Case report

Clinical characterization and whole genome sequence-based typing of two cases of endophthalmitis due to Listeria monocytogenes

MARIA GORI[1], SILVIA BIANCHI[1], GIULIA CICERI[1], ALESSIA LAI[2], MARCO TONELLI[3], NICOLA CLEMENTI[3], MASSIMO CLEMENTI[3], NICASIO MANCINI[3], CLARA FAPPANI[1], GIANGUGLIELMO ZEHENDER[2,4], ANTONELLA AMENDOLA[1,4], MIRELLA PONTELLO[4], ELISABETTA TANZI[1,4]

[1] Department of Health Sciences, University of Milan, Milan, Italy; [2] Department of Biomedical and Clinical Sciences, Luigi Sacco Hospital, University of Milan, Milan, Italy; [3] Microbiology and Virology Unit, Vita-Salute San Raffaele University, Milan, Italy; [4] EpiSoMI CRC-Coordinated Research Center, University of Milan, Milan, Italy

Key words

Listeriosis • Endophthalmitis • Surveillance • Whole-genome sequencing • Virulence

Summary

Endophthalmitis due to Listeria monocytogenes is a rare form of listeriosis. Here, we report two cases that occurred in patients with different medical history, a 46-years-old woman with no comorbidities and an elderly man with several comorbidities. There was no history of trauma or surgery in either patient suggesting an endogenous origin. Despite antibiotic treatment, both patients showed poor visual acuity outcomes. Subtyping clinical isolates using whole genome sequencing could allow to characterise Listeria monocytogenes strains involved in rare clinical manifestation, such as in unusual anatomical sites, even in immunocompetent patients.

Introduction

Listeria monocytogenes (*Lm*) is a major foodborne pathogen that can cause a number of life-threatening clinical syndromes, most frequently sepsis and meningitis, particularly in immunocompromised patients [1, 2]. Endophthalmitis is a serious intraocular infection of the vitreous cavity resulting from exogenous or endogenous spread of microorganisms into the eye and is an exceedingly rare form of listeriosis [3-6]. Indeed, since the publication of the first case in 1967 [7], less than 50 cases have been reported, almost all after the year 2000 in Europe or North America [6]. Although rare, *Lm* endophthalmitis deserves attention and was recently included in the case definition of invasive listeriosis by the European Centre for Disease Prevention and Control (ECDC) [8]. Notification of invasive listeriosis has been mandatory in Italy since 1990, and in 2018 the national rate (0.29 per 100,000 inhabitants) was lower than in most European Union countries [9]. In the Lombardy Region (more than one-sixth of the Italian population), where the national mandatory notification system has been integrated since 2005 with a laboratory-based network, a significantly higher incidence (0.75 per 100,000 inhabitants) was observed in the same year [10]. Starting from January 2019, all *Listeria monocytogenes* isolates have been analysed by whole-genome sequencing (WGS), in collaboration with the Istituto Superiore di Sanità (ISS). To date, a total of 1,070 cases of invasive listeriosis were recorded by the Regional Reference Laboratory (RRL), and only two cases (referred to as case A and case B) of endophthalmitis (0.2%) were detected in the period 2018-2019. The aim of this study was to report the two cases of endophthalmitis and characterized the clinical isolates by using WGS data.

Case Report

Since the clinical record was not available, information on case A was only provided by the epidemiological investigation form. A 46-year-old woman presented to a hospital emergency room (ER) in Brescia in September 2018 with photophobia, pain in the left eye, and nausea. She reported having initial symptoms of redness and a slightly blurred vision a few days before. There was no history of previous trauma, infection, or surgery. Her medical history was unremarkable. She was initially treated for an anterior uveitis with ophthalmic drops of steroids and antibiotics for one week, but a worsening of symptoms, with pulsating pain, reduced vision, and increasing ocular pressure (> 21 mmHg) was reported. Both blood and aqueous humour cultures were performed. After 24 hours, *Lm* was identified in both blood culture and aqueous humour by VITEK MS system (Biomerieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing was performed by E-test on Mueller-Hinton Fastidious Agar (Biomerieux, Marcy l'Étoile, France) and showed resistance to erythromycin and trimethoprim/sulfamethoxazole. The woman decided to leave the hospital against the medical advice ten days after admission. After ten months, the ocular inflammation was resolved, but reduced vision and photophobia persisted.
The second case was represented by an 85-year-old man presented to an ER in Milan in January 2019, reporting pain in his left eye, occurring one week after an episode of non-

Tab. I. Clinical and molecular characteristics of case A and case B.

Case	Age/Sex	Underlying medical conditions	Ocular symptoms	Blood culture	Other positive cultures	Serotype	Lineage	Sequence Type/Clonal Complex	Virulence
A	46/F	None	Photophobia, pain, redness, blurred vision	Positive	Aqueous humor	1/2a	II	ST20/CC20	LIPI-1, LIPI-2
B	85/M	Renal insufficiency, hypertension, type 2 diabetes	Pain, palpebral edema, blurred vision	Positive	Vitreous humor	4b	I	ST4/CC4	LIPI-1, LIPI-2, LIPI-3, LIPI-4

febrile gastroenteritis. The patient suffered from chronic renal failure and other comorbidities (hypertension, mild type 2 diabetes mellitus, and right eye glaucoma). His medical history was also significant for a grade 9 prostate adenocarcinoma. He was dismissed from the ER with steroids and antibiotic eye drops (dexamethasone-netilmicin and cyclopentolate) to be administered in both eyes. Four days later, due to the worsening of his left eyesight, the patient came back to the ER. The presence of periorbital oedema and hyperaemia was observed, and his vision was limited to light perception and hand movement. An increase in the intraocular pressure (30 mmHg) and a retinal detachment in the left eye were detected. Systemic antimicrobial therapy with ceftriaxone and eye drops (ampicillin, vancomycin, voriconazole, moxifloxacin and tobramycin) were prescribed. A vitrectomy of the left eye and blood culture were performed. After less than 24 hours, both vitreous humour and blood culture tested positive for *Lm*, which was identified by using VITEK MS system (Biomerieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing showed no resistance to all tested antibiotics. On this basis, ampicillin (2 g q4h) and cotrimoxazole (15 mg/kg q6h) were added to ceftriaxone. The antibiotic therapy was administered for 7 weeks, totally. After one month, the patient was discharged from the hospital. No visual improvement was observed.

As required by the national surveillance system, the clinical isolates of *Lm* from the two patients were referred to the RRL for molecular subtyping. The isolates were typed with WGS, as requested by. DNA was extracted from pure cultures using the GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA), spectrophotometrically quantified and controlled for quality. Purified DNA was then processed with the Nextera XT sample preparation kit (Illumina, Inc., San Diego, California, USA) and genomic libraries were sequenced on the Illumina MiSeq platform (Illumina, Inc., San Diego, California, USA) with 2 x 250 base pairs paired end runs. Lineage, Sequence Type (ST), Clonal Complex (CC) and virulence information were obtained from the genome sequence using the Listeria database hosted by the Institut Pasteur, France [11]. Conventional serotyping was performed at the ISS in Rome, using *Listeria* antisera (Denka Seiken Co., Ltd., Tokyo, Japan). The strain recovered from patient A was classified as serotype 1/2a, lineage II, ST20, CC20. Patient B's strain resulted serotype 4b, lineage I, ST4, CC4. Both strains harboured the two major *Listeria* pathogenicity islands (LIPI) LIPI-1 and LIPI-2. Isolate B carried also LIPI-3 and LIPI-4. Clinical characterization and WGS-based typing results are listed in Table I.

Discussion

Listeria monocytogenes is an extremely rare and serious cause of endophthalmitis, often with a poor outcome. It spreads mostly by hematogenous dissemination, although it is not easy to attribute the source of infection, as in the cases reported in this study [5, 12]. Considering that the present cases were not related to surgical procedures or trauma, it can be hypothesized that *Lm* has entered the eye from the bloodstream, following the ingestion of contaminated food. This hypothesis could be supported by the observation of an episode of gastroenteritis in patient B one week prior to the onset of the visual problem. A comparison between our cases and those reported in the literature shows that symptoms, age of the patient, and course of the disease observed here are in line with previous studies. Indeed, in the literature, a wide range of age (24-88 years) and different clinical and immunological conditions were described [3, 5, 6]. Moreover, *Lm* endophthalmitis may occur both in immunocompetent (such as case A) and immunocompromised patients (such as case B), [3, 6]. In correspondence with the previous findings, in both cases the final visual outcome was poor [3-6]. Regarding case A, the outcome could also be influenced by the poor ocular penetration of several antimicrobials [6, 12]. Interestingly, the resistance to trimethoprim/sulfamethoxazole was also observed in a recently published case of *Lm* endophthalmitis [12]. WGS-based typing revealed that isolate A belongs to ST20 and CC20, which appear to be rare, frequently isolated from the environment [13] and, remarkably, previously associated with eye infections [11]. Although this strain resulted clinically relevant in an immunocompetent subject, CC20 has not been previously described as a hypervirulent clone [11, 13]. Moreover, no other isolate with this ST has been recorded in the RRL database to date. The isolate B belonged to ST4 and CC4, typically associated with clinical cases [11, 14] and, remarkably, previously isolated from a case of endophthalmitis occurred in Austria in 2018 [15]. CC4 is reported to be a hypervirulent clone, strongly associated with central nervous system (CNS) infection due to the presence of LIPI-4, which is the first factor specifically implicated in *Lm* elective tropisms for the CNS [11, 14, 15]. Since the

optic nerve is considered part of the CNS, its involvement in ocular form of listeriosis is not surprising. Strain B also harboured LIPI-3, only detected in lineage I strains, which can enhance the haemolytic and cytotoxic activity of *Lm* [13, 14].

WGS is a powerful tool for the characterization of *Lm*, as it allows an exceptional subtyping resolution by using the complete genome to determine molecular characteristics of the circulating strains, even in unusual anatomical sites and in not-at-risk subjects. The unexpected finding of a strain not previously classified as hypervirulent in an immunocompetent subject represents a further confirmation of the versatile nature and unpredictability of *Lm*.

Acknowledgments

The authors would like to thank Antonio Piro at for his help in patient A data collection; Danilo Cereda, Sabrina Senatore and Marco Mentasti for retrieving the epidemiological data from the regional database MAINF; Monica Gianfranceschi, Antonietta Gattuso and Alfonsina Fiore for their kindly support and precious collaboration; Daniela Colzani for her support in laboratory analysis.

Conflict of interest statement

The authors declare no conflict of interest.

Author contributions

Conceptualization and study design, MG, ET and MP; molecular subtyping analysis, MG, SB, GC, AL and CF; data collection and clinical investigation, MT, NC and NM; data analysis, MG, SB, GC, AL, MT, NC and CF; writing–original draft preparation, MG, SB, GC, MT and MP; writing–review and editing, NM, AA, GZ and ET; supervision, ET, MP and MC; All authors have read and agreed to the published version of the manuscript.

References

[1] Hernandez-Milian A, Payeras-Cifre A. What is new in listeriosis?. Biomed Res Int 2014;2014:358051. https://doi.org/10.1155/2014/358051

[2] Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 2010;16:16-23. https://doi.org/10.1111/j.1469-0691.2009.03109.x

[3] Betriu C, Fuentemilla S, Méndez R, Picazo JJ, García-Sánchez J. Endophthalmitis caused by Listeria monocytogenes. J Clin Microbiol 2001;39:2742-4. https://doi.org/10.1128/JCM.39.7.2742-2744.2001

[4] Jackson TL, Eykyn SJ, Graham EM, Stanford MR. Endogenous bacterial endophthalmitis: a 17-year prospective series and review of 267 reported cases. Surv Ophthalmol 2003;48:403-23. https://doi.org/10.1016/s0039-6257(03)00054-7

[5] Bajor A, Luhr A, Brockmann D, Suerbaum S, Framme C, Sedlacek L. Listeria monocytogenes endophthalmitis - case report and review of risk factors and treatment outcomes. BMC Infect Dis 2016;16:332. Published 2016 Jul 16. https://doi.org/10.1186/s12879-016-1680-2

[6] Chersich MF, Takkinen J, Charlier C, Leclercq A, Adams PE, Godbole G, Altmeyer U, Friesema IHM, Labbé Sandelin L, Jenkin L, Fontana L, Aldigeri R, Venter F, Luchters SMF, Lecuit M, Cimino L. Diagnosis and Treatment of Listeria monocytogenes Endophthalmitis: A Systematic Review. Ocul Immunol Inflamm 2018;26:508-17. https://doi.org/10.1080/09273948.2016.1276788

[7] Goodner EK, Okumoto M. Intraocular listeriosis. Am J Ophthalmol 1967;64:682-6. https://doi.org/10.1016/0002-9394(67)92848-6

[8] European Commission. Commission implementing decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0945&from=EN (accessed on 23 November 2021)

[9] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021. The European Union One Health 2019 Zoonoses Report. EFSA Journal 2021;19:6406, 286 pp. https://doi.org/10.2903/j.efsa.2021.6406

[10] Gori M, Ciceri G, Bianchi S, Cereda D, Senatore S, Gramegna M, Amendola A, Pontello M, Tanzi E. Laboratory-based surveillance of invasive listeriosis in Northern Italy over a fourteen-year period: epidemiological and clinical results. J Prev Med Hyg 2020;61:E167-E172. https://doi.org/10.15167/2421-4248/jpmh2020.61.2.1473

[11] Institute Pasteur BIGSdb-*Lm* database. Available online: https://bigsdb.pasteur.fr/listeria/ (accessed on 28 January 2021)

[12] Hita LM, Moreno-Torre IC, Jiménez JB, García-Hidalgo CF, Suay VG, Mari JMN. *Listeria monocytogenes*, a rare cause of endophthalmitis. Rev Esp Quimioter 2020;33:216-7. https://doi.org/10.37201/req/097.2019

[13] Rychli K, Stessl B, Szakmary-Brändle K, Strauß A, Wagner M, Schoder D. Listeria monocytogenes Isolated from Illegally Imported Food Products into the European Union Harbor Different Virulence Factor Variants. Genes (Basel) 2018;9:428. Published 2018 Aug 23. https://doi.org/10.3390/genes9090428

[14] Maury MM, Tsai YH, Charlier C, et al. Uncovering Listeria monocytogenes hypervirulence by harnessing its biodiversity [published correction appears in Nat Genet. 2017 Mar 30;49(4):651] [published correction appears in Nat Genet. 2017 May 26;49(6):970]. Nat Genet 2016;48:308-13. https://doi.org/10.1038/ng.3501

[15] Cabal A, Allerberger F, Huhulescu S, Kornschober C, Springer B, Schlagenhaufen C, Wassermann-Neuhold M, Fötschl H, Pless P, Krause R, Lennkh A, Murer A, Ruppitsch W, Pietzka A. Listeriosis outbreak likely due to contaminated liver pâté consumed in a tavern, Austria, December 2018. Euro Surveill 2019;24:1900274. https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900274

Received on July 15, 2021. Accepted on December 6, 2021.

Correspondence: Elisabetta Tanzi – E-mail: elisabetta.tanzi@unimi.it

How to cite this article: Gori M, Bianchi S, Ciceri G, Lai A, Tonelli M, Clementi N, Clementi M, Mancini N, Fappani C, Zehender G, Amendola A, Pontello M, Tanzi E. Clinical characterization and whole genome sequence-based typing of two cases of endophthalmitis due to Listeria monocytogenes. J Prev Med Hyg 2022;63:E139-E141. https://doi.org/10.15167/2421-4248/jpmh2022.63.1.2261