Journal of Critical Care 35 (2016) 185–190

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.jccjournal.org

Investigation of hemophagocytic lymphohistiocytosis in severe sepsis patients☆

Burcin Halacli, MD [a], Nese Unver, PhD [b], Sevil Oskay Halacli, PhD [c], Hande Canpinar, PhD [d], Ebru Ortac Ersoy, MD [a], Serpil Ocal, MD [a], Dicle Guc, MD, PhD [d], Yahya Buyukasik, MD [e], Arzu Topeli, MD [a,*]

[a] Hacettepe University Faculty of Medicine, Department of Internal Medicine, Medical Intensive Care Unit, Ankara, Turkey
[b] The University of Texas, MD Anderson Cancer Center, Houston, USA
[c] Hacettepe University Faculty of Medicine, Department of Pediatrics, Division of Immunology, Ankara, Turkey
[d] Hacettepe University Faculty of Medicine, Department of Basic Oncology, Ankara, Turkey
[e] Hacettepe University Faculty of Medicine, Department of Internal Medicine, Division of Hematology, Ankara, Turkey

ARTICLE INFO

Keywords:
Sepsis
Cytopenia
Hemophagocytosis
Natural killer
Interleukin-2 receptor

ABSTRACT

Purpose: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening condition characterized by uncontrolled inflammation and has common clinical and laboratory features with sepsis. The aim of this study was to investigate patients treated with severe sepsis who had bicytopenia for the presence of HLH.
Materials and methods: Patients with severe sepsis who were non-responsive to treatment and developed at least bicytopenia were included. Peripheral blood samples were collected and stored for later evaluation for natural killer (NK) activity and soluble interleukin-2 receptor levels. Diagnostic criteria of HLH were retrospectively analyzed.
Results: Seventy-five of 382 patients (20%) were followed as severe sepsis and septic shock. Among them, 40 patients had bicytopenia. Twenty-six of 40 patients were excluded due to the presence of active solid or hematological malignancies. Three patients died before fulfillment of HLH criteria and one patient denied to give consent. All of the remaining 10 patients had at least five of the eight criteria according to criteria of the Histiocyte Society. Only one of 10 patients was diagnosed as HLH and received treatment during intensive care unit stay. None of the 10 patients survived.
Conclusions: This study emphasizes to consider the possibility of HLH and the need of rapid assessment of patients with severe sepsis who had bicytopenia and were resistant to treatment in intensive care.
© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH), which is classified as familial and acquired/secondary, is a life-threatening condition characterized by uncontrolled inflammation, prolonged fever and cytopenia [1]. HLH has common clinical and laboratory features with systemic inflammatory response syndrome, sepsis, and severe sepsis [2]. According to diagnostic criteria published by Histiocyte Society for HLH, fever, splenomegaly, at least bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, high serum ferritin level, low or absence of natural killer (NK) cell activity, and increased soluble interleukin-2 receptor (sIL-2R or sCD25) level are accepted parameters for evaluation which also appear to be consistent with sepsis (Table 1). Five of the 8 criteria are considered sufficient for diagnosis [3] and the latter two parameters are especially diagnostic for HLH [4].

It is thought that underlying cause of HLH may be severe uncontrolled inflammatory response in septic patients. Therefore, we cannot view HLH as a separate disease from severe sepsis but rather as a consequence of a severe uncontrolled inflammatory response heralding the inability to control the infectious trigger. Therefore, it is difficult to distinguish HLH during the course of sepsis.

It is well known that some cells and cytokines play crucial roles in the pathogenesis of immunity and inflammation. NK cells have an activity on modulation of immune system by impairment of signal-related

Abbreviations: aPTT, activated partial thromboplastin time; APACHE, acute physiology and chronic health evaluation; BAL, bronchoalveolar lavage; CVID, common variable immune deficiency; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; DIC, disseminated intravascular coagulation; EBV, ebstein barr virus; HLH, hemophagocytic lymphohistiocytosis; ICU, intensive care unit; INR, international normalized ratio; LDH, lactate dehydrogenase; LOS, length of stay; MOF, multiple organ failure; NK, natural killer; SOFA, sequential organ failure assessment; sIL-2R, soluble interleukin-2 receptor.

☆ Conflicts of interest disclosure: None of the authors has any financial or any other conflict of interest related to the content of this manuscript.

* Corresponding author at: Hacettepe University Faculty of Medicine, Department of Internal Medicine, Medical Intensive Care Unit, 06100, Sihhiye, Ankara, Turkey. Tel.: +90 312 305 20 68; fax: +90 312 305 23 02.

E-mail addresses: burcin.halacli@yahoo.com (B. Halacli), unvernese@yahoo.com (N. Unver), seviloskay@gmail.com (S.O. Halacli), hcanpina@hacettepe.edu.tr (H. Canpinar), ebru.ortac@hacettepe.edu.tr (E.O. Ersoy), drserpilgocmen@yahoo.com (S. Ocal), dguc@hacettepe.edu.tr (D. Guc), ybuyukas@hacettepe.edu.tr (Y. Buyukasik), atopeli@hacettepe.edu.tr (A. Topeli).

http://dx.doi.org/10.1016/j.jcrc.2016.04.034
0883-9441/© 2016 Elsevier Inc. All rights reserved.

Table 1
Diagnostic criteria of HLH [3]

1. Fever
2. Splenomegaly
3. At least bicytopenia
4. Hypertriglyceridemia (fasting triglyceride level ≥265 mg/dL) or hypofibrinogenemia (fibrinogen <1.5 g/L)
5. Hemophagocytosis demonstrated in bone marrow or other tissues
6. Serum ferritin ≥500 μg/L (ng/dL)
7. Low or absent NK cell activity
8. Increased soluble CD 25 (soluble interleukin-2 receptor) (according to adult level) [20]

antigen specific T cell function for protection of the body from over reaction in the process of inflammation. sIL-2R has inhibitory effect on the response of immune system to pathogens [5]. Generally, in patients with HLH, number of NK cells is normal. However, dysfunctional NK cell activity (deficiency or absence) may be seen in all forms of HLH [6].

Previously published studies including large case series and/or autopsy studies reveal that the etiology of secondary HLH comprises multi-inflammatory processes such as bacterial, viral, fungal, parasitic infections; autoimmune and rheumatologic illnesses [2,7]. HLH is one of the commonly encountered diseases in pediatric age and that is why majority of studies in the field of intensive care about HLH are in pediatric critically-ill patients [8]. Data regarding especially secondary HLH in adults is scarce because we do not have any routine laboratory parameters enabling estimation of HLH in the differential diagnosis. Hereby, our aim was to investigate underlying and/or accompanying possible HLH in patients with severe sepsis who had at least bicytopenia.

2. Materials and methods

2.1. Patients

Patients (>18 years old) hospitalized with severe sepsis in medical intensive care unit (ICU) between June 2011 and September 2012 were prospectively included to study and evaluated retrospectively for HLH diagnosis. This study was approved by Hacettepe University Ethical Committee (Approval date and reference number 12.05.2011, FON 10/25-23). Written informed consents were received from patients or their formal relatives.

Severe sepsis was diagnosed as sepsis associated with tissue hypoperfusion or sepsis-induced organ dysfunction according to Surviving Sepsis Guidelines [10]. Severe sepsis cases who were non-responsive to treatment and who developed at least bicytopenia were recruited. Unresponsiveness to treatment was accepted as continuing hemodynamic and metabolic instability and fever in spite of appropriate treatment with compliance to sepsis protocol according to Surviving Sepsis Guidelines [10]. Hemoglobin level <9 g/dL, absolute neutrophil level <1000 /mm^3 and platelet level <100,000 /mm^3 were considered compatible with cytopenia according to the diagnostic criteria published by Histiocyte Society for HLH (Table 1) [3]. Patients with alternative etiologies for cytopenia (eg, nutritional factor deficiencies, hypersplenism, and hematopoietic disorders), patients with active cancer and those who did not provide informed consent were excluded from the study.

Demographic findings, Acute Physiology and Chronic Health Evaluation (APACHE) II score, admission and last-day Sequential Organ Failure Assessment (SOFA) scores, comorbidities, day of ICU admission, and length of stay (LOS) in ICU and hospital were recorded. Culture results of blood, catheter, urine, bronchoalveolar lavage and the other sites were noted in order to determine source of infection. Laboratory parameters such as complete blood count; triglyceride and fibrinogen levels; international normalized ratio; activated partial thyromboplastin time; peak lactate dehydrogenase; ferritin and viral serology; and load levels for Cytomegalovirus (CMV), Ebstein-Barr virus (EBV), and Parvovirus were tested. During follow-up, bone marrow aspiration and biopsy specimens were obtained in patients with ongoing cytopenias. They were assessed by an experienced hematologist (author YB) and a hematopathologist. In addition, peripheral blood samples were collected from patients and also seven normal controls in order to determine normal ranges according to adult age and stored for later testing for sIL-2R levels and NK activity.

2.2. Laboratory analysis

2.2.1. Measurement of soluble IL-2R level

Five-milliliter blood samples from patients and controls were taken into tubes and serum was separated and stored in −80 °C. Soluble IL-2R level was measured by enzyme-linked immunosorbent assay (ELISA) en masse as described by the manufacturer (Ebioscience, San Diego, CA). The standards in the content of kit were prepared to the wells of the ELISA, and "standard curve" has been created. After addition of secondary antibody, incubation and washing procedures were performed. In order to measure the specific binding, substrate was added and the coloring reaction was stopped at the end of the incubation period. Color change was evaluated with a plate reader (Spectramax Plus, Molecular Devices, USA).

2.2.2. Analysis of cytotoxic activity of NK cells [9]

Five milliliters of heparinized blood from each patient and control group were collected. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque" (density 1.077) (Sigma, St. Louis, CA, USA). After centrifugation for 10 minutes at 700*g* at room temperature, mononuclear cell layer was collected. Following centrifugation for 10 minutes 250*g* at room temperature, cell pellet was resuspended in RPMI (Rosewell Park Medium) containing 10% fetal bovine serum (Biological Industries, Israel), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL; Biochrom, Berlin, Germany). PBMC were frozen through the use of cryopreservation solution containing fetal bovine serum plus 10% dimethyl sulfoxide. After thawing, the PBMC were cultured overnight at 37 °C, 5% CO_2. K562 cell line was cultured in RPMI medium containing 10% fetal bovine serum, L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL). K562 cells were labeled with CFSE (final concentration: 1 μmol/L) (*CellTrace CFSE Cell Proliferation Kit*, *Molecular Probes*; Invitrogen, Carlsbad, CA) and used as target cells. Pre-stained K562 cells were co-cultured with PBMC cells (effector cells) at a ratio of NK cells to target cells of 25:1 for 4 hours at 37°C. After co-culture, dead cells were labeled with 7-AAD (Ebioscience, San Diego, CA). The percentage of 7-AAD positive CFSE-labeled K562 cells were analyzed by flow cytometry. Thus, the cytotoxicity of the effector cells was evaluated according to percentage of dead target cells.

2.2.3. Flow cytometric analysis of $CD3^-CD16^+/56^+$ (NK) cells

$CD3^-/CD16^+CD56^+$ antibody (BD Biosciences, San Jose, CA) was used for labeling of NK cells. After overnight incubation of PBMC as described previously, NK cells were gated according to their forward-light scatter and side-light scatter values in EPICS XL-MCL flow cytometer (Beckman Coulter, Fullerton, CA). The percentage of positive cells was evaluated in comparison to the appropriate isotype-matched antibody controls (IgG1, IgG2a).

2.3. Statistical analysis

Descriptive statistical values of the patients were expressed as number (percentage (%)), median and minimum-maximum (min-max). sIL-2R levels in patients and healthy subjects were compared by Mann-Whitney *U* test. Statistical Packages for the Social Sciences v17.0 (SPSS Inc, Chicago, IL) software was used for statistical analyses. $P < .05$ was considered as statistically significant.

3. Results

Study flowchart is seen in Fig. 1. Among 382 hospitalized patients in the ICU during study period, 75 patients (20%) were followed for severe sepsis and septic shock. Forty of them had at least bicytopenia associated with

Download English Version:

https://daneshyari.com/en/article/2764417

Download Persian Version:

https://daneshyari.com/article/2764417

Daneshyari.com