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Case report

Investigation of the pathogenesis of massive hemolysis in a case of *Clostridium perfringens* septicemia

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Summary. Massive hemolysis is a rare, usually fatal complication of *Clostridium perfringens* septicemia. Of all toxins produced by the bacterium, phospholipase C (PLC) is believed to be the most likely cause of hemolysis. An influence of neuraminidase has often been suspected. In the present study, a case of *C. perfringens* septicemia with acute massive intravascular hemolysis is described. It led to death within 4 h of admission to the hospital. While the course of events was comparable to previously reported cases, we succeeded in gaining deeper insight into the pathogenesis by monitoring serum anti-T titer and quantifying serum PLC activity during the course of the disease. We excluded an effect of neuraminidase by a negative direct antiglobulin test, a negative anti-T lectin test, and a steady serum anti-T titer of 1 in 32. Serum PLC activity, on the other hand, showed a nearly fivefold increase (6.0 to 27.3 U/l), which is consistent with the hypothesized dominant role of this enzyme.

Key words: *Clostridium perfringens* – Hemolysis – Phospholipase C – Neuraminidase – T-antigen

Introduction

Clostridium perfringens is capable of inducing a wide spectrum of clinical manifestations, ranging from asymptomatic patients with an incidental positive blood culture to massive intravascular hemolysis, shock, and death [1, 6]. Of the various toxins produced by *C. perfringens*, PLC (alpha-lecithinase) and neuraminidase (sialidase) are held responsible for causing significant hemolysis in vivo [6, 11]. In one case additional proteolyic action of other toxins was proposed [16].

PLC acts upon red blood cells directly by disruption of their cell membrane, caused by hydrolyzing sphingomyelin and lecithin [6, 13]. Neuraminidase exposes the T-cryptantigen on normal red cells by removal of N-acetyl neuraminic acid, enabling binding of anti-T antibodies present in the serum of all subjects except infants [4, 7, 11, 17]. Whereas the hemolytic potential of anti-T antibodies reacting with exposed T-antigen on red blood cells has been demonstrated [5, 12], an involvement of neuraminidase in the massive hemolysis seen in *C. perfringens* septicemia has been doubted [9, 14]. Among the 25 comparable cases we found reported in the literature from 1959 onward, the direct antiglobulin test and the anti-T lectin test were reported to be positive only in one case [9]. Serum PLC was demonstrated in two cases with qualitative methods [3, 15]. In the present study, we describe a case of acute, massive hemolysis caused by *C. perfringens* septicemia. In addition to previously reported findings, we were able to describe the anti-T titer and to determine PLC activity in serum and urine.

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Materials and methods

A complete blood count (RBC, WBC and PLT), hemoglobin (HGB), hematocrit (HCT), red cell indices (MCV, MCH, MCHC), and blood cell histograms based on the size distribution of cells were obtained using a Coulter S-Plus STKR. All serological tests were performed by means of gel centrifugation (Diamed-ID microtyping cards, Diamed AG, Switzerland) [10]. T-activation was tested with anti-T lectin from *Arachis hypogaea* (Biotest Diagnostics). To determine the anti-T titer, serial twofold dilutions of the patient's serum were incubated with T-activated RBC prepared with *C. perfringens* neuraminidase (Sigma Chemicals Co.). A turbidimetric method developed by Jolivet-Reynaud et al. [8] was slightly modified for quantification of PLC activity in the serum.

Case report and results

A previously healthy 84-year-old woman sought medical attention because of indisposition and increasing abdominal girth. Physical examination revealed jaundice with massive abdominal distention, abdominal tenderness, elevated blood pressure of 170/80 mm Hg, an elevated pulse rate of 100/min, and moderate dysp-

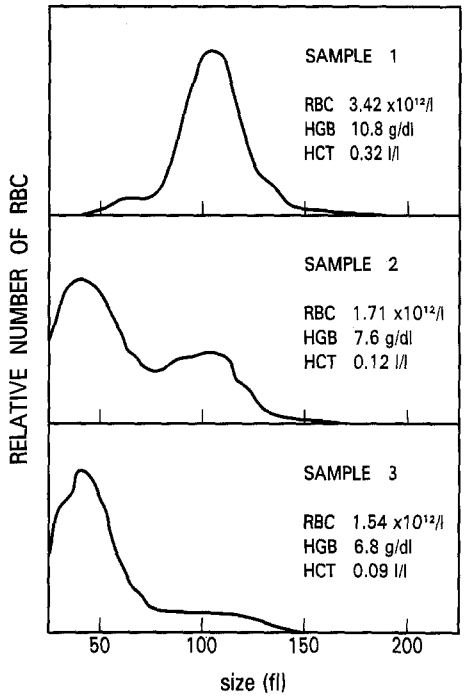

Fig. 1. Coulter red blood cell histograms of the three EDTA blood samples drawn within 3 h

nea. Her body temperature was 37° C. Blood samples were sent to the laboratory: Coulter analysis showed a leukocytosis of 16.5×10^9/l, mild anemia (RBC 3.42×10^{12}/l, HGB 10.8 g/dl, HCT 0.32 l/l) with normal MCV and MCHC. In retrospect, a minute elevation of the red cell histogram in the 60-nm region (Fig. 1) represented fragmented RBC. Routine coagulation tests were within the normal limits. Reddish discoloration of serum samples suggested hemolysis, which was confirmed by the results of blood chemistry: total bilirubin (TB) 21.0 mg/dl (359 μmol/l); direct bilirubin (DB) 7.2 mg/dl (123 μmol/l); lactate dehydrogenase (LDH) 1344 U/l; and a potassium level of 9.0 mmol/l.

A direct antiglobulin test of the patient's erythrocytes was negative. The C-reactive protein concentration was 23.6 mg/dl. Blood cultures later grew *C. perfringens*, *Escherichia coli*, and *enterococci*. The urine showed massive hemoglobinuria. In spite of antibiotic therapy the condition of the patient deteriorated rapidly; her body temperature rose to 38° C and she became unresponsive. A second set of blood samples drawn 90 min after the first one reflected the dramatic course of hemolysis: hematocrit had fallen to 0.12 l/l, the RBC count was 1.71×10^{12}/l, and hemoglobin 7.6 g/dl. The RBC histogram showed an abnormal population of seemingly microcytic cells, which in fact represented fragmented red cells and ghosts. These artifacts and the inclusion of plasma hemoglobin in the hemoglobin value accounted for the MCV of 68 fl and for the reported MCHC of 658 g/l. TB had risen to 32.0 mg/dl (547 μmol/l), DB to 12.0 mg/dl (205 μmol/l), and LDH to 4400 U/l.

After approximately another 90 min the last blood samples were drawn. Shortly thereafter the patient died. CBC results corresponded with the continued hemolysis. The red cell histogram revealed the preponderance of fragmented erythrocytes and ghosts (Fig. 1). TB amounted to 34.0 mg/dl (581 μmol/l), DB to 14.0 mg/dl (239 μmol/l) and LDH to 6120 U/l.

Serological findings. The antiglobulin and anti-T lectin tests were consistently negative. The anti-T titer remained at a steady normal level of 1 in 32 in all samples.

PLC activity. Quantification of PLC in the serum showed a considerable activity of 6.0 U/l already in the first sample. This activity rose to 10.1 and 27.3 U/l in the second and third serum sample, respectively. The activity in urine was 2.6 U/l (within a volume of 60 ml).

Remarkable results of the autopsy were septic alteration of organs, especially of the liver and spleen, and distention of the small and large intestines. As no site of entry of the infection was found, the intestine was assumed to be the portal of entry, which would be consistent with the combination of bacteria growing in the blood culture. Clostridia-like rods were found in the bone marrow only; signs of gas productioin were not detectable.

Discussion

No clinical finding specifically alerted to acute hemolysis, which was brought into the focus by hemolyzed samples and massive hemoglobinuria. The hematopathological findings of Coulter analysis mirror the dramatic course of events and at the same time demonstrate the limitations of numeric results and histograms, in which RBC ghosts and fragments appear merely as microcytic RBC (Fig. 1). RBC count and HCT consequently under-represent hemolysis. The potassium level of 9 mmol/l without corresponding alterations of the ECG at the time of the sample collection would suggest additional hemolysis in vitro.

Serological findings. The negative direct antiglobulin and anti-T lectin tests argued against T-activation. This assumption was confirmed by a steadily normal anti-T titer, a decrease of which is believed to be the most sensitive indicator of T-activation [5, 14].

PLC activity. The low activity of the enzyme in the urine voided shortly after admission is consistent with animal experiments in which intravenously administered PLC was found in urine only after it had destroyed the renal tubuli. PLC is thought to be totally reabsorbed after glomerular filtration by an intact tubulus [2].

The course of PLC activity in the patient's serum was remarkable; it rose from 6.0 to 27.3 U/l, this nearly fivefold increase occurring over the 3-h period between admission and the last blood collection. The only laboratory parameter showing a comparable increase was LDH.

Although the absolute figures of enzyme activity are difficult to interpret, the dramatic increase correlating with the course of the disease is consistent with the often-suspected dominant role of PLC in the pathogenesis of hemolysis in clostridial septicemia. Based on our results, hemolysis due to T-activation by neuraminidase can be excluded.

References

1. Becker RC, Giuliani M, Savage RA, Weick JK (1987) Massive hemolysis in *Clostridium perfringens* infections. J Surg Oncol 35:13–18
2. Carlsen E, Hetland O, Lindboe CF (1983) The effect of phospholipase C in sheep. Scand J Clin Lab Invest 43:445–451

3. Chaplin H, Glazer H, Hockett R, Krewson L, Love P, Murphy K (1990) Abdominal pain, total intravascular hemolysis, and death in a 53-year-old woman (clinicopathologic conference). Am J Med 88:667–674
4. Friedenreich V (1930) The Thomsen hemagglutination phenomenon. Production of a specific receptor quality in red corpuscles by bacterial activity. Levin & Munksgaard, Copenhagen
5. Gray JM, Beck ML, Oberman HA (1972) Clostridial-induced type-I polyagglutinability with associated intravascular hemolysis. Vox Sang 22:379–383
6. Hatheway CL (1990) Toxigenic clostridia. Clin Microbiol Rev 3:66–98
7. Hübener G (1925) Untersuchungen über die Haemagglutination mit besonderer Berücksichtigung scheinbarer Abweichungen vom Gruppenschema. Z Immun Forsch 45:223–236
8. Jolivet-Reynaud C, Moreau H, Alouf JE (1988) Assay methods for alpha toxin from *Clostridium perfringens*: phospholipase C. Methods Enzymol 165:293–299
9. Judd WJ, Oberman HA, Flynn S (1982) Fatal intravascular hemolysis associated with T-polyagglutination. Transfusion 22:345–346
10. Lapierre Y, Rigal D, Adam J, Josef D, Meyer F, Greber S, Drot C (1990) The gel test: a new way to detect red cell antigen-antibody reactions. Transfusion 30:109–113
11. Lind PE, McArthur NR (1947) The distribution of "T" agglutinins in human sera. Aust J Exp 25:247–250
12. Loghem JJ van, Van der Hart M, Land ME (1955) Polyagglutinability of red cells as a cause of severe hemolytic transfusion reaction. Vox Sang 5:125–128
13. Mera CL, Freedman MH (1984) Clostridium liver abscess and massive hemolysis. Unique demise in Franconi's aplastic anemia. Clin Pediatr 23:126–127
14. Mollison PL, Engelfriet CP, Contreras M (1987) Blood transfusion in clinical medicine. Blackwell Scientific, Oxford
15. Moore A, Gottfried EL, Stone PH, Coleman M (1976) *Clostridium perfringens* septicemia with detection of phospholipase C activity in the serum. Am J Med Sci 271:59–63
16. Simpkins H, Kahlenberg A, Rosenberg A, Tay S, Panko E (1971) Structural and compositional changes in the red cell membrane during *Clostridium welchi* infection. Br J Haematol 21:173–182
17. Thomsen O (1927) Ein vermehrungsfähiges Agens als Veränderer des isoagglutinatorischen Verhaltens der roten Blutkörperchen, eine bisher unbekannte Quelle der Fehlbestimmungen. Z Immun Forsch 52:85–107