

NOTES

Compartmentalization of Interleukin-6 Response in a Patient with Septic Meningococcal Peritonitis[∇]

Alexandre Leite de Souza,^{1*} Jaques Sztajn bok,¹ Maristela Marques Salgado,² Carla C. Romano,³ Maria das Graças Adelino Alkmin,² Alberto J. S. Duarte,³ and Antonio Carlos Seguro^{1,4}

Intensive Care Unit, Emílio Ribas Institute of Infectology, São Paulo, Brazil¹; Department of Immunology, Immunology and Microbiology Service, Adolfo Lutz Institute, São Paulo, Brazil²; Department of Dermatology/LIM56, University of São Paulo School of Medicine, São Paulo, Brazil³; and Department of Nephrology, Laboratory of Basic Research, University of São Paulo School of Medicine, São Paulo, Brazil⁴

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We report the first case of *Neisseria meningitidis*-induced septic peritonitis diagnosed by PCR assay of peritoneal fluid. Concentrations of interleukin-6 were notably higher in the peritoneal fluid than in the blood. PCR diagnosis of septic meningococcal peritonitis and the pathogenesis of the disease are discussed.

Case report. A previously healthy 6-year-old girl presented with a 24-h history of fever, headache, and intense myalgia. In the 2 h preceding her examination, she had developed petechiae on her abdomen, arms, face, and legs, accompanied by a deteriorating level of consciousness. Upon examination, petechial lesions were noted on both palpebral conjunctivae, and a dramatic progression from petechiae to confluent ecchymoses was observed on her skin, apparently fitting the clinical profile of disseminated intravascular coagulation. Her vital signs were as follows: axillary temperature, 38°C; pulse, 160 beats/min; respiration, 40 breaths/min; and blood pressure, 80/60 mm Hg. She was positive for Kernig's sign and Brudzinski's sign, and her Glasgow coma scale score was 13. The rest of the examination was unremarkable. The white blood cell (WBC) count was 28,000 WBCs/mm³ (93% polymorphonuclear neutrophils, 3% lymphocytes, 4% monocytes). The hemoglobin concentration was 9.3 g/dl, and the platelet count was 58,000 cells/mm³. Coagulation studies demonstrated an international normalized ratio of 2.12 and an activated partial-thromboplastin time of 55 s. Abnormal laboratory values included the following serum levels: creatine kinase, 784 U/liter; albumin, 2.7 g/dl; aspartate aminotransferase, 106 U/liter; and alanine aminotransferase, 63 U/liter. The acid/base response to this clinical profile was consistent with acidemia, metabolic acidosis, and respiratory alkalosis (arterial pH, 7.28; partial O₂ pressure, 96 mm Hg; bicarbonate, 15 mmol/liter; base deficit, -3 mmol/liter; partial CO₂ pressure, 26 mmol/liter). A computed tomography scan of the brain was normal, and a lumbar puncture was performed. The cerebrospinal fluid (CSF) was cloudy and contained 1,680 WBCs/mm³ (80% polymorphonuclear neutrophils, 15% lymphocytes, 5% monocytes), 104 mg/dl of protein, and 27 mg/dl of glucose. Gram staining of the CSF showed gram-negative

diplococci, and *Neisseria meningitidis* serogroup C was detected by using a latex agglutination test. Blood samples and CSF samples were collected for culture. Counterimmunoelectrophoresis results for *Neisseria meningitidis* serogroup C in the serum were positive. The preliminary diagnosis was meningitis accompanied by meningococemia. Therefore, antibiotic treatment was initiated with ceftriaxone (100 mg/kg of body weight/day) and dexamethasone (0.6 mg/kg/day for 4 days). In addition, supportive measures to maintain homeostasis, including electrolyte replacement, fluid reposition, vitamin K administration, and transfusion of fresh-frozen plasma, were implemented. The patient's close contacts received chemoprophylaxis.

Initially, the general status of the patient improved. However, 48 h after admission, the patient's headache and myalgia subsided and were replaced by severe abdominal pain. Upon examination, the patient's bowel sounds were found to be diminished, and the abdomen was tender and distended. A conventional X ray of the abdomen showed abnormal gas distribution in the bowel and generalized dilation of the bowel loops, although there was no fluid level or free air under the diaphragm. Appendicitis was suspected, and a midline laparotomy, including complete exploration of the abdominal cavity, was performed. The peritoneal cavity contained a moderate amount of yellowish exudate. The peritoneum appeared markedly inflamed, although the appendix was normal. An appendectomy was performed in the usual fashion. The clinical suspicion of appendicitis was later confirmed by histopathological findings, including alterations to the serous membrane that were indicative of peritonitis, and many gram-negative diplococci were present within the peritoneal specimen (Fig. 1). The peritoneal fluid contained 1,320 WBCs/mm³ (79% polymorphonuclear neutrophils, 15% lymphocytes, 6% monocytes), 2,100 mg/dl of protein, and 128 mg/dl of glucose. Gram and Ziehl-Neelsen staining revealed no microorganisms. The sample of peritoneal fluid obtained during a laparotomy performed after the patient had received five doses of ceftriaxone was negative, as were the cultures of CSF and blood. However,

* Corresponding author. Mailing address: Rua da Consolação, 2270 Ap 304, CEP 01302-001, São Paulo, SP, Brasil. Phone: 55 11 3066 7292. Fax: 55 11 30882267. E-mail: alexandre@emilioribas.sp.gov.br.

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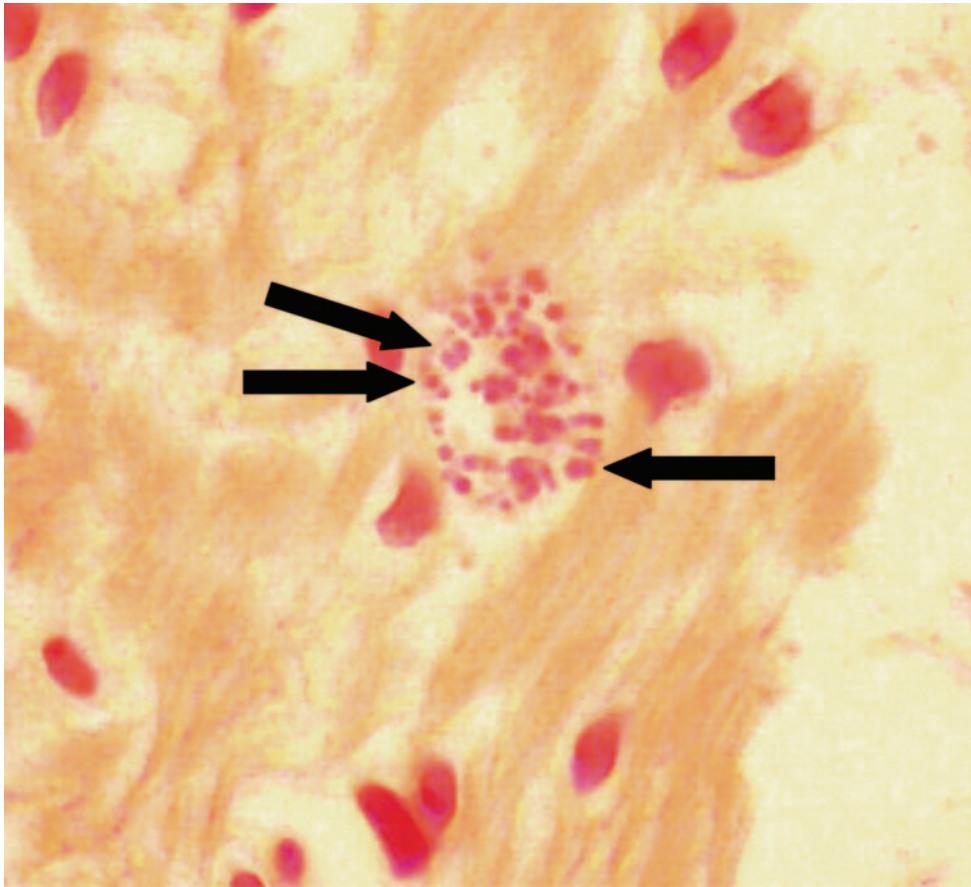


FIG. 1. Specimen obtained from a biopsy of the patient's peritoneum (hematoxylin and eosin stain; original magnification, $\times 1,000$). The arrows indicate gram-negative diplococci.

PCR results with the peritoneal fluid were positive for *N. meningitidis* serogroup C. The antibiotic therapy was therefore continued for an additional 10 days.

PCR amplification of *N. meningitidis* serogroup C genes in peritoneal fluid was performed for three specific regions (5a): the capsular transport gene (*ctrA*), the polysialyltransferase gene (*siaD*) for serogroup B (*siaD_B*), and *siaD* for serogroup C (*siaD_C*). The following pairs of primers were used: *ctrA1*, 5' ATG CGG TGG CTG CGG TAG GT 3', and *ctrA2*, 5' CCG GCG AGA ACA CAA ACG ACA AG 3'; B1, 5' GGA TCA TTT CAG TGT TTT CCA CCA 3', and B2, 5' GCA TGC TGG AGG AAT AAG CAT TAA 3' (for *siaD_B*); and C1, 5' TCA AAT GAG TTT GCG AAT AGA AGG T 3', and C2, 5' CAA TCA CGA TTT GCC CAA TTG AC 3' (for *siaD_C*) (8). PCR conditions were based on those published by Taha (17). The amplification was performed with an Eppendorf thermocycler (Mastercycler Personal model; Hamburg, Germany). Two whole-cell suspensions of the *N. meningitidis* clinical strains Nm573/03 (for *siaD_B* PCR) and Nm576/03 (for *siaD_C* PCR), with optical densities at 620 nm of 0.2, in 0.02% phosphate-buffered saline (PBS) sodium azide (inactivated at 56°C for 30 min and stored at 4°C), were utilized as positive controls. The PCR products were electrophoresed on an agarose gel containing 22 μ g ethidium bromide/50 ml of gel (15 min at 40 V/cm and 30 min at 80 V/cm) and sized with a 100-bp DNA

ladder (Invitrogen, Carlsbad, CA). A single product of 523 bp for *ctrA*, 450 bp for *siaD_B*, or 250 bp for *siaD_C* was interpreted as a positive result. Sample-positive PCR results for *ctrA* and *siaD_C* can be seen in Fig. 2.

In addition, commercially available enzyme-linked immunosorbent assay kits were used to measure cytokine levels in the CSF and blood collected upon the patient's admission, as well as in peritoneal fluid and blood recovered during laparotomy. Antibody-matched pairs and respective standards were purchased from Endogen and used according to the manufacturer's recommendations. The detection limit was 10 pg/ml. Briefly, the microplate was coated with anticytokine monoclonal antibody (4 mg/ml; R&D Systems) and incubated overnight at 4°C. The plate was washed in PBS-0.02% Tween 20 (Sigma) and blocked with PBS-4% bovine serum albumin for 2 h at room temperature. Then, the plate was washed five times with PBS-0.02% Tween 20, the standard recombinant cytokine (at concentrations of 1,000 to 10 pg/ml) and the supernatants were added in duplicate, plus biotinylated anticytokine monoclonal antibody (0.25 mg/ml; R&D Systems), and the plate was incubated for 2 h at room temperature. The microplate was washed again, and the reaction was revealed by the addition of streptavidin-peroxidase (diluted 1:200) for 30 min at 37°C. After the plate was washed, tetramethylbenzidine was added, and the plate was incubated for 30 min in the dark. The reaction was

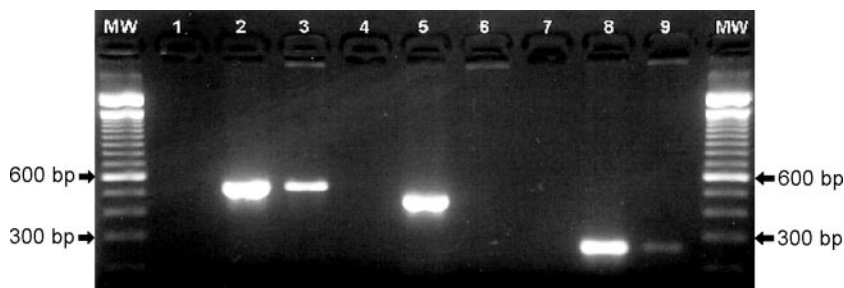


FIG. 2. PCR amplification of the capsular transport gene (*ctrA*) for *N. meningitidis* (523-bp amplicon) is shown in lanes 1, 2, and 3, corresponding to the negative and positive controls (strain Nm573/03) and the peritoneal fluid, respectively. PCR amplification of the polysialyltransferase gene (*siaD*) for *N. meningitidis* serogroup B (450-bp amplicon) is shown in lanes 4, 5, and 6, representing the negative and positive controls (strain Nm573/03) and the peritoneal fluid, respectively. PCR amplification of the *siaD* gene for *N. meningitidis* serogroup C (250-bp amplicon) is shown in lanes 7, 8, and 9, pertaining to the negative and positive controls (strain Nm576/03) and peritoneal fluid, respectively. Lanes MW, 100-bp DNA ladder (0.8 μ l/well).

stopped with 1 M sulfuric acid, and the development of color was read at 450 nm in a microplate reader (Bio-Rad reader). The results are shown in Table 1.

On day 7, the patient developed painful arthritis in both knees and ankles. Examination of the joints revealed signs of inflammatory activity but no clinical evidence of effusion. A presumptive diagnosis of polyarthritis (nonseptic) was made, and aspirin (50 mg/kg/day) was added to the antibiotic therapy for 5 days.

Based on all of the available clinical and laboratory evidence, the patient was diagnosed with severe sepsis and coagulopathy caused by *Neisseria meningitidis*, complicated by purulent peritonitis and probable nonseptic polyarthritis. The evolution was favorable in the postoperative period as well as over the long term.

Meningococcal disease is an infection caused by *Neisseria meningitidis* and has an epidemic and overwhelming nature. It is found the world over and presents a constellation of clinical profiles: meningitis, sepsis, severe sepsis, and septic shock (7). In patients with meningococcal infection, atypical presentations such as complications involving abdominal organs, which may converge to an acute abdomen, have also been well documented (1, 2). The case presented herein illustrates an atypical phenomenon in a child who developed purulent peritonitis caused by *Neisseria meningitidis* serogroup C. We have also discussed some aspects of the pathogenesis of meningococcal

peritonitis and recommend the use of PCR techniques for the diagnostic evaluation of septic meningococcal peritonitis.

Discussion. *Neisseria meningitidis* has been implicated in various conditions that result in an acute abdomen: peritonitis (1), splenic rupture (2), cholecystitis (4), pelvic inflammatory disease (3), mesenteric adenitis (11), ileitis (11), and pericarditis (6). Peritonitis caused by *Neisseria meningitidis* is rare but affects adults (1) and children alike (11, 12) and has been linked to serogroups B (5), C (11), W135 (1), and Z (12). Few cases of meningococcal peritonitis have been reported (9).

Our patient presented with septic peritonitis, which was confirmed by PCR results positive for *Neisseria meningitidis* serogroup C in the peritoneal fluid. How penetration into the peritoneal compartment occurs is not clearly understood. We propose the following sequence of events. After invasion of the bloodstream, *N. meningitidis* initiates hematogenous seeding and migrates independently into the meningeal and peritoneal compartments, where it triggers the host inflammatory response that leads to an acute abdomen. Our findings support the existence of this pathophysiological pathway, as evidenced by the meningococci found in the blood, the CSF, and the peritoneal fluid by using counterimmunoelectrophoresis, a latex agglutination test, and PCR techniques, respectively. In addition, the inflammatory response was confirmed by measuring cytokine concentrations in peritoneal fluid, CSF, and blood (Table 1), as well as by performing inflammatory cell counts. The concentration of interleukin-6 (IL-6) was higher in the peritoneal fluid than in the serum. This cytokine pattern suggests a compartmentalized inflammatory response in which IL-6 is probably synthesized by activated resident cells and/or inflammatory cells that have migrated from the bloodstream to the peritoneal compartment (5b, 10, 15, 16). We recently reported a case of purulent pericarditis that presented a similar cytokine pattern (5b).

Several aspects of this case make it potentially noteworthy, in particular the fact that it illustrates the importance of considering peritonitis within the constellation of the clinical profiles of meningococcal disease. In addition, it demonstrates the potential of *Neisseria meningitidis* to penetrate a wide range of compartments other than the leptomeninges. To our knowledge, this is the first case of septic meningococcal peritonitis diagnosed by PCR analysis of peritoneal fluid. This is also the

TABLE 1. Concentration of cytokines determined by enzyme-linked immunosorbent assay in a patient with septic meningococcal peritonitis

Cytokine ^a	Concn (pg/ml)			
	Day 1		Day 2	
	Serum	CSF	Serum	Peritoneal fluid
L-selectin			3,900	3,900
IL-6	226	132	87	984
IL-10	176	163	65	52
IFN- γ			1,963	997
TNF- α			51	44

^a IFN- γ , gamma interferon; TNF- α , tumor necrosis factor alpha.

first case in which IL-6 activation has been identified in the peritoneal fluid of a patient with meningococcal infection. In conclusion, immunological and molecular techniques are powerful tools for the diagnostic evaluation and clarification of meningococcal septic phenomena, especially after the administration of antibiotics (5a, 5b). The use of these tools could provide new clues regarding the stormy interaction between the host and the catastrophic infection caused by *Neisseria meningitidis*.

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