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Endothelial Injury in Paediatric Sepsis-associated Acute Kidney Injury

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Abstract

Background: Sepsis-associated acute kidney injury (SA-AKI) is an acute kidney injury in the presence of sepsis without other significant contributing factors explaining acute kidney injury (AKI). Circulating syndecan-1, angiopoietin-2, and urinary neutrophil gelatinase-associated lipocalin levels can potentially cause neglected AKI to become acute kidney disease (AKD) and even chronic kidney disease (CKD). Therefore, studying its levels in the early phase of AKI is important, especially in SAAKI.
Objectives: This study aims to analyze the levels of syndecan-1, angiopoietin-2, and urine neutrophil gelatinase-associated lipocalin (uNGAL) as other modalities that might detect AKI earlier in children with SAAKI.
Methods: This study enrolled 23 children between one month and 18 years with sepsis. Blood samples were collected from all patients at the baseline, 12, 24, and 48 hours after admission to assess serum creatinine, syndecan-1, angiopoietin-2, and a urine sample was collected to measure uNGAL levels. We used the Wilcoxon signed-rank test to compare each biomarker with the time of measurement and the Mann-Whitney test to compare the levels of biomarkers with the incidence of SA-AKI.
Results: The highest median value for uNGAL was 78.30 (3.20 - 24098.40) in the 12th-hour measurement; for syndecan was 2.92 (0.06 - 83.00) in the baseline measurement, slightly decreased and continued to increase up to 48 hours, and for Ang-2 was 4159.60 (17.60 - 226428.00) in the 12th-hour measurement. The incidence of SA-AKI based on the pediatric risk-injury-failure-loss-end stage (pRIFLE) criteria were that five children (21.7%) had AKI in the risk/injury phase at baseline, 24-hour, 48-hour observations, and also six children (26.1%) at 12-hour observation.
Conclusion: There was an increase in levels of syndecan-1 and ang-2 in children with severe sepsis, especially in the first 24 hours.

Keywords: Eendothelial Injury, Syndecan-1, Angiopoietin-2, uNGAL, Ppediatric Sepsis-Associated Acute Kidney Injury

1. Background

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, represents a severe disorder, and is a significant cause of death among children even in advanced countries; approximately 40% of the inflicted patients experience septic shock during hospitalization. Sepsis is associated with altered microhemodynamics and heterogeneous local perfusion, micro thrombosis, endothelial dysfunction, alteration of permeability, and interstitial fluid shift (1, 2). Different etiologies are involved in sepsis, such as haemato-oncology, neurology, gastro-hepatology, cardiology, pediatric, surgery, and skin infections. Furthermore, various infections can cause sepsis, and an infected umbilical cord is one of these causes that can lead to cellulitis, omphalitis, and eventually sepsis (3).

The endothelial glycocalyx is a complex layer that coats the luminal surface of the microvascular endothelium (1, 4). The inflammatory insult caused by infection and excessive resuscitation fluids can damage endothelial glycocalyx. This leads to capillary leakage, inflammation, platelet aggregation, coagulation, and loss of vascular tone (5, 6). Increased vascular tone and permeability are associated with leakage of fluids and proteins to extravascular space and cause edema. Circulating levels of syndecan-1 and angiopoietin-2 are related to endothelial damage and glycocalyx degradation. These markers increase in septic conditions and lead to organ dysfunction (1, 2, 7-10).

One of the hallmarks of circulation disruption in severe sepsis is a decrease in blood flow to vital organs, such as the brain and kidneys. Decreased renal blood flow (RBF) can cause acute kidney injury (AKI) (11). This condition is a frequent complication in patients with sepsis, called sepsis-associated AKI (SA-AKI), with a high morbidity and mortality rate. The incidence of AKI due to sepsis ranges between 26 to 50% in developed nations

(12-14).

Urinary neutrophil gelatinase-associated lipocalin (uNGAL) is the most prominent marker of renal epithelial injury that is important as an initial marker in sepsis patients with AKI. This examination can show the onset of AKI in pediatric patients at the intensive care unit approximately two days before serum creatinine levels increase (15-17).

2. Objectives

This study aimed to measure the levels of Syndecan-1, Angiopoietin-2, and uNGAL as markers of endothelial glycocalyx injury in pediatric sepsis-associated AKI conditions. Considering the limitations of previous studies linking syndecan-1 and angiopoietin-2 with SAAKI in children, this study seems imperative. Since both syndecan-1 and angiopoietin are glycocalyx proteins, it is important to investigate whether all SAAKI in children has the potential to cause secondary nephrotic syndrome, which of course, has the potential to develop into chronic kidney disease. Therefore, it may be used as an initial sepsis marker and the right therapeutic target.

3. Methods

This was a cross-sectional study conducted from May 2017 to August 2017. Our study protocol has been reviewed and released by the Health Research Ethical Committee of the Faculty of Medicine, Universitas Padjadjaran. Before recruiting the subjects, we provided information about this study to parents or guardians. We enrolled subjects and performed further examinations provided that they had signed an agreement. Eligible subjects were children with sepsis according to the specific criteria in the emergency department, High Care Unit (HCU), and Paediatric Intensive Care Unit (PICU) at Hasan Sadikin General Hospital, age older than one month to 18 years, and good nutritional status. They would terminate the study if their parents decided or the subjects died before completing the sampling. Sepsis screening in this study was based on the criteria of quick sepsis organ failure assessment (qSOFA) and quick pediatric logistic organ dysfunction-2 (qPELOD-2). Acute kidney injury classification is a risk, injury, failure, and end-stage renal disease (RIFLE) criteria, then modified to pediatric RIFLE (p-RIFLE) by calculating levels of estimated creatinine clearance and urine output. Exclusion criteria included patients with previous kidney disorders. The sample was consecutively collected, with the total number of samples being 23, which was calculated using a relationship analysis formula.

All samples were given fluid resuscitation based on the guidelines for pediatric sepsis (The American College of Critical Care Medicine-Pediatric Advanced Life Support (ACCM-PALS) 2017), i.e., initial fluid resuscitation was given up to 60 mL/kg and continued with maintenance fluid (18).

3.1. Laboratory Samples

Measurement of syndecan-1 and angiopoietin-2 levels was conducted on venous blood samples. Sterile syringes were inserted into a vein, and 3 - 5 mL of blood was drawn. The blood was poured into labeled ethylenediaminetetraacetic acid (EDTA) tubes; the enzyme-linked immunosorbent assay (ELISA) method was employed.

To measure uNGAL, 5 - 10 mL urine samples were collected, labeled, and stored at -20°C before ultimately being examined with an R&D kit using the ELISA method. Both blood and urine samples were examined four times, at 0, 12, 24, and 48 hours after the sepsis diagnosis.

3.2. Statistical Analysis

The characteristics of the research subjects and the marker levels were presented in numbers and percentages for categorical data (gender). Numerical data (age, levels of uNGAL, angiopoietin-2, and syndecan-1) were presented in mean, standard deviation, median, minimum, and maximum values. Wilcoxon signed-rank test was used to compare each biomarker at the time of measurement, and the Mann-Whitney test was employed to compare biomarkers levels with the incidence of SA-AKI. Data analysis was performed using Statistical Product and Service Solution (SPSS) for Windows program version 18.0, with the sample size being calculated using this formula:

$$n = 0.5 \left(Z_{1-\frac{\alpha}{2}}\right)^2 \sigma^2$$

Notes: $Z_{1-\frac{\alpha}{2}}$ = Standard normal variate; σ = Standard error/precision

4. Results

4.1. Patient Characteristics

During this study, 190 children were diagnosed with sepsis, 36 subjects were enrolled, and 13 were dropped out because of death. Therefore, the final number of subjects was 23. The general characteristics of the subjects are presented in Table 1. Age, sex, and height were not proven to be confounding variables in this study (P-value $>$ 0.05). The causes of sepsis in the present study were bronchopneumonia and infection-related oncologic diseases.

Table 1. Characteristics of the Subjects [a]

Characteristics	Patients (n = 23)	P-Value (Toward uNGAL)	P-Value (Toward GFR)
Age, y	6.52 ± 4.630 [7 (1 - 14)]	0.562	0.182
Sex		0.740 [b]	0.525 [b]
Male	11 (47.8)		
Female	12 (52.2)		
Height, cm	107.19 ± (27.275) [108 (67 - 160)]	0.612	0.345
Etiology			
Haemato-oncology	14 (60.9)		
Neurology	4 (17.4)		
Gastro-hepatology	2 (8.7)		
Cardiology	1 (4.3)		
Infection	1 (4.3)		
Pediatric surgery	1 (4.3)		

[a] Values are expressed as mean ± SD [median (min-max)] or No. (%). P = significance (P < 0.05)
[b] Based on the Mann-Whitney test.

Table 2. Description of Urine NGAL, Syndecan-1, Angiopoietin-2 (Ang-2) at 0 Hours, 12 Hours, 24 Hours, and 48 Hours [a]

Variables	Baseline (0)	12 h	24 h	48 h
uNGAL, ng/mL	25.50 (3.90 - 24629.60)	78.30 (3.20 - 24098.40)	60.30 (4.80 - 17593.60)	53.50 (2.80 - 3189.60)
Syndecan-1, ng/mL	2.92 (0.06 - 83.00)	2.07 (0.03 - 115.90)	2.34 (0.05 - 118.00)	2.55 (0.05 - 52.40)
Angiopoeitin (Ang-2), pg/mL	3964.70 (19.60 - 59813.00)	4159.60 (17.60 - 226428.00)	3466.60 (38.80 - 226428.00)	3131.50 (24.30 - 226514.00)

[a] Values are presented as median (range).

4.2. The Levels of uNGAL, Syndecan-1, and Angiopoietin-2

Table 2 tabulates the values of uNGAL, syndecan-1, and angiopoietin-2 four times (0, 12, 24, and 48 hours). The highest median value was 78.30 (3.20 - 24098.40) in the 12th-hour measurement for uNGAL, 2.92 (0.06 - 83.00) in the baseline measurement, slightly decreased, and continued to increase up to 48 hours for syndecan-1, and 4159.60 (17.60 - 226428.00) in the 12th-hour measurement for ang -2. The comparison test using the Wilcoxon (Table 3) among three biomarkers with several measurement times demonstrated no significant differences between the four measurement times. However, only a-2 on the measurements of 12 hours vs. 48 hours (P = 0.068) and 24 hours vs. 48 hours (P = 0.029) had a significant comparison with a tendency to decrease.

4.3. Correlation Between Biomarkers of Sepsis and Acute Kidney Injury

Table 4 shows the incidence of SAAKI based on the pRIFLE criteria: 5 children (21.7%) had AKI in the risk/injury phase at baseline, 24-hour, and 48-hour observations, and 6 children (26.1%) at 12-hour observation. Comparison of the levels of uNGAL, syndecan-1, and ang-2 based on SAAKI in Table 5 shows that uNGAL was the only biomarker that had a significant effect on SAAKI events at four-time observations, with the following P-values: 0.046, 0.008, 0.001, and 0.009.

Table 5 shows the cut-off value for uNGAL in diagnosing SAAKI in our study. This marker can be used earlier on the 0-hour observation with 80% sensitivity and 72.2% specificity with the area under the ROC curve (AUC) of 0.8. The best AUC value was 0.956, found in 24-hour observation with 80% sensitivity and 100% specificity.

5. Discussion

Under normal circumstances, the glycocalyx layer on the blood vessel endothelium maintains homeostasis of the vasculature, including controlling vascular permeability and microvascular tone, preventing microvascular thrombosis, and regulating leukocyte adhesion. However, if sepsis occurs, there will be degradation via inflammatory mechanisms such as metalloproteinases, heparanase, and hyaluronidase. Reactive oxygen species and pro-inflammatory cytokines activate these sheddases. This condition leads to capillary

Table 3. Comparison of uNGAL, Syndecan-1, and Ang-2 as Sepsis Biomarkers from Various Measurements

Comparison Between Measurement Times, h	P-Value [a]		
	uNGAL	Syndecan-1	Angiopoetin
0 vs. 12	0.316	0.783	0.879
0 vs. 24	0.064	0.191	0.784
0 vs. 48	0.274	0.513	0.330
12 vs. 24	0.465	0.181	0.584
12 vs. 48	0.447	0.323	0.068
24 vs. 48	0.919	0.891	0.029

[a] Based on the Wilcoxon test

Table 4. The Incidence of SAAKI

	Time Measurement			
	Baseline (0)	12 h	24 h	48 h
Injury/risk	5 (21.7)	6 (26.1)	5 (21.7)	5 (21.7)
Normal	18 (78.3)	17 (73.9)	18 (78.3)	18 (78.3)

[a] Values are expressed as No. (%).

Table 5. Comparison of the Levels of uNGAL, Syndecan-1, and Ang-2 Based on SAAKI

Variables	pRIFLE Criteria		P-Value [a]
	Injury/Risk (n = 5)	Normal (n = 18)	
1. uNGAL			
Baseline (0)	325.2 (15.5 - 24629.6)	20.9 (3.9 - 685.0)	0.046 [b]
12 h	552.9 (17.5 - 24098.4)	24.5 (3.2 - 173.4)	0.008 [b]
24 h	11178.9 (120.6 - 17593.6)	41.4 (4.8 - 389.3)	0.001 [b]
48 h	892.6 (102.5 - 2817.6)	36.6 (2.8 - 3189.6)	0.009 [b]
2. Syndecan-1			
Baseline (0)	2.93 (1.04 - 38)	2.36 (0.06 - 83.0)	0.745
12 h	1.65 (1.12 - 115.9)	2.22 (0.03 - 42.65)	1.0
24 h	22.34 (0.98 - 53.2)	2.5 (0.05 - 118)	1.0
48 h	2.09 (0.46 - 42.54)	2.59 (0.05 - 52.4)	0.914
3. Ang-2			
Baseline (0)	35095 (3588.3 - 58660)	2251.6 (19.6 - 59813)	0.150
12 h	8938.9 (813.8 - 226428)	3513 (17.6 - 57124)	0.473
24 h	18130 (3304.6 - 226426)	2874.9 (38.8 - 226428)	0.199
48 h	5782.3 (2848.6 - 52580)	2656.5 (24.3 - 226514)	0.290

[a] Based on the Mann-Whitney test
[b] Significant

leakage, inflammation, platelet aggregation, coagulation, and loss of vascular tone (19)

The results of this study showed that pediatric patients with sepsis had the highest median syndecan-1 level of 2.92 (0.06 - 83.00) ng/mL in the baseline measurement, slightly decreased, and continued to increase up to 2.55 (0.05 - 52.40) ng/mL at the 48th-hour observation. Previous research at the pediatric ICU (PICU) Cipto Mangunkusumo Hospital, Indonesia, showed that the level of syndecan-1 in pediatric sepsis was 83.40 (10.10 - 2257.91) ng/mL compared to the controls 27.7 ng/mL and that this value increased in the first three days and began to decline on the seventh day (20). Another research on the adult population at an intensive care unit (ICU) in Sweden showed that the median level of syndecan-1 in septic shock patients was 246 (180 - 496) ng/mL compared to controls 26 (23 - 31) ng/mL, which were not correlated with mortality (21). The levels of syndecan-1 in our study did not show significant differences between the four observation times. This can be caused by biomarker examinations were only carried out on the initial day of sepsis, and more extended observations were needed. The level of syndecan-1 was in a wide range, which is the same as in previous studies. It can occur due to the variety of disease severity. Glycocalyx damage can range from discrete disturbances in the composition of the entire luminal layer to excessive destruction and degradation, with the loss of the entire glycocalyx. Furthermore, the difference in glycocalyx thickness, basal turnover, speed, and degradation severity can affect this marker's level (20).

Research in an adult population with severe sepsis in China showed a correlation between increased levels of syndecan-1 with fluid overload. Positive fluid balance at 24 hours was significantly higher in sepsis patients who died than in those who survived (4). This was related to two phases of sepsis, the early-ebb phase and the late-flow phase. During the ebb phase, there was low cardiac output, inadequate tissue perfusion, and the patient was cold and clammy, which occurred in the early days. The ebb consists of a phase of resuscitation, optimization, and stabilization. In the initial phase, it was necessary to administer a fluid bolus of 60 mL/kg with the aim of early goal-directed fluid management, and the outcome was positive fluid balance, and normal range means arterial pressure (MAP)(22). It is crucial to carry out close monitoring in that initial phase. The inflammatory process and decreased tissue perfusion due to sepsis can cause glycocalyx degradation and can be aggravated by the administration of excessive fluids. Intravascular hypervolemia causes the release of atrial natriuretic peptide and rapidly causes matrix metalloproteinases-mediated digestion of the endothelial glycocalyx. The capillary leak inherent to sepsis promotes the extravasation of large amounts of fluid, inducing relative central hypovolemia that often requires further fluid administration. Capillary leak causes excessive fluid accumulation in the interstitial part, generating anasarca and end-organ edema, causing organ dysfunction. Therefore, stabilizing glycocalyx could be a new therapeutic target for patients with sepsis (4, 23).

The Angiopoietin protein is a family of endothelium-derived angiogenic factors that potently affect the vascular endothelium. Angiopoietins interacted with their cognate tyrosine kinase receptor, Tie2, expressed on the luminal endothelium. When bound by Angiopoietin-1 (Ang-1), Tie2 endothelial signaling promotes quiescence by enhancing cell survival and regulating cell adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM- 1). Endothelial injury due to inflammation or hypoxia stimulates the exocytosis of endothelial Weibel-Palade bodies and the release of Angiopoietin-2 (Ang-2), competitive antagonists of Ang-1 (24). In this study, we found that the highest median levels of Ang-2 occurred at the 12th observation at 4159.60 (17.60 - 226428.00) pg/mL and had a tendency to decrease significantly on the measurements of 12 h vs. 48 days (P = 0.068) and 24 h vs. 48 days (P = 0.029). A previous study stated that the median plasma level of Ang-2 was significantly higher among young infants (0-59 days) with suspected sepsis compared to those who survived (5.4 ng/mL or 5.400 pg/mL vs. 3.3 ng/mL to 3.300 pg/mL) (24). Another study by Melendez et al. (25) demonstrated that the median level of Ang-2 was higher in those with pediatric septic shock vs. those with sepsis, 8,235 pg/mL vs. 5,659 pg/mL. This value was associated with a high mortality rate (24). However, until now, there has been no cut-off value of Ang-2 for healthy and septic populations.

One of the hallmarks of circulation disruption in severe sepsis is a decrease in blood flow to vital organs such as kidneys that causes AKI (11). Neutrophil gelatinase-associated lipocalin (NGAL), a 25 kDa protein produced by injured nephron epithelia, is one of the most prominent markers of renal epithelial injury (26). In contrast to serum creatinine and urinary output, which are measures of kidney function, NGAL is specifically induced in the damaged nephron and appears two days earlier than the increase in creatinine levels. This test can also be used as a marker of multiple organ dysfunction syndromes (MODS), similar to C-Reactive Protein (CRP), procalcitonin, or IL-6 (27, 28). Until now, no reference value has been set for uNGAL levels. Urine neutrophil gelatinase-associated lipocalin is the only biomarker that has significant effects on SA-AKI events at four-time observations compared with syndecan-1 and Ang-2 in

Table 6. Cut Off Value for uNGAL to Predict SAAKI

Time, h	Cut Off Point	Sensitivity, %	Specificity, %	Area Under ROC, %	P-Value
0	> 38.7	80.0	72.2	80.0	0.019
12	> 173.4	66.7	100	86.3	< 0.001
24	> 389.3	80.0	100	95.6	< 0.001
48	> 96.5	100.0	72.2	87.8	< 0.001

our study, with following P-values of 0.046, 0.008, 0.001; and 0.009. The highest level of uNGAL was 1901.35 ± 4786,354 ng/mL, which occurred in the first 24 hours. Table 6 showed that the marker could be used earlier on the 0-hour observation with 80% sensitivity and 72.2% specificity (cut-off point > 38.7 ng/mL) with the area under the ROC curve (AUC) of 0.8. The best AUC value was 0.956, found in 24-hour observation with 80% sensitivity and 100% specificity (cut-off point > 389.3 ng/mL). This result resembles previous research in 2010 - 2014, which concluded that the peak increase in uNGAL levels occurred at hours 6 to 12 with a limit of more than 126 ng/mL, and concluded that this level could be used to detect AKI (28). Another research conducted in Iran in 2012 on pediatric patients treated in PICU concluded that the optimum value of uNGAL for detecting AKI on treatment days to 1 to 3 days later was 65.82 ng/mL - 66.81 ng/ml (29). It is expected that these markers can be used earlier in diagnosing and managing SA-AKI so that mortality and morbidity rates for pediatric sepsis can be decreased. Syndecan and angiopoietin-2 levels were immediately high when the child was clinically diagnosed with SAAKI, but after that, they did not increase significantly even though SAAKI was still ongoing in contrast to uNGAL, which, apart from having high levels immediately when a child is diagnosed with SAAKI, levels also increase in children with SAAKI whose condition has not improved. Syndecan, angiopoietin-2, and uNGAL can be used as early screening modalities for SAAKI, but only uNGAL can be used to monitor whether or not an SAAKI is improving.

This study has several limitations; there were few samples and a lack of data regarding the amount of fluid resuscitation had been given to each patient, the degree of sepsis, and also a measurement of a fluid overload until the end of the study (48 hours). Our study did not measure biomarker levels in healthy populations as a cut-off point. Therefore, further research is needed with a larger population by assessing the cut-off of these biomarkers.

5.1. Conclusions

There was an increase in levels of glycocalyx degradation markers, such as syndecan-1 and Ang-2, in pediatrics with severe sepsis, especially in the first 24 hours. Besides, the level of uNGAL, a parameter of kidney function disturbance, also increased in the first 24 hours. So, these biomarker levels can be considered early diagnosis and therapeutic targets in sepsis.

Footnotes

Authors' Contribution: DH conceptualized the idea and supervised the research, FM and AW collected data and wrote the manuscript, and DR provided the research funds and supervised the research and manuscript drafting.

Conflict of Interests: The authors declare no conflicts of interest.

Data Reproducibility: The information provided in this research is included as a supplemental file during submission and can be accessed by readers upon request. It is openly available for anyone who desires to review it.

Ethical Approval: Our study protocol has been reviewed and released by the Health Research Ethical Committee of the Faculty of Medicine, Universitas Padjadjaran. (1159/UN6.KEP/EC/2017)

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References

1. Chelazzi C, Villa G, Mancinelli P, De Gaudio AR, Adembri C. Glycocalyx and sepsis-induced alterations in vascular permeability. *Crit Care*. 2015;**19**(1):26. [PubMed ID: 25887223]. [PubMed Central ID: PMC4308932]. https://doi.org/10.1186/s13054-015-0741-z.
2. Martin L, Koczera P, Zechendorf E, Schuerholz T. The Endothelial Glycocalyx: New Diagnostic and Therapeutic Approaches in Sepsis. *Biomed Res Int*. 2016;**2016**:3758278. [PubMed ID: 27699168]. [PubMed Central ID: PMC5028820]. https://doi.org/10.1155/2016/3758278.
3. Siroosbakht S, Aarabi N, Rezakhaniha B. Bathing or Not Bathing: Which Is Better for Umbilical Cord Separation Time and Bacterial Colonization in Neonates? *Arch Pediatr Infect Dis*. 2020;**9**(2). https://doi.org/10.5812/pedinfect.104100.
4. Wu X, Hu Z, Yuan H, Chen L, Li Y, Zhao C. Fluid Resuscitation and Markers of Glycocalyx Degradation in Severe Sepsis. *Open Med (Wars)*. 2017;**12**:409–16. [PubMed ID: 29318186]. [PubMed Central ID: PMC5757348]. https://doi.org/10.1515/med-2017-0059.

5. Seratin F, Widiasta A, Adrizain R, Hilmanto D. An evaluation of kidney dysfunction as a common symptom of typhoid infection in an endemic country: A rare case study. *IDCases*. 2022;**29**. e01580. [PubMed ID: 35942255]. [PubMed Central ID: PMC9356191]. https://doi.org/10.1016/j.idcr.2022.e01580.
6. Risan NA, Permana DA, Widiasta A, Dewi MM, Hilmanto D. Successful Therapy of Infarct Stroke with Nephrotic Syndrome in Children: A Rare Case Report. *J Compr Pediatr*. 2023;**14**(2). https://doi.org/10.5812/compreped-135106.
7. Murphy LS, Wickersham N, McNeil JB, Shaver CM, May AK, Bastarache JA, et al. Endothelial glycocalyx degradation is more severe in patients with non-pulmonary sepsis compared to pulmonary sepsis and associates with risk of ARDS and other organ dysfunction. *Ann Intensive Care*. 2017;**7**(1):102. [PubMed ID: 28986821]. [PubMed Central ID: PMC5630541]. https://doi.org/10.1186/s13613-017-0325-y.
8. Sallisalmi M, Tenhunen J, Yang R, Oksala N, Pettila V. Vascular adhesion protein-1 and syndecan-1 in septic shock. *Acta Anaesthesiol Scand*. 2012;**56**(3):316–22. [PubMed ID: 22150439]. https://doi.org/10.1111/j.1399-6576.2011.02578.x.
9. Widiasta A, Wahyudi K, Sribudiani Y, Rachmadi D. The level of transforming growth factor-beta as a possible predictor of cyclophosphamide response in children with steroid-resistant nephrotic syndrome. *Biomedicine (Taipei)*. 2021;**11**(3):68–75. [PubMed ID: 35223413]. [PubMed Central ID: PMC8823489]. https://doi.org/10.37796/2211-8039.1205.
10. Widiasta A, Wahyudi K, Nugrahapraja H, Sribudiani Y, Rachmadi D. The Unique Difference Between Serum Level of Soluble Urokinase Plasminogen Activator Receptor (suPAR) in Steroid-Resistant Nephrotic Syndrome Children Treated with an Alkylating Agent and Calcineurin Inhibitors. *J Compr Pediatr*. 2021;**12**(2). https://doi.org/10.5812/compreped.109912.
11. Song JW, Zullo J, Lipphardt M, Dragovich M, Zhang FX, Fu B, et al. Endothelial glycocalyx-the battleground for complications of sepsis and kidney injury. *Nephrol Dial Transplant*. 2018;**33**(2):203–11. [PubMed ID: 28535253]. [PubMed Central ID: PMC5837430]. https://doi.org/10.1093/ndt/gfx076.
12. Ashraf M, Shahzad N, Irshad M, Hussain SQ, Ahmed P. Pediatric acute kidney injury: A syndrome under paradigm shift. *Indian J Crit Care Med*. 2014;**18**(8):518–26. [PubMed ID: 25136191]. [PubMed Central ID: PMC4134626]. https://doi.org/10.4103/0972-5229.138156.
13. Li L, Bonventre JV. Endothelial Glycocalyx: Not Just a Sugar Coat. *Am J Respir Crit Care Med*. 2016;**194**(4):390–3. [PubMed ID: 27525457]. [PubMed Central ID: PMC5441087]. https://doi.org/10.1164/rccm.201603-0624ED.
14. Alobaidi R, Basu RK, Goldstein SL, Bagshaw SM. Sepsis-associated acute kidney injury. *Semin Nephrol*. 2015;**35**(1):2–11. [PubMed ID: 25795495]. [PubMed Central ID: PMC4507081]. https://doi.org/10.1016/j.semnephrol.2015.01.002.
15. Fodor R, Grigorescu B, Veres M, Orlandea M, Badea J, Hlavathy K, et al. Plasma Neutrophil Gelatinase Associated Lipocalin (NGAL) - Early Biomarker for Acute Kidney Injury in Critically Ill Patients. *J Crit Care Med (Targu Mures)*. 2015;**1**(4):154–61. [PubMed ID: 29967824]. [PubMed Central ID: PMC5953298]. https://doi.org/10.1515/jccm-2015-0023.
16. Bagshaw SM, Bennett M, Haase M, Haase-Fielitz A, Egi M, Morimatsu H, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med*. 2010;**36**(3):452–61. [PubMed ID: 19956924]. https://doi.org/10.1007/s00134-009-1724-9.
17. Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. *Atherosclerosis*. 2012;**222**(1):8–14. [PubMed ID: 22137664]. https://doi.org/10.1016/j.atherosclerosis.2011.10.044.
18. Kawasaki T. Update on pediatric sepsis: a review. *J Intensive Care*. 2017;**5**:47. [PubMed ID: 28729906]. [PubMed Central ID: PMC5518149]. https://doi.org/10.1186/s40560-017-0240-1.
19. Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care*. 2019;**23**(1):16. [PubMed ID: 30654825]. [PubMed Central ID: PMC6337861]. https://doi.org/10.1186/s13054-018-2292-6.
20. Saragih RA, Pudjiadi AH, Tambunan T, Satari HI, Aulia D, Bardosono S, et al. Correlation between urinary albumin to creatinine ratio and systemic glycocalyx degradation in pediatric sepsis. *Med J Indones*. 2018;**27**(3):194–200. https://doi.org/10.13181/mji.v27i3.2156.
21. Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. *Shock*. 2008;**30**(6):623–7. [PubMed ID: 18497712]. https://doi.org/10.1097/SHK.0b013e3181777da3.
22. Jaehne AK, Rivers EP. Early Liberal Fluid Therapy for Sepsis Patients Is Not Harmful: Hydrophobia Is Unwarranted but Drink Responsibly. *Crit Care Med*. 2016;**44**(12):2263–9. [PubMed ID: 27749314]. [PubMed Central ID: PMC5113226]. https://doi.org/10.1097/CCM.0000000000002145.
23. Malbrain M, Van Regenmortel N, Saugel B, De Tavernier B, Van Gaal PJ, Joannes-Boyau O, et al. Principles of fluid management and stewardship in septic shock: it is time to consider the four D's and the four phases of fluid therapy. *Ann Intensive Care*. 2018;**8**(1):66. [PubMed ID: 29789983]. [PubMed Central ID: PMC5964054]. https://doi.org/10.1186/s13613-018-0402-x.
24. Wright JK, Hayford K, Tran V, Al Kibria GM, Baqui A, Manajjir A, et al. Biomarkers of endothelial dysfunction predict sepsis mortality in young infants: a matched case-control study. *BMC Pediatr*. 2018;**18**(1):118. [PubMed ID: 29571293]. [PubMed Central ID: PMC5866512]. https://doi.org/10.1186/s12887-018-1087-x.
25. Melendez E, Whitney JE, Norton JS, Silverman M, Harju-Baker S, Mikacenic C, et al. Systemic Angiopoietin-1/2 Dysregulation in Pediatric Sepsis and Septic Shock. *Int J Med Sci*. 2019;**16**(2):318–23. [PubMed ID: 30745813]. [PubMed Central ID: PMC6367536]. https://doi.org/10.7150/ijms.27731.
26. Djalil DL, Widiasta A, Ghrahani R, Hilmanto D, Rachmadi Dedi. Therapeutic plasma exchange as a promising therapeutic modality in the management of acute cytokine storms and secondary prevention of chronic kidney disease in children during pandemic. *Int J Pharm Res*. 2021;**13**(2). https://doi.org/10.31838/ijpr/2021.13.02.259.
27. Singer E, Marko L, Paragas N, Barasch J, Dragun D, Muller DN, et al. Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. *Acta Physiol (Oxf)*. 2013;**207**(4):663–72. [PubMed ID: 23375078]. [PubMed Central ID: PMC3979296]. https://doi.org/10.1111/apha.12054.
28. Zwiers AJ, de Wildt SN, van Rosmalen J, de Rijke YB, Buijs EA, Tibboel D, et al. Urinary neutrophil gelatinase-associated lipocalin identifies critically ill young children with acute kidney injury following intensive care admission: a prospective cohort study. *Crit Care*. 2015;**19**(1):181. [PubMed ID: 25895828]. [PubMed Central ID: PMC4422047]. https://doi.org/10.1186/s13054-015-0910-0.
29. Merrikhi A, Gheissari A, Mousazadeh H. Urine and serum neutrophil gelatinase-associated lipocalin cut-off point for the prediction of acute kidney injury. *Adv Biomed Res*. 2014;**3**:66. [PubMed ID: 24627874]. [PubMed Central ID: PMC3950801]. https://doi.org/10.4103/2277-9175.125847.