



## Research Article

# RAPID IDENTIFICATION OF A *LISTERIA MONOCYTOGENES* STRAIN CAUSING SEPSIS AND MENINGITIS IN A NON-CIRRHOTIC PATIENT WITH CHRONIC HEPATITIS C INFECTION NOT TREATED: A CASE REPORT

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### ABSTRACT

We present a case of a 61-year old Caucasian woman who developed sepsis and meningitis caused by *Listeria monocytogenes*. Her medical history included chronic hepatitis C not treated, malnutrition, and previous alcohol abuse. Rapid identification of *Listeria monocytogenes* strain with Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry system (MALDI-TOF) allowed early diagnosis. The possible pathophysiological association is discussed, along with the review of literature.

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## INTRODUCTION

*Listeria monocytogenes* is a Gram-positive rod-shaped bacterium and a facultative intracellular pathogen causing a broad spectrum of severe invasive diseases, mainly in immunocompromised individuals: gastroenteritis, sepsis, meningitis, encephalitis, and miscarriage, or stillbirth (Allerberger et al., 2010; Hernandez-Milian, 2014; Arslan et al., 2015; Barocci et al., 2015; Gaini, 2015). Patients with chronic conditions, including alcoholism (Carrillo-Esper et al., 2013), diabetes mellitus (Samra et al., 1984), and cancer (Rivero et al., 2003; Chavada et al., 2014; Itoga et al., 2015) are at high risk. *Listeria monocytogenes* is known to infect humans through contaminated food, such as meat, ready-to-eat seafood, raw seafood, unpasteurized milk, ice creams, cheese, and fresh vegetables (Hernandez-Milian, 2014; Arslan et al., 2015). It has been documented that the incidence of invasive listeriosis from different countries ranges between from 0.1 to 1 case per 100,000 inhabitant/ year, and an increase in the incidence has been reported in several European countries (Hernandez-Milian, 2014; Madeo et al., 2015).

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The mortality is high and variable, ranging between 20 and 50 % depending on the countries. In the United States (USA) and in Europe, *Listeria monocytogenes* is the second cause of death among foodborne diseases (Hernandez-Milian, 2014; Swaminathan, 2007). The disease has significant clinical manifestation, such as bloodstream infection, infection of central nervous system and maternal fetal listeriosis, and important clinical symptoms, such as fever, myalgia, general malaise, headaches, abdominal pain, vomiting and diarrhea (Hernandez-Milian, 2014; Arslan et al., 2015). The majority of human cases have been caused by serotypes 1/2a, 1/2b, 1/2c, and 4b, among the 13 serotypes of *L. monocytogenes* (Hernandez-Milian, 2014). In Italy, 92 % of listeriosis cases have been identified to belong to serovars 1/2a, 1/2b, and 4b, and in particular, in Lombardy region with 16 % of the total population the most frequent serotypes identified are 1/2a (52.2 %) and 4b (38.8%) (Pontello et al., 2012; Mammina et al., 2013). Listerial infection is often misdiagnosed since clinical symptoms are similar to other etiologic agents causing bacteremia, and in immunocompromised individuals host factors can masque symptoms and consequently it is difficult to diagnose an infection by *L. monocytogenes* (Hernandez-Milian, 2014; Arslan et al., 2015). Due to these facts, it is very important to establish an early microbiological diagnosis of

listeriosis, allowing correct treatment and ensuring an optimal patient outcome. In this study, we report a case of meningitis and sepsis caused by a strain of *L. monocytogenes* isolated at the Desio Hospital (Lombardy, Italy), associated with previous alcohol abuse, chronic hepatitis C, and malnutrition (overweight). A rapid identification of *L. monocytogenes* strain isolated in positive blood cultures with Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry system (MALDI-TOF) permitted an improvement of patient outcome.

### Case report

On November 4, 2015, a 61-year old Caucasian woman was admitted to the emergency room of our hospital with deterioration of her mental status and with a two-day history of fever. The patient's medical history included arterial hypertension, thyroidectomy, cholecystectomy, overweight, and chronic hepatitis C not treated. She had no history of diabetes mellitus, but history of alcohol abuse. In 2009, she was admitted to the emergency room for acute intoxication. On presentation, she appeared ill, but headache, photophobia, and nausea or vomiting were not present. Her vital signs were as follows: blood pressure, 140/80 mmHg; heart rate 94 beats/min; body temperature 39.4 °C. Examination of the heart, lungs and abdomen was normal. Neurological assessment disclosed nuchal rigidity without other abnormalities. Laboratory tests showed a mild anemia with haemoglobin value of 9.3 g/dl and haematocrit of 27.5 %; white blood cell count of 8,400/mm<sup>3</sup>; low platelet count of 89,000/mm<sup>3</sup>. The blood electrolytes were outside normal limits: sodium was 129 mMol/l and potassium 2.9 mMol/l.

The aminotransferase level of GOT was higher than normal range (46 U/L), while aminotransferase level of GPT was normal (19 U/L); total serum bilirubin was high 2.4 with direct 1.6 mg/dl. Moreover, value of C-reactive protein was elevated, about 17 mg/l, while procalcitonin was normal, 0.41 ng/ml. Urine sediment analysis showed the presence of bacteria in small concentration. Urinary *Streptococcus pneumoniae* and *Legionella pneumophila* antigen tests were negative. Abdominal ultrasound and computed tomography (CT) confirmed chronic liver disease. Antimicrobial therapy with ceftriaxone 2 g started. On November 5, due to the persistence of high fever (39.6 °C) and severe clinical situation, laboratory tests were repeated. We observed an increasing of white blood cell count to 11,100/mm<sup>3</sup>, and a decreasing of platelet count to 77,000/mm<sup>3</sup>. Total serum bilirubin increased to 3.4 with direct 2.4 mg/dl, and prothrombin time out of normal range (18.3 seconds). Values of C-reactive protein and procalcitonin were increased to 49.59 mg/l and 0.99 ng/ml, respectively, indicating a possible blood infection. Moreover, the presence of different symptoms, such as confusion, difficulty to concentrating, sleepiness, and sensitivity to light, confirmed a possible meningitis. Blood cultures started. Lumbar puncture showed an opalescent and xanthochromic colour of cerebrospinal fluid (CSF), containing 303 mg/dl protein, 1 mg/dl glucose (concomitant serum glucose was 115 mg/dl), 350/mm<sup>3</sup> of red blood cells, 840/mm<sup>3</sup> of white blood cells with 60 % polymorphonuclear cells and 40 % mononuclear. Cultures on agar plates started. Rapid tests for meningitis were carried out, but the antigens of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* were not detected.

A Gram-staining of the CSF revealed the presence of gram-positive bacilli. Antibiotic spectrum was widened with vancomycin 1 g and ampicillin 3 g. On the morning of November 6, blood cultures resulted positive, and initial Gram-staining disclosed gram-positive bacilli, as previously identified in CSF. After only a 4-hour incubation of 5 drops of blood-broth medium on PolyViteX chocolate agar plates, identification of bacteria was carried out using Vitek<sup>®</sup> Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry system (bioMérieux). The pathogen identified both from blood cultures than from cultures of CSF was a *Listeria monocytogenes* strain. As control, bacteria identification was confirmed by VITEK<sup>®</sup> GP (Gram-positive cocci and non-spore-forming bacilli) colorimetric identification cards, considered as the reference method. Etest<sup>®</sup> method (bioMérieux) was performed to determine the susceptibility profile of the isolated *Listeria monocytogenes* from blood cultures and from cultures of CSF. The strain were sensitive to ampicillin (MIC ≤ 0.50 µg/ml), penicillin (MIC ≤ 0.38 µg/ml), and erythromycin (MIC ≤ 0.38 µg/ml). The patient was brought to the department of Infectious diseases of San Gerardo Hospital, Monza (Italy). Her condition progressively improved and she was discharged without any symptoms one month later. *Listeria monocytogenes* isolates from blood and CSF were sent to the regional reference laboratory of Lombardy at the University of Milan (Mammaia et al., 2013). Pulse-field gel electrophoresis (PFGE) was carried out using the PulseNet protocol with the *AscI* and *Apal* enzymes (Graves and Swaminathan, 2001). Molecular analysis revealed that this strain of *Listeria monocytogenes* was different from recent isolates detected in Lombardy. However, the National Institute of Health has not yet performed serotyping.

### DISCUSSION

Listeriosis is a disease that account for 10 % of community acquired meningitis, and it usually occurs in newborn, pregnant woman, elderly patients, and immunosuppressed patients (Hernandez-Milian, 2014; Arslan et al., 2015; Swaminathan et al., 2007). The present clinical case had several clues for discussion. Our patient is an in compromised immune status as she has a chronic hepatitis C not treated and history of alcohol abuse. T cell-mediated immunity is essential, and it is the predominant host resistance against *Listeria monocytogenes* infection (Pamer, 2004). In chronic hepatitis C, it is typically observed an interference and a dysfunction of the responses of virus-specific T cells, as described in previous reports (Lee et al., 2010; Schmidt et al., 20138). In our case, the possible weak and limited immune reaction may have caused increased susceptibility to *Listeria monocytogenes* infection. *Listeria monocytogenes* has the ability to survive for long periods in the environment, and to proliferate in temperatures ranging from 1 to 45°C. Moreover, this bacterium is part of fecal flora and it is a common foodborne pathogen. In our case, the focus of the infection was unclear, however we can speculate that the patient acquired infection from food, and being a compromised host was prone to infection. In fact, intestinal epithelial structure functions as a mechanical barrier to protect the organism from excessive peristalsis and digestive juices, and it is an immunologic barrier from pathogenic microbes. However, the intestinal mucosa becomes hyper-permeable under pathological conditions, such as malnutrition and hepatic disorders, leading to invasion by pathogens (Cossart and

Sansonetti, 2004). We suggest that our patient may have been susceptible to *Listeria monocytogenes* infection due its altered host immune-responsiveness from intestinal tract, although a direct synergy is difficult to demonstrate. Literature data regarding *Listeria monocytogenes* infection in immunocompromised patients are widely known (Hernandez-Milian, 2014). Vander *et al.*, presented a case of meningitis caused by *L. monocytogenes* in a non-cirrhotic patient with chronic hepatitis C treated by interferon alfa and ribavirin (Vander, 2003). Carrillo-Esper *et al.* reported a case of rhombencephalitis infection by *L. monocytogenes* in an old man with cirrhosis (Carrillo-Esper, 2013). Gaini presented a case of *L. monocytogenes* meningitis associated to an autoimmune hepatitis and IgG4 subclass deficiency (Gaini, 2015). Dias *et al.* (2016) described a case of meningitis caused by *L. monocytogenes* isolated from CSF of an immunocompromised patient, as he was old and a chronic alcoholic (Dias, 2014). Our case is the first report that the combination between chronic hepatitis C not treated and malnutrition may have contributed to listerial infection. Meningitis and sepsis due to *L. monocytogenes* is an important public health and recognition of the symptoms caused by listerial infection is vital in allowing early diagnosis. The importance of a rapid identification of listeriosis using MALDI-TOF MS system in addition to microbiological standard procedures plays an essential role to ensure an optimal patient outcome without neurological consequences. Moreover, we believe that patients suffering from HCV infection not treated and having a condition of malnutrition should be considered more susceptible to *L. monocytogenes* infection, since their depressed cellular immunity.

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#### Conflict of Interest

The authors declare that they have no conflict of interest.

#### Ethical approval

Informed consent to publish was obtained from all individual participants included in the study.

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