The association between hypoalbuminemia and microcirculation, endothelium and glycocalyx disorders in children with sepsis.

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Abstract

Background

Endothelial inflammation and activation in sepsis can alter albumin synthesis and increase its loss. In these patients, hypoalbuminemia tends to be associated with worse outcomes. The consequences of hypoalbuminemia for the microcirculation of children with sepsis are unknown. We evaluated the association between hypoalbuminemia and microcirculation disorders, endothelial activation and glycocalyx degradation in this group of patients.

Methods

This was an observational, analytical, prospective cohort study in children with sepsis hospitalized in the pediatric intensive care unit (PICU). The primary outcome was the association between hypoalbuminemia and microcirculation disorders using a perfused boundary region (PBR) > 2.0 µm on sublingual video microscopy or plasma biomarkers (syndecan-1, angiopoietin-2). The secondary outcomes were the association between hypoalbuminemia, microcirculation disorders, the inflammatory response and the need for mechanical ventilation.

Results

Out of the 1,180 admissions to the PICU during the study period, 125 patients with sepsis were included. The median age was 2.0 years (IQR 0.5–12.5), and the main focus of infection was respiratory. Children with hypoalbuminemia had more abnormal microcirculation with a higher PBR flow corrected (2.16 µm [IQR 2.03–2.47] vs. 1.92 [1.76–2.28]; p = 0.01) and more 4–6 µm capillaries recruited (60% vs. 40%; p = 0.04). The low albumin group that had the worst PBR had the most 4–6 µm capillaries recruited (rho 0.29; p < 0.01), 48% higher Ang-2 (p = 0.04), worse annexin A5 (p = 0.03) and no syndecan-1 abnormalities (p = 0.21). Children with hypoalbuminemia and a greater percentage of blood volume in their capillaries needed mechanical ventilation more often (56.3% vs. 43.7%; aOR 2.01 95% CI 1.38–3.10 ;p < 0.01). Children with corrected hypoalbuminemia had improved PBR levels (aOR: 0.17: p5% CI 0.04–0.75; p = 0.02), shorter PICU stays (p = 0.01) and lower mortality (p = 0.02).

Conclusions

In children with sepsis, an association was found between hypoalbuminemia and microcirculation changes, vascular permeability and greater endothelial glycocalyx degradation. Hypoalbuminemia correction was associated with a recovered glycocalyx, shorter hospital stay and lower mortality.

Introduction
Sepsis is a clinical syndrome characterized by potentially fatal organ dysfunction secondary to a
dysregulated host response to infection [1]. Despite advances in diagnosis and early recognition, it still
has a high mortality rate, especially in middle and low-income countries [2]. It is common for multiple
organ failure to be the final common pathway to death for the majority of these patients. The
cardiovascular system is one of the most commonly involved organs both in macro and microcirculation.
Damage to the microcirculation in infectious diseases like sepsis is characterized by endothelial
activation and glycocalyx degradation associated with a major inflammatory response [3–6].

The glycocalyx is a negatively charged carbohydrate-rich layer covering all endothelial cells. It is made up
of proteoglycans, glycosaminoglycans and glycoproteins. It was first described by Luft et al. in 1966
using special stains that allowed it to be seen on electron microscopy [7, 8]. It is responsible for
maintaining vascular permeability and mechanotransduction and for modulating the inflammatory
response, as well as sustaining an endothelial “antiadherent” phenotype. Its components can be
measured with plasma biomarkers (syndecan-1, endocan) or sublingual dark field video microscopy
(Handheld Vital Microscopes - HVM) [8]. With the devices available today, the glycocalyx can be assessed
indirectly in adults and children with sepsis with a high inter and intra-observer correlation index and
good reproducibility in emergency room and intensive care settings [5, 9, 10]. Identifying endothelial
glycocalyx injury in patients with sepsis can be useful and may be related to unsatisfactory outcomes
like respiratory failure, organ failure and death [10–12].

In addition, endothelial inflammation and dysfunction in these patients may decrease albumin synthesis
and increase its loss. There are several consequences of this hypoalbuminemia including abnormal
transport of hormones and other substances, abnormalities in the ability to regulate osmotic pressure, or
diminished antioxidant activity. The level of serum albumin has been associated with outcomes in
critically ill patients and those with sepsis [13–17]. Due to its negative charge, albumin is electrostatically
repelled by the glycocalyx, which helps keep it within the intravascular space. However, in disease states,
albumin moves to the interstitial space, and it is unclear whether it can alter capillary Starling forces. The
current theories mention that the sub-glycocalyx (protein-free) space is one of the most important
determinants in the fluid balance between the intravascular and interstitial space [5, 18]. Animal models
have shown that when albumin is completely eliminated from the intravascular space, 100% of the
endothelial glycocalyx is degraded [19–22]. The consequences of hypoalbuminemia for the endothelium
and endothelial glycocalyx in children with sepsis are unknown. Our hypothesis is that low albumin levels
alter the microcirculation and foster glycocalyx degradation and, therefore, the inflammatory response,
which may lead to worse clinical outcomes. The objective of our study was to evaluate the association
between serum albumin levels and microcirculation changes, glycocalyx degradation and the clinical
outcomes of interest.

Methods

Study design and context
An observational, analytical, prospective cohort study was carried out in children hospitalized in the PICU of Fundación Cardio-Infantil in Bogotá, Colombia, between January 2021 and June 2022. This study was approved by the hospital’s ethics and research committees (CEIC-0366-0022) and all the parents or guardians signed informed consent prior to being included in the protocol. This study was carried out according to the protocol and the International Conference on Harmonization Good Clinical Practice Guideline.

All children from one month to 18 years old with sepsis or septic shock who were admitted to the PICU due to clinical deterioration and on whom sublingual video microscopy and albumin serum levels were conducted within six hours of admission were included. Patients who had received 5% albumin boluses for fluid resuscitation within the 24 hours prior to admission to the study; those with hyperglycemia, diabetic ketoacidosis, head trauma, or continuous renal support therapy; those in the postoperative period following cardiovascular surgery and those with a history of chronic kidney disease (defined as a glomerular filtration rate less than 60 mL/min per 1.73 m² for more than three months) were excluded.

All children with a clinical syndrome characterized by potentially fatal organ dysfunction caused by a dysregulated host response to infection were considered to have sepsis. Septic shock was defined as sepsis with particularly severe circulatory, cellular and metabolic abnormalities, according to the recently recommended definitions [2]. Hypoalbuminemia was defined as serum albumin less than 3.0 gr/dL [13, 17]. Children with low albumin during their PICU stay received replacement with 20% albumin at a dose of 1–2 gm/kg/day at the discretion of the attending physician. The severity of all patients was evaluated using the PIM-2 scale. Acute kidney injury was defined as abnormal creatinine for the patient's height, according to the Schwartz formula. Hyperchloremia was defined as serum chloride greater than 110 mEq/L, and hypernatremia as serum sodium greater than 150 mEq/L. Semiquantitative elevated procalcitonin (PCT) was defined as greater than 2.0 gm/dL, elevated C-reactive protein (CRP) as 4 mg/dL, elevated ferritin as greater than 500 mg/dL, and abnormal D-dimer as more than 1.5 mg/L.

**Microcirculation, Endothelial Activation And Glycocalyx Degradation Measurement**

The definition of microcirculation used was the one recommended in the consensus on sublingual microcirculation [10]. Microvessels were defined as vessels with a diameter < 20 µm, including arterioles, capillaries, and venules. Capillaries were defined as vessels < 10 µm in diameter in which a single file of red blood cells (RBCs) can be observed. The main characteristics of venules are that they are vessels that collect blood from other vessels, and they have more RBCs in the lumen than the single-file RBCs seen in capillaries. The microcirculation and endothelial glycocalyx degradation were assessed in vivo using dark field video microscopy (Glycocheck System ® - Microvascular Health Solutions Inc 2014, Salt Lake City, UT, USA) within six hours of admission to intensive care. The measurement was repeated 24 hours after being included in the study. This device measures sublingual microcirculation by evaluating 4 to 25 µm diameter vessels, using a dark field camera (CapiScope, HVCS, KK Technology United Kingdom) which...
emits stroboscopic green light diodes which detect RBCs by reflection. The machine amplifies the image 325 times with 720 pixel resolution and establishes 23 frames per second. The software (Glycocheck System®) analyzes the measurements from high-quality images (in terms of movement, intensity and focus). In order to do this, it defines 10 µm vascular segments and records 40 frames (300 green segments, which are the ones with complete measurement). The operator would move the camera to five or ten different positions and could take up to 3,000 vascular segments. The measurements were analyzed by the machine's software independently of the examiner and the investigators. This system analyzes the data and reports what has been termed the perfused boundary region (PBR) in µm, which is inversely related to the endothelial glycocalyx dimensions. In healthy individuals, the normal PBR is considered to be less than 2.0 µm [9]. In addition, the video microscope measures the percentage of capillary blood volume (PPV -proportion of perfused blood vessels over the total number of vessels) and the capillary density of 4–6 µm vessels (CD 4-6s).

Angiopoietin-2 (Ang-2 / Human Angiopoietin 2 ELISA Kit ANG 2; ab99971, Abcam Lab, Cambridge, United Kingdom) was used as the biomarker for endothelial activation and increased vascular permeability. Plasma syndecan-1 (Human Syndecan-1 ELISA Kit CD138; ab46506; Abcam Lab, Cambridge, United Kingdom) was processed as the endothelial glycocalyx degradation biomarker, and a level under 80 mg/dL was considered normal [12]. Annexin A5 (ab119503 – Annexin V Human ELISA Kit: Abcam Lab, Cambridge, United Kingdom) was measured to evaluate cell death. All biomarker measurements were done in duplicate according to the manufacturer's instructions, simultaneously and within six hours of admission to intensive care. The samples were 100 µl of citrated plasma which were centrifuged for 30 minutes at 1,000 rpm, and the samples were stored at (-) 20°C for later processing using the enzyme-linked immunosorbent assay (ELISA) method in the immunology laboratory at Fundación Cardioinfantil-IC, Bogotá.

Demographic analysis variables along with clinical data on macrocirculation perfusion (heart rate, arterial pressure, pulse pressure) and microcirculation perfusion (capillary refill) were gathered on admission to the PICU and 24 hours later. In addition, the need for vasoactive support and the severity of the disease according to the Pediatric Index of Mortality – 2 (PIM-2) and Pediatric Logistic Organ Dysfunction-2 (PELOD-2) were also gathered within six hours of admission. Laboratory tests were conducted on admission and 24 hours later, including serum albumin, electrolytes, serum lactate, creatinine, D-dimer and inflammatory biomarkers (ferritin, C-reactive protein and procalcitonin).

All these biomarkers were processed according to the standard processes in the hospital’s main laboratory within 60 minutes of being drawn. Arterial blood was obtained from an arterial line (radial or femoral) and venous blood was drawn through a central venous catheter located in the subclavian or internal jugular vein, at the cavoatrial junction, and the tests were performed at all the study times (Rapidlab 1265, 15630 series/Siemens 2010 ® gas analyzer).

Outcomes
The primary outcome was the association between hypoalbuminemia and microcirculation changes, endothelial activation and glycocalyx degradation measured with sublingual video microscopy and plasma biomarkers (Ang-2, syndecan-1). The secondary outcomes were the association between microcirculation abnormalities in patients with hypoalbuminemia and the presence of an inflammatory response or need for mechanical ventilation.

**Statistical Methods**

Descriptive statistics were derived reporting each cohort with or without hypoalbuminemia. The categorical variables were reported as proportions and compared using Pearson’s Chi² or Fisher’s exact test according to the count per cell. For continuous variables, data were reported as means or medians according to their distribution, with their associated measures of dispersion. A bivariate analysis was performed according to the variable’s distribution using Student’s t-test for equal variances in the case of two variables. The Wilcoxon test was used to evaluate the differences in non-normally distributed biomarkers from dependent groups. Confounding factors were controlled for in the design, using the exclusion criteria to restrict patients who might have other reasons to explain the endothelial glycocalyx damage previously described in the literature, like ketoacidosis or trauma [5, 8, 11]. Confounding (especially disease severity assessed by the PIM-2 scale, malnutrition, the use of vasoactive medications and the use of 20% albumin infusions) was also controlled for in the statistical analysis plan by carrying out multivariate analysis using logistic regression. The variables which fulfilled the Hosmer-Lemeshow criteria on bivariate analysis and which had biological plausibility were included in the model. The model was constructed using the forward method and was adjusted with the Omnibus test. Two-sided analyses were performed with a p value less than or equal to 0.05 considered to be statistically significant.

Analysis was performed using SPSS (IBM® version 26 statistical package). Confounding factors were controlled for in the design, using the exclusion criteria to restrict patients who might have other reasons to explain el daño del glicocálix endothelial previously described in the literature as ketoacidosis or trauma [5, 8, 11].

**Results**

During the study period, 125 patients with sepsis or septic shock were included (Table 1). The median age was two years (IQR 0.5–12.5). The participants were similarly distributed by sex (46% females). Of these, 48 children (38.4%) had hypoalbuminemia. The main causes of PICU admission were respiratory and gastrointestinal problems. Altogether, 57.6% of the patients had septic shock, with no difference in terms of severity with regard to the serum albumin level. We had 14 patients (11.2%) admitted to intensive care for SARS-CoV-2 related diseases including multisystemic inflammatory syndrome associated with COVID-19 (MIS-C).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n = 125</th>
<th>Hypoalbuminemia n = 48</th>
<th>Normal albumin n = 77</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (IQR)</td>
<td>2.0 (0.5–12.5)</td>
<td>1.95 (0.5–13.5)</td>
<td>2.0 (0.66–11.1)</td>
<td>0.604</td>
</tr>
<tr>
<td>Weight, kg (IQR)</td>
<td>10.9 (6.7–30.0)</td>
<td>10.6 (6.45–30)</td>
<td>11.0 (6.8–30.0)</td>
<td>0.798</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>58 (46.4)</td>
<td>24 (50)</td>
<td>34 (44.1)</td>
<td></td>
</tr>
<tr>
<td>Days in PICU</td>
<td>11 (6.0–19)</td>
<td>10.5 (7–19)</td>
<td>11 (6–21)</td>
<td>0.916</td>
</tr>
<tr>
<td>Focus of Infection (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td>41 (32.8)</td>
<td>15 (31.3)</td>
<td>26 (33.8)</td>
<td>0.554</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>2 (1.6)</td>
<td>2 (2.1)</td>
<td>1 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Genitourinary</td>
<td>7 (5.6)</td>
<td>2 (4.1)</td>
<td>5 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td>20 (16)</td>
<td>7 (14.6)</td>
<td>12 (16.9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis classification (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>72 (57.6)</td>
<td>32 (66.7)</td>
<td>40 (52)</td>
<td>0.105</td>
</tr>
<tr>
<td>Septic shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIM-2 (IQR)</td>
<td>18.1 (8.9–31.7)</td>
<td>17.7 (10.4–29.5)</td>
<td>18.3 (8.1–32)</td>
<td>0.615</td>
</tr>
<tr>
<td>PELOD-2 score (IQR)</td>
<td>8 (3–10)</td>
<td>8 (3–9)</td>
<td>8 (4–10)</td>
<td>0.383</td>
</tr>
<tr>
<td>Lactate mmol/L (IQR)</td>
<td>1.20 (0.82–1.78)</td>
<td>1.12 (0.81–1.69)</td>
<td>1.31 (0.88–1.85)</td>
<td>0.247</td>
</tr>
<tr>
<td>Glucose mg/dL (IQR)</td>
<td>109 (91–138)</td>
<td>111 (90.2–138.7)</td>
<td>109.2 (92–136.3)</td>
<td>0.979</td>
</tr>
<tr>
<td>Ferritin mg/dL (IQR)</td>
<td>431.1 (179.6–</td>
<td>409.6 (292.3–628.3)</td>
<td>201.4 (96.9–604.5)</td>
<td>0.272</td>
</tr>
<tr>
<td>C-reactive protein mg/dL (IQR)</td>
<td>5.1 (2.0–9.8)</td>
<td>7.8 (2.1–17.9)</td>
<td>3.4 (1.9–6.3)</td>
<td>0.025</td>
</tr>
<tr>
<td>D-dimer mg/L (IQR)</td>
<td>3.1 (1.51–6.2)</td>
<td>3.1 (1.3–4.9)</td>
<td>2.8 (1.2–4.3)</td>
<td>0.463</td>
</tr>
<tr>
<td>Procalcitonin g/dL (IQR)</td>
<td>1.2 (0.4–5.2)</td>
<td>1.9 (0.4–7.9)</td>
<td>0.9 (0.3–4.7)</td>
<td>0.120</td>
</tr>
<tr>
<td>Creatinine mg/dL (IQR)</td>
<td>0.4 (0.4–0.6)</td>
<td>0.4 (0.4–0.7)</td>
<td>0.5 (0.4–0.6)</td>
<td>0.023</td>
</tr>
<tr>
<td>Vasoactive score (IQR)</td>
<td>12.3 (4.5–28.5)</td>
<td>7 (4–20)</td>
<td>10 (4–20)</td>
<td>0.484</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Total n = 125</td>
<td>Hypoalbuminemia n = 48</td>
<td>Normal albumin n = 77</td>
<td>P value</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>---------</td>
</tr>
<tr>
<td>Mechanical ventilation (%)</td>
<td>76 (60.8)</td>
<td>26 (54.1)</td>
<td>50 (64.9)</td>
<td>0.392</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>13 (10.4)</td>
<td>4 (8.3)</td>
<td>9 (11.6)</td>
<td>0.765</td>
</tr>
</tbody>
</table>


**Microcirculatory Changes Associated With Serum Albumin**

Children with sepsis were found to have microcirculatory changes associated with the serum albumin level (Table 2). Of the patients with a serum albumin under 3.0 gm/dL in the first 24 hours, 76% (19/25) had a PBR flow corrected greater than 2.0 µm. Children with hypoalbuminemia had more 4-6-micron capillaries recruited than patients with normal albumin (60% vs. 40%; p = 0.04), with no differences in the percentage of blood volume in the capillary (p = 0.42) (Fig. 1). A moderate correlation was found between PBR flow corrected and the number of CD 4-6s recruited (rho 0.28; p < 0.01). In fact, those with the worst PBR had the highest number of CD 4-6s recruited (rho 0.29; p < 0.01).
Table 2
Microcirculation changes associated with the serum albumin level.

<table>
<thead>
<tr>
<th>Microcirculation evaluation variables using video microscopy or serum biomarkers</th>
<th>Total n = 125</th>
<th>Hypoalbuminemia n = 48</th>
<th>Normal albumin n = 77</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBR µm (SD)</td>
<td>2.15 (1.97–2.29)</td>
<td>2.15 (2.03–2.40)</td>
<td>2.13 (1.95–2.26)</td>
<td>0.98</td>
</tr>
<tr>
<td>PBR flow corrected µm (IQR)</td>
<td>2.07 (1.78–2.42)</td>
<td>2.16 (2.03–2.47)</td>
<td>1.92 (1.76–2.28)</td>
<td>0.01</td>
</tr>
<tr>
<td>Worst PBR µm (IQR)</td>
<td>3.26 (2.99–3.61)</td>
<td>3.36 (3.09–3.62)</td>
<td>3.2 (2.95–3.48)</td>
<td>0.31</td>
</tr>
<tr>
<td>4–6 µm capillary density (IQR)</td>
<td>36.8 (18.8–67.1)</td>
<td>41.2 (24.1–59.6)</td>
<td>29.6 (15.0–49.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Percentage of capillary blood volume (IQR)</td>
<td>63.3 (18.9–83.4)</td>
<td>74.6 (45.5–87.2)</td>
<td>66.5 (34.1–83.3)</td>
<td>0.24</td>
</tr>
<tr>
<td>Syndecan-1 ng/mL (IQR)</td>
<td>104.1 (62.1–192.1)</td>
<td>116.8 (63–217.7)</td>
<td>103.9 (85.1–132.6)</td>
<td>0.21</td>
</tr>
<tr>
<td>Angiopoietin-2 ng/mL (IQR)</td>
<td>11.6 (7.1–23.9)</td>
<td>15.9 (8.3–24)</td>
<td>10.7 (7.1–24.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Endocan ng/mL (IQR)</td>
<td>2.4 (0.9–3.7)</td>
<td>2.1 (0.8–3.2)</td>
<td>2.8 (1.8–3.8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Annexin A5 ng/mL (IQR)</td>
<td>3.1 (2.1–9.7)</td>
<td>3.3 (2.1–11.9)</td>
<td>1.8 (2.1–6.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>C02 delta</td>
<td>4.8 (2.7–6.9)</td>
<td>4.7 (2.8–6.6)</td>
<td>5.6 (2.7–6.9)</td>
<td>0.838</td>
</tr>
</tbody>
</table>

*PBR: perfused boundary region; C02 delta: venous-arterial C02 difference.

We found no relationship between the syndecan-1 level and serum albumin levels (p = 0.21). On admission, patients with hypoalbuminemia had 48% higher Ang-2 (p = 0.04) and annexin A5 (p = 0.03). Twenty-four hours after admission the microcirculation disorders persisted in patients with low albumin. The hypoalbuminemic group’s PBR was higher (2.2 IQR :1.81–2.49) than that of the group with a normal albumin level at 24 hours (1.79 IQR: 1.75–1.84) (p = 0.04).

There were no differences in mortality between the groups with normal or low albumin (p = 0.76). Nor were there differences in mortality when the PBR was abnormal (p = 0.11) prior to correcting the
hypoalbuminemia. The patients with hypoalbuminemia who died had a similar number of small capillaries (CD 4–6 microns) recruited (50% vs. 44%; p = 0.85) but a greater percentage of capillary blood flow (p = 0.04) than those who survived. Patients with hypoalbuminemia did not have a longer PICU stay (p = 0.91), nor differences in PIM-2 (p = 0.61) or PELOD-2 (p = 0.38). The group with hypoalbuminemia and a high PBR did not have a longer PICU stay (p = 0.76), or greater need of mechanical ventilation (p = 0.87).

In this regard, the patients who required mechanical ventilation (MV) had a higher percentage of blood volume in the capillaries (p < 0.01), higher Ang-2 levels (p < 0.01), metabolic acidosis (p < 0.05) and need for vasoactive support (p < 0.05). Children with MV did not have more syndecan-1 abnormalities (p = 0.16). We found a correlation between the days of MV and Ang-2 levels (rho (-) 0.33; p < 0.05), percentage of blood volume in the capillaries (rho (-) 0.31; p < 0.01), density of small capillaries recruited (rho 0.20; p = 0.03) and PBR level (rho (-) 0.20; p = 0.03). Patients with hypoalbuminemia who had a greater percentage of blood volume in their capillaries (above the 75th percentile) needed MV more often (67/119:56.3% vs. 52/119 43.7%; aOR 2.01 95% CI 1.38–3.10 ;p < 0.01).

Children with hypoalbuminemia and elevated PCT had abnormal PBR flow corrected for each capillary more often (56% vs. 22.3%; p < 0.05). These patients with high PCT did not have more 4–6 µm capillaries recruited, regardless of the albumin level (p = 0.61). Patients with normal albumin and high CRP had a greater blood volume percentage in the recruited blood capillaries (72% vs. 44%; aOR 3.18 95% CI 1.12–3.12; p = 0.02) with no alterations in PBR (p = 0.42) nor more 4-6-micron capillaries recruited (p = 0.92). Likewise, children with ferritin under 500 mg/dl had less blood volume in the recruited capillaries (60.8% vs. 26.7%; aOR 0.23 95% CI 0.10–0.53; p < 0.01), which was correlated with normal capillary refill (rho 0.23; p = 0.01). Patients with a capillary refill time of less than two seconds had more small capillaries (CD4-6) recruited (rho 0.30; p < 0.01). (p = 0.92).

The group of patients in whom hypoalbuminemia was corrected with a 20% albumin infusion (Fig. 2) had a less abnormal PBR flow corrected at 24 hours than those who did not receive 20% albumin replacement [54.1% (20/37) vs. 65.6% (42/22); aOR: 0.17; p5% CI 0.04–0.75;p = 0.02]). The group of patients in whom PBR normalization was achieved with an albumin infusion had lower mortality than patients in whom it was still abnormal (0% vs. 30%; aOR 1.42; 95% CI 1.71–1.91; p = 0.02) (Fig. 2). In this group, patients who did not receive 20% albumin infusion replacement and had hypoalbuminemia had a higher risk of glycolcalyx injury on video microscopy (aOR 4.48 95% CI 1.48–13.54; p < 0.01), regardless of age, PIM-2 and VIS (Fig. 2). In addition, patients who received albumin replacement had more 4–6 µm capillaries recruited at 24 hours than the group that did not receive replacement (p = 0.01). The group with albumin replacement had less positive fluid balances (p = 0.03). The lack of hypoalbuminemia correction was associated with PICU stays longer than 14 days (aOR 2.97 95% CI 1.27–6.92; p = 0.01).

Discussion

In this study, we found that patients with sepsis have microcirculation changes associated with hypoalbuminemia. These children have endothelial glycolcalyx degradation more often, a greater density
of small 4–6 µm capillaries recruited, and increased redistribution of blood flow toward the microcirculation. In addition, we found that children with hypoalbuminemia have more elevated biomarkers of increased endothelial permeability, greater inflammatory response and require mechanical ventilation more often. The group who received albumin replacement for hypoalbuminemia showed recovery of the microcirculation and glycocalyx variables and had a shorter PICU stay and lower mortality.

The microcirculation is the terminal effector site of the cardiovascular circulatory system, where the supply of oxygen to the tissues and elimination of metabolic waste are coupled and controlled. In the capillaries, the passage of fluid from the intravascular to the interstitial space is controlled by hydrostatic pressures, capillary oncotic pressure and sub-glycocalyx pressure [5, 6, 18]. In the microcirculation, albumin, besides regulating the flow of liquid between the different spaces, can have immunomodulating effects in patients with sepsis. Studies in animal models have found that normal albumin levels favor sphingosine-1-phosphate transport in the red blood cells and platelets [22, 23]. This sphingolipid has a high capacity for eliminating the matrix metalloproteinases responsible for degrading the glycocalyx and magnifying the inflammatory response in patients with sepsis [24–26]. In our study, we found greater glycocalyx degradation in children with sepsis and hypoalbuminemia, which was associated with a greater inflammatory response and elevated cell death biomarkers.

In sepsis, hypoalbuminemia has been associated with worse outcomes, including an up to 23 times greater risk of death. That is, for each 2.5 gm/dl below its normal value, the odds of death increase 24–56% [13–17]. Recently, with artificial intelligence techniques and machine learning-based models which perform non-linear analyses, low albumin has been found to be one of the most useful serum biomarkers for predicting survival in critically and chronically ill patients [17]. Albumin, besides being the most important protein for maintaining the colloid-osmotic pressure of plasma, is responsible for carrying many endogenous molecules (anti-inflammatory and immunomodulatory molecules) and exogenous molecules (like antibiotics) [14, 15]. It has important anti-inflammatory and antioxidant properties [20, 21]. The group with the lowest albumin in our study, in addition to being associated with microcirculation disorders, had a greater inflammatory response and worse outcomes, like a greater need for mechanical ventilation. This could be explained by increased recruitment of small pulmonary capillaries in children with hypoalbuminemia associated with increased vascular permeability due to sepsis (we found elevated angiopoietin-2), which would favor more capillary leakage toward the alveolar units, a higher risk of pulmonary edema, and therefore, a longer duration of mechanical ventilation support.

In an animal model of hemorrhagic shock, 5% albumin resuscitation, which is similar to the body's protein content, restores the glycocalyx when compared with crystalloids [27]. These effects may be mediated by greater sphingosine-1-phosphate release from the red blood cells and the platelets. In a recent study, Fernández-Sarmiento et al. [28] found that, in children with sepsis, saline solution was associated with greater endothelial glycocalyx degradation, and that patients with hypoalbuminemia and albumin infusion replacement had a lower risk of microcirculation disorders (aOR 0.56 95% CI 0.31–0.98). Serum
albumin replacement may have beneficial effects on the respiratory, cardiovascular, and neurological systems as well as the circulatory status [16].

In this regard, the Saline versus Albumin Fluid Evaluation (SAFE) study compared the impact on mortality in critically ill adults of receiving 0.9% normal saline solution versus 4% albumin as replacement fluid during fluid resuscitation [29]. No differences were found in terms of mortality, but patients who received fluid resuscitation with albumin required a 30% lower volume of fluids during resuscitation. Later, the ALBIOS study evaluated the effect of simultaneous administration of 20% albumin and crystalloids to maintain blood albumin levels equal to or greater than 3.0 gm/dL [30]. Patients who had better albumin levels had their vasopressors or inotropes discontinued sooner, less positive balances, and greater macrocirculation stability. Recently, Raghunathan K et al. [31] found that patients with sepsis and acute kidney injury who received albumin infusions had shorter hospital stays than those who did not receive replacement (hazard ratio, 1.83; 95% CI, 1.56–2.15; p < 0.001). Albumin replacement and maintaining levels greater than 3.0 gm/dL in critically ill patients has been considered a cost-effective approach, showing an almost 20% reduction in care costs for patients with septic shock [32]. In our study, we found that patients who received albumin replacement experienced endothelial glycocalyx degradation recovery, and this group had a shorter hospital stay and lower mortality. Our hypothesis is that maintaining albumin levels above 3 gm/dL stabilized the endothelial glycocalyx and decreased its degradation, and therefore fewer glycocalyx degradation products were released to potentially magnify inflammation and behave as damage-associated molecular patterns (DAMPs) [33–35].

There is increasing evidence of the association between hypoalbuminemia and clinical outcomes in critically ill patients. Leite H et al. found that a 1.0 gm/dL increase in serum albumin on admission was related to a 73% reduction in the risk of death (HR 0.27; 95% CI 0.14–0.51; p < 0.01) [36]. A 1 gm/dL increase in serum albumin was independently associated with a 33% greater probability of early discharge from the PICU (HR 1.33; 95% CI 1.07–1.64; p = 0.008) and an increase in ventilator-free-days (OR 1.86; 95% CI 0.56–3.16; p = 0.005). In our study, we found that patients in whom hypoalbuminemia was corrected had less positive fluid balances and a shorter PICU stay. We should carry out clinical studies evaluating the role of albumin infusions in outcomes like endothelial dysfunction, glycocalyx degradation and possible patient subgroups who could benefit more than others, decreasing the time on mechanical ventilation or mortality.

**Limitations**

We consider that our study has several limitations. First, it was performed at a single reference center for highly complex patients. This could lead to the included patients being much more ill. However, the severity and organ dysfunction scales were similar in the groups with and without hypoalbuminemia, despite changes found in the microcirculation. In addition, we did not differentially analyze patients with malnutrition. This group could have underlying conditions associated with chronic microcirculation changes and worse outcomes. We tried to control for this variable in the multivariate analysis. However,
we are not aware of studies evaluating how nutritional status affects end-tissue perfusion and microcirculation. Finally, due to the available budget, we did not measure cytokines nor conduct long-term follow up of the acid-base status or chloride levels in our patients to determine if there was a relationship between albumin levels, microcirculation changes, and glycocalyx degradation which could be explained by an abnormal inflammatory response or acid-base balance or hyperchloremia [37, 38].

Conclusion

In children with sepsis, an association was found between hyopalbuminemia and microcirculation changes. These patients have endothelial glycocalyx degradation more often, greater small capillary recruitment, and increased blood flow redistribution toward the microcirculation. In addition, the group with hypoalbuminemia had elevated endothelial activation, inflammatory response and apoptosis biomarkers. These microcirculation disorders in patients with hypoalbuminemia were associated with a greater need for and duration of mechanical ventilation, a longer hospital stay and greater mortality.

Abbreviations

Ang-2: angiopoietin -2
C.I.: confidence interval
C02 delta: venous-arterial C02 difference
CD-4-6: capillary density of 4-6 µm vessels
CRP: C-reactive protein
IQR: interquartile range
MV: mechanical ventilation
PBR: perfused boundary region
PICU: pediatric intensive care unit
PPV- proportion of perfused vessels
PCT: procalcitonin

Declarations

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

JFS designed the study. JFS, MPS, RHS, SB, VC and CD conducted the study. JFS supervised the study. JFS and MPS provided critical consultancy on the study implementation. JFS analyzed the data. JFS, RHS, MPS and CD interpreted the data. JFS and CD had full access to the data. JFS, RHS, MPS and CD drafted the manuscript. All authors reviewed the manuscript for important intellectual content and approved the final version.

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Ethics approval and consent to participate

This study was approved by the hospital’s ethics and research committees (CEIC-0366-0022) from Fundación Cardioinfantil-IC and all the parents or guardians signed informed consent prior to being included in the protocol. The ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments were respected.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to disclose.

References


Figures
Figure 1. Alterations in the microcirculation of children with hipoalbuminemia. PBR: perfused boundary region. PBSC: percentage of blood volume in the capillary.

Figure 1

See image above for figure legend.
Figure 2. Alterations in microcirculation in patients who received hypoalbuminemia correction. A. Percentage of patients with PBR corrected for impaired flow and correction of hypoalbuminemia. B. Percentage of capillaries with a density of 4-6 microns recruited and correction of hypoalbuminemia. C. Fluid overload greater than 10% and correction of hypoalbuminemia. D. Angiopoietin-2 and correction of hypoalbuminemia. Yes: correction of hypoalbuminemia. No: no correction of hypoalbuminemia. PBR: perfused boundary region. CD 4-6: capillary density 4-6 microns.

**Figure 2**

See image above for figure legend.