Rapid diagnosis of *Streptococcus pneumoniae*-induced haemolytic-uraemic syndrome

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Introduction

Haemolytic-uraemic syndrome (HUS) is caused in many cases by an enteral infection with enterohaemorrhagic *Escherichia coli* (EHEC), *Shigella dysenteriae* type 1 or *Citrobacter freundii* producing Shiga-toxin, or by hereditary or acquired defects of the regulation of the complement cascade[1-4]. In rare cases infections with bacteria not producing Shiga-toxin, such as *Streptococcus pneumoniae* and *Clostridium perfringens* (e.g. in pneumonia, necrotising enterocolitis, sepsis or gas gangrene), can also cause life-threatening haemolysis, coagulation disorder, and acute renal failure[5-9]. Through the effect of the enzyme sialidase, these bacteria can unmask a normally hidden antigen on the red blood cell surface. This cryptic antigen, called T-antigen, was first described *in vitro* by Thomsen and Friedenreich[10,11]. The pathogenic part of a potential reaction of the corresponding antibody (of the IgM-class) in human plasma (anti-T) with the T-antigen *in vivo* is a source of controversy, since the optimal temperature of this antibody is lower than body temperature and other bacterial toxins (e.g. lecithinase in several *Clostridium spp.*) are also very strong haemolysins[12,13]. Independently of the pathogenic mechanism, detection of T-antigen activation on red blood cells can be of differential diagnostic value in acute haemolysis[9]. Here we report the case of life-threatening HUS in a child, in whom the combination of immunohaematological findings in the blood grouping laboratory and immunological detection of bacterial antigens in the patient's body fluids led to the correct diagnosis of the underlying disease within only a few hours.

Case report

The 9-month old male patient had been a preterm neonate, born at 36(+6) weeks' gestation and had suffered from sepsis without a known focus 6 weeks after birth. The baby's subsequent development had been slightly delayed, but without other severe diseases. He had not yet received any recommended vaccinations.

On admission the mother reported that the boy had suffered from cold and fever for some days, but not from vomiting or diarrhoea. His skin was pale, but without petechiae. His body temperature reached 40 °C. The patient weighed 9.6 kg (between 50-75th percentile) and his height was 80 cm (90-97th percentile). Auscultation of the lungs revealed reduced breathing sounds over the right lung, vesicular crepitations on both sides and tachypnoea. There were no signs of impairment of other organs; in particular there were no abdominal signs of relevance, no meningism or other neurological symptoms. The chest X-ray showed shadowing of the whole right hemithorax due to a combination of pulmonary infiltrates and atelectasia. Ultrasound revealed chambered pleural effusion around the right lung. On admission the white blood cell count was greatly increased (34.0×10^9/L) with 79% segmented neutrophils and 7% bands. The patient had mild anaemia (haemoglobin 10.3 g/dL) and a normal platelet count (520×10^9/L). C-reactive protein (CRP) was strongly increased (409 mg/L), creatinine was normal (0.4 mg/dL). Antibiotic therapy was started immediately with cefuroxime i.v. and was combined with clindamycin i.v. on day 5 as the fever continued.

On day 6 the child's general condition deteriorated dramatically. In particular, his skin was very pale. The laboratory examination revealed massive inflammation (white blood cell count 20.3×10^9/L; C-reactive protein 273 mg/L; procalcitonin 23.4 µg/L; interleukin-6 163.3 ng/L), severe anaemia (haemoglobin 3.0 g/dL; haematocrit 8.7%; red blood cell count 1.1×10^{12}/L; fragmented cells 29/1,000 red blood cells), haemolysis (lactate dehydrogenase 3,278 U/L; haptoglobin <8 mg/dL; potassium 6.0 mmol/L), acute renal failure (creatinine 2.6 mg/dL; urea 179 mg/dL) and coagulopathy (partial thromboplastin time 53 seconds; platelet count 43×10^9/L; D-dimer 14.5 mg/L). On this day a polyspecific direct antiglobulin test (DAT) of the patient's red blood cells was performed together with blood grouping (O CCD.ee K-; antibody screening test negative) and showed a positive result (strength: 1+ on a scale from 0 to 4+). For further differentiation of the positive polyspecific DAT, monospecific DAT

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with anti-IgG, anti-IgM, anti-IgA, anti-C3b and anti-C3d were performed and showed a positive result with anti-C3d (strength: 2+) (the DAT were done using the gel card centrifugation method, Diamed AG, Cressier FR, Switzerland). T-antigen was demonstrated on red blood cells in two blood samples taken at different times on day 6 (strength: both 4+) (tube spin method using lectin from *Arachis hypogea*, Sigma-Aldrich Chemie GmbH, Munich, Germany). To determine the cause of the T-antigen activation, immediately after the immunohaematological examinations in the blood grouping laboratory, the patient's urine was tested for soluble pneumococcal antigen using a rapid immunochromatographic test (BinaxNOW® *Streptococcus pneumoniae*, Alere GmbH, Cologne, Germany) and showed a positive result. The diagnosis of *Streptococcus pneumoniae*-induced atypical HUS was made based on the combination of the clinical symptoms (acute haemolysis, thrombocytopenia, acute renal failure) and the laboratory findings (T-antigen activation, pneumococcal antigen detection).

The patient was immediately admitted to the intensive care unit for mechanical ventilation (for 23 days) and for peritoneal dialysis (for 18 days). The circulation was stabilised by catecholamines for 24 days. Antibiotic therapy was continued with cefotaxime and clindamycin for altogether 21 days, and for a part of this time teicoplanin was added. Altogether the patient received five transfusions of red blood cell concentrate and one transfusion of an apheresis platelet concentrate. On day 36 the child left the intensive care unit and was discharged home from the hospital on day 45 in a good general condition (haemoglobin 10.2 g/dL; creatinine 0.4 mg/dL).

In contrast to the above mentioned immunological test for pneumococcal antigen, the results of the other microbiological examinations were unremarkable: examination of a throat swab for influenza A and influenza B virus RNA using polymerase chain reaction analysis was negative as was examination of the patient's faeces for Shiga-toxin using an enzyme-linked immunosorbent assay. An aerobic blood culture taken on day 4 showed no growth of bacteria or fungi. Also in tracheal aspirate and in gastric fluid taken on day 7 there was no growth of bacteria or fungi. No pathogenic bacteria were found in throat swabs taken on days 4 and 6 and in a nose swab taken on day 6, presumably because antibiotic therapy had already been started.

Discussion

Most cases of HUS in children are caused by EHEC, but in about 5% of cases in children pneumococcal infections are found[14]. The incidence of HUS following invasive *Streptococcus pneumoniae* infections is estimated to be about 0.5%[14]. The pathophysiology of pneumococcal HUS is not yet completely resolved, although T-antigen activation was already described in patients with pneumococcal HUS more than 40 years ago[5]. T-antigen on the surface of red blood cells, platelets and glomerular endothelial cells is normally hidden by sialic acid. It can be unmasked by the enzyme sialidase produced by some micro-organisms such as *Streptococcus pneumoniae* and *Clostridium perfringens*[5-9]. Although the pathogenic role of the corresponding anti-T antibody in human plasma is the subject of lively debate[12,13], modification of the surface of red blood cells or cells in the kidney by bacterial enzymes might be of relevance in the pathogenesis of pneumococcal HUS[15].

In the case described above the test for T-antigen activation on red blood cells was ordered in the context of an episode of acute severe haemolysis, which might have been caused by an infection with a sialidase-producing pathogen, but also by incompatible drugs (e.g. antibiotics) or by autoimmune mechanisms (e.g. in the case of a *Mycoplasma pneumoniae* infection). These latter differential diagnoses would have made a change of therapy necessary. The uncovered T-antigen on the patient's red blood cells was demonstrated by a simple agglutination technique using *Arachis hypogea* lectin[16]. Together with the clinical findings of anaemia, haemolysis, and acute renal failure, this lead to the suspicion of an atypical HUS. The rapid test for pneumococcal antigen in the urine then allowed *Streptococcus pneumoniae* to be identified as the causative agent only a few hours after the beginning of the severe haemolysis. A positive DAT has also been described in other patients with *Streptococcus pneumoniae*-associated atypical HUS[17]. Other differential diagnoses, such as a typical HUS caused by EHEC, a necrotising enterocolitis or an influenza A virus infection[1,7,18], could be excluded by the lack of typical clinical symptoms and by the negative laboratory results.

In contrast to bacterial culture methods, which need an incubation period of at least 1 day and often more and which can produce false-negative results because of antibiotic treatment before specimen collection, immunological tests can detect some infections rapidly and with little expenditure. Such tests can accelerate and simplify also the diagnosis of pneumococcal infections. The sensitivity and specificity reported in the literature for the pneumococcal antigen test that we had used in our case are satisfactory (75 and 95%, respectively, in adults)[19], even though false positive results can be seen in children with heavy colonisation with *Streptococcus pneumoniae*[20].

In conclusion, the case reported above demonstrates that a simple immunohaematological test for T-antigen

activation on red blood cells together with testing the patient's urine for soluble pneumococcal antigen by a rapid immunological assay can provide an important contribution to quick diagnosis in some cases of atypical HUS.

Keywords: haemolytic-uraemic syndrome, *Streptococcus pneumoniae*, T-antigen, pneumococcal antigen, haemolysis.

The Authors declare no conflicts of interest.

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