLH (1145.0; range 0.0-8955.0 IU/ml, SD 1663.1 IU/ml). Highest levels of sIL-2R were found in the infection/inflammation group (2921.5; range 328.0-8955.0 IU/ml, SD 2044.2 IU/ml), followed by malignancy (1425.0; range 322.0-4110.0, SD 1366.3 IU/ml), autoimmunity (1103.5; range 0.0-2872.0 IU/ml, SD 807.0 IU/ml), and transplantation (787.0; range 142.0-3070.0 IU/ml, SD 1065.3 IU/ml). The difference was statistically significant between the infection/inflammation and autoimmunity groups ($p<0.01$) and between infection and transplantation groups ($p<0.05$).

Level of sIL-2R above 2400 IU/ml was found in 9/10 patients with HLH (sensitivity of analysis 90.0%) and 9/35 (25.8%) of patients who did not have HLH, yielding a specificity of 74.2%. Of the nine patients with high sIL-2R and no HLH, five (55.6%) were in the infection/inflammation group, three (33.3%) in the malignancy group, and one (11.1%) in the transplantation group. Of the five children in the first group, one had culture-confirmed staphylococcal septicemia, while four had a febrile condition of unknown origin. Of these, one was eventually diagnosed with multisystemic inflammatory syndrome (MIS-C) as a consequence of COVID-19. All five of the aforementioned children fully recovered – three with glucocorticoids with IVIG (with or without IgM enrichment), and one without any anti-inflammatory treatment. Two of the three children with malignant disease (acute lymphoblastic leukemia) died – one of febrile neutropenia and consequent septicemia, the other due to disease progression. The third child recovered with glucocorticoids alone. The only patient with high sIL-2R in the transplantation group had positive serological and virological findings for EBV and was successfully treated.

Fever, Splenomegaly and Cytopenias

Fever was present in all ten patients with HLH and 20/35 (57.1%) patients without HLH. It was found in 16/18 (88.9%) children with infection/inflammation, 5/10 (50.0%) of children with autoimmunity, 7/11 (63.6%) of those with malignancy, and 2/6 (33.3%) of those with transplanted kidney. Among the patients with high sIL-2R and no HLH, 7/9 (77.8%) were febrile.

An enlarged spleen was found in 6/10 (60.0%) of children with HLH and 12/35 (34.3%) without HLH. In the infection/inflammation group, splenomegaly was present in 10/18 (55.6%); in the autoimmunity group, in 3/10 (30.0%); in the malignancy group, in 3/11 (27.3%); and in the transplantation group, in 1/6 (16.7%). The frequency of splenomegaly among children with high sIL-2R and no HLH was 6/9 (66.7%).

Bi- or pancytopenia existed in 7/10 (70.0%) patients with HLH and 16/35 (45.7%) patients without HLH. In the subgroup of the latter with high sIL-2R levels, bi- or pancytopenia was found in 4/9 (44.4%) children. Among patient groups, those with malignancy had the highest frequency of bi/pancytopenia (8/11, 72.7%), followed by transplantation (4/6, 66.7%), autoimmunity (4/10, 40.0%), and infection/inflammation (7/18, 38.9%).

Hemophagocytosis

Hemophagocytosis was observed in the bone marrow in 4/10 (40.0%) of children with HLH and 1/35 (2.9%) without HLH. The child with hemophagocytosis did not have sIL-2R level above the cutoff. All four patients with hemophagocytosis and HLH were in the infection/inflammation group, while the patient with hemophagocytosis without HLH belonged to the transplantation group.

Fibrinogen and Triglycerides

Median fibrinogen level of children with HLH was 1.44 g/l (range 0.80-4.90 g/l, SD 1.41 g/l) and of those without HLH 3.60 (range 1.13-14.30 g/l, SD 3.08 g/l; $p<0.01$). Fibrinogen was lowest in the infection/inflammation group (median 2.05; range 0.80-8.00 g/l, SD 1.97 g/l), followed by the autoimmunity (median 3.05 g/l; range 1.16-14.30 g/l, SD 4.49 g/l), malignancy (median 3.96 g/l; range 1.13-14.00 g/l, SD 3.49 g/l) and transplantation (median 4.27 g/l; range 3.40-5.11 g/l, SD 0.67 g/l) groups. None of these differences were statistically significant.

Median level of triglycerides was 3.07 mmol/l (range 1.60-9.14 mmol/l, SD 2.53 mmol/l in children with HLH and 2.29 mmol/l (range 0.45-10.78 mmol/l, SD 2.45 mmol/l) in children without HLH. This difference was not statistically significant. By group, the highest triglyceride level was in the infection/inflammation group (median 3.15 mmol/l; range 0.86-10.06 mmol/l, SD 2.74 mmol/l), followed by the autoimmunity (median 2.63 mmol/l; range 0.45-10.78 mmol/l, SD 3.17 mmol/l), transplantation (median 2.54 mmol/l; range 1.17-6.48 mmol/l, SD 1.92 mmol/l) and malignancy groups (median 1.99 mmol/l; range 0.63-3.20 mmol/l, SD 0.74 mmol/l). The only significant difference was between infection/inflammation and malignancy groups ($p<0.05$).

In total, 8/10 (80.0%) children with HLH had hypofibrinogenemia and/or hypertriglyceridemia, as did 28/35 (80.0%) children without HLH and 8/9 (88.9%) children without HLH who had sIL-2R above 2400 IU/ml.

Ferritin

Serum ferritin level was above 500 ng/ml in 8/10 (80.0%) children with HLH and 26/35 (74.2%) children without HLH. In the subgroup of the latter with high sIL-2R, hyperferritinemia was present in 7/9 (77.8%). Median level of ferritin in children with HLH

was 1734.0 ng/ml (range 155.6-5001.0 ng/ml; SD 1825.2 ng/ml), compared to 1490.0 (range 34.2-7985.2 ng/ml; SD 2069.8 ng/ml) in children without HLH. This difference was not statistically significant. The highest ferritin level was found in patients with malignancy (median 2699.7 ng/ml; range 1351.9-5401.7 ng/ml, SD 1660.9 ng/ml), followed by autoimmunity (median 1495.4 ng/ml; range 34.2-7378.7 ng/ml, SD 2311.8 ng/ml), transplantation (median 1296.8 ng/ml; range 295.8-3507.8 ng/ml, SD 1203.1 ng/ml), and infection/inflammation (median 762.0 ng/ml; range 50.1-7985.2 ng/ml, SD 2094.0 ng/ml). Statistically significant were the differences between infection/inflammation and malignancy ($p<0.01$) and between malignancy and transplantation ($p<0.05$).

C-Reactive Protein

In patients with HLH, CRP levels were somewhat higher (median 87.2 mg/l; range 7.3-196.9 mg/l, SD 74.5 mg/l) compared to patients without HLH (median 33.5; range 0.6-349.8 mg/l, SD 99.2 mg/l). However, this difference was not statistically significant. Highest CRP levels were found in malignancy (median 150.6 mg/l; range 1.2-323.8 mg/l, SD 100.3 mg/l), followed by infection/inflammation (median 53.5 mg/l; range 1.4-349.8 mg/l, SD 101.9 mg/l), transplantation (median 14.2 mg/l; range 1.2-45.3 mg/l, SD 16.3 mg/l), and autoimmunity (median 9.7 mg/l; range 0.6-171.0 mg/l, SD 60.7 mg/l). Statistical significance is reached between autoimmunity and malignancy ($p<0.05$) and transplantation and malignancy ($p<0.01$). Nine patients with high sIL-2R and no HLH had median CRP level of 58.7 mg/l (range 4.5-349.8 mg/l, SD 109.7 mg/l). This was not significantly different from the HLH group.

Procalcitonin

Procalcitonin levels were available for only three of the ten HLH patients and were 0.47, 0.48 and 0.51 ng/ml, respectively (median 0.48 ng/ml, SD 0.02 ng/ml). Procalcitonin levels

were also available for 19 patients without HLH (median 0.32 ng/ml; range 0.06-4.99 ng/ml, SD 1.43 ng/ml). The highest median procalcitonin level was in children with malignancy (0.59 ng/ml; range 0.25-2.06 ng/ml, SD 0.75 ng/ml; N=8), followed by infection/inflammation (0.49 ng/ml; range 0.19-4.99 ng/ml, SD 2.13 ng/ml; N=7), transplantation (0.28 ng/ml; range 0.25-0.32 ng/ml, SD 0.04 ng/ml; N=3) and autoimmunity (0.24 ng/ml; range 0.06-0.54 ng/ml, SD 0.20 ng/ml; N=4). None of these differences were statistically significant; however, this mainly reflects small sample sizes.

Cerebrospinal Fluid Findings

Of children with HLH, 8/10 (80.0%) had normal cytological and biochemical CST findings. Two children (20.0%) had a pathologic finding: one had leukocytosis with predominance of lymphocytes (83%) and marked proteinorachia (1159.0 g/l), while the other had moderate amounts of protein (0.4 g/l) with no cellular elements. Moderate leukocytosis and proteinorachia were also found in 2/35 (5.7%) children without HLH, none of whom had sIL-2R above 2400 IU/ml.

Transaminases

In total, 9/10 (90.0%) children with HLH and 17/35 (46.8%) children without HLH had elevated serum levels of aspartate-aminotransferase (AST, above 36 U/l) and/or alanine-aminotransferase (ALT, above 68 U/l). In children without HLH and sIL-2R>2400 IU/ml, 7/9 (77.8%) had elevated AST and/or ALT. This finding was present in 14/18 (77.8%) children in the infection/inflammation group, 5/10 (50.0%) in the autoimmunity group, 7/11 (63.6%) in the malignancy group, and 1/6 (16.7%) in the transplantation group.

Blood Coagulation Defect

A prolonged APTT (>35 seconds) was found in 4/10 (40.0%) children with HLH and 5/30 (16.7%) children without HLH for whom this analysis was performed. In patients without HLH who had sIL-2R above 2400 IU/ml, APTT was measured in 7 children and was prolonged in 3 (42.8%). This abnormality was noted in 6/15 (40.0%) patients with infection/inflammation, 1/8 (12.5%) patients with autoimmunity, 2/11 (18.2%) patients with malignancy and none of the six patients with a transplanted kidney.

DISCUSSION

Primary HLH was not diagnosed in our patient series. This may partly be due to the unavailability of genetic testing in all patients but one. Notably, no sharp demarcation exists between primary and secondary HLH, since primary HLH is often initiated by an infection or other trigger [5] and secondary HLH is often associated with a genetic predisposition [6]. Primary and secondary HLH are, however, quite distinct in their response to treatment and prognosis: in the absence of timely diagnosis and hematopoietic stem cell transplantation, patients with primary HLH may experience only a brief remission and their long-term prognosis is dismal, while secondary HLH can be cured by immunosuppressive agents [7]. For the purpose of this analysis, we decided to group patients according to the type of underlying condition or trigger (infection/hyperinflammatory state, autoimmune disease, pre-existing malignancy, organ transplantation). Most of our HLH patients belonged to the infection/inflammation group. We chose to form such a heterogenous group because causative agent or trigger is rarely identified. Of the 18 children in this group, only five had a defined or probable infection. An infectious trigger was also present in three of the ten patients with autoimmune disease, one of the eleven children with malignancies, and two of the six children with transplanted kidney. Since all children suspected of HLH underwent

extensive bacteriological, virusological, and (if necessary) parasitological and mycological investigations, a small percentage of patients with an identified microbiological agent in our series may be an additional sign of the magnitude of challenge faced by clinicians in the attempt to uncover the trigger of secondary HLH.

Macrophage activation syndrome, a variant of HLH, most often accompanies systemic juvenile idiopathic arthritis (sJIA) [8] and systemic lupus erythematosus (SLE) [9], but can be encountered in a wide range of autoimmune disorders [10]. The only child in our series with autoimmune disease who was diagnosed with HLH suffered from ANCA-positive systemic vasculitis. The remaining patients (not diagnosed with HLH) in this group had autoimmune hemopathies (5), sJIA (2), SLE (1) and polymyositis/dermatomyositis (1). Since all received immunosuppressive treatment, we cannot exclude the possibility that some of them – less than five HLH-2004 criteria notwithstanding – really exhibited a sort of incomplete, decapitated, or abortive form of MAS.

In children, as in adults, HLH can arise in various hematological malignancies [11, 12]. Although only one of eleven children with malignancies in this series was diagnosed with HLH, additional three had sIL-2R level above 2400 IU/ml, two of whom even formally satisfied HLH-2004 criteria. In this group, the main differential diagnostic problem is febrile neutropenia due to malignancy itself and its treatment. Some children with malignancy display a sort of inflammatory syndrome that may overlap with HLH, but responds promptly to glucocorticoid treatment. In such children, measurement of plasma sIL-2R levels may aid differential diagnosis. Diagnosis of HLH in pediatric oncology is in many ways a peculiar diagnostic problem, necessitating a specific approach [13]. Finally, although none of our patients with a transplanted organ had HLH, the investigation of sIL-2R in this group may be additionally justified by the fact that rising sIL-2R levels could be a harbinger of transplant rejection [14].

In our patient series, measurement of sIL-2R alone (with the HLH-2004-prescribed cutoff of 2400 IU/ml) displayed a sensitivity of 90.0% and specificity of 74.3% in the diagnosis of HLH. True sensitivity could, however, be even higher, given that the only patient with HLH and sIL-2R below the cutoff value was investigated early in the course of the disease. The specificity observed in our series was also in broad agreement with published data [15]. From the differential diagnostic perspective, the group of nine patients who had sIL-2R above 2400 IU/ml and no HLH comes to attention. One child in this group had Kawasaki disease and another had MIS-C, while four had an acute inflammatory condition of unknown cause. This resonates well with literature data indicating that the specificity of sIL-2R measurement in the diagnosis of HLH could be significantly improved by the exclusion of patients with inflammatory conditions known to be accompanied by high sIL-2R levels (such as Kawasaki disease, SIRS, MIS-C) [16, 17, 18]. MIS-C is documented to be associated with extremely high plasma levels of proinflammatory cytokines, and consequently very high levels of sIL-2R [19, 20]. Similar appears to be true of SIRS of any etiology [21]. This is important because treatment of choice in these conditions significantly differs from that of HLH: the above hyperinflammatory states respond well to intravenous administration of immunoglobulins, with or without IgM enrichment [22].

Most often satisfied HLH-2004 criteria in our patients were febrility, cytopenias and hyperferritinemia, and this confirms their importance in the triage of patients suspected to have HLH. Hemophagocytosis was noted in the bone marrow in only four patients (40%), which is by no means unusual [23], and in just one child without HLH, even though this phenomenon may be encountered in a wide range of other conditions [24]. Notably, CRP level does not appear to be of great assistance in the diagnosis of HLH. The same applies to procalcitonin levels, APTT and CST findings. However, these tests are indispensable in the vigilance for potential complications.

Although the function of cytotoxic lymphocytes is intimately connected to the pathogenesis of HLH, appropriate laboratory tests are not routinely performed in our institution (or indeed available) at this moment. Thus, we were able to obtain data on cytotoxic lymphocyte function in just three patients, all with normal findings. The inability to perform this analysis places the treating physician into a difficult situation to diagnose HLH based on five of seven, rather than five of eight criteria, potentially reducing the sensitivity, and to a lesser degree also the specificity of analysis. Similarly, genetic analysis was performed in just one child with HLH, uncovering two variants of undetermined significance in UNC13D gene. This corresponds to secondary HLH arising upon variable genetic predisposition [25]. Although costly, extensive genetic testing of HLH patients could be expected to uncover such predisposition in many more instances.

The cure rate of children with HLH in our series (60.0%) is in broad agreement with literature data [26]. Time elapsed before the diagnosis of HLH (median 2½ weeks) may also be considered acceptable, but it can – and should – be improved by timely and judiciously performing appropriate laboratory tests, including plasma sIL-2R measurement and cytotoxic lymphocyte functional analyses.

CONCLUSION

Soluble IL-2 receptor measurement is valuable in children suspected to have HLH. Sensitivity and specificity of this analysis can be further improved by strict patient selection and a comprehensive diagnostic approach.

ACKNOWLEDGEMENTS

The authors wish to express utmost gratitude to laboratory technicians Branislav Đorđević and Marija Lazović for their indispensable support. The presented data are part of

specialist academic thesis of Marija Ćazić (University of Belgrade Faculty of Biology, 2022).

Conflict of interest: None declared.

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