

Risk factors for poor short-term outcomes of children with dialysis-dependent hemolytic uremic syndrome in Kazakhstan : a retrospective cohort study

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ABSTRACT

Background: Diarrhea-associated Hemolytic Uremic Syndrome (D+HUS) is one of the important causes of pediatric Acute Kidney Injury (AKI) consequences of which may lead to long-term renal impairment, such as development of CKD/ESRD. This single-center study aimed to investigate the risk factors for poor short-term outcomes in Kazakhstani children with D+HUS who required dialysis during the course of the disease from years 2008-2024.

Methods: Data of 89 pediatric patients with clinically diagnosed D+HUS who underwent dialysis was analysed retrospectively. The outcomes of interest included absence of kidney function recovery and acute-phase death. Risk factors were identified with the use of the linear and logistic regression models.

Results: Acute-phase mortality rate in our study was 6.7% (n=6), and the rate of renal function non-recovery was 10.1% (n=9). Sepsis and leukocytosis $>15\ 000/L$ on 10th day after dialysis initiation were identified as risk factors for acute-phase mortality in our study. For renal non-recovery, prolonged anuria (>10 days) and dialysis (>20 days), presence of sepsis, neurological involvement, leukocytosis on admission and on 10th day after dialysis start $>15\ 000/L$, and ionized calcium levels $<1.1\ mmol/l$ were identified as risk factors.

Conclusion: In our study, the main determinants for acute-phase mortality were sepsis and leukocytosis. Prolonged anuria and dialysis, increased leukocyte count on admission and 10th day of dialysis, along with extra-renal involvement (sepsis and neurological involvement) were predictors of poor renal outcome during the acute phase of the disease.

INTRODUCTION

Hemolytic uremic syndrome (HUS) is one of the important causes of pediatric acute kidney injury (AKI) that is diagnosed based on the presence of a clinical triad of thrombotic microangiopathy, hemolytic anemia and AKI (Noris & Remuzzi, 2005). Classification of the disease is based on etiological agent, with typical being due to disease emergence following a Shiga-toxin producing E.Coli (STEC) infection, whereas atypical HUS (aHUS) arises due to genetic and subsequent functional alterations in the complement system (Fakhouri et al., 2017). A central role in STEC-HUS is attributed to shiga-toxins (stx1/stx2) exerting their effects via binding on Gb3 receptors in target tissues, including the kidneys (Zumbrun et al., 2010). The toxins cause endothelial injury by induction of necrosis or apoptosis of the endothelial cells

(Bauwens et al., 2011), induction of platelet activation by binding and internalization in platelets (stx1) (Karpman et al., 2001). Platelet involvement and endothelial injury in the pathogenesis are crucial steps related to development of thrombotic microangiopathy (TMA), a cornerstone responsible for renal and extra-renal damage, as well as for the clinical picture seen in STEC-HUS. The neurological involvement in STEC-HUS is one of the common findings in affected patients, and the presentation is variable: encephalopathy, seizures and stroke may develop in the acute phase of the disease as manifestations of neurological involvement (Brown et al., 2021). Cardiac involvement rate in STEC-HUS varies between 1.9-14% (Luna et al., 2020; Oualha et al., 2018; Sanders et al., 2021). Cardiac involvement may manifest as myocarditis (Tanné et al., 2022), cardiac arrest and pericardial effusion (Sanders et al., 2021). From gastrointestinal involvement, variants include bowel stenosis, rectal prolapse and bowel perforation (Giordano et al., 2024). Mortality rates from STEC-HUS are up to 4% in the acute period, and detrimental consequences to kidney function include development of Chronic Kidney Disease (CKD) or End-Stage Renal Disease (ESRD), hypertension and proteinuria (Spinale et al., 2013). Risk factors for poor short-term outcomes in D+HUS reported in the literature include anuria lasting >10 days (Oakes et al., 2008), dialysis duration being >10 days (Alconcher et al., 2023, VanSickle et al., 2018), as well as leukocytosis (Mody et al., 2015), and presence of neurological involvement (Brown et al., 2021; Oakes et al., 2006).

The purpose of this study was the investigation of risk factors for poor short-term outcomes (death/kidney function non-recovery) in Kazakhstani pediatric patients affected with D+HUS requiring dialysis support.

METHODS

This is a single-center retrospective cohort study where laboratory and clinical data was investigated from a population of pediatric patients hospitalized with a diagnosis of typical HUS to the Mother and Child Center, Astana city, in the period between years 2008 - 2024 in the acute phase of typical HUS. The medical records of patients diagnosed with typical HUS were retrieved for analysis. Patients who did not receive dialysis or had missing data were excluded from the analysis. The data was collected from medical records from first admissions from primary hospital and our center. The collected data included demographic data (age, gender, place of residence), laboratory values (common blood count, biochemistry) on admissions to regional hospital, our

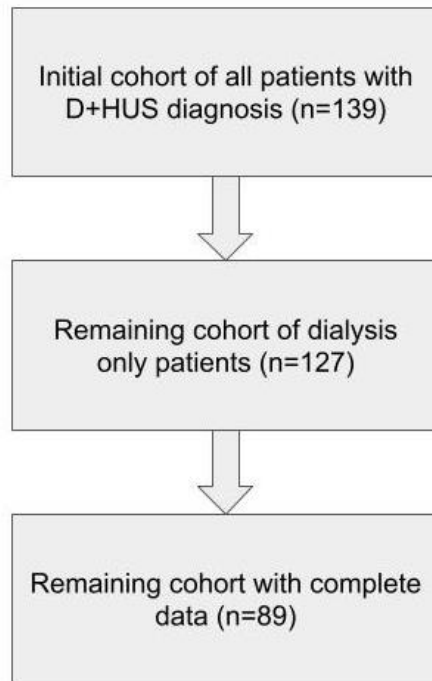
center, 10 days after initiation of dialysis and on discharge, along with dialysis status, type and duration. Other variables included extra-renal involvement - gastrointestinal (excluding diarrhea and vomiting), CNS involvement (seizures, coma, encephalopathy, ischemic/hemorrhagic stroke), and cardiovascular involvement (arrhythmias, carditis, need for vasopressor support) on onset, 10 days after dialysis start and on discharge, and respiratory involvement on disease onset (including pneumonia and other viral/bacterial upper and lower respiratory tract infections). The outcomes of interest were acute phase non-recovery of renal function defined by requirement of dialysis by discharge day, or acute phase death. Notably, non-recovery of kidney function was a composite outcome that has included those patients who died, as well as those who were on dialysis by discharge moment.

Statistical Analysis

For continuous variables, Kolmogorov - Smirnov test was used for normality assessment for guidance of descriptive statistics reports, with normally distributed variables being reported as Mean $\pm$ SD, and non-normally distributed variables being reported as Median (IQR). Logistic regression models were used for the estimation of associations between independent variables and outcomes. In addition, linear regression models were used for the analysis of relationships between continuous variables and outcomes. All logistic regression models were then adjusted to age and gender. Statistical significance was defined as p-value <0.05 . Results were reported as Odds Ratios (OR) for logistic regression models with 95% confidence intervals. All statistical analysis was performed using STATA 19 software. The study was approved by the Institutional Ethics Board of the University Medical Center on 12.02.2025, protocol #2025/01/ПЭ, assigned number 2025/03-002.

RESULTS

We have identified 139 patients with D+ HUS in our study. However, 50 patients were excluded from analysis due to not receiving dialysis or lacking data. The remaining cohort of 89 patients was analyzed.



The median age in our cohort was 1.7 years (1.12-2.83), and females comprised 51.7% of the group (n=46). The time to the development of decreased urine output (anuria or oliguria) was 4 days (3-5), and median duration of anuria/oliguria was 9 days (6-14). Neurological involvement during the entire disease course was present in 52.81% of the group. Sepsis has developed in 9 patients (10.11%), and 4 of these patients died. The acute phase mortality rate in our cohort was 6.74%. Three patients (3.37%) still required dialysis by the moment of discharge.

Table 1. Baseline cohort characteristics	
Characteristic	Cohort (n=89)
Age (years)	1.703 (1.123-2.839)
Body mass index, kg/m ²	15.9 (14.6 - 17.6)
Gender (F), (%)	46 (51.69)
Diarrhea, n %	84 (94.38)
Bloody diarrhea, %	44 (49.44)

Edema on admission, %	75 (85.23)
Hypertension on admission, %	50 (58.82)
Neurological involvement at onset, %	30 (33.71)
Gastrointestinal involvement at onset, %	43 (48.31)
Cardiovascular involvement at onset, %	4 (4.49)
NSAID ¹ use at onset, %	10 (11.24)
Antibiotic use at onset, %	27 (30.34)
Time to anuria/oliguria, days	4 (3-5)
Total days of anuria/oliguria	9 (6-14)
Dialysis type	
Peritoneal dialysis	25 (28.09)
Hemodialysis	28 (31.46)
Continuous renal replacement therapy	5 (5.62)
Mixed	31 (34.83)
Days to dialysis	2 (2-3)
Dialysis duration, days	14 (7-21)
eGFR ² in admission, ml/min/1.73m ²	11.42 (8.5-16.91)
LABORATORY VALUES ON ADMISSION	
Hemoglobin, g/l	75.5±16.75
Hematocrit, %	22.1 ± 5
White blood cells, 10 ⁹	13 (9.89-18.38)

Platelets, 10 ⁹	78 (43-125)
C-reactive protein, g/l	14.5 (7.07-35.5)
Duration of hospital stay, days	25 (20-33)
Sepsis during hospitalization, %	9 (10.11)
Died	4
Alive	5
Neurological involvement at any point, %	47 (52.81)
eGFR ² on discharge, ml/min/1.73m ²	
Outcome	
Alive	80 (89.89)
Died	6 (6.74)
Dialysis-dependent	3 (3.37)

Table 1. Baseline cohort characteristics

According to the results of univariate analysis for the kidney function recovery outcome, 8 variables have shown statistically significant association with the outcome. The results are demonstrated in table 2.

Variable	N	Univariate Odds Ratio [95% Confidence Interval] kidney function recovery	P value	Adjusted Odds Ratio [95% Confidence Interval]	P value
Age <1 y.o.	70/89	0.29 [0.07, 1.20]	0.088		
Body Mass Index, kg/m ²	73/73	1.18 [0.85, 1.64]	0.311	1.15 [0.81, 1.64]	0.433
Diarrhea	84/89	quasi separation		quasi separation	

Bloody diarrhea	44/89	0.26 [0.05, 1.32]	0.104	0.27 [0.05, 1.41]	0.119
Fever	40/89	0.98 [0.24, 3.91]	0.975	0.96 [0.23, 3.95]	0.952
Edema	75/88	0.57 [0.10, 3.08]	0.511	0.41 [0.07, 2.54]	0.337
Hypertension	50/85	1.18 [0.26, 5.32]	0.824	1.01 [0.22, 4.73]	0.989
Anuria days before dialysis >2 days	67/89	1.17 [0.22, 6.08]	0.855	1.44 [0.25, 8.13]	0.682
Anuria duration >10 days	43/89	10.29 [1.23, 86.14]	0.032	15.23 [1.62, 143.06]	0.017
Days to anuria/oliguria from disease onset, >3 days	35/89	0.75 [0.17, 3.22]	0.699	0.87 [0.19, 3.96]	0.862
Respiratory involvement	2/89	quasi separation		quasi separation	
Neurological involvement	30/89	2.75 [0.68, 11.12]	0.156	1.91 [0.40, 9.02]	0.412
Gastrointestinal involvement	43/89	0.5 [0.12, 2.14]	0.35	0.37 [0.08, 1.74]	0.209
Cardiac involvement	4/89	quasi separation		quasi separation	
NSAIDs use ¹	10/89	quasi separation		quasi separation	
Antibiotic use	27/89	0.63 [0.12, 3.24]	0.579	0.53 [0.10, 2.91]	0.467
Hemoglobin on admission < 65 g/l	9/68	quasi separation		quasi separation	

Platelets on admission < 80 000/l	21/61	1.16 [0.25, 5.44]	0.844	1.20 [0.25, 5.84]	0.817
White blood cells on admission, > 15 000/l	37/61	1.71 [0.30, 9.67]	0.539	4.64 [0.53, 40.32]	0.164
GFR on admission, < 15 ml/min/1.73m ²	44/63	2.31 [0.25, 21.21]	0.46	2.79 [0.28, 28.21]	0.385
Hemoglobin < 65 g/l UMC	25/89	1.32 [0.30, 5.73]	0.713	1.13 [0.25, 5.21]	0.873
Hematocrit > 23% UMC	36/89	1.97 [0.49, 7.93]	0.377	2.35 [0.55, 9.99]	0.248
White blood cells UMC, > 15 000/l	31/89	4.4 [1.01, 19.03]	0.047	4.54 [1.01, 20.33]	0.048
Platelets < 80 000/l UMC	47/89	0.41 [0.95, 1.75]	0.228	0.38 [0.08, 1.70]	0.204
Na <130 mmol/l	55/89	0.75 [0.19, 3.01]	0.685	0.62 [0.15, 2.64]	0.518
K <3.5mmol/l / >5mmol/l	33/89	0.83 [0.19, 3.58]	0.806	0.85 [0.19, 3.87]	0.838
Ca ion <1.1mmol/l	22/89	2.76 [0.67, 11.35]	0.161	3.62 [0.77, 17.12]	0.104
Urea >30 u/l	37/89	0.37 [0.07, 1.88]	0.229	0.32 [0.06, 1.72]	0.184
ALT >35 U/L	45/89	2.10 [0.49, 8.99]	0.316	1.74 [0.39, 7.81]	0.468
AST >35 U/L	72/89	0.81 [0.15, 4.28]	0.802	0.71 [0.12, 4.03]	0.700
Total protein < 45 g/L	33/88	0.82 [0.19, 3.51]	0.785	0.68 [0.15, 3.14]	0.622

C-reactive protein > 10 g/L	52/89	2.72 [0.53, 13.93]	0.229	3.09 [0.58, 16.51]	0.186
Hemoglobin 10 days < 65 g/l	10/89	2.57 [0.45, 14.55]	0.285	3.43 [0.55, 21.45]	0.188
White blood cells 10 days > 15 000/l	14/89	9.86 [2.23, 43.60]	0.003	9.69 [2.09, 44.84]	0.004
Hematocrit 10 days > 23%	66/89	1.19 [0.23, 6.18]	0.839	1.21 [0.23, 6.54]	0.820
Platelets 10d < 80 000/l	12/89	1.94 [0.35, 10.70]	0.445	1.89 [0.33, 10.80]	0.475
Na 10d <130 mmol/l	16/88	4.46 [1.05, 19.06]	0.043	3.75 [0.78, 17.96]	0.099
K 10d <3.5mmol/l / >5mmol/l	32/89	0.87 [0.20, 3.78]	0.863	0.74 [0.16, 3.35]	0.697
Ca ion 10d <1.1mmol/l	17/82	6.36 [1.26, 31.87]	0.024	7.07 [1.27, 39.27]	0.025
Urea 10d >30 u/l	2/88	11.28 [0.63, 200.55]	0.099	19.00 [0.87, 415.60]	0.061
Total protein 10 days < 45 g/L	15/84	4.06 [0.80, 20.50]	0.09	3.88 [0.75, 20.15]	0.107
NSAIDs use at hosp	25/89	0.71 [0.14, 3.66]	0.681	0.54 [0.10, 2.97]	0.476
Dialysis duration >20 days	25/89	6.42 [1.46, 28.16]	0.014	8.84 [1.74, 44.89]	0.009
Hospital stay duration > 20 days	68/88	quasi separation		quasi separation	

Neurological involvement at hospital	36/89	6.15 [1.20, 31.61]	0.029	5.59 [1.07, 29.24]	0.042
Neurological involvement at any point	47/89	quasi separation		quasi separation	
HTN therapy or HTN at discharge	51/89	6.88 [0.82, 57.62]	0.075	9.10 [0.98, 84.49]	0.052
Sepsis	9/89	12 [2.43, 59.22]	0.002	14.92 [2.62, 84.81]	0.002

Table 2. Factors associated with absence of kidney function recovery

Anuria lasting >10 days has shown a statistically significant association with the outcome OR=10.29, 95% CI 1.23, 86.14, p=0.032, which has remained and increased after adjustment for age and gender OR=15.23, 95% CI 1.62, 143.0, p=0.017. Same pattern was present for dialysis duration - with >20 days of dialysis yielding an OR=6.42, 95% CI 1.46, 28.16, p=0.014 before adjustment and OR=8.84, 95% CI 1.74, 44.89, p=0.009 after adjustment for age and gender. From electrolytes, sodium and ionized calcium value abnormalities on the 10th day after initiation of dialysis have shown statistically significant associations. Hypocalcemia (ionized) <1.1mmol/l has shown an OR=6.36, 95% CI 1.26, 31.87, p=0.024 before adjustment, and OR=7.07, 95% CI 2.27, 39.27, p=0.025 after adjustment. Same for hyponatremia, with the resulting OR=4.46, 95% CI 1.05, 19.06, p=0.043. However, here the statistical significance was lost after adjustment OR=3.75, 95% CI 0.78, 17.96, p=0.099. Leukocytosis on admission to our hospital has shown OR=4.4, 95% CI 1.01, 19.03, p=0.047, adjusted OR=4.54, and on 10th day after dialysis start OR=9.86, 95% CI 2.23, 43.60, p=0.003, adjusted OR=9.69. Among extra-renal involvement, neurological involvement during hospitalization OR=6.15, 95% CI 1.20, 31.6, p=0.029 (adjusted OR=0.042), and sepsis OR=12, 95% CI 2.43, 59.22, p=0.002 (adjusted OR=14.92) have shown a strong association with the outcome.

For univariate analysis for the death outcome, the results are demonstrated in table 3.

Variable	N	Univariate Odds Ratio [95% CI] death	P value	Adjusted Odds Ratio [95% CI]	P value
Age <1 y.o.	70/89	0.24 [0.04, 1.29]	0.097		
Body Mass Index, kg/m ²	73/73	1.06 [0.67, 1.70]	0.794	0.96 [0.53, 1.72]	0.882
Diarrhea	84/89	quasi separation		quasi separation	
Bloody diarrhea	44/89	0.49 [0.08, 2.82]	0.422	0.49 [0.08, 2.99]	0.443
Fever	40/89	1.24 [0.24, 6.52]	0.797	1.26 [0.23, 6.92]	0.790
Edema	75/88	0.86 [0.09, 7.99]	0.892	0.69 [0.06, 7.37]	0.758
Hypertension	50/85	1.05 [0.17, 6.66]	0.956	0.82 [0.12, 5.64]	0.840
Anuria days before dialysis >2 days	67/89	1.69 [0.19, 15.33]	0.639	2.05 [0.20, 20.51]	0.540
Anuria duration >10 days	43/89	5.92 [0.66, 52.90]	0.111	8.20 [0.83, 81.10]	0.072
Days to anuria/oliguria from disease onset, >3 days	35/89	0.29 [0.03, 2.58]	0.266	0.31 [0.03, 2.97]	0.312
Respiratory involvement	2/89	quasi separation		quasi separation	
Neurological involvement	30/89	2.07 [0.39, 10.96]	0.39	1.11 [0.16, 7.67]	0.912
Gastrointestinal involvement	43/89	0.51 [0.09, 2.95]	0.454	0.35 [0.05, 2.26]	0.268
Cardiac involvement	4/89	quasi separation		quasi separation	
NSAIDs use ¹	10/89	quasi separation		quasi separation	

Antibiotic use	27/89	1.16 [0.20, 6.75]	0.869	0.92 [0.15, 5.79]	0.929
Hemoglobin on admission < 65 g/l	9/68	quasi separation		quasi separation	
Platelets on admission < 80 000/l	21/61	2.05 [0.38, 11.21]	0.405	2.26 [0.37, 13.56]	0.374
White blood cells on admission, > 15 000/l	37/61	1.33 [0.22, 7.91]	0.752	3.47 [0.38, 31.52]	0.269
GFR on admission, < 15 ml/min/1.73m ²	44/63	0.86 [0.07, 10.06]	0.902	1.41 [0.11, 18.34]	0.791
Hemoglobin < 65 g/l UMC	25/89	1.30 [0.22, 7.61]	0.768	0.98 [0.15, 6.43]	0.986
Hematocrit > 23% UMC	36/89	1.51 [0.29, 7.96]	0.624	1.85 [0.32, 10.59]	0.489
White blood cells UMC, > 15 000/l	31/89	1.96 [0.37, 10.37]	0.426	1.98 [0.36, 10.92]	0.432
Platelets < 80 000/l UMC	47/89	0.42 [0.07, 2.43]	0.335	0.35 [0.05, 2.19]	0.261
Na <130 mmol/l	55/89	0.60 [0.11, 3.14]	0.542	0.49 [0.09, 2.77]	0.418
K <3.5mmol/l / >5mmol/l	33/89	1.77 [0.33, 9.31]	0.502	2.21 [0.38, 12.91]	0.378
Ca ion <1.1mmol/l	22/89	1.57 [0.27, 9.25]	0.615	2.28 [0.33, 15.59]	0.401
Urea >30 u/l	37/89	0.26 [0.03, 2.33]	0.230	0.22 [0.02, 2.06]	0.183
ALT >35 U/L	45/89	2.05 [0.36, 11.80]	0.422	1.65 [0.26, 10.18]	0.592
AST >35 U/L	72/89	1.19 [0.13, 10.94]	0.875	0.95 [0.10, 9.43]	0.967

Total protein < 45 g/L	33/88	0.82 [0.14, 4.76]	0.827	0.70 [0.11, 4.45]	0.711
C-reactive protein > 10 g/L	52/89	1.46 [0.25, 8.41]	0.673	1.63 [0.27, 9.88]	0.597
Hemoglobin 10 days < 65 g/l	10/89	4.69 [0.74, 29.73]	0.101	7.30 [0.90, 58.87]	0.062
White blood cells 10 days > 15 000/l	14/89	14.6 [2.36, 90.24]	0.004	14.53 [2.19, 96.43]	0.006
Hematocrit 10 days > 23%	66/89	0.64 [0.11, 3.79]	0.628	0.64 [0.10, 3.94]	0.627
Platelets 10d < 80 000/l	12/89	1.27 [0.14, 11.94]	0.833	1.22 [0.12, 12.03]	0.861
Na 10d <130 mmol/l	16/88	2.43 [0.40, 14.58]	0.332	1.96 [0.27, 13.95]	0.502
K 10d <3.5mmol/l / >5mmol/l	32/89	0.33 [0.04, 3.01]	0.329	0.25 [0.03, 2.48]	0.239
Ca ion 10d <1.1mmol/l	17/82	4.2 [0.55, 32.28]	0.168	4.19 [0.45, 38.91]	0.207
Urea 10d >30 u/l	2/88	16.2 [0.88, 298.94]	0.061	27.53 [1.12, 677.01]	0.042
Total protein 10 days < 45 g/L	15/84	quasi separation		quasi separation	
NSAIDs use at hosp	25/89	1.30 [0.22, 7.61]	0.768	0.97 [0.16, 6.06]	0.975
Dialysis duration >20 days	25/89	2.77 [0.52, 14.77]	0.232	3.43 [0.58, 20.17]	0.172
Hospital stay duration > 20 days	68/88	quasi separation		quasi separation	

Neurological involvement at hospital	36/89	quasi separation		quasi separation	
Neurological involvement at any point	47/89	quasi separation		quasi separation	
HTN therapy or HTN at discharge	51/89	4.02 [0.45, 35.94]	0.213	6.12 [0.58, 64.53]	0.132
Sepsis	9/89	31.2 [4.56, 213.49]	<0.05	66.88 [5.32, 839.57]	0.001

Table 3. Factors associated with acute-phase mortality.

For the death outcome, strong associations were observed with leukocytosis on 10th day after dialysis start, similar to the absence of kidney function recovery outcome, with an OR=14.6 95% CI 2.36, 90.24, $p=0.004$, and OR=14.53, 95% CI 2.19, 96.43, $p=0.006$ after adjustment for age and gender. In addition, for our cohort, sepsis has shown the strongest association with acute-phase mortality - the OR increased after adjustment for age and gender from OR = 31.2, 95% CI 4.56, 213.49, $p = < 0.05$ till OR=66.88, 95% CI 5.32, 839.57, $p = 0.001$. The last parameter that has demonstrated statistically significant result after adjustment is serum urea values on 10th day of RRT initiation with OR=16.2, 95% CI 0.88, 298.94, $p=0.061$ before adjustment and OR=27.53, 95% CI 1.12, 677.01, $p=0.042$.

DISCUSSION

In this study, we report a cohort of 89 dialyzed pediatric patients with D+ HUS. We analyzed the clinical and laboratory data, and their association with poor short-term outcomes.

The distinguishing feature of our cohort is that there was a 100% requirement for renal replacement therapy (RRT) in our patients. The acute phase mortality rate in our study was 6.74%, , which falls into the range reported in several studies (Mody et al., 2015, Robitaille et al., 2012, Zagożdżon et

al., 2024, Ali et al., 2017). The deaths occurred twice as much in females vs males (66% vs 33% of deaths). The rate of non-recovery of kidney function on the acute phase was 10.1% in our cohort.

Similarly to other studies, prolonged anuria and dialysis duration were associated with poor renal outcomes in our study as well, reflecting the ongoing renal insult requiring prolongation of supportive measures. In addition, anuria > 10 days has also demonstrated borderline significance in association with mortality after adjustment ($p=0.072$). For anuria duration, a defined cutoff in our study was >10 days. In current literature, the span for a cutoff point for worse prognosis varies between 7-10 days of anuria (Zhao et al., 2017, Viennet et al., 2023, Oakes et al., 2008). Dialysis duration in our study had a cutoff of >20 days. In the current literature, the cutoff values vary: Rosales et al. (2012) used 7-22 days, VanSickle et al. (2018) and Alconcher, Lucarelli, Bronfen, et al. (2023) used 10 days. In spite of the difference in cutoffs used, our findings regarding anuria and RRT align with existing literature evidence.

There was also an association observed between both outcomes and persistent uremia > 30mmol/L on 10th day of RRT initiation, which reached statistical significance only with the composite outcome after adjustment for age and gender. Urea has been reported to be associated with mortality in a study by Chávez-Íñiguez et al. (2023) on adult patients. As for pediatric patients, BUN was used instead of Urea in a study by Choi et al. (2017) showing an association with mortality, although the reported odds ratio for BUN was only 1.032. The possible mechanism behind association with both outcomes is the biological effect of uremic toxins - induction of inflammatory response, neurotoxicity and endothelial damage (Herget-Rosenthal et al., 2009).

When it comes to hemoglobin and hematocrit levels, they are seen as surrogate markers of hemoconcentration. In the context of D+HUS, diarrhea (sometimes with concurrent vomiting) is the main reason for dehydration, subsequent hemoconcentration and intravascular volume depletion, which adds to the renal damage by contributing to renal hypoperfusion (Basile et al., 2012), besides the intra-renal microthrombi and endothelial injury that already contribute to hemodynamic compromise of the kidneys (Leisring et al., 2023). One of the differences in our study is that we did not get higher hemoglobin levels on the 10th day of RRT initiation as a risk factor for poor renal outcome. It is lower hemoglobin that was associated with increased risk of

poor renal function (OR 1.32) in our study. The cutoff we used was 65 g/l, compared with other studies where 95g/l (Loos et al., 2021), 10g/dL (VanSickle et al., 2018), 108 g/L (Alconcher et al., 2018), and 112g/L (Ardissino et al., 2014) cutoffs were used. The cutoff value that we used could be an explanation to our result.. On the other hand, hematocrit >23% on admission did show an increase in the risk of poor renal outcomes (OR 1.97) in our study, same as in other researchers' findings (Alconcher et al., 2023, Oakes et. al., 2006). However, considering that both parameters did not reach statistical significance in our cohort and taking into account the limitations of our study, the findings are to be interpreted cautiously.

Our results have also demonstrated a statistically significant association between elevated WBC count on the 10th day of dialysis initiation with both outcomes, similarly to other studies (Oualha et al., 2018, Oakes et al., 2006), even if our study used a lower threshold of WBC count (15 000/l), compared to other authors. The results are clinically plausible, considering that one of the major roles in the pathogenesis of the D+HUS is attributed to neutrophil, macrophage and monocyte activation, along with a profound inflammatory response (Michael et al., 2022, Bowen & Coward, 2017). Leukocytosis on admission to our hospital has demonstrated a statistically significant effect as well, but the association was observed only with the composite outcome, and no association between admission values and mortality was observed. Notably, even though we used two time points, both correspond to the acute phase of the disease. The association persisted after adjustment for both predictors (OR 4.54 for admission values, and OR 9.69 for values on 10th day after dialysis start). Notably, the significance for admission values for WBC is borderline, compared to values on 10th days after dialysis start, likely due to the second parameter being a reflection of continuing inflammatory response.

Interestingly, hyponatremia on 10th day after RRT initiation was another risk factor for poor renal outcomes in univariate analysis, however it has lost its significance with adjustment for age and gender. A study specifically for pediatric patients with D+HUS highlighting hyponatremia as a risk factor for mortality was done by Alconcher et al. (2018), however, unlike in our model, it has shown to be associated specifically with death as an outcome, whereas in our case death is a part of composite outcome. The finding itself can be explained by the fact that hyponatremia is an expected finding in AKI due to impairment of free water excretion (Adrogué et al., 2022).

Neurological involvement is a well-known disease severity marker in D+HUS that is often associated with a more complicated course of the disease (Luna et al., 2020). Özdoğan et al. (2017) reported higher creatinine levels in patients with neurological involvement, showing indirect association with renal malfunction as well, but without a statement on prognosis of renal outcome. In our case, the association between neurological involvement during hospital stay and composite outcome was strong (OR=6.15), similarly to findings observed by other colleagues. Such association of the neurological involvement with the composite outcome in our study is a clinically plausible finding explained by the pathophysiology of the shiga-toxin action: its ability to bind to neurons, as well as by an inflammatory response induction due to cytokine release and microvessel damage (Michael et al., 2022). In addition, even though in our study the attempt to estimate the association between total neurological involvement (at onset and during hospitalization) in the acute phase of the disease and acute-phase death resulted in quasi separation and quantitative estimation of risk was not possible, the presence of quasi separation itself indicates a strong clinical association: all patients without neurological involvement at any point of the acute phase of the disease reached kidney recovery, and out of all 9 patients who did not recover their renal function, 100% experienced neurological involvement at some point.

Hypocalcemia during AKI has been associated with short-term mortality in a study by Afshinnia et al. (2013) in critically ill patients with AKI on RRT. In our case, the values on the 10th day after dialysis initiation have shown the strongest association among the two time-points used. This could probably be reflecting the ongoing inflammatory response and renal insult, considering the underlying pathophysiology of hypocalcemia in AKI being hyperphosphatemia, along with inflammation-induced changes in CaSR (Leaf & Christov, 2018).

The strong association of sepsis with both outcomes in our study could be explained by the fact that sepsis is a hyperdynamic shock that contributes to the AKI by further reducing the eGFR due to elevated RBF and increased efferent arteriole diameter. Besides that, dysregulated inflammatory response getting superimposed on already present AKI likely worsens the critical condition further and increases the risk for both death and kidney damage (Fani et al., 2017). Interestingly, there are no studies highlighting sepsis as a cause of acute-phase mortality specifically in dialyzed pediatric

D+HUS patients. Luna et al. (2020) have mentioned that 63.6% of their cohort has developed septic shock. However, it is not clear whether all of these 63.6% of patients underwent dialysis. It is also not specified if the septic proportion of the cohort are among those 27,3% who have died. In our study, sepsis was present in 10.1% of the cohort. Sepsis was also responsible for 66.7% of the deaths in our cohort, compromising $\frac{2}{3}$ of all deaths in total. Jenssen et al. (2016) have also mentioned sepsis occurrence in their cohort being 29% of dialyzed children with D+HUS. They also mention the 5% fatality in their D+HUS cohort, however the cause was not specified.

In conclusion, we have identified several key predictors of both mortality and renal non-recovery in our study of 89 dialysis-dependent Kazakhstani pediatric D+HUS patients. Our analysis confirmed that mortality is independently predicted by sustained and intense inflammation - presence of sepsis and leukocytosis $> 15\ 000/L$ present at day 10 after initiation of RRT. Furthermore, persistent uremia $> 30\ mmol/L$ at 10th day of dialysis start served as an independent predictor for mortality as well. Regarding renal prognosis, anuria > 10 days and dialysis > 20 days, persistent electrolyte imbalances by 10th day of RRT (hyponatremia $< 130\ mmol/l$ and hypocalcemia $< 1.1\ mmol/L$), inflammatory markers (leukocytosis $> 15\ 000/L$ on admission and 10th day of RRT) and systemic involvement (sepsis and neurological involvement during hospital stay) were independent predictors for poor renal recovery.

The study warrants sepsis as an important cause of acute-phase mortality, highlighting the importance of early detection and management of secondary infections to improve short-term survival of patients with complicated D+HUS cases.

LIMITATIONS

This study has several limitations. The primary issue of our study is the small sample size, which affects the statistical power of our analysis and generalizability of our results. The design of the study being retrospective made us dependent on completeness of the medical records, which, due to lacking information on some crucial clinical parameters, has led to a reduction in sample size and limited us with the inclusion of the critically important clinical variables. Another limitation of our study is that the diagnosis of D+HUS was made clinically without the confirmation of infection with STEC. The likely explanation is that a large proportion of our cohort has been

receiving antibiotics by the moment of stool sampling, leading to false negative results. Due to these limitations, the findings of our study are to be interpreted with caution.

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