538

HUMAN NEUTROPHIL GELATINASE-ASSOCIATED PROTEIN (N-GAL) AND PROINFLAMMATORY CYTOKINES AS BIOMARKERS OF DISEASE SEVERITY IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME (HUS) AND SHIGATOXIN-PRODUCING E.COLI (STEC) INFECTION

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BACKGROUND

Hemolytic Uremic Syndrome (HUS) is a complication of Shigatoxin producing *E.coli* (STEC) infection which presents a characteristic triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure.

Preliminary *in vitro* and experimental animal studies demonstrated that Shigatoxins (STXs) induce the secretion of proinflammatory cytokines.

Human neutrophil gelatinase-associated protein (N-gal) has been reported as a marker of acute kidney injury.

OBJECTIVES

- To dose serum levels of IL-8, TNF-α, IL-6, IL-1β and N-gal in children with STEC-associated infection and HUS in order to determine the role of these cytokines as biomarkers of renal injury and severity.
- To establish the proinflammatory profile related to HUS.

METHODS

Prospective study between 2017 and 2020 was performed.

Three groups of patients < 18 years were included: bloody diarrhea (BD), HUS requiring dialysis (HUSD) and HUS with no dialysis requirement (HUSND), all of them with presence of STX in stool.

Blood samples were collected at diagnosis (T1) and at 7-10 days (T2).

An immunoassay was used for detection of TNF-α, IL-1β, IL-6, and IL-8 (Bio-Plex Pro Human®, BioRad). An immunoassay (Anti-Lipocalin-2-ABCAM) was used for N-gal detection.

Data were analysed using the χ2 test for categorical variables. The Kruskal–Wallis test and Dunnett multiple comparison post-test were used to evaluate differences of continuous variables among multiple groups. A p value <0.05 was considered statistically significant for all the tests performed. Statistical analyses were performed using Stata 13 software (StataCorp LP, College Station, TX) and graphics were made with GraphPad Prism 8.0.

Principal component analysis (PCA) was performed with R Statistical Language version 4.1.0.

RESULTS

- We collected serum samples from 38 children in our study. We included 14 children, who presented bloody diarrhea and STXs in their faeces. In addition, we included 24 children with STEC associated HUS,12 of whom required dialysis (HUSD) and 12 did not (HUSND). Moreover, we included 11 healthy children's serum samples (HC). Demographic and clinical characteristics are described in Table 1.

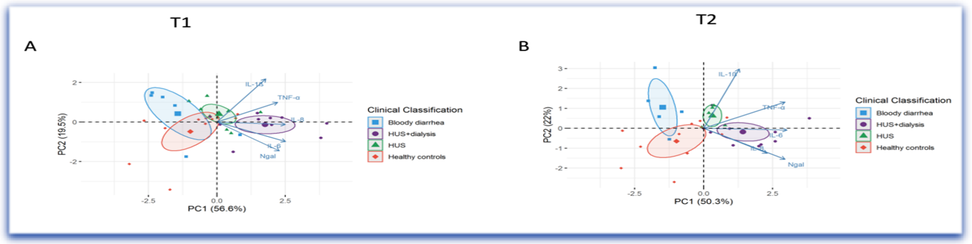
Table 1. Demographic and Clinical Characteristics of the Pediatric Population

Demographic and clinical characteristics	Total (n=38)	Bloody diarrhea (n=14)	HUS with dialysis (n=12)	HUS without dialysis (n=12)	*P*[a]
Age in months median (IQR)	24 (20-40)	31 (24-57)	21 (19-32)	25 (20-43)	0.31
Age in months range	9-168	10-168	9-41	9-120	-
Female n (%)	21 (55.2)	7 (50)	10 (83.3)	4 (33.3)	0.04
Dialysis days median (IQR)	-	-	8(5-11)	-	-

QR: interquartile range; [a] *P*-values were calculated using the Kruskal-Wallis test for non-categorical variables and Chi-square test for categorical variables.

- We elaborated a principal component analysis for 5 markers (IL-1 β, IL-8, IL-6, TNF-β and N-gal) at T1 and T2 among the 4 study groups (BD, HUSD, HUSND and HC), to determine cytokine profile related to HUS associated STEC (Fig.1). Tumor necrosis factor-alpha, N-gal y IL-8 were mostly associated with PC1 and IL-1 β presented the greatest contribution to PC2 (Fig.1 A). At T1 a separation among the study groups was evidenced in PC1. In contrast, no separation was observed in PC2 (Fig.1 A). Along PC1 at T1, we observed a significant differentiation between HUSD and the other groups. At T2 we observed a separation among the study groups in PC1 such as the one observed at T1. However, unlike T1, a differentiation began to occur in axis PC2. This axis was more strongly associated with IL-1beta.

Figure 1 . Principal component analysis according Clinical Outcome



- We elaborated a univariate analysis for each cytokine tested (IL-1 β, IL-8, IL-6, TNF-α) among the 4 patient study groups. We analysed pediatric patients (BD, HUSD and HUSND) and healthy children's serum samples at T1 and T2 (Fig. 2). At T1 increased levels of IL-8, IL-6 and TNF- α were identified in HUSD patients compared to BD patients and healthy children (Fig. 2 A, C and E). In contrast, we observed an increase only in IL-8 levels from HUSD patients as compared to those from HUSND patients.
- At T2 an increase in IL-8, IL-6 and TNF- α levels was detected in HUSD compared to BD and healthy children. However, no increase was evidenced in cytokine levels as compared to HUSND patients (Fig.2B, D and F). When we analysed the 4 study markers between T1 and T2, we observed a decrease in IL-8 levels only in HUSD patients at T2.
- At both T1 and T2 we evidenced an increase in N-gal levels from HUSD patients compared to those from BD, HUSND and HC (Fig.3 A and B). We did not find differences in N-gal levels between T1 and T2 in the study group patients.

Figure 2 . Cytokine levels according Clinical Outcome

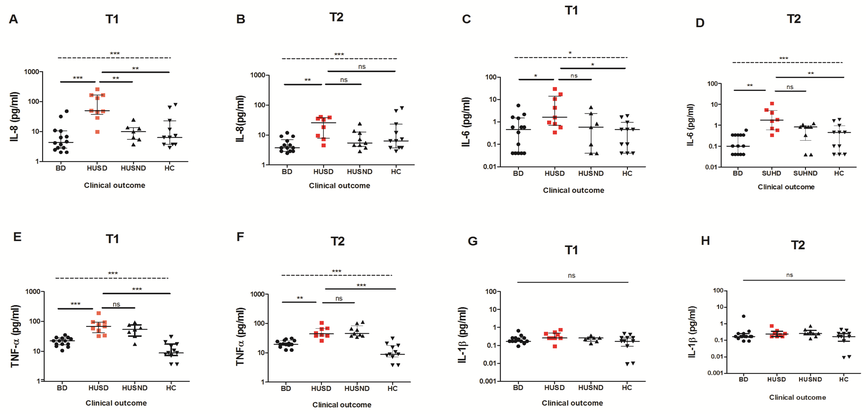
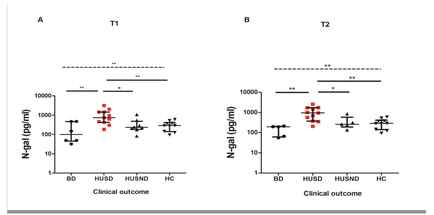


Figure 3 . N-gal levels according Clinical Outcome



- **IN CONCLUSION**, we observed higher serum IL-8 levels in HUSD patients than HUSND only at T1. In addition, we evidenced lower serum IL-8 levels in HUSD patients at T2 than at T1. We observed higher N-gal values in HUSD patients than HUSND at T1 and T2.
- Interleukin 8 and N-gal may be considered as potential serological markers of renal damage and dialysis requirement.
- Children with HUSD exhibited an immune profile different from that of HUSND, BD and HC.