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Diagnosis of disseminated toxoplasmosis by PCR analysis of ascitic fluid in a patient with haematologic malignancy

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Toxoplasma gondii can cause severe life-threatening infections in immunocompromised patients. Involvement of lung or central nervous system occurs alone or in association with dissemination of the parasite to other body parts [1]. Acute infection often results from the reactivation of dormant *T. gondii* bradyzoites contained within cysts after a primary infection, and serum antibodies may be unreliable in differentiating between active and chronic infection. To overcome this difficulty, detection of *T. gondii* DNA by PCR has been applied to blood, bronchoalveolar lavage and cerebrospinal fluid samples [2,3]. We report a case of active toxoplasmosis that was diagnosed by PCR analysis of the ascitic fluid in a patient treated with pyrimethamine.

In September 1995, a 50-year-old man with idiopathic myelofibrosis and evidence of past *T. gondii* infection (serum IgG antibody titer at 50 IU/mL with no specific IgM) presented a serologic toxoplasmic reactivation with a 20-fold increase in anti-*T. gondii* IgG and appearance of specific IgA (index = 20, Platelia Sanofi Diagnostic Pasteur) without any clinical evidence of disease. Upon treatment with pyrimethamine (100 mg/day) and sulfadiazine (4 g/day), IgG antibodies returned to their initial values and IgA disappeared. Sulfadiazine was thus discontinued after 6 weeks of treatment, and the patient was maintained on pyrimethamine (100 mg/day until June 1996 and then 50 mg/day) as secondary prophylaxis. In September 1996, the patient was admitted with significant splenomegaly and ascites. Osteomedullary biopsy showed diffuse myelofibrosis without signs of blast crisis. A splenectomy was performed. Pathologic examination of the spleen revealed acute leukemic transformation and local infarction. A few days after splenectomy, the patient presented with fever and relapse of the ascites. Analysis of the peritoneal fluid showed a total protein content of 39 g/L, a leukocytosis of 1.8×10^6/L (granulocyte neutrophils 77%; macrophages, 23%), and rare peritoneal cells. No malignant cells were observed. Peritoneal cultures were negative for bacteria, fungi and viruses. Toxoplasmic serologic values remained unchanged. *T. gondii* DNA was detected in the ascitic fluid by PCR-ELISA (Boehringer Mannheim), using a technique that targets the B1 gene of *T. gondii* and includes dUTP and uracyl DNA glycosylase to prevent carryover contaminations, as well as a positive internal control to avoid false negative results due to PCR inhibitors [4]. Subsequent PCR analysis of the spleen parenchyma that had been frozen at −80°C after splenectomy was also positive for *T. gondii*. The patient's clinical status improved when therapy with sulfadiazine (4 g/day) and pyrimethamine (100 mg/day) was initiated. His condition remained stable and he was treated as an outpatient with sulfadiazine, pyrimethamine and hydroxycarbamide (1 g/day).

Serologic screening for anti-*T. gondii* antibodies is part of the regular follow-up scheme for immunocompromised patients at our institution. Since the appearance of specific IgA may occur prior to clinical infection in immunocompromised patients [5], and given the IgA index of the sample (20 is the maximum value with the Platelia Sanofi Diagnostic Pasteur assay),

the patient was initially treated for toxoplasmosis based on serologic data. During the second toxoplasmic episode, it is unclear whether toxoplasmic reactivation was initiated by the surgical procedure or had already started before surgery. The patient was receiving broad-spectrum antibacterial therapy at the time of sampling of the peritoneal fluid. Therefore, negative culture for bacteria cannot rule out the possibility of a bacterial peritonitis, although the cell count of the peritoneal fluid makes this hypothesis unlikely. Whilst detection of DNA by PCR is not absolute evidence for the presence of viable *T. gondii*, rapid clinical improvement with resolution of the fever after institution of specific antiprotozoal therapy supports the diagnosis of active toxoplasmosis. The fact that the patient was treated with pyrimethamine at the time of reactivation confirms previous reports that pyrimethamine alone is not always efficient in preventing toxoplasmosis in immunocompromised patients [6], and further emphasizes the importance of DNA amplification, since diagnosis of toxoplasmosis is unlikely to be established by mice inoculation or cell culture in a patient receiving antiprotozoal therapy.

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Surveillance of meningococcal infections in Belgium

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Meningococcal disease remains an important public-health problem in both developing [1] and industrialized countries [2]. Before 1990, the annual incidence of meningococcal disease in Belgium fluctuated around one case per 100 000 inhabitants. However, since the early 1990s an increase in the incidence of meningococcal disease has been seen.

Meningococci are subdivided into serotypes based on the immunologic properties of several outer-membrane proteins and lipopolysaccharides [3], and development of monoclonal antibodies against those antigens has significantly contributed to a better understanding of the epidemiology of meningococcal disease [3].

In Belgium, serogroup B is the predominant serogroup causing meningococcal disease, followed by serogroup C. No effective vaccine is currently available for protection against group B meningococcal disease, because the B capsular polysaccharide is not immunogenic in humans [4]. The recent emergence of penicillin-resistant meningococcal strains [5,6] may create additional therapeutic problems.

In the present investigation we describe the spread in Belgium of a serogroup B strain which was first reported in The Netherlands in the early 1980s [7]. Isolates were characterized by traditional serotyping, and their susceptibility patterns to a panel of antimicrobial agents were determined. Extensive phenotyping (multilocus enzyme electrophoresis) and genotyping (PCR and pulsed-field gel electrophoresis analyses) were performed on these and other strains [8].

Meningococcal infections are notifiable in Belgium. Trends of meningococcal disease have been derived from epidemiologic and laboratory data sets that are complementary but are maintained separately. On the one hand, the Federal Belgian Health Inspection receives physician notifications; data include age, gender, date and location. On the other hand, the Belgian Meningococcal Reference Center at the Scientific Institute for Public Health–Louis Pasteur (Brussels) receives isolates of *Neisseria meningitidis* from