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Could Monocyte Human Leukocyte Antigen-DR Expression help for Pediatric Sepsis Patients?

Could Monocyte Human Leukocyte Antigen-DR Expression help as a prognostic indicator of Sepsis

Eslam Adel Mohammed¹, Mervat Atfy Mohammed¹, Amal Ahmed Zidan², Marwa Zakaria Mohammed¹

¹ Department of Pediatrics, Faculty of Medicine, Zagazig University, Egypt

² Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt

Corresponding author: Eslam Adel Mohammed

E-mail: Dreslamadel@gmail.com, iabdelgawad@Zu.edu.eg

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Abstract

Background: Fever, capillary leakage, and organ failure are symptoms of the proinflammatory surge that characterizes the initial host immunological response to sepsis in children. On the other hand, innate and adaptive immune cells become hyporesponsive as a result of the concomitant anti-inflammatory response. Serious cases of this reaction, known as immunoparalysis, can lead to organ failure for an extended period of time, an increased likelihood of nosocomial infections, and even mortality in infants and adults with septic shock. Reductions in absolute cell counts, expression of human leukocyte antigen (HLA)-DR on circulating monocytes, and whole blood ex vivo stimulated cytokine production capacity are laboratory indicators of sepsis-induced immune suppression. Immunostimulatory treatments, such granulocyte macrophage colony-stimulating factor (GM-CSF), show potential in patients with sepsis-induced immunoparalysis, in contrast to anti-inflammatory medicines, which have generally failed to improve outcomes from both pediatric and adult sepsis. Improving outcomes for children with septic shock requires more research into immunoparalysis risk factors and the design and implementation of treatment trials targeting specific immunophenotypes to optimize innate and adaptive immune function. The association between low mHLA-DR expression and deleterious outcomes, deserves to be assessed and confirmed in multicenter studies.

Keywords: Pediatric Sepsis Patients, Monocyte Human Leukocyte Antigen-DR

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Introduction:

The hallmark symptoms of sepsis, including fever, rapid heart rate, shortness of breath, leakage of capillaries, and malfunction of organs, are usually caused by the body's inflammatory reaction to an infection. Septic adults have been the focus of multiple treatment trials that have sought to address this aspect of the host response by reducing the proinflammatory surge through the use of anti-inflammatory medications. Researchers began looking into the counter-regulatory immune response to sepsis after most of these experiments failed to improve outcomes. Several researchers in the past 20 years have found that certain patients with sepsis have an excessively strong compensatory anti-inflammatory reaction. This condition, known as "immunoparalysis," has

serious consequences, such as an increased risk of nosocomial infections, chronic organ failure, and even death.

It is possible to improve the local environment for combating infections by localizing the production of proinflammatory cytokines and chemokines to infected tissues. The symptoms of sepsis occur when these mediators enter the bloodstream systemically, though. An innate immune response by the host to the invading pathogen is thus primarily responsible for the pathophysiological aspects of acute sepsis. Leukocytes, including monocytes, dendritic cells, macrophages, and neutrophils, make up the innate immune system. Cell surface receptors that are constitutively expressed allow these cells to recognize and respond to pathogen-associated molecular patterns (PAMP), such lipopolysaccharide (LPS). One of the most well-known examples of this is the toll-like receptor (TLR) family. Many different chemicals expressed by pathogens but not by human cells can be recognized by members of this family of PAMP receptors. When activated, innate immune cells attack pathogens by enzymatic activity or reactive oxygen species after they have been ingested. Class II major histocompatibility complex (MHC) molecules, such human leukocyte antigen (HLA)-DR, are laden with antigenic peptides digested by innate immune cells from pathogens. To the adaptive immune system's lymphocytes, these antigens can subsequently be delivered. Finally, chemokines draw additional leukocytes to the region along concentration gradients, and proinflammatory cytokines modify the local environment to enhance infection clearance. These molecules are produced by activated innate immune cells. It is not necessary to have been exposed to a pathogen before, and the innate immune response ought to be consistent in strength with every encounter.

T cells and B lymphocytes make up the adaptive immune system. In most cases, but not all, these cells need to connect with an innate immune cell that carries an antigen in order to become activated. A stronger and faster immune response is elicited with successive antigen exposures; this response is also highly antigen specific. It is the plasma cells that arise from activated B cells that secrete antibodies. There are two main types of T cells, CD4+ helper T cells and CD8+ cytotoxic T cells, which are distinguished by the expression of specific markers on their cell surfaces. By developing lytic enzymes, cytotoxic T cells facilitate cell death. The cytokines produced by helper T cells regulate and sustain the immunological response. Various subtypes of naïve helper T cells can develop in response to signals received from antigen-presenting cells and the activating cytokine milieu. The proinflammatory cytokines produced by T helper (H)-1 cells include interferon (IFN)- γ and interleukin (IL)-2. T H 2 cells elaborate IL-10, IL-4, and IL-13, which stimulate anti-inflammatory and autoimmune/allergic responses. T regulatory T cells (Treg) produce IL-10, transforming growth factor (TGF)- γ , and directly suppress inflammatory processes through cell-contact-mediated inhibition, whereas IL-17, which generates T H 17 cells, is highly proinflammatory.

The clinical manifestation of the systemic proinflammatory response is the systemic inflammatory response syndrome (SIRS). Sepsis, like most inflammatory diseases, shares the clinical phenomenology of fever, rapid heart rate, shallow breathing, and leaky capillaries. But there's a counter-regulatory system for every big biological route. The proinflammatory response has a "off switch" in the compensatory anti-inflammatory response syndrome (CARS) (Fig. 1). Though it lacks a clinical phenotype, the CARS reaction does exhibit a number of noteworthy physiological features. Initiating innate and adaptive leukocytes down-regulates intracellular signaling and hyporesponsiveness to stimuli within minutes to hours of activation of the proinflammatory response. The purpose of this dormant phase is to control the inflammatory response and, hopefully, prevent cytokine systemic spillover. A second point to consider is that leukocyte cell death can happen either when the cells are initially activated (like neutrophils) or after they undergo apoptosis (like lymphocytes). Finally, IL-10 and other anti-inflammatory mediators can be engineered by innate and adaptive leukocytes that manage to survive. Although short and mild

CARS responses are probably good for the host, severe and protracted responses can become pathogenic. The name used to describe this condition in an ICU context is immunoparalysis, and it is a significant type of acquired immune deficiency.

Immunoparalysis in Sepsis

In experimental settings, the CARS response can be measured, and many methods for diagnosing immunoparalysis in individuals with septic shock have been tried. Monocytes in circulation should display high levels of the antigen-presenting molecule HLA-DR. As part of the CARS response, HLA-DR molecules are internalized, and this can be measured with flow cytometry. While being healthy, more than 90% of monocytes in circulation should be positive for HLA-DR. On the other hand, if the expression level of monocyte HLA-DR (mHLA-DR) is less than 30%, there is an increased risk of nosocomial infection and death.²³⁴ Another approach, also utilizing flow cytometry, permits the quantification of HLA-DR molecules per cell. In investigations conducted on adults, immunoparalysis was defined as having 8,000 molecules per cell or more, with 5,000 molecules per cell or less indicating the highest level of suppression.⁵⁶ One new study hints that the shift in mHLA-DR expression over time might be more significant than absolute thresholds when it comes to unfavorable outcomes from pediatric sepsis, but the exact thresholds remain unclear. In a study involving 30 infants with septic shock, Manzoli et al. discovered that death occurred when mHLA-DR levels did not rise by 1,000 molecules per cell within one week of the start of sepsis.⁷

To find patients whose leukocytes are hyporesponsive, another approach to diagnosing immunoparalysis is to stimulate entire blood outside of the body. The potential of circulating innate immune cells to react to an unfamiliar stimuli is measured by the whole blood ex vivo LPS-induced tumor necrosis factor (TNF)- α production capacity assay. The supernatant is used to assess TNF α levels in this assay, which involves adding whole blood to a tube that already contains standardized LPS stimulation reagent and then incubating it for a specific amount of time. When incubated with LPS, the blood of healthy youngsters should release a significant amount of TNF α . Immunoparalysis is characterized by a marked decrease in the TNF α response, which is prominent in CARS. Using the TNF α response to assess innate immune activity does not provide any special weight to TNF α or the TLR4/CD14 pathway that LPS signals through. Instead, because of TNF α 's favorable kinetics and relative ease of testing, it is employed as a stand-in for the innate immune cell's broader reactivity. A TNF α response below about 200 pg/mL has been linked to higher chances of nosocomial infection, prolonged organ dysfunction, and mortality in both single- and multicenter studies of children with septic shock, according to the most commonly reported method of this kind of testing.^{2 8 9} The TNF α response thresholds linked to negative consequences can differ based on the LPS type, blood volume, incubation circumstances, and outcome in question.

The CARS reaction may potentially have an effect on the adaptive immune system. After sepsis, lymphocytes often die off, leading to symptoms like peripheral lymphopenia and splenic lymphocyte depletion; both conditions have been linked to complications including multiple organ dysfunction syndrome (MODS) and even mortality in septic children and adults.^{ten, eleven, and twelve} Similarly, sepsis-related complications have been linked to a decrease in lymphocyte responsiveness. Using peripheral blood leukocytes, Wong et al. assessed the expression of 100 genes related to adaptive immunity and glucocorticoid receptor activation using mRNA profiling in the early stages of juvenile septic shock.¹³ There were two endotypes, or patterns of gene expression, that they found in the cohort. One had a lot more silenced genes, while the other had a lot more active genes. In children with a more repressed endotype, the risks of mortality and extended MODS were considerably higher. Another indicator of sepsis outcomes is protein-level lymphocyte suppression. Ex vivo stimulation is another method that adaptive immune testing can use to

measure cellular responsiveness. Phytohemagglutinin (PHA) or anti-CD3/anti-CD28 antibodies are suitable lymphocyte stimulants that can be used in place of LPS. Readouts from lymphocyte stimulation in this setting have included multiple cytokines. However, in septic children and adults, a decreased ability to produce IFN γ in response to ex vivo stimulation has been linked to the development of infectious complications, prolonged organ failure, and death.[9, 11, 14]

There is substantial debate among experts as to when the immune system's innate and adaptive systems are suppressed. Previous research on innate immune activity in individuals with septic shock found that samples taken later in the illness's progression (days 3-4) were more indicative of worse outcomes than those taken very early (days 1-2). Although very early samples were sparse in that cohort, the first study of the TNF α response in children with MODS indicated an elevated risk of nosocomial infection and death in participants whose immunoparalysis lasted beyond day 3 of MODS. Early on, within 1 to 2 days after sepsis begins, both single-center and multicenter studies have demonstrated that substantial suppression of the TNF α response and/or lymphocyte responsiveness is associated with an elevated risk of protracted organ failure and death. 8 to 9-As far as reversibility and consequences are concerned, the relevance of early vs late immunoparalysis is unknown.

Globally, septic shock is still a leading cause of death and disability in children, even with improvements in critical care management [1]. When it comes to adults, the fact that supplementary medicines haven't been able to lessen the severity of sepsis suggests that the original theories about the disease's biology were probably wrong [2]. As a result of its powerful systemic inflammatory response and associated anti-inflammatory process, sepsis significantly disrupts immunological homeostasis in adults, according to current consensus. Patients who initially survive sepsis may have reduced resistance to secondary acquired infections due in large part to sepsis-induced immunological changes, which can become dangerous as most immune functions are weakened [3]. Abnormalities in the immune system's innate and adaptive mechanisms define this immunosuppressive condition. Specifically, mHLA-DR expression is reduced and patients typically have significant lymphopenia. Reduced expression of the latter has been linked to higher rates of death and nosocomial infections in adults, and it continues to be the most important criterion for patient monitoring [4-6]. Here, similar to cancer immunotherapy, targeted treatments are being considered for adult septic patients with the goal of reviving their immune responses (e.g., GM-CSF, Interleukin-7, anti-PD-1/-L1) [7-10]. Fewer data exist for children with septic shock compared to adults. There is a lack of information regarding how this changed immune response affects secondary infections in children who have septic shock. A trustworthy biomarker to identify children at greatest risk of secondary acquired illness or mortality is necessary for the design of therapeutic approaches to restore immune function in children. At this time, kids do not have this marking. It is uncommon for pediatric results to be able to pinpoint septic shock. They include a wide range of reasons for admission to the pediatric intensive care unit (PICU), including surgery and organ dysfunctions [11-14], or they are specific to preterm newborns [15, 16].

Patients in critical care may require immunosuppression for an extended period of time, which can raise their risk of secondary infections [1-4]. Between eleven and nineteen percent of children admitted to the pediatric intensive care unit (PICU) get secondary infections while there, which increase the likelihood of complications, length of hospital stay, and financial burden [5-7, 8].

Most investigations have concentrated on adult patients with sepsis and septic shock [3, 15-22], although immunosuppression has been proven in critically ill patients following trauma, surgery, or stroke [9-14].

One useful biomarker for estimating immunosuppression in critically ill patients is monocytic human leukocyte antigen-DR (mHLA-DR) expression. In adults, a number of small studies have

linked a prolonged reduction in mHLA-DR expression to secondary infection acquisition and mortality [13, 19, 20, 23, 24], although one research failed to find a connection [25]. Lower mHLA-DR values were discovered among non-survivors in the largest study that has ever been conducted, which included over 400 adult patients. Nevertheless, the authors came to the conclusion that mHLA-DR expression lacked the discriminative power necessary to be used as a predictive parameter [26].

Limited research has shown that lower mHLA-DR levels are associated with an increased risk of subsequent infection or death in children who are critically unwell after surgery [27], critically ill children for various causes [28], and children with septic shock [16, 18]. Reducing mHLA-DR expression was associated with death in 30 pediatric septic patients, according to Manzoli et al. [18], but not with secondary infections. Critically sick children with a variety of infectious diseases, including those that may have been contracted in the community or while in the hospital, are admitted to our pediatric intensive care unit. Better understanding of the relationship between mHLA-DR expression and mortality and secondary infections in these kids should help doctors choose patients who would respond well to immunosuppressant drugs.

Multiple therapies aimed at reducing inflammation and sepsis-related mortality were developed in the 1980s and 1990s, but the studies failed to demonstrate a survival benefit [4], [5], [6]. Also in the 1980s, other studies suggested that late mortality from surgery, sepsis, or trauma in the ICU may be associated with an acquired immune deficiency state, which was later termed immunoparalysis [7], [8]. Thus, immunoparalysis is characterized by a persistent and markedly compensatory anti-inflammatory response leading to impaired innate immune function [8].

Prolonged periods of reduced monocytic human leukocyte antigen-DR (mHLA-DR) expression correlates with poor prognosis in sepsis. In fact, an HLA-DR expression level of < 30% was associated with a 12% survival rate in adult surgical patients, whereas a survival rate of 88% was observed in patients with mHLA-DR expression > 30% [9], [10].

Despite the significant reduction in mortality from sepsis with the use of early goal-directed therapy [11], [12] and the development of new cardiopulmonary support technologies, the incidence of sepsis in intensive care units has increased in the last decade and mortality rates from severe sepsis are still high [13], [14].

Novel therapeutic and monitoring approaches have the purpose of reducing mortality from sepsis modulation of the immune system and have shown promising results so far [15], [16].

The quantification of mHLA-DR expression has been used as a diagnostic tool for immunoparalysis, but multicenter comparisons are lacking because methods are not reproducible across centers. The Becton Dickinson (BD) QuantiBRITE Anti HLA-DR/Anti-Monocyte reagent is a standardized flow cytometric tool that addresses this issue [17], enabling the development of reliable protocols for identifying immunoparalysis and the potential implementation of therapeutic measures to reverse this pathological state of immunosuppression and reduce mortality in septic patients.

Conclusion

The immune response to sepsis is highly dynamic and has both pro- and anti-inflammatory components. The presence of high levels of systemic inflammation, with accompanying symptoms of SIRS, does not rule out the presence of a compensatory anti-inflammatory response which may in itself be pathologic. Early attempts at immunomodulation in sepsis were likely flawed due to their singular focus on reduction of the proinflammatory response without measuring resultant effects on immune cell function. As in adults, septic shock in pediatric patients induced marked alterations in immune parameters in accordance with the occurrence of a state of

immunosuppression. The association between low mHLA-DR expression and deleterious outcomes, deserves to be assessed and confirmed in multicenter studies.

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