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Prolonged suppression of monocytic human leukocyte antigen–DR expression correlates with mortality in pediatric septic patients in a pediatric tertiary Intensive Care Unit☆,☆☆,★

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ABSTRACT

Introduction: Immunoparalysis is a syndrome with no clinical symptoms that occurs in some septic patients. Monocytic human leukocyte antigen–DR (mHLA-DR) expression has been used to identify patients in immunoparalysis and prolonged periods of reduced mHLA-DR expression have been correlated with a poor prognosis in sepsis. However, there is a lack of studies investigating mHLA-DR expression in pediatric septic patients.
Aim: To determine if mHLA-DR expression correlates with mortality in pediatric septic patients using the QuantiBRITE Anti HLA-DR/Anti-Monocyte,a Bechton Dickinson novel reagent that standardizes flow cytometry values.
Methods: We determined mHLA-DR expression in 30 patients with severe sepsis or septic shock admitted to the pediatric intensive care unit at Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, between January 2013 and February 2015. mHLA-DR expression was quantified between days 3 to 5 and 5 to 7 after the onset of sepsis and the ΔmHLA-DR (mHLA-DR2 − mHLA-DR1) was calculated. We also measured mHLA-DR levels in 21 healthy control patients.
Results: Mean mHLA-DR expression was significantly lower in septic patients than in controls ($P = .0001$). Mortality was 46% in patients with negative ΔHLA-DR or <1000 mAb/cell and 7% in patients with positive ΔHLA-DR or >1000 mAb/cell. Mean ΔmHLA-DR levels were significantly different between survivors and non-survivors ($P = .023$).
Conclusion: ΔHLA-DR correlates with mortality in pediatric patients with septic shock or severe sepsis. This is the first study to have used the QuantiBRITE Anti HLA-DR/Anti-Monocyte reagent to quantify monocyte HLA-DR expression in pediatric septic patients.

1. Introduction

The release of pro-inflammatory mediators in severe cases of sepsis leads to an inflammatory state that is termed systemic inflammatory response syndrome [1–3], which is followed by a compensatory anti-inflammatory response. Thus, proper immunologic balance between pro- and anti-inflammatory responses is necessary in recovery from sepsis.

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 [4–6]. 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].

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Table 1
Clinical characteristics of septic patients admitted to the PICU at HCFMUSP, São Paulo, Brazil, between January 2013 and February 2015 who were included in the study

	Survivors (n = 23)	Non survivors (n = 7)
Gender	65% boys 35% girls	57% boys 43% girls
Source of infection	43% pulmonary 21% hematologic 17,3% abdominal 13% skin 4% urinary	57% pulmonary 14% hematologic 14% abdominal 14% urinary
Comorbidities	30% neurologic 26% gastrointestinal tract alterations 26% onco-hematologic 4,3% immunologic 13,7% other	42% neurologic 14% gastrointestinal tract alterations 14% immunologic 14% pulmonary 14% metabolic

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.

In this study, we quantified mHLA-DR expression in patients with severe sepsis or septic shock admitted to a tertiary pediatric intensive care unit (PICU). Using the QuantiBRITE Anti HLA-DR/Anti- Monocyte reagent, we aimed to determine the mHLA-DR expression profile and the incidence of immunoparalysis in pediatric septic patients, the association between mHLA-DR expression and mortality, and the correlation between mHLA-DR expression and predictors of favorable outcome (mechanical ventilation-free days and vasoactive agent-free days).

2. Methods

In this prospective cohort study, we quantified mHLA-DR expression and determined the incidence of immunoparalysis in patients with severe sepsis or septic shock (as defined in the 2005 International Consensus Conference on Pediatric Sepsis [2]) who were admitted to the PICU at Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, between January 2013 and February 2015.

The study was approved by the research ethics committee at Hospital das Clinicas da Faculdade de Medicina da USP and patients were enrolled after their parents or legal guardians signed an informed consent form.

All patients admitted to the PICU during the study period with a diagnosis of severe sepsis or septic shock were included in the study. Patients with septic shock who died less than 48 hours after admission to the PICU, patients with end stage hepatic failure, patients in palliative care, patients suspected of brain death, patients with severe leukopenia (due to the technical difficulty of isolating monocytes from these samples), and patients who had already participated in the study during their hospital stay were not included in the study.

Patients were followed up for 45 days and assigned to two groups: "non-survivors" and "survivors" (patients who were discharged and patients who survived for 45 days of hospitalization but who remained hospitalized at HCFMUSP). We established a 45-day length of stay (LOS) because some patients remained in the PICU for longer periods even after recovery from sepsis.

Blood was collected between days 3 and 5 after diagnosis of severe sepsis or septic shock, and another sample was taken 2 to 4 days after the first one. Samples were stored in EDTA tubes at 4°C and analyzed within 2 to 8 hours of collection, which did not significantly affect HLA-DR expression according to some authors [18]. In addition, data from patients' medical records were also obtained.

Blood samples were also taken from 21 control patients between five months and 16 years of age with no known diagnosis of immunodeficiency or active infection at the time of collection.

The expression of mHLA-DR was compared between septic patients and controls and among septic patients (survivors versus non-survivors and secondary infection versus no infection) using the Mann-Whitney *U* test with a significance level of 5% ($P < .05$).

The variation in mHLA-DR expression from the first to the second blood sample (ΔmHLA-DR) was also compared. We calculated the difference in mHLA-DR expression between the second and first blood sample (mHLA-DR2 − mHLA-DR1) for each patient and compared the ΔmHLA-DR between survivors versus non-survivors and secondary infection versus no infection using the Mann-Whitney *U* test ($P < .05$).

Assuming an alpha error of 5%, a power of 80%, an exposure prevalence of 30%, an 80% mortality in the immunoparalysis group, and a 15% mortality in the group without immunoparalysis, the sample required for this trial was 25 patients. In our study, 30 patients with severe sepsis or septic shock were included.

The correlations between the variables evaluated were determined using the Spearman's rank correlation coefficient. Specifically, the correlation of mHLA-DR and ΔmHLA-DR expression with mortality risk assessment scores upon PICU admission (Pediatric Index of Mortality 2 [PIM-2] [19] and Pediatric Risk of Mortality [PRISM] [20]), and secondary endpoints (mechanical ventilation- and vasoactive drug-free days).

The method used was the Mann-Whitney *U* test ($P < .05$) to evaluate the association between mortality and the following secondary end points: mortality risk assessment scores (PIM/PRISM), age, and LOS in the PICU.

A receiver operating characteristic (ROC) curve was constructed to determine the best method to predict mortality between ΔmHLA-DR, mHLA-DR in the first blood sample (mHLA-DR d3_5), and mHLA-DR in the second blood sample (mHLA-DR d5_7).

The mHLA-DR expression was quantified by flow cytometry using a FACSCalibur flow cytometer and the QuantiBRITE Anti HLA-DR/Anti-

Table 2
Clinical and laboratorial characteristics of septic patients admitted to the PICU at HCFMUSP, São Paulo, Brazil, between January 2013 and February 2015 who were included in the study

	Survivors (n = 23)	Nonsurvivors (n = 7)	*P* (Mann-Whitney)
Median age (months)	99 (IQR 69.5)	28.5 (IQR 51.9)	0.053
Median PIM 2	5.9 (IQR 19.2)	26.8 (IQR 64.9)	0.078
Median PRISM	5.3 (IQR 11.2)	15.9 (IQR 51.3)	0.48
Median total leucocytes d3-5	10600 (IQR 7375)	13900 (IQR 8042)	0.27
Median total leucocytes d5-7	10845 (IQR 5858)	13500 (IQR 7657)	0.525
Median total lymphocytes d3-5	1604 (IQR 3209)	1580 (IQR 1760)	0.75
Median total lymphocytes d5-7	1846 (IQR 2281)	2660 (IQR 4044)	0.698
Median delta lymphocytes	436 (IQR 1969)	−326 (IQR 3086)	0.782

*IQR = interquartile range.