Association of traumatic brain injury with extracranial organ dysfunction and systemic inflammation: a potential cause of subclinical acute kidney injury in transplant donors

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OBJECTIVES AND AIM

Extracranial organ dysfunction (EOD) is a typical feature of critically ill patients with traumatic brain injury (TBI). TBI patients are potential candidates for organ donation. EOD is associated with worse outcome and with systemic inflammation. Previous studies demonstrated that circulating inflammatory mediators may induce renal tubular epithelial cells (TEC) dysfunction and apoptosis, typical features of subclinical acute kidney injury (AKI). Development of TEC damage in donors may enhance the incidence of delayed graft function (DGF) early after transplantation. The aim of this study was to evaluate subclinical AKI in TBI studying the effects of patients' plasma on TEC.

METHODS

We enrolled 23 TBI patients recording APACHE II and RIFLE. Plasma and urine samples were drawn to evaluate plasma levels of inflammatory cytokines (IL-1beta, IL-6, IL-8, TNF-alpha, TNF-RI, TNF-RII), Neutrophil Gelatinase-Associated Lipocalin (pNGAL) and urine immunoelectrophoresis for Retinol Binding Protein (RBP) and alpha1-Microglobulin (α 1-M).
In vitro, we studied the effects of patients' plasma on TEC evaluating:

- neutrophil adhesion;
- cell polarity (trans-epithelial electrical resistance-TEER);
- apoptosis (TUNEL assay);
- gene and protein expression of NGAL, ZO-1 and megalin.

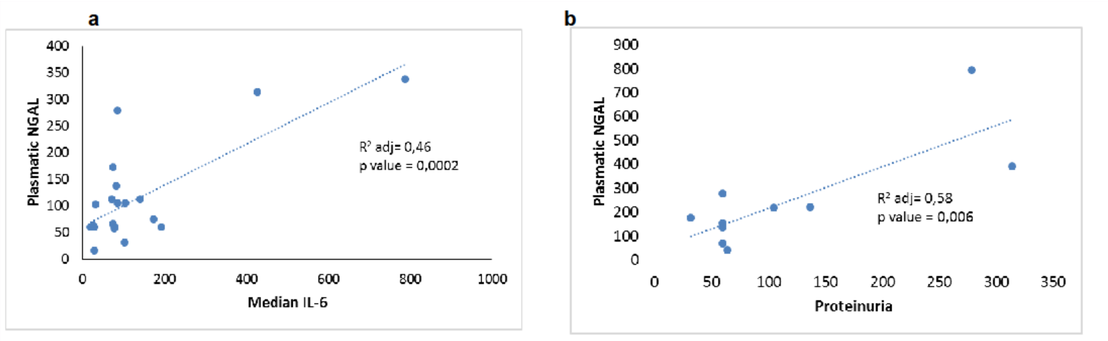

Figure 1: (a) Correlation between Median IL-6 and plasmatic NGAL (n=23). **(b)** Correlation between proteinuria and plasmatic NGAL (n=8).

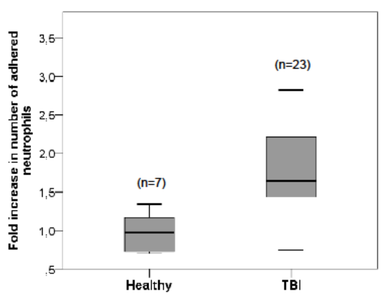

Figure 2: Box plot representing neutrophil adhesion on PTEC incubated with 10% of TBI patients' plasma compared to control group . Median and extreme values within each group are shown. Comparison between groups was made by unpaired t test (*p=0,001).

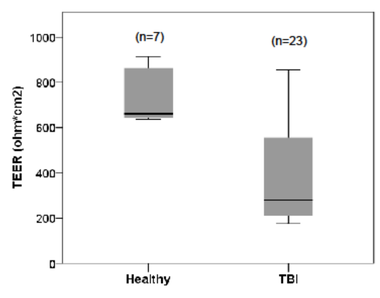

Figure 3: Change of cell polarity induced by TBI plasma stimulation for 24h on PTEC. Median and extreme values within each group are shown. Comparison between groups was made by test U Mann-Whitney, unpaired. (*p=0.003)

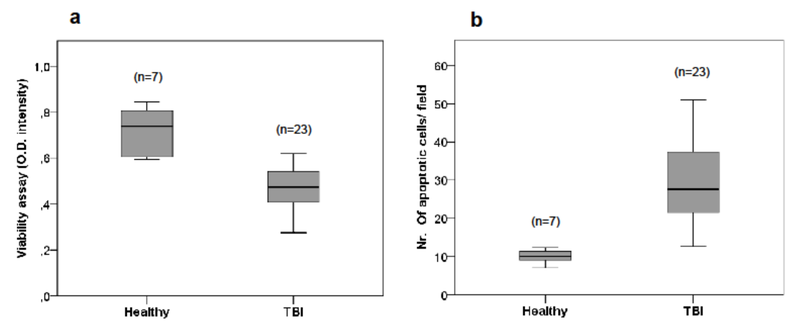

Figure 4: Effect of PTEC stimulation for 24h with TBI plasma on cell viability **(a)** and apoptosis **(b)**
Comparison between groups was made by unpaired t test (*p=0,0001).

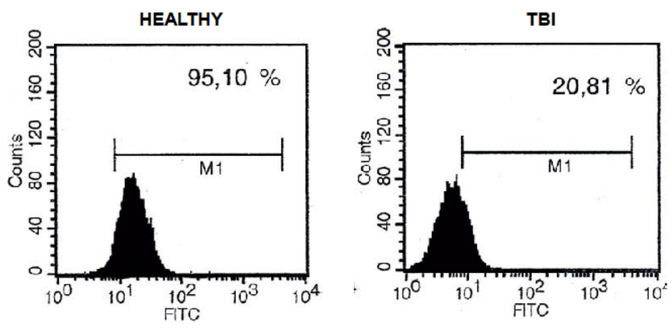

Figure 5: Representative FACS analysis of ZO-1 expression on PTEC incubated with healthy or TBI plasma.

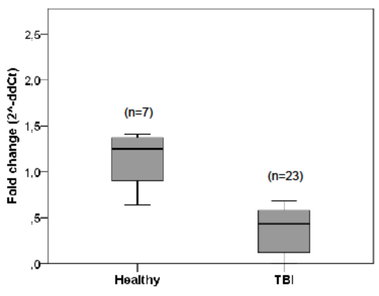

Figure 6: qRT-PCR analysis of Megalin transcript level in PTEC normalized to GAPDH expression. Comparison between groups was made by test U Mann-Whitney, unpaired. (*p=0.008)

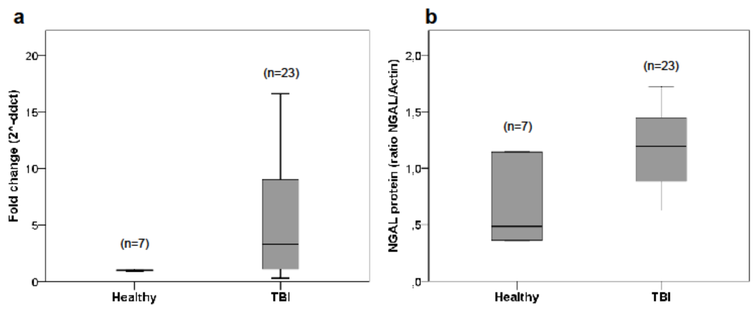

Figure 7 : (a) qRT-PCR analysis of NGAL transcript level (median and extreme values). Comparison between groups was made by KRUSKAL-WALLIS (*p<0,05). **(b)** box plot of the NGAL/Actin expression ratios in stimulated PTEC. Data were analyzed using a U test Mann-Whitney, unpaired. (*p=0,009)

RESULTS

TBI patients' characteristics were: age 40±19 years, GCS 5±3, APACHE II 18±13, elevated levels of inflammatory cytokines, in particular IL-6 was 160 pg/ml (20-789).
A significant correlation between IL-6 and APACHE II (p=0.032), duration of endocranic hypertension (p <0.0001) or of hypoxia (p=0.042) and between TNF-RII and duration of endocranic hypertension (p =0.045) or of hypoxia (p=0.013) was found (not shown). A substantial correlation was observed between plasmatic NGAL and IL-6 (p=0.0002) **(Fig.1a)**, urine protein/creatinine ratio (p=0.006) **(Fig.1b)**, and the low molecular weight proteins α1-M (p=0.02) and RBP (p=0.02) (not shown). Only one TBI patient was included in the classical RIFLE criteria for AKI.
A significant increase of pNGAL levels (199±84 ng/ml) and a 5-to-7-fold increase of urine RBP and α1-M was observed. TBI plasma increased neutrophil adhesion to TEC **(Fig.2)**, altered cell polarity **(Fig.3)**, and triggered apoptosis **(Fig.4)**.
TBI plasma-induced TEC dysfunction was confirmed by down-regulation of the tight junction protein ZO-1 **(Fig.5)**, and the endocytic receptor megalin **(Fig.6)**, and by the increase of gene and protein NGAL expression. **(Fig.7)**.

CONCLUSIONS

In TBI patients that are potential candidates for organ donation, the severity of primary insult (APACHE II) and the duration of secondary insult (endocranic hypertension, hypoxia) lead to systemic inflammation.
Our *in vitro* data on TEC sustained that circulating inflammatory mediators are responsible for TEC dysfunction and apoptosis.
Moreover, plasma levels of IL-6 and NGAL may be accurate biomarkers of subclinical AKI that may increase DGF incidence after kidney transplantation.

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