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ORIGINAL ARTICLE

Relationship between red blood cell transfusion requirements and severity of renal disease during the acute stage of hemolytic uremic syndrome

Carlos J. Cobeñas[1] · Paula S. Bresso[1] · Laura L. Lombardi[1] · Oscar R. Amoreo[1] · Javier D. Ruscasso[1] · Ana P. Spizzirri[1] · Ângela del C. Suarez[1] · Javier H. Zalba[1] · Ricardo C. Rahman[1] · Paula Risso[2]

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Abstract
Background We performed a retrospective evaluation of patients with diarrhea-associated hemolytic uremic syndrome (D + HUS) with the aims of: (1) determining the rate of red blood cell (RBC) transfusions; (2) establishing the relationship between need for RBC transfusion and severity of renal involvement; (3) determining whether precise measurements of lactic dehydrogenase (LDH) levels can predict the rate of hemolysis and severity of renal disease.
Methods A total of 288 patients with D + HUS were retrospectively divided into three groups based on dialysis treatment: group 1, no dialysis treatment (144 patients); group 2, dialysis for 1–10 days (67 patients); group 3, dialysis for ≥11 days (77 patients).
Results Of the patients in groups 1, 2 and 3, 73.6, 86.5 and 83.1 %, respectively, required at least one RBC transfusion. The number of RBC transfusions in groups 1, 2 and 3 was 163, 107 and 162, respectively. Comparison of the groups revealed that the number of RBC transfusions was significantly higher in patients in groups 2 and 3 than in those in group 1 ($p = 0.0001$). Most RBC transfusions (94.2 %) occurred during the first 2 weeks of the disease. The median peak LDH level was 2091 U/l in 32 patients with no RBC transfusion (group A), 3900 U/l in 73 patients with one transfusion (group B) and 6378 U/l in 62 patients with two or more transfusions (group C). Patients who received two or more RBC transfusions had a significantly higher median peak LDH level than those who did not receive RBC transfusions or received only one transfusion. This difference was also observed between patients who received only one RBC transfusion and those who did not receive any transfusions ($p < 0.00001$). Comparison of LDH levels on admission and peak LDH levels among patients in groups A, B and C revealed that 28/32 patients in group A, 56/73 patients in group B and 33/62 patients in group C had a stable LDH level, suggesting that patients with a stable LDH level require fewer RBC transfusions ($p \leq 0.006$). Finally, we evaluated the possibility of an association between peak LDH levels and the degree of renal disease. The median peak LDH level in patients of group 1, 2 and 3 was 3538 (range 756–9373), 5165 (451–9205) and 7510 (1,145–16,340) U/l, respectively. Patients with >10 days of dialysis (group 3) had the highest LDH levels, followed by patients with 1–10 days of dialysis (group 2) and then by patients with no dialysis requirements (group 1) ($p < 0.00001$).
Conclusions The rate of RBC transfusion was higher in patients with the most severe renal injury, and most were performed during the first 2 weeks of the disease. Patients with stable LDH levels seemed to require fewer RBC transfusions. Median peak LDH levels were significantly higher in the group of patients with the most severe renal disease.

Keywords Hemolytic uremic syndrome · Microangiopathic hemolytic anemia · Red blood cell transfusions · Lactic dehydrogenase levels · Renal disease

✉ Carlos J. Cobeñas
carlos.cobenas@hotmail.com

[1] Hospital de Niños "Sup. Sor María Ludovica", La Plata, Buenos Aires, Argentina

[2] Cátedra de Bioestadística Bayesiana y Clásica, Carrera de Microbiología Clínica e Industrial, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

Introduction

Diarrhea-associated hemolytic uremic syndrome (D + HUS) constitutes the leading cause of acute renal failure in Argentina [1]. This disease is characterized by microangiopathic hemolytic anemia (MAHA), transient thrombocytopenia and acute renal disease, with variable degrees of central nervous system (CNS) involvement [2]. MAHA is mainly associated to the mechanical damage to red blood cells (RBC) as they pass through the renal microcirculation affected with the thrombotic microangiopathy that characterizes this disorder [3]. The severity of the disease is related to renal, colonic and CNS involvement, and the degree of anemia can be variable, ranging from a mild decrease in hematocrit values to profound anemia that sometimes requires repeated RBC transfusions [3–6]. However, the intensity and recurrences of hemolytic anemia does not account for the severity of the disease. It has been stated that there is often no correlation between the severity of hemolysis and the degree of acute renal disease [7]. Some patients may present with less severe forms of renal and CNS involvement but still require multiple RBC transfusions, while others may have acute renal failure that requires >10 days of renal replacement therapy (RRT) or repeated seizures, but with mild anemia. Very few publications have dealt with this specific aspect of the disease.

The aims of the retrospective study reported here were: (1) to determine the rate of RBC transfusions in patients with different degrees of acute renal disease; (2) to establish the relationship between RBC transfusions and severity of renal involvement; (3) to determine whether precise measurements of lactic dehydrogenase (LDH) levels can predict the rate of hemolysis and severity of renal disease

Patients and methods

Our study was a retrospective evaluation of patients with D + HUS whose disease was managed at the Nephrology Department, Hospital de Niños "Superiora Sor María Ludovica", La Plata (Argentina), from December 2002 to December 2011. For this type of study formal consent is not required. Patients with any suggestion of atypical HUS (familial or recurrent disease, known genetic predisposition) were excluded from our analysis. Data were collected from the medical files on age at diagnosis, gender, severity of the acute renal disease, modality of dialysis therapy (peritoneal or hemodialysis) and magnitude of hemolytic anemia. The severity of acute renal injury was assessed based on the number of days of dialysis. Indications for dialysis in HUS patients did not change in our institution over the study period and included anuria lasting for >24 h, hyperkalemia, metabolic acidosis refractory to conservative measures, severe neurologic involvement (seizures, coma) and hypervolemia or patients with uremic symptoms despite maintenance of urine output [5]. Lactate dehydrogenase (LDH) levels (on admission and peak levels) were also evaluated. Patients whose LDH level did not rise after admission were considered as having "stable LDH". Patients were divided into three groups based on the number of days of dialysis required: group 1, no need for dialysis during the acute stage; group 2, 1–10 days of dialysis; group 3, ≥11 days of dialysis). The magnitude of anemia was evaluated according to the number of RBC transfusions needed during the acute stage. In our institution, patients with a diagnosis of HUS are considered for a RBC transfusion whenever they have: a hematocrit level of ≤18 % or a hematocrit level of between 18 and 20 % associated with hemodynamic instability or extreme pallor, as judged by our staff of experienced pediatric nephrologists assisting HUS patients. A RBC transfusion consisted of 10 ml/kg body weight. The number of RBC transfusions per patient and the percentage of patients who were transfused were calculated for each group of patients. We also analyzed the timing of RBC transfusions in terms of those performed during the first, second and at or after the third week (which we refer to as late transfusions). The relationship between LDH level and need for RBC transfusions was evaluated by calculating the median LDH levels on admission for patients with no need for RBC transfusions (group A) and for those who required one and two or more RBC transfusions (groups B and C, respectively). LDH levels were considered to be normal when they fell within the range 338–820 U/l. Finally, median peak LDH levels were determined according to the severity of renal disease (groups 1, 2 and 3).

Statistical analyses

The normality of the data was tested with the Shapiro–Wilk test. The homocedasticity of variances was calculated using the Levene test. In absence of these criteria ($p<0.05$), we performed the non-parametric Kruskall–Wallis test (H value) to compare the median value among groups. The Dunn–Bonferroni test was selected as a post hoc methodology.

The Fisher exact test was run to compare proportions and nominal data. The level of significance in all cases was $\alpha \leq 0.05$ (p value<0.05). Statistical data were analyzed with InfoStat [8] and EPIDAT 4.1 [9] software.

Results

A total of 288 patients with a diagnosis of D + HUS whose disease was managed at our institution during the study period were evaluated. Mean age on admission was 34.4 [standard deviation (SD)=31.8, range:5–172] months. Of these patients, 149 (51.7 %) were girls. Patients were divided in groups according to the severity of renal disease, as previously mentioned, resulting in 144 (50.0 %) patients in group 1, 67 (23.3 %) patients in group 2 and 77 (26.7 %) patients in group 3. Among the 144 patients who required dialysis, only 19

(6.7 % of the whole group) received hemodialysis therapy. Of the 144 patients in group 1, 106 (73.6 %) were transfused; in groups 2 and 3, 58 of 67 (86.5 %) patients and 64 of 77 (83.1 %) patients, respectively, were transfused. A total of 432 RBC transfusions were performed in the whole group of 288 patients (1.5 transfusions per patient).

The number of RBC transfusions in each group was determined, revealing that there were 163 transfusions in the 144 patients in group 1, 107 transfusions in the 67 patients in group 2 and 162 transfusions in the 77 patients in group 3. Analysis of the rate of RBC transfusions per severity of kidney injury in all three groups demonstrated that the number of RBC transfusions was significantly higher in patients of groups 2 and 3 than in those of group 1 (H value 17.08, $p = 0.0001$; Table 1). Patients with more severe renal disease required more RBC transfusions than patients with less severe renal dysfunction.

We analyzed the timing of RBC transfusions and found that 407 RBC transfusions (94.2 %) were performed during the first 2 weeks of the disease, while only 25 (5.8 %) were performed at or after the third week. Most RBC transfusions were performed during the first week of the disease in all groups, although in the group with the most severe renal disease this difference was less evident.

We also evaluated the relationship between renal disease and late transfusions. The rate of late transfusions was 1/163 (0.6 %) in group 1, 1/107 (0.9 %) in group 2 and 23/162 (14.2 %) in group 3 (Table 2). These results show that almost all patients who were given RBC transfusions at or after the third week belonged to the group of patients with the most severe renal disease.

LDH serum levels obtained at or after the third week of disease were available in only 14/16 patients receiving late transfusions. The median LDH level in this group of patients was 7120.5 (1145–16,340) U/l. These results show persistently high LDH levels in patients with late RBC transfusions, probably a reflection of some degree of persisting hemolytic activity.

LDH levels (both on admission and peak values) had been recorded in the medical charts of 167 patients. We calculated median peak LDH levels according to RBC transfusion requirements. The results of these calculations are shown in Table 3. The median LDH level was 2,091 (range: 451–10,350) U/l in 32 patients who required no RBC transfusion (group A), 3900 (range 1017–14,951) U/l in 73 patients with only one RBC transfusion (group B) and 6378 (range 1145–16,340) U/l in 62 patients who received two or more transfusions (group C).

Our results show that the median peak LDH level differed significantly among groups of patients (H value 33.36, $p<0.00001$). Patients who received two or more RBC transfusions had statistically significant higher median peak LDH levels than those who did not receive any transfusion or received only one transfusion. This difference was also observed between patients who received only one RBC transfusion and those who did not receive any RBC transfusion at all (Table 3).

We compared LDH levels on admission and peak LDH levels in groups A, B and C. Patients with stable LDH levels were included in this analysis, i.e, those whose LDH did not rise after admission. In the group who did not receive RBC transfusions, 28 of 32 patients had stable LDH levels, whereas in patients with one RBC transfusion and in those with two or more RBC transfusion, the LDH level was stable in 56 of 73 patients and in 33 of 62 patients, respectively. Our results suggest that patients with stable LDH levels can be expected to require fewer RBC transfusions ($p \leq 0.006$; Table 4).

Finally, we evaluated peak LDH level and its relationship with the degree of renal disease, based on the number of days

Table 2 Timing of red blood cell (RBC) transfusions according to the severity of renal dysfunction

Groups	Week of disease	RBC transfusions and proportions (%)
1	First	149/163 (91.4)
	Second	13/163 (8.0)
	Third	1/163 (0.6)
2	First	88/107 (82.3)
	Second	18/107 (16.8)
	Third	1/107 (0.9)
3	First	96/162 (59.3)
	Second	43/162 (26.5)
	Third	18/162 (11.1)
	Longer than third	5/162 (3.1)

Table 1 Differences between red blood cell transfusion requirements according to renal dysfunction

Groups[a] (no. of patients)	RBC transfusions (n)	Median RBC transfusions (n)	Average rank
1 (n = 144)	163	1	124.75 a
2 (n = 67)	107	1	157.23 b
3 (n = 77)	162	2	170.35 b

Different lowercase letters following values indicate significance at $p \leq 0.05$ between groups

RBC, Red blood cells

[a] Patients were categorized in groups according to the severity of renal dysfunction which in turn was based on number of days of dialysis required: group 1, no need for dialysis during the acute stage; group 2, 1–10 days of dialysis; group 3, ≥11 days of dialysis. For more detail, see text

Table 3 Relationship between lactic dehydrogenase and red blood cell transfusions

Groups[a]	Cases (*n*)	Median LDH level (U/l)	Average rank
A	32	2,091	52.94 a
B	73	3,900	75.54 b
C	62	6,378	109.99 c

Different lowercase letters following values indicate significance at $p \leq 0.05$ between groups

LDH, Lactate dehydrogenase

[a] Patients were categorized into groups based on the need for RBC transfusion: group A, patients with no need for RBC transfusion; group B, those who required one RBC transfusion; group C, those who required than two RBC transfusions. For more detail, see text

of dialysis, categorizing patients in groups 1, 2 and 3, as mentioned above. Patients in group 1 (n=86), 2 (n=39) and 3 (n=42) had median peak LDH levels of 3538 (range 756–9373) U/l, 5165 (range 451–9205) U/l and 7510 (range 1145–16,340) U/l, respectively. Our results show significant differences in the median peak LDH level among the tree groups of patients (H value 39.64, p <0.00001). Patients with >10 days of dialysis had the highest LDH levels, followed by patients with 1–10 days of dialysis, who in turn had higher levels than patients with no dialysis requirement (Table 5). This pattern coincided with the LDH levels in terms of need for RBC transfusion.

Discussion

Hematologic disease in HUS is characterized by MAHA, in which fragmented RBC (schistocytes) are found in the peripheral blood, usually accompanied by thrombocytopenia. MAHA is usually associated with elevated LDH levels and seems to be related to mechanical damage to RBC as they pass through the renal microcirculation affected with a thrombotic microangiopathy [3]. Some patients present with profound and repeated episodes of hemolytic anemia, requiring several RBC transfusions, while others only have mild anemia and no need for transfusions during the acute stage of the disease. The focus of our evaluation was hemolytic activity during the acute phase of the disease—not anemia due to reduced RBC

Table 4 Relationship between lactic dehydrogenase level and red blood cell transfusion

Groups	Value contrasted (U/l)	Fisher test	*p*
A vs. B	28/32 vs. 56/73	0.11	0.290
A vs. C	28/32 vs. 33/62	0.34	0.001
B vs. C	56/73 vs. 33/62	0.23	0.006

$p \leq 0.05$ indicates significant difference between groups

Table 5 Relationship between median lactic dehydrogenase (LDH) peak level and renal disease

Groups	Median LDH peak value (U/l)	Average rank
1	3538	63.53 a
2	5165	90.08 b
3	7510	120.26 c

Different lowercase letters following values indicate significance at $p \leq 0.05$ between groups

production. However, the severity of the hemolytic anemia does not correlate with the severity of the disease. While extra-renal manifestations are the main cause of death during the acute stage [6, 10], long-term outcome is mainly related to the severity of renal involvement [4, 11, 12].

A relationship between hematologic and renal disease has not been clearly established. We evaluated the rate of RBC transfusions in patients with different degrees of renal disease and found that the number of RBC transfusions was significantly higher in patients with 1–10 and >10 days of dialysis than in patients with no need for dialysis. As only 6.7 % of all D+HUS patients in our study received hemodialysis, we do not believe that blood loss associated to this modality of RRT biased our results.

Serum levels of erythropoietin (EPO) may also account for the severity of anemia in HUS patients. Exeni et al. [13] showed an inadequate EPO synthesis relative to the severity of anemia in patients with HUS, which could have played a role in increasing the severity of hemolytic anemia in their patients. These authors also postulated that repeated RBC transfusions may have exacerbated this inadequate synthesis. A therapeutic role for recombinant EPO has been advocated, but while some authors have shown a reduction in the number of RBC transfusions in patients treated with EPO [14], Balestracci et al. [15] recently reported the lack of a favorable effect of EPO in this setting. We do not routinely evaluate serum EPO levels in our HUS patients, so due to the retrospective nature of our series we were unable to address this issue.

The severity of renal failure may also be associated to the persistence of reduced EPO levels, leading to increased transfusion requirements [13]. This association may explain why the group of patients with the most severe renal failure in our series had a significantly higher rate of RBC transfusions. Alternatively, the evaluation of LDH level showed that patients with more RBC transfusions had significantly higher levels of LDH than those with a lower transfusion requirement and also that LDH levels were significantly higher in patients with the most severe renal injury. Furthermore, LDH levels were still high at or after the third week of the disease in patients receiving late RBC transfusions. These findings suggest that, at least in part, the higher LDH levels we observed in patients with the most severe renal disease may have been associated to persistent

higher degrees of hemolysis, in addition to persistently reduced serum EPO levels, as mentioned above. Again, as we did not evaluate serum EPO levels or other markers of microangiopathic hemolytic anemia, with the exception of LDH levels, we may only speculate on this point.

Some interference may exist between acute kidney injury and LDH levels, particularly isoenzyme LDH1 [16–18]. It has been reported that in some cases of renal infarction, isoenzyme LDH1 levels may be elevated. We do not routinely evaluate LDH isoenzymes at our institution, and so we are not able to better clarify this point. However, due to the obvious hemolytic nature of anemia in this disorder, we do not believe (although it is only our speculation) that the elevated serum LDH levels could be of renal origin.

Blood losses associated to venous samples and hemodialysis must be taken into account [19]. However, as mentioned above, as only 6.7 % of our patient group received hemodialysis, we do not believe that blood loses associated to this type of RRT constituted any bias to our results. Regarding other venous punctures, including repeated blood losses in relation to laboratory evaluations, it is logical to assume that patients with a longer hospital stay probably have more blood samples taken. Although difficult to evaluate, we do not think that blood losses related to blood sampling could constitute a bias in our group of patients.

To the best of our knowledge, this is the first study which attempts to relate RBC transfusions and the severity of renal disease in patients with D+HUS. The finding of significantly higher LDH levels in patients with the most severe renal injury may account for, at least in part, a higher degree of hemolysis in this group. A role for persistently reduced serum EPO levels in patients with longer anuric periods also has to be taken into account. As we do not routinely evaluate serum EPO levels and due to the retrospective nature of our study, this point could not be properly addressed in our series.

In summary, 73.6, 86.5 and 83.1 % the patients in our study required at least one RBC transfusion in groups 1, 2 and 3, respectively. The rate of RBC transfusion was higher in patients with the most severe renal injury, and most were performed during the first week of the disease in all three groups of renal dysfunction. Median LDH levels were significantly higher in the group with the most severe renal disease.

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Conflict of interest The authors declare no conflict of interest.

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