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ORIGINAL ARTICLE

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

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Abstract

Objective: The objective of this study was to evaluate the association between serum albumin levels and microcirculation changes, glycocalyx degradation, and the clinical outcomes of interest.

Methods: Observational, prospective study in children with sepsis. The primary outcome was the association between hypoalbuminemia and microcirculation disorders, endothelial activation and glycocalyx degradation using a perfused boundary region (PBR) (abnormal >2.0 μm on sublingual video microscopy) or plasma biomarkers (syndecan-1, angiopoietin-2).

Results: A total of 125 patients with sepsis were included. The median age was 2.0 years (IQR 0.5–12.5). Children with hypoalbuminemia had more abnormal microcirculation with a higher PBR (2.16 μm [IQR 2.03–2.47] vs. 1.92 [1.76–2.28]; $p = .01$) and more 4–6 μm capillaries recruited (60% vs. 40%; $p = .04$). The low albumin group that had the worst PBR had the most 4–6 μm capillaries recruited (rho 0.29; $p < .01$), 48% higher Ang-2 ($p = .04$), worse annexin A5 ($p = 0.03$) and no syndecan-1 abnormalities ($p = .21$). 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: $p < .01$).

Conclusions: In children with sepsis, an association was found between hypoalbuminemia and microcirculation changes, vascular permeability, and greater endothelial glycocalyx degradation.

KEYWORDS
children, endothelium, fluids, glycocalyx, septic shock

Abbreviations: Ang-2, Angiopoietin-2; CD 4-6s, Capillary density of 4-6 μm; CRP, C-reactive protein; DPP4, Dipeptidyl-peptidase 4; ELISA, Enzyme-linked immunosorbent assay; MIS-C, Multisystemic inflammatory syndrome associated with COVID-19; PBR, Perfused boundary region; PCT, Procalcitonin; PELOD-2, Pediatric Logistic Organ Dysfunction-2; PICU, Pediatric Intensive Care Unit; PIM-2, Pediatric Index of Mortality-2; RBCs, Red blood cells.

1 | 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.

2 | MATERIALS AND METHODS

2.1 | 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.

2.2 | Patients eligibility

All children from 1 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 6 h of admission were included. Patients with hypoalbuminemia and with normal serum albumin levels were included to evaluate microcirculation in both cohorts. Patients who had received 5% albumin boluses for fluid resuscitation within the 24 h 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 1.73m^2 for more than 3 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.

2.3 | 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.*) within 6 h of admission to intensive care. The measurement was repeated 24 h after being included in the study. This device measures sublingual microcirculation by evaluating 4–25 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 3000 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.[8,9] The software calculates the dynamic lateral red blood cell movement in the permeable region of the glycocalyx layer, which is expressed as the PBR (in μm). This measurement may vary depending on the red blood cell velocity and flow, and therefore the software also measures what is known as PBR dynamic or flow corrected.[8,9] The flow-corrected evaluation is a complement to PBR and has been found to be higher in patients with sepsis compared with healthy controls.[8] In addition, the video microscope measures the percentage of capillary blood volume (PBV-proportion of perfused blood vessels over the total number of vessels) and the capillary density of 4–6 μm vessels (CD 4–6 s).

Angiopoietin-2 (Ang-2 / Human Angiopoietin 2 ELISA Kit ANG 2; ab99971, Abcam Lab) 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 ng/mL was considered normal.[12] Annexin A5 (ab119503 – Annexin V Human ELISA Kit: Abcam Lab) was measured to evaluate cell death. All biomarker measurements were done in duplicate according to the manufacturer's instructions, simultaneously and within 6 h of admission to intensive care. The samples were 100 μL of citrated plasma which were centrifuged for 30 min at 1000 rpm, and the samples were stored at (−) 20°C for later processing using the enzyme-linked immunosorbent assay (ELISA) method.

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 h 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 6 h of admission. Laboratory tests were conducted on admission and 24 h later, including serum albumin, electrolytes, serum lactate, creatinine, D-dimer, and inflammatory biomarkers (ferritin, CRP, and PCT).

2.4 | 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 (MV).

2.5 | 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 Chi^2 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).

3 | RESULTS

3.1 | Patients characteristics

During the study period, 125 patients with sepsis or septic shock were included (Table 1). The median age was 2 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).

3.2 | 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 h, 76% 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%; $p = .04$), with no differences in the percentage of blood volume (PBV) in the capillary ($p = .42$) (Figure 1A-C). A moderate correlation was found between PBR flow corrected and the number of CD 4–6 s recruited (rho 0.28; $p < .01$) (Figure 1B). In fact, those with the worst PBR had the highest number of CD 4–6 s recruited (rho 0.29; $p < .01$).

We found no relationship between the syndecan-1 level and serum albumin levels ($p = .21$), although the patients with hypoalbuminemia had more kidney injury that could affect the serum levels of this biomarker. On admission, patients with hypoalbuminemia had 48% higher Ang-2 ($p = .04$) and annexin A5 ($p = .03$). Twenty-four hours after admission the microcirculation disorders persisted in patients with low albumin. The hypoalbuminemic group's PBR flow corrected was higher (2.2 µm IQR 1.81–2.49) than that of the group with a normal albumin level at 24 h (1.79 µm IQR 1.75–1.84) ($p = .04$).

TABLE 1 Population characteristics.

Characteristic	Total $n = 125$	Hypoalbuminemia $n = 48$	Normal albumin $n = 77$	p Value
Age, years (IQR)	2.0 (0.5–12.5)	1.95 (0.5–13.5)	2.0 (0.66–11.1)	0.604
Weight, kg (IQR)	10.9 (6.7–30.0)	10.6 (6.45–30)	11.0 (6.8–30.0)	0.798
Female sex (%)	58 (46.4)	24 (50)	34 (44.1)	
Days in PICU	11 (6.0–19)	10.5 (7–19)	11 (6–21)	0.916
Focus of Infection (%)				0.554
Respiratory	55 (44)	22 (45.8)	33 (42.9)	
Gastrointestinal tract	41 (32.8)	15 (31.3)	26 (33.8)	
Genitourinary	2 (1.6)	2 (2.1)	1 (1.3)	
Central nervous system	7 (5.6)	2 (4.1)	5 (6.5)	
Other	20 (16)	7 (14.6)	12 (16.9)	
Sepsis classification (%)				0.105
Severe sepsis	53 (42.4)	16 (33.3)	37 (48)	
Septic shock	72 (57.6)	32 (66.7)	40 (52)	
PIM-2 (IQR)	18.1 (8.9–31.7)	17.7 (10.4–29.5)	18.3 (8.1–32)	0.615
PELOD-2 score (IQR)	8 (3–10)	8 (3–9)	8 (4–10)	0.383
Lactate mmol/L (IQR)	1.20 (0.82–1.78)	1.12 (0.81–1.69)	1.31 (0.88–1.85)	0.247
Glucose mg/dL (IQR)	109 (91–138)	111 (90.2–138.7)	109.2 (92–136.3)	0.979
Ferritin mg/dL (IQR)	431.1 (179.6–1698.8)	409.6 (292.3–628.3)	201.4 (96.9–604.5)	0.272
C-reactive protein mg/dL (IQR)	5.1 (2.0–9.8)	7.8 (2.1–17.9)	3.4 (1.9–6.3)	0.025
D-dimer mg/L (IQR)	3.1 (1.51–6.2)	3.1 (1.3–4.9)	2.8 (1.2–4.3)	0.463
Procalcitonin g/dL (IQR)	1.2 (0.4–5.2)	1.9 (0.4–7.9)	0.9 (0.3–4.7)	0.120
Creatinine mg/dL (IQR)	0.4 (0.4–0.6)	0.4 (0.4–0.7)	0.5 (0.4–0.6)	0.023
Vasoactive score (IQR)	12.3 (4.5–28.5)	7 (4–20)	10 (4–20)	0.484
Mechanical ventilation (%)	76 (60.8)	26 (54.1)	50 (64.9)	0.392
Mortality (%)	13 (10.4)	4 (8.3)	9 (11.6)	0.765

Abbreviations: PIM-2, pediatric index of mortality-2; PELOD-2, pediatric logistic organ dysfunction-2.

TABLE 2 Microcirculation changes associated with the serum albumin level.

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

Abbreviations: C02 delta, venous–arterial C02 difference; PBR, perfused boundary region.

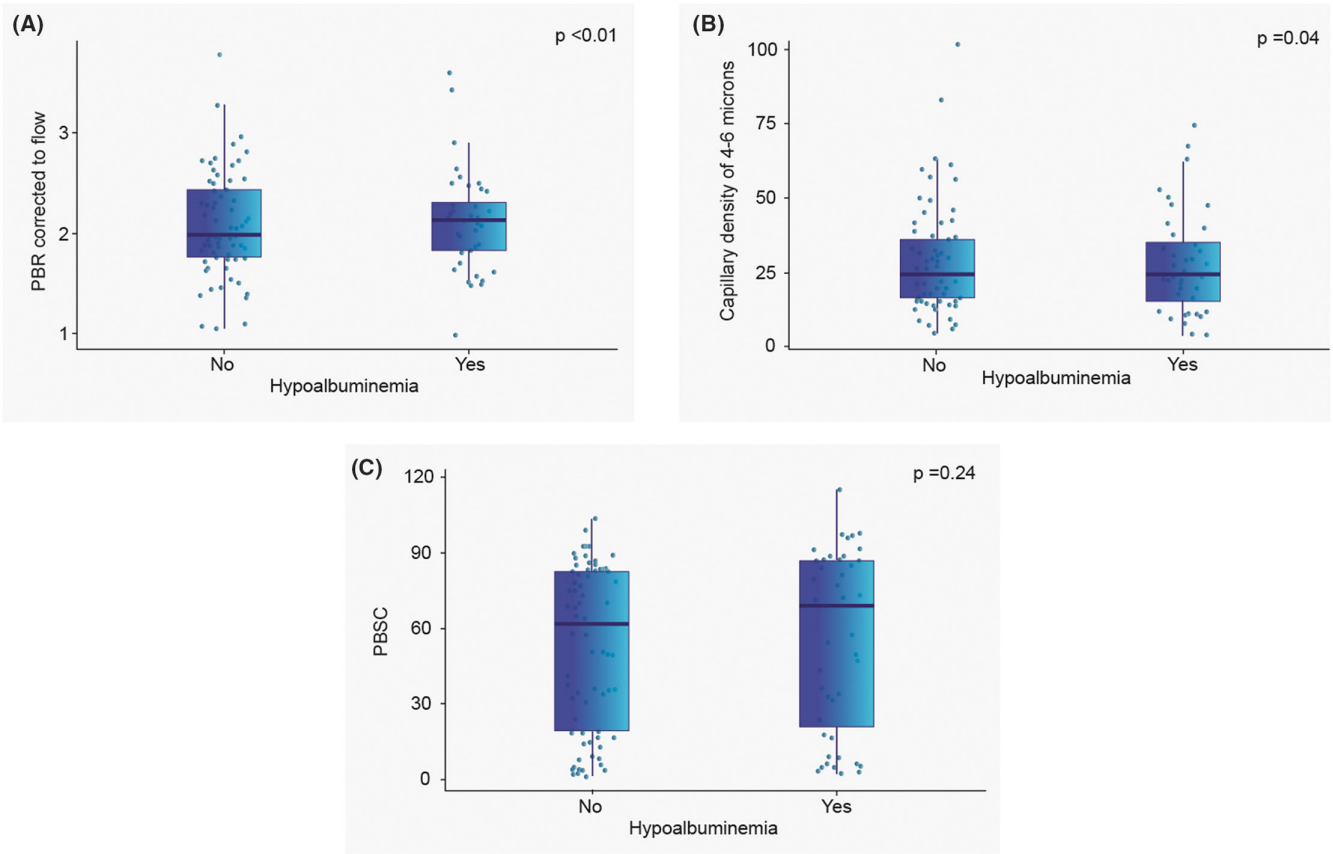

FIGURE 1 Alterations in the microcirculations in children with hypoalbuminemia. (A) Perfused Boundary region and hypoalbuminemia. (B) Capillary density of 4–6 microns and hypoalbuminemia. (C) Percentage of blood volume and hypoalbuminemia.

3.3 | Microcirculatory changes and inflammatory response

Of the total population, 57.6% (72/125) had elevated PCT. Specifically, the group with elevated PCT and simultaneous hypoalbuminemia had an abnormal PBR flow corrected more often than patients with normal PCT. (56% [20/36] vs. 22.3% [8/36]; $p<.05$). There was also a negative correlation between albumin levels and PCT on admission (rho: [−] 0.16; $p=.04$) and after 24 h (rho [−] 0.20; $p=.04$). These patients with high PCT did not have more 4–6 μm capillaries recruited, regardless of the albumin level ($p=.61$). We found a negative correlation between albumin levels and CRP on admission (rho: (−) 0.22; $p=.01$) and after 24 h (rho [−] 0.26; $p<.01$). 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; $p=.02$) with no alterations

in PBR ($p = .42$) nor more 4-6-micron capillaries recruited ($p = .92$). Likewise, children with ferritin under 500 mg/dL had less blood volume (PBV) in the recruited capillaries (26.7%vs. 60.8%; aOR 0.23 95% CI 0.10–0.53; $p < .01$), which was correlated with normal capillary refill (rho 0.23; $p = .01$).

3.4 | Microcirculatory changes and clinical outcomes of interest

There were no differences in mortality between the groups with normal or low albumin ($p = .76$). The patients with hypoalbuminemia who died had a similar number of small capillaries (CD 4–6 microns) recruited (50% vs. 44%; $p = .85$) but a greater percentage of capillary blood flow ($p = .04$) than those who survived. Patients with hypoalbuminemia did not have a longer PICU stay ($p = .91$), nor differences in PIM-2 ($p = .61$) or PELOD-2 ($p = .38$). The group with hypoalbuminemia and a high PBR did not have a longer PICU stay ($p = .76$), or greater need of MV ($p = .87$).

In this regard, the patients who required MV had a higher percentage of blood volume (PPV) in the capillaries ($p < .01$), higher Ang-2 levels ($p < .01$), metabolic acidosis ($p < .05$) and need for vasoactive support ($p < .05$). 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; $p < .05$), percentage of blood volume in the capillaries (rho [−] 0.31; $p < .01$), density of small capillaries recruited (rho 0.20; $p = .03$) and PBR level (rho [−] 0.20; $p = .03$). Patients with hypoalbuminemia who had a greater percentage of blood volume in their capillaries (above the 75th percentile) needed MV more often (56.3% [10/18] vs. 44.4% [8/18]; aOR 2.01 95% CI 1.38–3.10; $p < .01$) (Table 3).

The group of patients in whom hypoalbuminemia was corrected with a 20% albumin infusion (43%; 21/48) had a less abnormal PBR at 24 h than those who did not receive 20% albumin replacement (54.1% vs. 65.6%; aOR: 0.17 95% CI 0.04–0.75; $p = .02$). In addition, patients who received albumin replacement had more 4–6 μm capillaries recruited at 24 h than the group that did not receive replacement ($p = .01$). Patients who did not receive 20% albumin infusion replacement and had hypoalbuminemia had a higher risk of glycocalyx injury on video microscopy (aOR 4.48 95% CI 1.48–13.54; $p < .01$), regardless of age, PIM-2, and VIS (Figure 2A,B). The group with albumin replacement had less positive fluid balances ($p = .03$) (Figure 2C,D). The lack of hypoalbuminemia correction was associated with PICU stays longer than 14 days (aOR 2.97 95% CI 1.27–6.92; $p = .01$). 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; $p = .02$) (Figure 2).

TABLE 3 Multivariate analysis of the factors associated with microcirculation alterations in children with sepsis and hypoalbuminemia evaluated through video microscopy.

Variables	aOR	95% CI	*p* Value*
c-reactive protein >4 mgr/dL	0.91	0.44–1.92	0.81
Hyperchloremia >110 mEq/L	3.73	1.19–11.65	0.02
PICU stays longer than 14 days	2.97	1.27–6.92	0.01
20% albumin infusion	0.17	0.04–0.75	0.02
Mechanical ventilation	2.01	1.38–3.10	<0.01
Mortality[a]	1.42	1.71–1.91	0.02

Note: *Adjusted for PIM-2, age, malnutrition, vasoactive score.
Abbreviation: PBR, perfused boundary region.
[a]Persistence of altered PBR despite correction of hypoalbuminemia. Adjusted for PIM-2, age, vasoactive score.

4 | DISCUSSION

In this study, we found that patients with sepsis have microcirculation changes associated with hypoalbuminemia. These children have endothelial glycocalyx 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 MV 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 RBCs 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 nonlinear 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

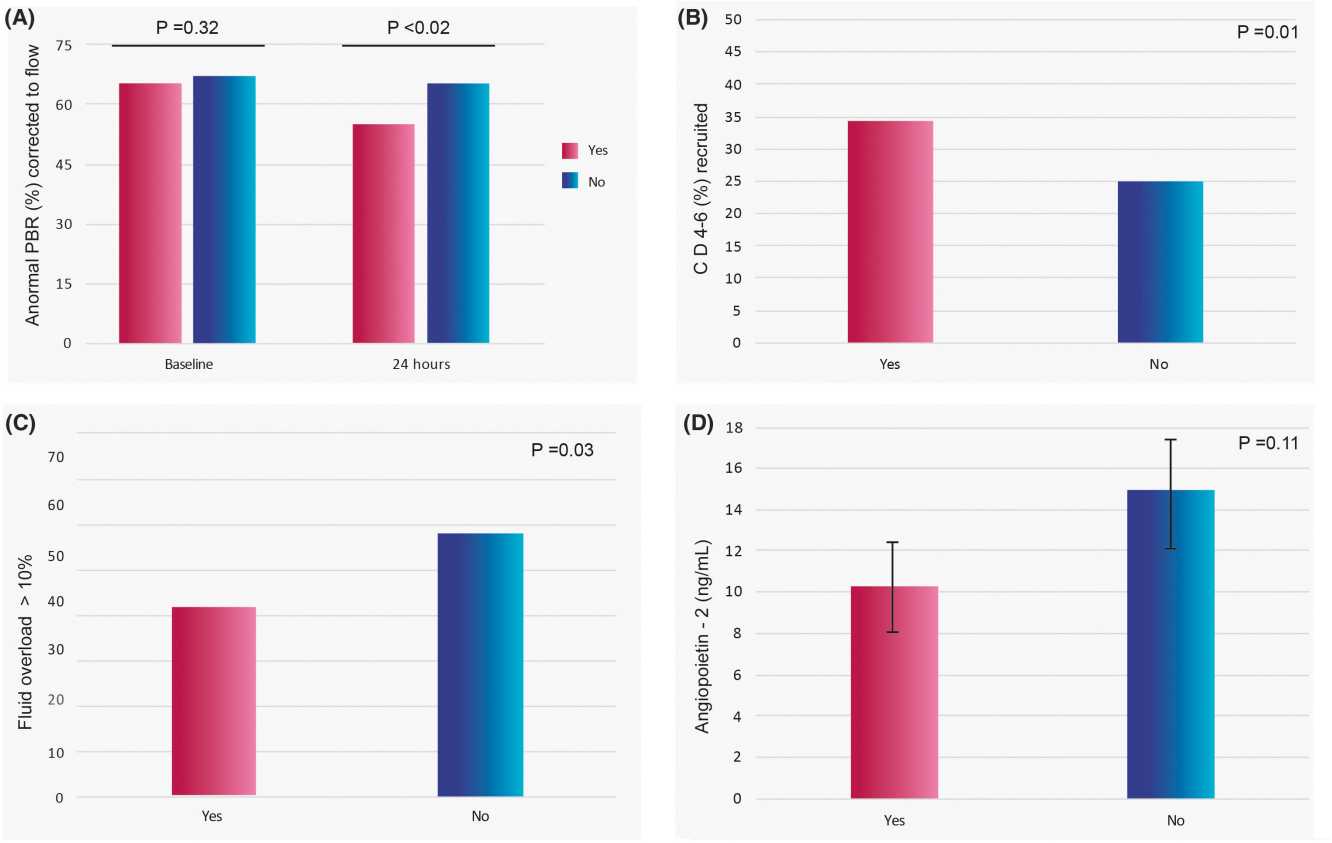

FIGURE 2 Alterations in microcirculation in patients who underwent hypoalbuminemia correction. (A) Perfused boundary region corrected to flow on admission and at 24 hours in patients with and without hypoalbuminemia correction. (B) Percentage of Capillary density of 4–6 microns. (C) Fluid balance percentage greater than 10% with and without hypoalbuminemia correction. (D) Angiopoietin-2 and hypoalbuminemia correction.

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 MV. 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 MV support. We also found a higher frequency of acute kidney injury in the group with hypoalbuminemia, which could favor albuminuria and predispose to worsening and greater glycocalyx degradation in sepsis.

As has been stated, serum albumin has anti-inflammatory and antioxidant properties.[20,21] In this study, we found that patients with higher PCT levels had more glycocalyx degradation, lower serum albumin, and elevated angiopoieitin-2. Recently, Brabenec L et al. found that elevated PCT was associated with microvascular dysfunction in patients after heart surgery, favoring increased vascular permeability.[27,28] These findings were confirmed with an animal model in which endothelial junction damage was induced by injecting intravenous PCT in a preclinical model. They identified how PCT-induced damage requires activation of a dipeptidyl-peptidase 4 (DPP4) which, when inhibited with medications, was correlated with reduced capillary leakage and improved vascular integrity. This is the first study in human beings that suggests that PCT may be the cause of increased inflammation and capillary leakage in inflammatory states. In addition, they found that modulating elevated PCT levels could have protective effects on the vascular barrier. The findings of our study in children with sepsis are along the same lines. Hyperprocalcitoninemia was associated with low albumin, glycocalyx degradation and increased vascular permeability biomarkers (Ang-2). These mutually associated phenomena may favor the inflammatory response, with consequences for the microcirculation and microvascular permeability. In fact, we believe that the presence of hypoalbuminemia in sepsis may condition a compensatory response in the microcirculation leading initially to greater capillary recruitment, contrary to what is described in adults. This may be a temporary compensatory microvascular change in the early stages of the disease. As sepsis advances, this compensatory capacity for capillary recruitment may be lost, resulting in low vascular density which, if it persists after 48 h of PICU admission, has been associated with higher mortality.[29] Studies are needed to examine these aspects further and conduct medium-term follow up of the microvascular changes associated with sepsis.

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.[30] These effects may be mediated by greater sphingosine-1-phosphate release from the RBCs and the platelets. In a recent study, Fernández-Sarmiento et al.[31] 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.[32] 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.[33] 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.[34] 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; $p < .001$). 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.[35] 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).[36–38] This hypothesis must be confirmed in future clinical trials or studies with an appropriate design for determining causality.

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; $p < .01$).[39] 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; $p = .008$) and an increase in ventilator-free-days (OR 1.86; 95% CI 0.56–3.16; $p = .005$). In our study, we found that patients in whom hypoalbuminemia was corrected had less positive fluid balances and a shorter PICU stay.

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. However, the group with normal serum albumin had significantly more acute kidney injury. This involvement could have affected the serum levels of syndecan-1, which we did not find to be different between the two groups.[40] 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. Another limitation of our study is that PBR is an indirect measure of glycocalyx damage. Although the equipment has low inter and intra-observer variability, it is unknown if the measurements could be affected by fluid boluses or the use of vasopressors. In addition, we found an altered PBR with elevated inflammation biomarkers or a greater need for MV. While the groups in which we found this association are small, they had statistical significance, and we must continue to study the relationship between the inflammatory response and microcirculation abnormalities. 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.[41,42]

5 | CONCLUSION AND PROSPECT

In children with sepsis, an association was found between hypoalbuminemia 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 MV, a longer hospital stay and greater mortality. 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 MV or mortality.

6 | PERSPECTIVES

Children with hypoalbuminemia have important changes in microcirculation, increased endothelial activation, glycocalyx degradation, and elevation of inflammatory biomarkers. These microcirculation disorders were associated with a greater need for and duration of

mechanical ventilation, a longer hospital stay and greater mortality. We must develop clinical trials in children with sepsis that evaluate the efficacy and safety of correcting hypoalbuminemia as well as its clinical impact.

AUTHOR CONTRIBUTIONS

Jaime Fernández-Sarmiento designed the study. Jaime Fernández-Sarmiento, María Paula Salazar, Ricardo Hernández-Sarmiento, Sofia Barrera, Valeria Castilla and Catalina Duque conducted the study. Jaime Fernández-Sarmiento supervised the study. Jaime Fernández-Sarmiento and María Paula Salazar provided critical consultancy on the study implementation. Jaime Fernández-Sarmiento analyzed the data. Jaime Fernández-Sarmiento, Ricardo Hernández-Sarmiento, María Paula Salazar and Catalina Duque interpreted the data. Jaime Fernández-Sarmiento and Catalina Duque had full access to the data. Jaime Fernández-Sarmiento, Ricardo Hernández-Sarmiento, María Paula Salazar and Catalina Duque drafted the manuscript. All authors reviewed the manuscript for important intellectual content and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Weiss SL, Peters MJ, Alhazzani W, et al. Surviving sepsis campaign international guidelines for the Management of Septic Shock and Sepsis-Associated Organ Dysfunction in children. *Pediatr Crit Care med*. 2020;21(2):e52-e106.
2. Fernández-Sarmiento J, De Souza DC, Martinez A, et al. Latin American consensus on the Management of Sepsis in children: Sociedad Latinoamericana de Cuidados Intensivos Pediátricos [Latin American pediatric Intensive Care Society] (SLACIP) task force: executive summary. *J Intensive Care med*. 2022 Jun;37(6):753-763.
3. Fernández-Sarmiento J, Flórez S, Alarcón-Forero LC, et al. Case report: endothelial glycocalyx damage in critically ill patients with SARS-CoV-2-related multisystem inflammatory syndrome (MIS-C). *Front Pediatr*. 2021 Sep;6(9):726949.
4. Chelazzi C, Villa G, Mancinelli P, De GAR, Adembri C. Glycocalyx and sepsis-induced alterations in vascular permeability. *Crit Care*. 2015;18(1):1-7.
5. Fernández-Sarmiento J, Salazar-Peláez LM, Carcillo JA. The endothelial glycocalyx: a fundamental determinant of vascular permeability in sepsis. *Pediatr Crit Care med*. 2020;21(5):e291-e300.
6. Donati A, Damiani E, Domizi R, Romano R, Adrario E, Pelaia P. Alteration of the sublingual microvascular glycocalyx in critically ill patients. *Microvasc Res*. 2013;90:86-89.
7. Luft J. Fine structures of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc*. 1966;25(6):1773-1783.
8. Rovas A, Sackarnd J, Rossaint J, et al. Identification of novel sublingual parameters to analyze and diagnose microvascular dysfunction in sepsis: the NOSTRADAMUS study. *Crit Care*. 2021;25:112. doi:10.1186/s13054-021-03520-w
9. Rovas A, Lukasz AH, Vink H, et al. Bedside analysis of the sublingual microvascular glycocalyx in the emergency room and intensive care unit - the GlycoNurse study. *Scand J Trauma Resusc Emerg med*. 2018;26(1):16. doi:10.1186/s13049-018-0483-4
10. Ince C, Boerma EC, Cecconi M, et al. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. *Intensive Care med*. 2018 Mar;44(3):281-299.
11. Patterson EK, Cepinskas G, Fraser DD. Endothelial glycocalyx degradation in critical illness and injury. *Front med (Lausanne)*. 2022;8(9):898592.
12. Fernández-Sarmiento J, Molina CF, Salazar-Pelaez LM, et al. Biomarkers of glycocalyx injury and endothelial activation are associated with clinical outcomes in patients with sepsis: a systematic review and meta-analysis. *J Intensive Care med*. 2023;38(1):95-105.
13. Goldwasser P, Feldman J. Association of serum albumin and mortality risk. *J Clin Epidemiol*. 1997;50(6):693-703.
14. Furukawa M, Kinoshita K, Yamaguchi J, Hori S, Sakurai A. Sepsis patients with complication of hypoglycemia and hypoalbuminemia are an early and easy identification of high mortality risk. *Intern Emerg med*. 2019;14(4):539-548.
15. Saucedo-Moreno EM, Fernández-Rivera E, Ricárdez-García JA. Hypoalbuminemia as a predictor of mortality in abdominal sepsis. *Cir Cir*. 2020;88(4):481-484.
16. Yin M, Si L, Qin W, et al. Predictive value of serum albumin level for the prognosis of severe sepsis without exogenous human albumin administration: a prospective cohort study. *J Intensive Care med*. 2018;33(12):687-694.
17. Qiu W, Chen H, Dincer AB, Lundberg S, Kaeberlein M, Lee SI. Interpretable machine learning prediction of all-cause mortality. *Commun med (Lond)*. 2022;3(2):125.
18. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res*. 2010;87(2):198-210. doi:10.1093/cvr/cvq062
19. Zeng Y, Adamson RH, Curry FR, et al. Sphingosine-1-phosphate protects endothelial glycocalyx by inhibiting sindecano-1 shedding. *Am J Physiol Heart Circ Physiol*. 2014;306:H363-H372.
20. Aldecoa C, Llau JV, Nuvials X, Artigas A. Role of albumin in the preservation of endothelial glycocalyx integrity and the microcirculation: a review. *Ann Intensive Care*. 2020;10(1):85. doi:10.1186/s13613-020-00697-1
21. Iba T, Levy JH. Derangement of the endothelial glycocalyx in sepsis. *J Thromb Haemost*. 2019;17(2):283-294. doi:10.1111/jth.14371

22. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol*. 2012;226(4):562-574. doi:10.1002/path.3964
23. Gräler MH. The role of sphingosine 1-phosphate in immunity and sepsis. *Am J Clin Exp Immunol*. 2012;1(2):90-100.
24. Camerer E, Regard JB, Cornelissen I, et al. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J Clin Invest*. 2009;119(7):1871-1879.
25. Adamson RH, Clark JF, Radeva M, Kheirolomoom A, Ferrara KW, Curry FE. Albumin modulates S1P delivery from red blood cells in perfused microvessels: mechanism of the protein effect. *Am J Physiol Heart Circ Physiol*. 2014;306:H1011-H1017.
26. Wu X, Hou J, Li H, et al. Inverse correlation between plasma sphingosine-1-phosphate and ceramide concentrations in septic patients and their utility in predicting mortality. *Shock*. 2019;51(6):718-724.
27. Brabenec L, Müller M, Hellenthal KEM, et al. Targeting procalcitonin protects vascular barrier integrity. *Am J Respir Crit Care med*. 2022 Aug 15;206(4):488-500.
28. Sullivan RC, Schmidt EP. Procalcitonin: a mediator of microvascular dysfunction during critical illness. *Am J Respir Crit Care med*. 2022;206(4):375-376.
29. Top AP, Ince C, de Meij N, van Dijk M, Tibboel D. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. *Crit Care med*. 2011;39(1):8-13.
30. Torres LN, Chung KK, Salgado CL, Dubick MA, Torres Filho IP. Low-volume resuscitation with normal saline is associated with microvascular endothelial dysfunction after hemorrhage in rats, compared to colloids and balanced crystalloids. *Crit Care*. 2017;21:160.
31. Fernández-Sarmiento J, Salazar-Peláez LM, Acevedo L, et al. Endothelial and glycocalyx biomarkers in children with sepsis after one bolus of unbalanced or balanced crystalloids. *Pediatr Crit Care med*. 2023;24:213-221. doi:10.1097/PCC.0000000000003123
32. The Safe study investigators. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. *N Engl J med*. 2004;350(22):2247-2256.
33. Caironi P, Tognoni G, Masson S, et al. ALBIOS study investigators. Albumin replacement in patients with severe sepsis or septic shock. *N Engl J med*. 2014;370(15):1412-1421.
34. Raghunathan K, Kempker JA, Davis EA, et al. Early albumin infusion is associated with greater survival to discharge among patients with sepsis/septic shock who develop severe acute kidney injury among patients with sepsis/septic shock who develop severe acute kidney injury. *Crit Care Explor*. 2022;4(12):e0793. doi:10.1097/CCE.0000000000000793
35. Guidet B, Ghout I, Ropers J, Aegerter P. Economic model of albumin infusion in septic shock: the EMAISS study. *Acta Anaesthesiol Scand*. 2020;64(6):781-788.
36. Kremer H, Baron-Menguy C, Tesse A, et al. Human serum albumin improves endothelial dysfunction and survival during experimental endotoxemia: concentration-dependent properties. *Crit Care: 1414 med*. 2011;39:1414-1422.
37. Fernández-sarmiento J, Schlapbach LJ, Acevedo L, et al. Endothelial damage in sepsis: the importance of systems biology. *Front Pediatr*. 2022;10:1-10.
38. Tiwari LK, Singhi S, Jayashree M, Baranwal AK, Bansal A. Hypoalbuminemia in critically sick children. *Indian J Crit Care med*. 2014;18(9):565-569.
39. Leite HP, Rodrigues da Silva AV, de Oliveira Iglesias SB, Koch Nogueira PC. Serum albumin is an independent predictor of clinical outcomes in critically ill children. *Pediatr Crit Care med*. 2016;17(2):e50-e57. doi:10.1097/PCC.0000000000000596
40. Jing Z, Wei-Jie Y, Yi-Feng ZG, Jing H. Downregulation of Syndecan-1 induce glomerular endothelial cell dysfunction through modulating internalization of VEGFR-2. *Cell Signal*. 2016;28(8):826-837.
41. Omron E, Omron R. A physicochemical model of crystalloid infusion on acid-base status. *J Intensive Care med*. 2010;25(5):271-280.
42. Yuos NM, Bellomo R, Hegarty C, et al. Association between a chloride-liberal vs chloride-restrictive intravenous fluid administration strategy and kidney injury in critically ill adults. *JAMA*. 2012;308(15):1566-1572.

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