POSTDIARRHEAL SHIGA TOXIN-MEDIATED HEMOLYTIC UREMIA SYNDROME SIMILAR TO SEPTIC SHOCK

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Abstract The inflammatory response of host endothelial cells is included in the development of vascular damage observed in enterohemorrhagic Escherichia coli (EHEC) infection, resulting in hemolytic uremic syndrome (HUS). The response to a non-conventional treatment for a group of D+ HUS (diarrhea positive HUS) patients, with clinical hemodynamic parameters of septic shock was evaluated in this prospective study (1999-2003). Twelve children 2.8 ± 0.6 years old, with D+ HUS produced by E. coli infection with serological evidence of Shiga toxin, presenting severe unstable hemodynamic parameters and neurological dysfunction at onset, were studied. The protocol included fresh frozen plasma infusions, methylprednisolone pulses (10mg/k/day) for three consecutive days and plasma exchange for five days, starting after admission to the intensive care unit (ICU). The twelve patients with increased pediatric risk of mortality (PRISM) score: 18 ± 2 after admission to intensive care unit (ICU), required dialysis for 17.4 ± 4 days, mechanical ventilator assistance for 10 ± 1 days and early inotropic drugs support for 10.5 ± 1 days. Neurological dysfunction included generalized tonic-clonic seizures lasting for 5.4 ± 1 days, n:8. Focal seizures were present in the remaining patients. Dilated cardiomyopathy was present in 6 children. Eight children suffered hemorrhagic colitis. Nine patients survived. Within one year of the injury, neurological sequelae, Glasgow outcome scale (GOS) 3 and 4, were present in two patients, chronic renal failure in one patient. We suggest that early introduction of this protocol could benefit D+ HUS patients with hemodynamic instability and neurological dysfunction at onset. Further studies are likely to elucidate the mechanisms involved in this early adverse clinical presentation of D+ HUS patients.

Key words: hemolytic uremic syndrome, multiple organ failure, unstable hemodynamic parameters, plasma infusions, corticosteroid therapy, plasmapheresis

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The term hemolytic-uremic syndrome (HUS) was introduced by Gasser et al. in 1955 to describe a devastating illness consisting of acute renal failure, accompanied by nonimmune hemolytic anemia and thrombocytopenia. The diarrhea positive HUS (D+ HUS) occurs in young children after a prodromal period of bloody diarrhea caused by Shiga toxin (Stx) from *Escherichia coli* (usually serotype O157-H7)²,³. Multiorgan failure involving the central nervous system (CNS), pancreas, heart or other systems or organs may accompany this disease. Although the clinical features of this D+ HUS are widely known, a clear understanding of its pathophysiology and treatment remain unclear. Endothelial injury has been recognized as the trigger event in the development of the microangiopathic process. Human endothelial cells stimulated with subinhibitory concentrations of Stxs elicited few, but reproducible changes in gene expression of chemokines and cell adhesion molecules. In addition, Stx can induce cytokine release from human brain endothelial cells (hBEC), which may contribute toward the characteristic CNS neuropathology. These findings indicate the important role of Stxs in inducing a multifaceted host inflammatory response. Nevertheless, the lipopolysaccharide (LPS) of enterohemorrhagic *E. coli* (EHEC) and Shiga toxin together substantially contribute to the pathophysiology of typical HUS. Endothelial cells may be activated by bacterial toxins and inflammatory mediators. Shiga toxins activate endothelial cells expressing the globotriosilceramide receptor (GB3), and tumor necrosis factor alfa (TNFα) amplifies the response augmenting endothelial GB3 expression. Elevated levels of antibodies to EHEC lipopolysaccharide (LPS) can be found in HUS patients. Results from clinical trials have found high circulating levels of IL-1β and TNFα in HUS. In spite of this, attempts to prove that patients with an acute episode of HUS have elevated systemic proinflammatory cytokine levels in comparison to those in children with infectious diarrhea, have failed. Recently, evaluation of anti-inflammatory cytokine profiles in order to monitor the balance between pro and anti-inflammatory immune activation, has been reported.

Overwhelming sepsis is accompanied by massive systemic endothelial cell activation. In response to bacterial endotoxin, LPS-activated macrophages induce increased TNFα and IL-1β. Trials of antiinflammatory agents, including corticosteroids, in patients with sepsis, have been published. The known cellular and molecular mechanisms involved in the antiinflammatory effects of corticosteroids include downregulation of the expression of cytokines (IL-1, IL-6 and TNFα), inhibition of intercellular adhesion molecule (ICAM-1) expression, and stabilization of vascular permeability in endothelial cells. In severe sepsis, the use of plasma exchange procedure to remove endotoxins and proinflammatory mediators released from monocytes/macrophages as free myoglobin and hemoglobin, has been successfully tried in smaller non controlled studies since 1984. In addition, while antiinflammatory therapy, including glucocorticoids, anti-TNF antibodies, and IL-1 receptor antagonist, makes sense during the initial hyperinflammatory phase, immune stimulation by removing inhibitory factors by plasmapheresis may be useful during immunoparalysis in sepsis.

The present prospective study reports a group of patients HUS D+ in the presence of clinical septic shock parameters and neurological dysfunction from the very beginning. Evaluation after a non-conventional therapeutic approach to these patients is analyzed hereinbelow.

**Material and Methods**

A total of one hundred forty-eight children D+ HUS were treated at the Pediatric Nephrology Department of Hospital Notti, Mendoza, Argentina during a period of five years (1999-2003). The diagnosis was based on the presence of a diarrhea prodrome, acute renal failure, hemolytic changes in peripheral blood film and thrombocytopenia.

From this group of one hundred eighty patients, at the present work, we prospectively studied twelve D+ HUS children (median age 2.8 ± 0.6 years, range 1 to 8 years) with clinical hemodynamic parameters of septic shock and multiple organ system failure in the first forty eight hours after admission. Signs of neurological dysfunction at this time were demonstrated in twelve patients. Serological evidence of Shiga toxin produced by *E. coli* infection was present in eleven patients. The twelve patients were admitted in the Intensive Care Unit (ICU).

The pediatric risk of mortality (PRISM) score was developed from the Physiological Stability Index (PSI), the resulting PRISM score consists of 14 routinely measured, physiological variables. To obtain an objective weighing of the variables, the PRISM score was applied to the twelve patients, the first day after admission in the Intensive Care Unit (ICU). After admission in the ICU, once the airway was clear and circulation adequate, the level of consciousness was determined by the Glasgow Coma or Responsiveness Scale (GCS). Cardiovascular evaluation included data from the standard method of 2 dimensionally directed M mode and doppler echocardiograms in the admitted ICU patients. Glasgow outcome score (GOS) was performed in the follow up of the patients.

**Microbiologic confirmed Shiga toxin Escherichia coli (STEC) infection**

Stool samples were inoculated directly, and after an enrichment step (37 °C, 4-6 h) in trypticase soy broth with cefixime and potassium tellurite (CT-TSB), into sorbitol- MacConkey agar (SMAC). A multiplex PCR was performed to detect Stx1 and Stx2 sequences in the confluent growth zone and in the isolated colonies. Colonies that do not ferment sorbitol were tested for agglutination with O157 antiserum. If Stx was detected, colonies were forwarded to the National Reference Laboratory (Instituto Nacional de Enfermedades Infecciosas-ANLIS Dr. Carlos G. Malbrán, Buenos Aires) for serotyping, biotyping and virulence factors characterization. Detection of *Stx2*-neutralizing antibodies was performed in the serum samples at the admission day and after fifteen days. The assay was performed in Vero cells using CD50 of reference Stxs Stx1-968 (Stx1); 1271-84(Stx 2) and E32511 (Stx2c).
Laboratory assessment

Coagulation parameters, total hemolytic complement, C3 and C4 fraction levels were evaluated after patients admission at Intensive Care Unit (ICU). Laboratory assessment of renal and hepatic function was also done. Blood and urine cultures, and routine peritoneal cultures were performed.

Treatment protocol

The Ethical Committee of Hospital Pediátrico Humberto Notti approved this study protocol and informed consent was obtained from the twelve children parents after explaining the side effects of the treatment.

The protocol included fresh frozen plasma infusions the first day (5 ml/k every eight hours) and methylprednisolone pulses (10 mg/k/day) from the first day and for three consecutive days. Plasma exchange with fresh frozen plasma (PEX/FFP) so as to replace one volemia and a half per day for five days was started from the very beginning as soon as the patients had stable hemodynamic parameters.

Statistical analysis

The normal distribution of the data from tables 1 and 2 was probed by Kolmourov-Smirnoff test and all variables were normally distributed. Results were given as means ± SEM. Statistical tests were performed by using GraphPad InStat version 3.00 for Window 95 (GraphPad Software Inc, San Diego, CA, USA).

Results

Isolation of E. coli O157-H7 was demonstrated in stool cultures from 3 patients. Fourfold or greater rises in Stx2-neutralizing antibody titer (1:4) were detected in eleven cases after fifteen days. The remaining patient showed isolation of E. coli O157-H7 in stool culture, evaluation of Stx2-neutralizing antibody titer could not be performed, the patient died ten days after his admission. Table 1 includes laboratory findings of the patients at presentation.

Erythrocytes fragmentation with severe hemolytic anemia (mean hemoglobin 7.1 ± 0.3g/dl), schistocytosis (8.4 ± 2.0%), and trombocytopenia (35 966 ± 7 925 mm³) were present.

High leukocyte count was demonstrated in the patients at admission, as a clinical marker of acute inflammation. The results of the hemostatic evaluation, showed a mean protrombin tromboplastin time (PTT) of the D+ HUS in normal limits, protrombin time (PT) was prolonged in the whole group. Elevated levels of fibrin breakdown products were evaluated and detected in six children. Normal C3 levels were demonstrated in the twelve patients.

Three patients were misdiagnosed as appendicitis on admission because of acute abdomen and surgery was performed. In the next 24 hours hematological signs of HUS were present.

At the time the twelve patients were admitted in ICU, hemodynamic parameters with decreased medium arterial pressure (MAP) and increased heart rate were shown (Table 2). Early inotropic support was required. The twelve children received dobutamine and dopamine in doses of 4-20 γ/kg/min. Milrinone in dose of 0.23 γ/kg/min (dose adjusted to renal clearance) was added to six patients. The twelve children were mechanical ventilator assisted since the admission to ICU. Eight children suffered from generalized tonic-clonic seizures. Focal seizures were present in the remaining four patients.

<table>
<thead>
<tr>
<th>TABLE 1.–</th>
<th>Laboratory parameters at onset in diarrhea positive hemolytic uremic syndrome (D+HUS) patients (n:12)</th>
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<tbody>
<tr>
<td></td>
<td>Hb [g/dl]</td>
</tr>
<tr>
<td>NV.</td>
<td>12 ±2</td>
</tr>
<tr>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
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<td>3</td>
<td>8.1</td>
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<td>7.9</td>
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<td>6.6</td>
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<td>4.9</td>
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<td>6.3</td>
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<td>11</td>
<td>8.9</td>
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<tr>
<td>12</td>
<td>8.8</td>
</tr>
<tr>
<td>Mean</td>
<td>7.1</td>
</tr>
<tr>
<td>SEM</td>
<td>0.3</td>
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</tbody>
</table>

Results are expressed as means ± SEM.
At onset, severe acute renal failure was present. Eleven patients required peritoneal dialysis and one patient hemodialysis.

Evaluation of PRISM allowed to obtain an objective assessment of these critically ill children with multiple organ system failure. Increased PRISM score: 18 ± 2, was obtained after admission in ICU. Treatment protocol was started after admission in ICU. Complications of plasma exchange treatment include only one episode of hemorrhage after subclavian catheter insertion.

All patients required respiratory assistance for a period of 10 ± 1.3 days. Three patients developed features of acute respiratory distress syndrome. Six patients suffered from cardiac manifestations included dilated cardiomyopathy with increased left ventricular diastolic dimension (LVEDD) 36 ± 0.4 in M-mode echocardiographic assessment. In Doppler ultrasonography, a prolonged relaxation time and decreased early (E), coupled with increased late atrial (A) flow velocity across the mitral valve, were demonstrated in these six patients. Left ventricular systolic dysfunction was shown in five patients, with a decreased left ventricular shortening fraction and decreased ejection fraction (EF): 20 ± 1.7% and 41 ± 3.2% respectively (Table 3). The patients were dialyzed between 8 and 25 days (mean. 17.4 ± 4 days).

Hemorrhagic colitis clinical signs were demonstrated in eight children in the short term follow up in ICU. Recent sigmoidoscopy findings, performed in one patient, included mucosal friability, edema, bowel wall thickening and petechiae. Hepatic dysfunction was demonstrated

<table>
<thead>
<tr>
<th>Patient N°</th>
<th>Age (years)</th>
<th>MAP (mm Hg)</th>
<th>Heart rate/min</th>
<th>Respiratory rate/min</th>
<th>Glasgow</th>
<th>PRISM</th>
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<tr>
<td>1</td>
<td>1.4</td>
<td>38</td>
<td>180</td>
<td>60</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>41</td>
<td>160</td>
<td>54</td>
<td>8</td>
<td>22</td>
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<tr>
<td>3</td>
<td>1</td>
<td>23</td>
<td>170</td>
<td>65</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>8.6</td>
<td>35</td>
<td>160</td>
<td>55</td>
<td>15</td>
<td>25</td>
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<tr>
<td>5</td>
<td>2</td>
<td>43</td>
<td>170</td>
<td>65</td>
<td>15</td>
<td>22</td>
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<tr>
<td>6</td>
<td>4</td>
<td>28</td>
<td>150</td>
<td>70</td>
<td>14</td>
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<td>7</td>
<td>2.4</td>
<td>49</td>
<td>180</td>
<td>50</td>
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<td>8</td>
<td>2.8</td>
<td>36</td>
<td>180</td>
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<tr>
<td>9</td>
<td>1.2</td>
<td>46</td>
<td>190</td>
<td>60</td>
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<tr>
<td>10</td>
<td>1.8</td>
<td>49</td>
<td>182</td>
<td>60</td>
<td>8</td>
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<tr>
<td>11</td>
<td>2</td>
<td>44</td>
<td>160</td>
<td>50</td>
<td>13</td>
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<td>12</td>
<td>2</td>
<td>48</td>
<td>140</td>
<td>54</td>
<td>9</td>
<td>21</td>
</tr>
</tbody>
</table>

Mean 2.86 ± 7.0 40 16 58 12 18

SEM 2.07 ± 0.7 14 6 2 1

TABLE 2.– Clinical parameters at intensive care unit (ICU) admission. Diarrhea positive uremic syndrome D+HUS patients (n:12)

<table>
<thead>
<tr>
<th>Patient N°</th>
<th>EF (%)</th>
<th>AF (%)</th>
<th>LVEDD (mm)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>27</td>
<td>35 (29±3)</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>16</td>
<td>38 (35±4)</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>17</td>
<td>35.5 (29±3)</td>
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<tr>
<td>4</td>
<td>60</td>
<td>40</td>
<td>31 (35±4)</td>
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<tr>
<td>5</td>
<td>40</td>
<td>22</td>
<td>36.7 (29±3)</td>
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<tr>
<td>6</td>
<td>63.9</td>
<td>33.9</td>
<td>31 (33±2)</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>42</td>
<td>32 (33±2)</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>22</td>
<td>35.2 (29±3)</td>
</tr>
<tr>
<td>9</td>
<td>70</td>
<td>39</td>
<td>31 (29±3)</td>
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<tr>
<td>10</td>
<td>70</td>
<td>39.6</td>
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<td>11</td>
<td>72</td>
<td>39</td>
<td>31 (29±3)</td>
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<tr>
<td>12</td>
<td>75</td>
<td>42</td>
<td>37 (33±2)</td>
</tr>
</tbody>
</table>

TABLE 3.– Measures of cardiac state for the twelve diarrhea positive hemolytic uremic syndrome (D+HUS) patients


GCS: Glasgow coma scale including the motor response, verbal response and eye opening response. Normal value: 15

MAP: medium arterial pressure

Data from the standard method of 2 dimensionally directed M mode echocardiograms were obtained

EF (ejection fraction) normal mean values: 60%

AF (shorting fraction) normal mean values: 28%

LVEDD (left ventricular end-diastolic dimension). Normal mean measurement ± SD related to body surface area (BSA) is included in the last column of the table.
through the serum increased levels of transaminases and prolonged prothrombin time.

Eight patients suffered from generalized tonic-clonic seizures for a period of 5.4 ± 1.7 days. Focal seizures were present in the remaining four patients. Computed tomography scans were abnormal in five patients. Presence of hypodense areas was demonstrated in one patient, ischemic areas in two patients and left parietal hemorrhage in two patients. Magnetic resonance imaging confirmed the ischemic areas and intraparenchymal parietal hemorrhage in these patients.

Only one patient developed a secondary infection in ICU, blood culture was positive for *Enterobacter cloacae*.

We did not include a control group. In a retrospective evaluation of three years 1996-1998, six patients from a total of seventy eight patients, presenting with D+ HUS, clinical hemodynamic parameters of septic shock (MAP: 54 ± 3 mmHg, heart rate: 182 ± 18 x min) and severe neurological compromise, were admitted in ICU. These six anuric patients 2.6 ± 0.6 years of age, PRISM: 24 ± 1, GCS: 6 ± 0.6 were supported only with mechanical ventilator assistance, inotropic drugs and dialysis. Five patients died after 3.6 ± 0.8 days of admission to ICU.

From the studied twelve patients, three patients died during the acute phase of the disease, two because of massive lung hemorrhage and the third one because of dilated cardiomyopathy with intense depression of the left ventricular function.

Nine patients survived the initial period. A prospective follow up was performed in these patients. Renal sequelae included chronic renal failure development in one patient, and presence of proteinuria in five patients at six and twelve months of follow up: 38 ± 9 mg/kg/day and 28 ± 7 mg/kg/day, respectively. Periodic blood pressure measurements showed blood pressure within normal limits for age and weight.

Neurological sequelae were evaluated by GOS after leaving ICU, at six and twelve months. At the time the survived nine patients left ICU, GOS 3 (persistent severe disability, conscious but disabled) was demonstrated in three patients, GOS 4 (moderate disability, disabled but independent) in two children, total recovery GOS 5 was shown in the other four patients. Within one year of the injury, GOS 3 was shown in one patient, and GOS 4 in another patient. Recovery of the remaining seven patients (GOS 5) was demonstrated after one year of follow up.

During the period of study, 1999-2003, the outcome of the remaining one hundred thirty-six patients out of the whole group revealed proteinuria: 30.7 ± 5.3 mg/k/day in fourteen children per twelve months of follow up, and 39.7 ± 8 mg/k/day in three children after 3.2 ± 1 years of follow up. Anuria for 9.4 ± 1.5 days was demonstrated during the acute phase of the disease in these seventeen patients. A high blood pressure level was demonstrated in one patient. Glomerular filtration rate was within normal limits in the entirety of the group. No neurologic sequelae were demonstrated.

**Discussion**

The present study has shown clinical evidence of an amplified acute inflammatory response, the magnitude of which predicts the short term, clinical outcome in D+ HUS patients.

To our knowledge, the present study is the first clinical report demonstrating intense compromise of hemodynamic parameters similar to septic shock with multisystemic organ injury, including severe neurological dysfunction, at the initial acute phase in D+ HUS patients.

Sepsis syndrome is very uncommon in children with Shiga toxin *Escherichia coli* (STEC), except in the setting of bowel infarction. Local production of cytokines in the gut might be important in the generation of intestinal inflammation because of their ability to activate intestinal endothelial cells. However, patients with uncomplicated STEC also have increased cytokines levels.

In our group, three patients required intestinal surgery. Histopathological study showed no evidence of bowel infarction. No differences were demonstrated in the early follow up of these three patients related to the whole studied group.

Presentation of sepsis in children with elevated levels of cytokines has been described. Defined as a systemic uncontrolled inflammatory response to an infection, in certain forms of sepsis, such as meningococcemia, circulating TNFα levels are high and correlate with mortality. In HUS, both LPS of EHEC and Shiga toxin have been shown to be immune stimulators and could play a key role in the individual innate immune response, characterized by proinflammatory and anti-inflammatory cytokines.

Although no measurements of blood levels of cytokines were performed during the present study as it has been demonstrated in septic shock, the patients showed severe instability of clinical hemodynamic parameters. Increased heart rate together with decreased mean blood pressure was present in these patients in response to the initial decrease in systemic vascular resistance hence, close hemodynamics monitoring was essential. Early administration of inotropic agents was required. Pulmonary support, with early institution of mechanical ventilation, was necessary in all patients. In addition to the early blocking effect on inflammatory mediators, inhibition of leucocyte migration has also been demonstrated by corticosteroid treatment. Sustained neutrophilia after *Stx2* injection related to renal injury, together with a subsequent increased neutrophil expression of CD11b, enhanced cytotoxic capacity, and greater adhesive properties, was shown in a murine model. In D+HUS, the high
peripheral blood neutrophil count at presentation, has been correlated with a poor prognosis\(^3\).

Herein, increased neutrophilia at onset is shown in the twelve patients.

Taking into account these effects of glucocorticoids, methylprednisolone in doses of 10 mg/kg for three days was included in our protocol.

A 2001 study showed that patients with persistent shock requiring vasopressors and prolonged mechanical ventilation may benefit from physiologic doses of corticosteroids\(^34\). The proposed explanation for the response to corticosteroids, in these patients with relative adrenal insufficiency, despite normal or elevated levels of circulating cortisol, could be the desensitization of corticosteroid responsiveness with down-regulation of adrenergic receptors\(^35\). However the authors proposed lower doses of corticosteroids than the one we have used in the present study.

Administration of higher doses (e.g. 30 mg of methylprednisolone per kilogram) has been reported not to improve survival among patients with sepsis and may worsen the outcome by increasing secondary infections\(^36\). From our group, only one patient had a secondary systemic infection, with a fatal outcome.

The severity of the trombocytopenia and anemia, with high levels of lactate dehydrogenase (LDH) and the persistent neurological signs and symptoms in this group of D+ HUS patients, allow us to demonstrate similar clinical presentation to adult thrombotic thrombocytopenic purpura\(^37\). Plasma exchange with fresh plasma infusions have been reported as the treatment in adult PTT, a microangiopathic disease due to deficiency of the specific von Willebrand Factor (VWF) cleaving protease (VWF-CP) ADAMTS 13\(^38, 39\). Similar treatment has been included in D+ HUS patients, allowing us to demonstrate similar clinical presentation to adult thrombotic thrombocytopenic purpura without any adverse effects.

In our studied D+HUS children group with normal limits of C3 levels and intense neurological compromise, PEX/FFP was included in the aggressive treatment. PEX/FFP therapy was started early on, presuming to remove remaining level of circulating toxins before their binding to cellular receptors.

Data reported in the literature related to plasmapheresis in D+ HUS are scanty\(^41, 42\). In a retrospective study that reviewed children over 5 years of age, plasmapheresis was performed in patients at risk of poor outcome\(^43\). Up to now, plasmapheresis has not been incorporated to the protocol treatment of D+ HUS children\(^44\).

In the outcome, nine patients survived, three of them, with severe sequelae after one year of follow up. Although signs of severe neurological compromise were shown during the acute phase, substantial improvement was demonstrated in the short follow up in seven patients as it has been reported in HUS\(^45\). Yet, two patients with structural neurological damage had persistent severe disability (GOS 3 and GOS 4, respectively).

The fact that during the period 1996-1998, five out of six D+HUS patients, affected with unstable hemodynamic parameters and multiple organ failure including severe neurological compromise died, stirred us to action and motivated us to look for a non conventional treatment of dealing with this disease.

Signs of reduced functional renal mass through the increased proteinuria in the remaining studied patients, were not different from the remaining one hundred thirty-six patients D+ HUS patients with no evidence of hemodynamic instability and neurological dysfunction at onset. In the whole group, lasting anuria was related to a poor renal prognosis as it has been previously reported by Spizzirri et al\(^46\).

We conclude from our results, that early introduction of the described therapeutic protocol, seems to be able to achieve positive results in severe multisystemic D+ HUS.

The adverse clinical outcome at short-term follow up, similar to septic shock, allows us to suggest an intense inflammatory amplified response after STx exposure in these patients.

Future studies will be required to elucidate the mechanisms involved in this early severe clinical presentation in D+ HUS patients.

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