Death caused by hemolytic-uremic syndrome.

Case series

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Abstract: Hemolytic-uremic syndrome is defined by a combination between renal failure, microangiopathic hemolytic anemia and thrombocytopenia; it usually appears in young children, is preceded by gastrointestinal enteritis and may involve other organs as well. The purpose of this article is to present a series of four cases of lethal hemolytic uremic syndrome and discuss its legal medicine consequences. For the first three cases an autopsy was not performed and the direct cause of death was considered brain involvement. In the fourth case an autopsy was performed and the direct cause of death was considered to be septic shock, secondary to diarrheal associated hemolytic uremic syndrome, caused by an enteropathogen E.Coli.

Key Words: hemolytic uremic syndrome, sudden unexpected death in infancy, E.Coli.

Hemolytic-uremic syndrome (HUS) is defined as a combination between renal failure, microangiopathic hemolytic anemia and thrombocytopenia; it usually appears in young children, is preceded by gastrointestinal enteritis and may involve other organs as well (especially the CNS) [1]. It was classified in D+HUS (diarrheal associated) and aHUS (atypical or non-diarrheal) [2]. D+HUS is more common and is usually associated with verocytotoxin-producing bacteria like E. Coli O157:H7. E. Coli is a normal commensal bacteria in the human gut and most strains produce an endotoxin. One of the most important virulence factors is a group of enterotoxins that leads to diarrheal disease, with some varieties leading to hemolytic uremic syndrome. Besides digestive disorders, E. Coli may also cause urinary tract infection, neonatal meningitis, and so on. E coli spreads by fecal-oral route, often from contaminated foods like undercooked beef and hamburgers [3].

The onset of symptoms in HUS occurs between 2 and 12 days after the contact with the infective agent; in E.Coli O157:H7 infections there usually are one to three days of non-bloody followed by bloody diarrhoea [4]. Renal involvement may include isolated hematuria,
proteinuria, and leads to acute renal failure in 55-70% of cases, with about 60% needing dialysis during the acute phase. Extrarenal symptoms may appear at the level of the CNS (irritability, encephalopathy, seizures, coma), GI tract (hemorrhagic colitis, bowel necrosis, colonic stricture, perforation or intussusception, hepatomegaly with elevated transaminases, pancreatic insufficiency with glucose intolerance), or heart (congestive heart failure in the acute phase, and cardiomyopathies, identifiable a few months after the onset of the symptoms) [5]. Atypical HUS is not caused by verocytotoxin producing bacteria, and have a poorer prognosis, with death rates up to 25% and progression to end-stage renal disease in up to 50% of cases.

The purpose of this article is to present a series of cases of lethal HUS and discuss its legal medicine consequences.

CASE SERIES

Case 1

An 8-month-old girl was referred to the Emergency Care Unit with a three-day history of bloody stools, diarrhea, and vomiting, and was hospitalized in the Intensive Care Unit for dehydration, severe anemia, thrombocytopenia and renal insufficiency. Upon physical examination, the patient was found pale. A rapid overview of the child’s clinical symptoms and laboratory data at admission suggested a case of post-diarrheal HUS. The disease evolved as progressive generalized edema, hyporeactivity, seizures, hypertension, the persistence of bloody stool emission, and anuria in correlation with marked leukocytosis (24000/mm³), microangiopathic changes consistent with hemolysis on peripheral blood smear (schisocytes and helmet cells), platelet count of 16000/mm³, metabolic acidosis (pH 6.9; HCO₃ 17.8 mmol/L; BE - 12.5 mmol/L), hyponatremia (116.8 mEq/L), and the rapid rise in blood urea and creatinine levels (200 mg/dL and 4 mg/dL). The treatment consisted of parenteral hydration, plasma and pancreatic exocrine insufficiency – reinforced markers of pancreatic inflammation, pancreatic injury, and pancreatic exocrine insufficiency – reinforced the diagnosis. Ultrasonography revealed an abnormal image of the pancreas, while important gastrointestinal bleeding led to shock status. Despite the complexity of the therapeutic schema – blood transfusions, antibiotics, anti-shock therapy, intubation, and mechanical ventilation – the child died from the rapid progression of HUS which caused multiple organ failure. Autopsy was not performed in this case.

Case 2

An 8-month-old girl was admitted in the Emergency Care Unit for seizures, generalized edema, pallor, and anuria, with a two-day history of intussusception successfully reduced by an air enema. On the same day, the child manifested bloody diarrhea with frequent episodes of mucoid hematochezia, and tenesmus associated with irritability. Laboratory tests – hemoglobin 7 mg/dL, urea 240 mg/dL, creatinine 4 mg/dL, leukocytosis 32000/mm³ and thrombocytopenia 30000/ mm³ – confirmed the HUS diagnosis. Over the next few days, repetitive seizures and aggravation of hemorrhagic colitis, significant elevation in amylase, lipase, glucose and hepatocellular enzyme levels, obvious reduction in size of kidneys, and signs of pancreatitis (confirmed by abdominal ultrasound) evinced severe complications.

A pulmonary edema, arrhythmia, and progressive neurological deterioration (4 points on Glasgow Coma Scale) imposed intubation and mechanical ventilation in the Intensive Care Unit, as continuation to the initial treatment consisting of peritoneal dialysis, parenteral administration of hydration, and medication. Death was confirmed following cardiopulmonary arrest. Autopsy was not performed in this case.

Case 3

A 10-month-old girl was hospitalized for hemorrhagic enterocolitis and recurrent seizures, three days after an initial simple antibiotic-associated diarrhea diagnosis. A short time after admission striking pallor, fever, and oliguria with minimal edema pointed to HUS. The blood test consisting of low hemoglobin (5.4 g/dL), high leukocytosis (24000/ mm³) and high azotemia values, trombocitopenia (45000 mm³) with normal coagulation parameters, elevated level of hepatocellular enzymes, and, especially, the marked increase in blood amylase (422 u/L), lipase (252 u/L), and high glucose serum levels (236 mg/dL) – all markers of pancreatic inflammation, pancreatic injury, and pancreatic exocrine insufficiency – reinforced the diagnosis. Ultrasonography revealed an abnormal image of the pancreas, while important gastrointestinal bleeding led to shock status. Despite the complexity of the therapeutic schema – blood transfusions, antibiotics, anti-shock therapy, intubation, and mechanical ventilation – the child died from the rapid progression of HUS which caused multiple organ failure. Autopsy was not performed in this case.

Case 4

Clinical data

An 18 months old girl was hospitalized for vomiting, soft, bloody faces, without fever, with the onset of symptoms about 48 hours before. At admission the patient was afebrile, sleepy, with the vesicular murmur present bilaterally, AV=100/min, warm extremities, bilious vomiting, discretely hyperemic pharynx, present diuresis, without signs of meningeal irritation. Serum
Creatinine was 0.6 mg/dL at admission but increased to 3.1 after 48 hours, with urea increasing from 46 to 130 mg/dL. After 48 hours the patient became comatose, with a BP of 98/76 mmHg, a pale skin, bloody vomiting, multiple bloody stools, acute renal failure, decreased platelet levels (59*10^3/u), decreased serum Na (130 mmol/L), high leukocyte count (25700/mm^3).

Stool samples revealed a positive reaction for enteropathogen E. Coli (EPEC). The patient was transferred to the ICU, and is put on dialysis but died in the same day.

**Autopsy findings**

The autopsy showed diffuse cerebral edema, pulmonary condensations, petechiae in the parietal peritoneum, mesenteric adenopathy. The small intestine was 296 cm and the colon was 54 cm long. The small intestine was greyish-pink with erosions and ulcerative areas (Fig.1). The serous layer was reddish-violet in the cecum, and partially on the transverse colon and the ascending colon. The mucosal layer of the colon was violet, with violent-black oval areas, well defined, diffuse, and erosive areas not surpassing the serous layer, oval, well rounded, with preeminent margins, with maximum diameters of 1/0.5 cm. The cecal appendix was about 4 cm, thickened, with a violent-black serosa layer and numerous reddish-violet spots located in the mucosa layer. Both kidneys present numerous reddish spots, located within the capsule, and violet-black spots, diffusely located within the cortical and medullar layers (Fig.2).

**Histopathology investigation**

**Tissue sampling and stains:**

Specimens of the stomach, jejunum, ileum, cecum, appendix and colon were taken for histopathology investigation.

Also, samples of brain, lungs, myocardium, liver and kidney were taken as well.

Other fragments were harvested from adrenal glands, pancreas, spleen, mesenteric lymph nodes and thymus.

Samples were taken after informed consent, using a protocol approved by the local Bioethics Committee, in accordance to generally accepted international practice.

The selected tissue samples were fixed in 10% neutral buffered formalin (pH - 7) for 24–48 hours and paraffin embedded. Sections were cut at 5 μm and stained with standard HE.

In addition, special stains such as elastic van Gieson, Mallory’s phosphotungstic acid-hematoxylin (PTAH) and Gram have been done.

**Immunohistochemistry:**

Immunohistochemical analysis (IHC) was done using sections displayed on slides treated first with poly-L-lysine. IHC was performed on 3 μm thick sections from formalin-fixed paraffin-embedded specimens, according to the indirect tristadial Avidin-Biotin-Complex method of Hsu [6], modified by Bussolati and Gugliotta [7].

Briefly, the procedure comprised: deparaffination in xylene and alcohol series, rehydration, washing in phosphate buffer saline (PBS), blocking with normal serum, for 20 min., incubation with primary antibody overnight then with standard labeled streptavidin-antibody biotin (LSAB kit, DAKO, Denmark), washing in carbonate buffer and developing in 3,3'-DAB hydrochloride/H_2O_2.

The antibodies used for IHC were: CD20, CD45RO, CD68, CD62-P, TRAF-1 (for details, see Table 1).

**Table 1. Antibodies used for IHC tests**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Producer</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>L26</td>
<td>RTU</td>
<td>Novocastra</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td>CD45RO</td>
<td>UCHL1</td>
<td>RTU</td>
<td>Novocastra</td>
<td>T lymphocytes</td>
</tr>
<tr>
<td>CD68</td>
<td>51H14</td>
<td>RTU</td>
<td>Novocastra</td>
<td>Macrophages</td>
</tr>
<tr>
<td>CD62-P</td>
<td>C34</td>
<td>RTU</td>
<td>Novocastra</td>
<td>Adhesion molecule</td>
</tr>
<tr>
<td>TRAF-1</td>
<td>7C11</td>
<td>RTU</td>
<td>Novocastra</td>
<td>Pro-inflammatory cytokine</td>
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</tbody>
</table>
Antigen retrieval techniques (thermal or enzymatic pretreatment) for some of the aforementioned antibodies were done, according to the producer’s specifications. Both positive and negative controls were used.

To ensure the reliability of the experimental study, internal quality control of histopathologic and IHC techniques were performed as a part of an implemented and certified quality assurance system (ISO 9001/2008). All slides were examined and photographed on a Zeiss AxioImager microscope (Gottingen, Germany). Digital images acquired with Zeiss Axio Vision program have been processed and analyzed with ACDSee Pro Photo Manager (Washington DC), running under Windows Vista.

RESULTS

In stomach and jejunum were found multiple foci of mucosal erosions associated with micro-hemorrhages in the corion.

In the ileum were noticed diffuse ulcerations with necrotic areas, hemorrhagic foci and marked mixed inflammatory infiltrate in mucosa and submucosa with micro-abscess formation, associated with fibrinous micro-thrombi in the capillaries (Fig.3).

The appendix showed acute purulent appendicitis with subsequent inflammatory reaction of the peritoneum.

The cecum and the colon have presented diffuse ulceration of the mucosa with necrotic debris at the surface, associated with marked acute inflammation and crypt micro-abscesses. In addition, multiple intramural hemorrhagic foci and serous acute inflammatory reaction were found.

PTAH stain has revealed many capillaries with micro-thrombosis

Figure 3. a) Ileum with extensive loss of villi due to ulceration and necrosis, associated with marked inflammatory infiltrate in the corion (inset: capillary micro-thrombus), HE, 100x; b) mixed inflammatory infiltrate with micro-abscess formation and capillaries with micro-thrombi (arrows), HE, 200x.

Figure 4. Colon with ulceration of the mucosa, blunt villi and capillaries with fibrinous micro-thrombosis, PTAH stain, 200x.

Figure 5. Interstitial lymphocytic pneumonia with atelectasis, HE, 100x.
in mucosa and submucosa (Fig.4). Gram stain has revealed very rare, isolated Gram negative bacilli.

The morphologic aspects were compatible with an acute ulcerative, necrotic and hemorrhagic enterocolitis due to a possible infectious nature.

All lymphatic organs (spleen, thymus, mesenteric lymph nodes) were reactive, showing lymphoid follicular hyperplasia, with prominent germinal centers and sinusal histiocytosis.

Interstitial lymphocytic pneumonia with diffuse pulmonary atelectasis was found in lungs (Fig.5).

Immunohistochemistry showed frequent B lymphocytes (CD20 positive) in the septa and many intra-alveolar macrophages (CD68 positive) (Fig.6); UCHL1 was negative. CD62-P (p-selectin) was positive in rare activated capillaries; TRAF-1 was negative.

In the kidney were found foci of acute tubular necrosis, interstitial hemorrhages and foci of acute inflammatory infiltrate with PMN (Fig.7). PTAH stain has revealed numerous fibrinous micro-thrombi in the glomerular capillaries (Fig.8). The adrenal glands showed interstitial hemorrhages in the medulla (Fig.9).

The aforementioned lesions were consistent with a shock kidney, in a hemolytic uremic syndrome.

The other organs were congested and edematous.

**Thanatochemistry**

Serum liquids and blood samples were taken for biochemistry analysis.

Toxicological screening was negative. Microbiological cultures conducted on samples obtained at the autopsy revealed a positive reaction in blood for Klebsiella pneumoniae in the blood (MDR, lactamase producing). Stool samples was negative for EPEC.

The cause of death was septic shock with intravascular disseminated coagulation secondary to a EPEC caused HUS.

**DISCUSSIONS**

HUS was considered as a relatively benign pathology, especially in small children [8]. However, a more recent study revealed this pathology to have an overall mortality of about 10%, main causes of death being CNS, cardiovascular or gastrointestinal complications, shock, sepsis, or hospital-related pathologies [9]. As it usually affects infants and may have a rapid course if left untreated, was sometimes cited as a cause of sudden infant death [10, 11]. For example Manton et al. published two cases of sudden unexpected death in infancy in which the etiology was...
of microbiological organisms is not always accurate [13], especially in cases in which the patient has previously received antibiotic treatment.

A fast and accurate diagnosis may have significant public health consequences, as a positive identification of the causal agent (usually contaminated cattle meat or dairy products) [14-19] may prevent further lethal contagions.

Brain involvement was considered to be the direct cause of death in the first three cases; as the families have refused the autopsy, we do not have objective proof to confirm it; the fourth, in which an autopsy was performed, identified acute renal lesions, signs of intravascular disseminated coagulation, and severe, necrotic intestinal lesions, suggesting that septic shock was the direct cause of death. Brain involvement was mild in this case (cerebral stasis and edema, without hemorrhages or infarctions, often described in the scientific literature [12].

The presence of small bowel and upper digestive tract lesions are rarely described in association with HUS. For example Gallo and Gianantonio identified, on 64 autopsied cases, only five infants with small bowel, one with stomach and two with esophagus lesions[20]. The fourth case in our series had both small bowel and gastric lesions.

Kidney involvement was severe in all cases, as suggested by clinical signs and symptoms and pathology examination (fourth case only). Gianantonio classified HUS associated kidney lesions in acute and chronic (later stages). Acute lesions were further classified in (1) fibrin thrombi and/or linear deposition of fibrin along the glomerular capillary walls, (2) fibrinoid necrosis of the interlobular arteries with thrombotic occlusion of their lumen, and (3) hemorrhagic lesions not related to thrombosis of the adjuvant vasa recta. Later (chronic) lesions are characterized by bilateral renal cortical necrosis, with marked centrilobular thickening, thickening of the capillary walls and glomerular

HUS, complicated with hemorrhagic brain infarcts [12]. The diagnosis may be suspected during the autopsy in a patient with that dies secondary to a gastrointestinal illness. The pathologist/forensic pathologist should examine thoroughly the colon, kidneys, brain, heart, abdominal lymph nodes and check for signs of intravascular disseminated coagulation, with infarction of both small and large vessels and hemorrhages in various locations. Microbiology should always be tempted (if was not done so during hospitalization), even if it is known that postmortem identification
fibrosis [21]. The fourth case has both type 1 and type 2 acute lesions, with an associated acute inflammatory, peritubular reactions.

HUS shares many clinical and pathological features with thrombotic thrombocytopenic purpura (TTP) but, while TTP is typically a disease of adults, HUS occurs usually in children. Both are determined by inappropriate activation of platelets associated with activation of coagulation system. This leads to formation of micro-thrombi in the vessels, especially in the kidney and lungs, resulting in ischemia and hypoxia. Activation of platelets also releases inflammatory mediators that cause damage to target organs, producing ARDS (acute respiratory distress syndrome), renal failure or both [22].

In a study performed on 56 autopsied cases with HUS and TTP in 70 years [23], the authors have shown that the differences in the character and distribution of arterial lesions found in their cases suggest that TTP and HUS are distinct patho-physiologic entities. Lesions in multiple organs, with cardiac involvement in all cases, characterized TTP, whereas patients with HUS had severe renal disease and infrequent lesions in other organs. In their review, the most useful histologic discriminator between TTP and HUS on autopsy material was the platelet-rich, fibrin-poor character of TTP lesions and the fibrin-rich character of HUS lesions as shown by PTAH staining.

IHC can be done in order to find and illustrate platelet aggregates with FVIII, activated capillaries with some adhesion molecules such as P-selectin (CD62-P) or E-selectin, in context of inflammation or other vascular markers such as CD34 or CD31, but non-specific for HUS. Therefore, PTAH seems more reliable than IHC when applied to autopsy tissues.

CONCLUSIONS

As HUS is often caused by microbial agents, a proper diagnosis and epidemiological survey is mandatory in order to limit potentially severe outbreaks. If an infectious lethal HUS is identified in a specific area associated with other, lethal or non-lethal cases, forensic autopsies should be mandatory in each death as it/they may be considered as suspect in accordance with Romanian law.

**References**