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LETTER TO THE EDITOR

LISTERIA MONOCYTOGENES IN A YOUNG PATIENT WITH NON HODGKIN'S LYMPHOMA: CASE REPORT

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***Listeria monocytogenes* is an intracellular food-borne pathogen, widely distributed in the environment, which rarely causes clinical infection in healthy people, but may cause severe disease in immunocompromised patients. A case of listeriosis is certified in an immunocompromised patient, thus confirming this microorganism to be an opportunistic human pathogen.**

Listeria monocytogenes is an intracellular food-borne pathogen widely distributed in the environment (soil, water and decaying vegetation) (1-2) which rarely causes clinical infection in healthy people, but may cause severe disease in immunocompromised patients. A 14-year-old girl was admitted to the Policlinico "Umberto I" in Rome, Italy, and Hodgkin's lymphoma was diagnosed. During hospitalization listeriosis was diagnosed in this patient, confirming that *Listeria monocytogenes* is an opportunistic human pathogen.

MATERIALS AND METHODS

C. D. a 14-year-old girl was admitted to the Policlinico "Umberto I" in Rome, Italy, in January 2004 because she had had a fever and a cough for more than 10 days and suffered from general fatigue, respiratory distress and thoracic pain. Initially the thoracic radiography showed extensive left pleural effusion, after which a computerized tomography (CT) of the thorax revealed a large mass in the anterior mediastinum, displacing the heart on the right and compressing the main left bronchus (causing a partial collapse of the left lung), and multiple pleural lesions with left pleural effusion. Finally, an excisional biopsy of the mediastinic mass and a bone marrow biopsy were performed, and the patient was diagnosed as having a T-cell lymphoblastic lymphoma (NHL). According to protocol, she was inserted with a central venous catheter (CVC) (Groshong). She had a history of preterm birth and prenatal hypoxic/ischaemic injury with neurological sequelae and poor neurodevelopmental outcomes. Thereafter, the patient was treated according to the AIEOP LNH97 protocol. The treatment was comprised of an induction phase, from February to April 2004, followed by consolidation phase, from May to June 2004. During the therapy she suffered from a deep hematologic toxicity (especially thrombocytopenia and neutropenia) causing delay between the chemotherapy cycles. At the end of July, a control CT showed persistence of disease. The patient then started a second line chemotherapy, the Memphis protocol, lasting from August to December 2004, based on Vincristine, Prednisone, Methotrexate, Mercaptopurine and Cytosina Arabinoside. During febrile neutropenia episodes, blood samples were taken and placed in Bactec (Becton Dickinson, USA) for culture on April 28, May 5, May 18, May 26 and June 15, 2004. The identification of microrganism was performed with Phoenix identification system (Becton

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Dickinson, USA), Api-Listeria system (bioMérieux, France) and API-CAUX system (bioMérieux, France). The antibiotic susceptibility of testing was performed by Phoenix identification system (Becton Dickinson, USA) and Kirby-Bauer method, on 7% sheep blood agar (Oxoid CM271; Oxoid, United Kingdom). Antifungal susceptibility testing was performed with ATB-Fungus system (bioMérieux, France).

RESULTS

The first four blood cultures resulted negative after seven days, but the June 15 blood culture became positive for *Staphylococcus epidermidis* (the results are shown in Table I). The patient was treated successfully with Teicoplanin (300 mg. i. v. every 12 h. for the first day and once daily for the following days) for 21 days. Two months later, the blood culture resulted negative, whereas the pharynx swab and the urine culture evidenced Candida species. The patient was treated with antifungal therapy for seven days with successful results. At the 4th cycle of Memphis weekly chemotherapy in October, on admission she presented febrile symptoms (temperature: 39.5°C) and referred night sweats; the physical examination revealed pharynx hyperemia, inflammation of the external ear canal and wheezes in all chest fields. The blood cell count showed 2,170 leukocytes per mm^3 with 90% neutrophils and 6.8% lymphocytes, 7.5 gr/dl of haemoglobin, 38,000 platelet per mm^3, associated with low levels of proteins and albumin. Blood sample, pharynx swab and urine culture were taken, after which an antibiotic regimen with Ceftriaxone (1.5 gr i.v. every day) plus Amikacin (450 mg i.v. every day) was started as an empiric therapy. Three days later, since the temperature was still febrile, Ceftriaxone was changed with Ceftazidime (1 gr i.v. every 8 h) and then Teicoplanin (300 mg i.v. every 12 h for the first day and once daily for the following days) was also added. On the 4thday, at microbial control the pharynx swab and the urine samples were negative, while the blood culture in Bactec resulted positive. The observation using light microscopy showed bacillus with characteristic tumbling motility, and the Gram stain revealed gram-positive bacillus, with typical disposition as Chinese letters. A subculture on Listeria selective agar base (Oxford formulation, Oxoid, United Kingdom) at 37°C in air was then performed, and after 24 h of incubation, grey, small, beta haemolytic colonies, 1 mm in diameter, were evident on plates. The catalase test and the hydrolysis of esculin were positive. The gram-positive bacilli with tumbling motility and typical diphtheroid morphology were subsequently identified as *Listeria monocytogenes* with Api-Listeria and Phoenyx identification system.

The antibiotic susceptibility testing was performed by Kirby-Bauer method and the results are shown in Table II. The patient was successfully treated with Ampicillin (1 gr. i.v. every 8 h.) for 10 days and Vancomicin (300 mg i.v. every 6 h) for 7 days, and all subsequent blood cultures were negative. Unfortunately, seven days after the end of therapy, the patient again became pyrexial. Four days later the blood culture resulted positive; the Gram stain revealed mycelial forms, therefore subcultures on 7% sheep blood agar and Sabouraud were carried out and incubated at 37°C in air. After 24 h the preliminary identification of *Candida spp.* was based on morphology of colonies and on Gram stain. This result was subsequently confirmed using API-CAUX system which identified the yeast as *Candida tropicalis*. Antifungal susceptibility testing was performed, and the strain resulted susceptible to Flucytosine (MIC<= 0.5) and Amphotericin B (MIC<= 0.5), but resistant to Fluconazole (MIC=2) and Itraconazole (MIC= 0.5). The patient was treated on Amphotericin B (90 mg/die) for 8 days. After therapy her symptoms vanished, and subsequently the blood culture, the pharynx swab and the urine cultures resulted negative for Listeria and other pathogens.

DISCUSSION

Listeria monocytogenes is an intracellular foodborne pathogen widely distributed in the environment (soil, water, and decaying vegetation) (1-2) which rarely causes clinical infection in healthy people.

Although widely distributed, L. monocytogenes is present in low numbers in most environmental habitats and is rarely a commensal organism among humans. Risk foods include raw milk, raw-milk products, such as soft cheese, raw fruits and vegetables, raw or undercooked meats and seafood, and ready-to-eat foods like bagged salads, hot dogs

Table I. *Antibiotic susceptibility of S.epidermidis isolated from the bloodstream (Phoenix system).*

Antibiotic	MIC (mg/liter)	
Clindamycin	<= 0.25	S
Linezolid	= 1	S
Rifampicin	<=0.5	S
Teicoplanin	<=2	S
Tetraciclin	<=0.5	S
Vancomicin	=1	S
Amoxacillin/Clavulanic Acid	>=1	R
Ciprofloxacin	>=2	R
Erytromycin	>=4	R
Oxacillin	>=2	R
Penicillin G	>=1	R
Trimethroprim/Sulfamethoxazole	>2/38	R

Table II. *Antibiotic susceptibility of L. monocytogenes isolated from the bloodstream (Kirby-Bauer method).*

Antibiotic	MIC (mg/liter)	
Ampicillinin	<= 0.25	S
Gentamicin	= 1	S
Cefepime	<=0.5	R
Teicoplanin	<=2	R
Tetraciclin	<=0.5	S
Vancomicin	=1	S
Moxifloxacin	>=1	S
Oxacillin	>=2	R
Erytromycin	>=4	S
Ceftazidime	>=2	R
Penicillin G	>=1	S
Trimethroprim/Sulfamethoxazole	>2/38	S

and deli meats. Because the incubation period of L. monocytogenes ranges from 3 to 70 days with an average of 3 weeks, it is often very difficult to identify the source of infection (3).

Common signs and symptoms of Listeriosis include fever, muscle aches, nausea, diarrhoea, headache, stiff neck, confusion, loss of balance, convulsions, premature birth and stillbirth. This microorganism may cause serious sporadic and epidemic food-borne disease, usually among people with lowered immune systems, particularly the elderly, pregnant women, neonates, patients under immunosuppressive therapy and patients with cancer, haematological malignancies, renal disease, AIDS or any other immunocompromising disease or condition (2, 4).

Factors such as active or progressive malignancy, antibiotic prophylaxis, use of broadspectrum antibiotics for other or presumed infections, a breach of integrity of the intestinal barrier, CVC, previous colonization, defective cell-mediated immunity in lymphomas and impaired macrophage-

monocyte function may influence the patient risk (5). Listeriosis in these cases usually appear as bacteriemia or meningitis, but other manifestations have also been described (6).

L. monocytogenes causes approximately 2,500 illnesses, 2,300 hospitalizations and 500 deaths in the United States per year and has a case-fatality rate of 20 percent (7). *Listeria monocytogenes* is a Gram positive, intracellular facultative, food-borne bacterium, responsible for life-threatening infections in humans and animals (8). The presence of listeriosis in certain risk groups, including immunocompromised hosts, has permitted to form the hypothesis that the host susceptibility has a determinant role in the epidemiology and clinical presentation of the infection (9-13). The correlation between listeriosis and cancer, first suggested by Louria et al. (14) and subsequently confirmed, emphasised the severity of clinical manifestations, the high fatality rate and the association with lymphoreticular malignancies (11-12, 15-18). Most infections occur after oral ingestion, with access to the systemic circulation after intestinal penetration; in fact, *L. monocytogene* infection takes place in the host's intestinal mucosal cells, while the preferential site for replication is the Peyer's patches (19). Then, bacteria proliferate within macrophages and a variety of normally non-phagocytic cells, such as epithelial, endothelial cells and hepatocytes (20). In all cell types, *L. monocytogenes* develops a characteristic intracellular life cycle which involves several virulence determinants according to the following steps: i) association with the host cells and internalisation; ii) early escape from the phagocytic vacuoles, mainly mediated by listeriolysin O, a pore-forming haemolysin; iii) multiplication in the host cell cytoplasm and directional intracellular mobility by the surface protein ActA (codified by *actA* gene) which induces actin polymerisation and spreads to neighbouring cells (18). Before reaching target cells at the site of infection, *L. monocytogenes* is subjected to different stresses, such as the low pH of the stomach (20), the deleterious effects of volatile fatty acids in the gut (20) and the acidic phagosomal environment of macrophages (22). Listeria has evolved different strategies for survival and proliferation at lethal acidic pH, including the acid tolerance response (ATR) (22-24). The main target organ is the liver, where the bacteria multiply inside hepatocytes. Early recruitment of polymorphonuclear cells leads to hepatocyte lysis and there to bacterial release. This causes prolonged septicemia, particularly in immunocompromised hosts, thus exposing the placenta and brain to infection. Despite bactericidal antibiotic therapy, the overall mortality is still high (25 to 30%) (25). In our case, the antibiotic therapy for *L. monocytogenes* was successful and the patient, even if immunocompromised for chemotherapy and for NHL, recovered. This confirms the efficacy of antibiotic therapy for opportunistic infections from *L. monocytogenes*. However, the immunological status of the host contributes to bacterial proliferation and, consequently, influences the outcome of the infection; it is therefore essential that the microbiological diagnosis of listeriosis must be performed as soon as possible.

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