

## *Listeria*-Associated Lymphadenitis: A Series of 11 Consecutive Cases and Review of the Literature

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We studied 11 cases of culture-proven *Listeria*-associated lymphadenitis reported to the French National Reference Center for *Listeria* from 1994 to 2019 and 8 additional published cases. *Listeria*-associated lymphadenitis is rare, but it is associated with a mortality as high as for invasive listeriosis, and it is frequently diagnosed with concomitant neoplasia.

**Keywords.** adenitis; cancer; *Listeria monocytogenes*.

*Listeria monocytogenes* (*Lm*) is responsible for listeriosis, a severe foodborne infection mostly reported in immunocompromised patients, where it mostly presents as septicemia and neuroinfection. It is also responsible for maternal-neonatal infection. Focal infections are rare, consecutive to hematogenous seeding or direct inoculation [1]. Among them, *Lm*-associated lymphadenitis have not been characterized. We undertook a national retrospective study of this presentation over the last 25 years and identified 11 cases and reviewed the 8 published cases.

### PATIENTS AND METHODS

#### Data Collection

French surveillance of human listeriosis relies on mandatory reporting since 1999 with strains submission to the French

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National Reference Center for *Listeria* [2]. Reporting exhaustiveness is 87% [3]. We retrospectively studied culture-proven *Listeria*-associated lymphadenitis cases reported from January 1994 to December 2019. All cases with mention of “lymphadenitis”, “lymph node infection”, or “abscess” were reviewed. A confirmed case was defined as a patient from whom *Listeria* was isolated by culture of a lymph node. Eleven cases were identified, their medical charts were reviewed, and bacterial isolates were sequenced.

#### Ethical Evaluation

Because of the observational nature of the study with all data collected as a part of French National Surveillance, this study did not require patients’ written consent nor formal Institutional Review Board approval, according to the French legislation.

#### Review of the Literature

We searched the PubMed database for reports between January 1966 and December 2019, using the terms “*Listeria*”, “listeriosis”, “lymphadenitis”, and “lymph node” without language restriction [4–10].

#### *Listeria monocytogenes* Characterization

Species identification was carried out with API-*Listeria* microgallery (bioM rieux, Marcy l’Etoile, France) before January 2017 and then matrix-assisted laser desorption ionization time-of-flight mass spectrometry [11]. Genome sequencing was performed as previously described [12]. Polymerase chain reaction (PCR) serotyping, multilocus sequence types (MLSTs), and core genome MLST profiles were deduced from genome assemblies using the BIGSdb-*Lm* platform (<https://bigsdb.pasteur.fr/listeria>) [12].

#### Histopathological Analyses

We studied 2 available case samples: 5- m thick sections of paraffin-embedded tissue specimens were performed. *Listeria* was labeled by immunohistochemistry using a polyclonal rabbit antiserum (R12) that detects *Lm* serotype 1/2a (*Listeria* O I/II) or 4b and *Listeria ivanovii* (*Listeria* O V/VI antiserum Seiken kit; Denka Seiken Co., Tokyo, Japan), and a goat antirabbit antibody coupled to peroxidase (EnVision+; Dako), followed by hematoxylin counterstaining. Images were captured on AxioImager A2 microscope with AxioCam ICc 1 digital camera (Zeiss).

For immunofluorescence staining, paraffin-embedded tissues were deparaffinized, and antigens were retrieved by citric acid buffer. Samples were stained with R12 antibody for *Lm* serotype I/IIa [1] or *Listeria* O V/VI for *L. ivanovii* and a goat antirabbit antibody conjugated with Alexa Fluor 546 (Invitrogen) together

with Hoechst 33342. Images were captured on a LSM710 confocal microscope (Zeiss).

## RESULTS

### Clinical Cohort

Among 7643 human cases collected between 1994 and 2019 in France, 11 were *Lm*-associated lymphadenitis (0.14%). Eight more published cases were identified, including 6 with some clinical data available. The 19 cases are listed in Table 1.

### Epidemiological Features

Ten patients (10 of 18, 56%) were male. Median age was 65 years (interquartile range [IQR], 57–79). Four patients (4 of 18, 22%) were  $\geq 80$  years old. Sixteen patients (16 of 18, 89%) presented 1–3 immunosuppressive comorbidities, either pre-existing ( $n = 12$ ) or revealed by listeriosis ( $n = 4$ ): diabetes was reported in 12 of 18 (67%); alcoholism and neoplasia were each reported in 4 of 18 (22%); neoplasia was diagnosed shortly before (case 8), concomitantly (cases 3 and 11), or shortly after (case 18) the diagnosis of lymphadenitis (no patient had received chemotherapy at lymphadenitis onset); myelofibrosis with chronic neutropenia and human immunodeficiency virus infection were also reported ( $n = 1$ , each).

### Clinical Features

Median time from first symptom to diagnosis was 4 weeks (IQR, 3–4 weeks;  $n = 10$ ). Lymphadenitis was isolated (9 of 15, 60%) or multiple (6 of 15, 40%). All involved a single unilateral area: cervical (14 of 19, 74%), inguinal (3 of 19, 16%), supraclavicular or iliac (1 of 19, 5% each). Lesions were bulky in 12 of 15 (80%). Suppuration was evidenced clinically and/or by computed tomography or ultrasound imaging in 7 of 16 (44%). Five patients (5 of 13, 38%) reported fever. No neurological involvement was reported. One patient with inguinal lymphadenitis exhibited concomitant intertrigo and lower limb cellulitis, and another with cervical adenitis had concomitant upper lobe consolidation pneumonia that resolved, respectively, with amoxicillin/clavulanate and amoxicillin/gentamicin. Another patient with iliac lymphadenitis had confirmed *Listeria*-associated appendicitis.

### Laboratory Characteristics and Microbiological Features

Median leucocyte count was  $10\ 150/\text{mm}^3$  (IQR, 6250–10 525;  $n = 6$ ), and median C-reactive protein level was 42 mg/L (IQR, 36–54;  $n = 5$ ). *Listeria* was isolated from pus or tissue culture in 17 of 17 cases. In 1 patient (case 10, iliac lymphadenitis), samples also grew *Streptococcus anginosus* and anaerobic flora. Blood cultures, when performed, were all negative (4 of 4). *Listeria monocytogenes* was identified in 10 of 11 (91%) cases, and *L. ivanovii* was identified in 1. Distribution of *Lm* clonal complexes was as follows: 2 isolates belonged to CC2 and CC4 hypervirulent clones, 2 belonged to hypovirulent clones CC9,

and 121 others belonged to clones with intermediate virulence (Table 1).

### Histopathological Findings

Histopathological reports were available for 8 patients and showed epithelioid granuloma in 6 of 8 (75%, including 2 with necrosis), chronic lymphadenitis or metastatic gynecological carcinoma without epithelioid granuloma (1 of 8, 13% each). *Listeria* could be identified in samples of 2 cases (cases 6 and 10), by immunoperoxidase staining (Figure 1A, B, E and F) and immunofluorescence (Figure 1C, D, G and H). Bacteria were localized mostly in cells having a morphology evocative of monocytes/macrophages and also in polymorphonuclear cells (Figure 1B and F).

### Treatment and Follow-up

Management relied on surgery in 16 of 16 cases (100%): puncture (6 of 16, 38%), surgical drainage (4 of 16, 25%), or excisional biopsy (6 of 16, 38%). Antibiotics were administered in 11 of 13 (85%) and relied on amoxicillin ( $n = 4$ ), amoxicillin-clavulanate ( $n = 2$ ), amoxicillin and gentamicin ( $n = 3$ ), cotrimoxazole or pefloxacin ( $n = 1$ , each), for a median of 21 days (IQR, 15–28;  $n = 10$ ). Thirteen patients (13 of 17, 76%) recovered; 1 reported protracted recurrence requiring prolonged antibiotics (6%). Three patients (3 of 17, 18%) died within 3 months, immediately after surgical procedure ( $n = 1$ , no autopsy performed) or as a consequence of concomitant neoplasia ( $n = 2$ , including 1 patient for whom autopsy findings revealed myocardial ischemia and bladder carcinoma).

## DISCUSSION

We studied the detailed clinical and microbiological features of *Lm*-associated lymphadenitis in a cohort of 19 patients, including 11 from the French cohort and the 8 previously published cases. Several conclusions can be drawn.

First, although rare, *Lm*-associated lymphadenitis is a genuine disease entity, whose mortality is as high as invasive listeriosis [15]. In line with this observation, most patients presented predisposing conditions associated with invasive listeriosis, namely, older age and immunosuppressive comorbidities, including diabetes, alcoholism, and ongoing neoplasia [15]. *Listeria*-associated lymphadenitis and cancer were diagnosed concomitantly in 4 patients, in the range reported in nonmaternal invasive listeriosis (22% versus 20%) [15]. None had received antitumoral chemotherapy at lymphadenitis onset, possibly reflecting tumor-associated immunosuppression. Indeed, studies in a BALB/c model of mammary carcinoma have shown that *Lm* survives and multiplies in the tumoral microenvironment while rapidly killed in healthy tissues [16]. This selective survival and growth is linked to the recruitment of myeloid-derived suppressor cells that produce interleukin-10 and tumor necrosis factor- $\beta$  that could help *Lm* escape the cellular immune

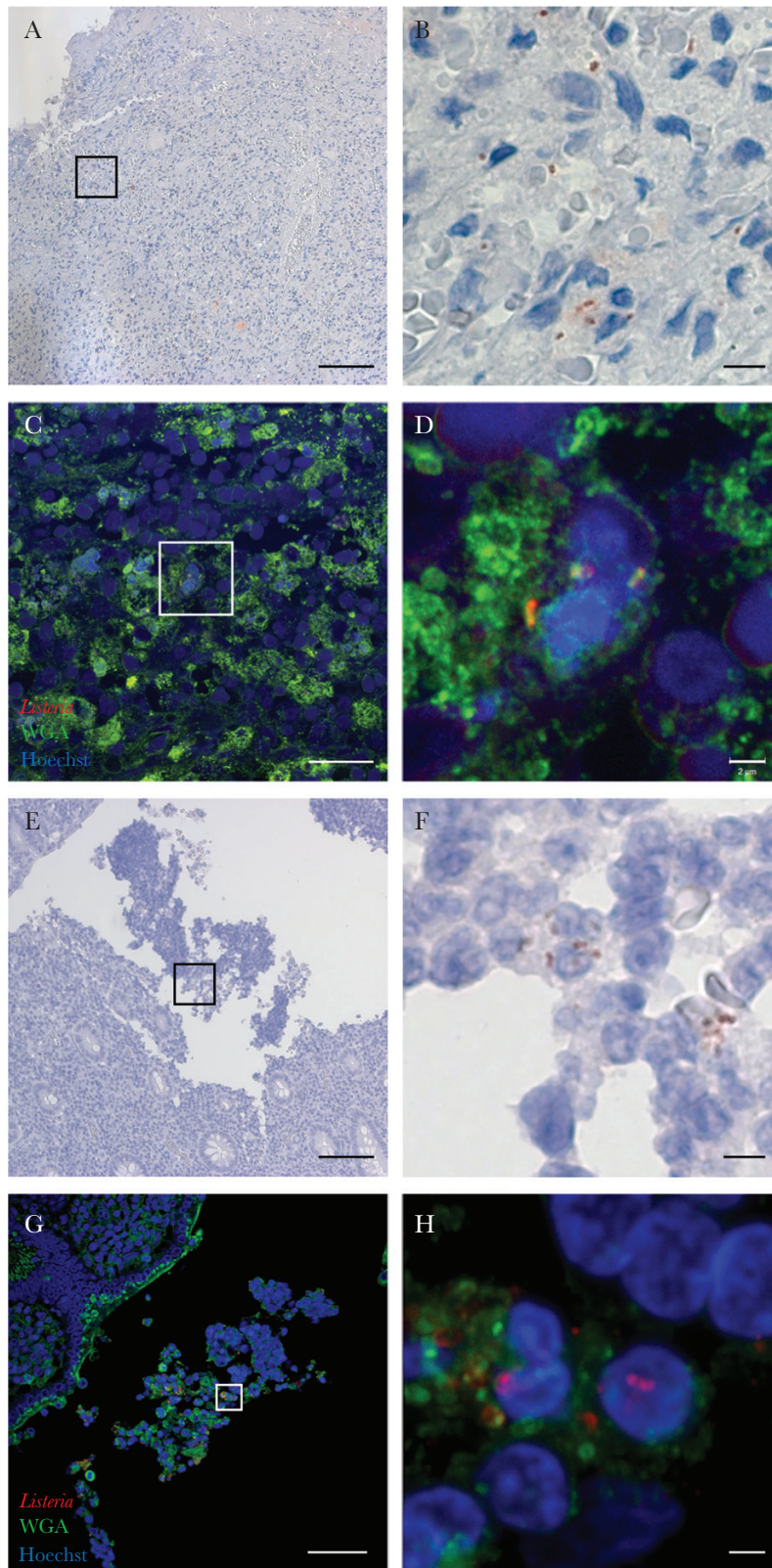
**Table 1. Features of 19 Patients With *Listeria monocytogenes*-Associated Lymphadenitis**

Reference	Patient No.	Age at Diagnosis, Sex, Underlying Diseases	Clinical and Imaging Features							Species and MLST Clonal Complex	Histopathology	Treatment, Surgery, Antibiotics	Outcome
			Symptoms Duration Before Diagnosis	Localization	Size, Number	Signs of Abscess	General and Associated Signs	Blood Cultures	Signs and Associated Signs				
This study	1	72-year-old man, Essential thrombocythemia, myelofibrosis, chronic neutropenia, unbalanced diabetes, prednisone 7.5 mg/day	3 weeks	Right cervical and under the chin	Bulky, multiple (CT scan)	No	Inflammatory (C) and necrotic (CT scan) mass	Pneumonia	Negative	<i>L monocytogenes</i> CC121	NP	Puncture, amoxicillin (26 days) + gentamicin (5 days)	Two recurrences treated with amoxicillin (3 then 4 weeks). Cure (4 months). Death (1 year).
This study	2	63-year-old man, uncontrolled diabetes (glycated hemoglobin 17.4%), tobacco smoke, alcoholism	1 month	Right cervical	Bulky (6.5 x 8 cm; CT scan), single			Weight loss (12 kg)	NP	<i>L monocytogenes</i> CC4	NP	Puncture, amoxicillin + gentamicin	Cure. Death (4 years, hepatocellular carcinoma)
This study	3	88-year-old woman, Parkinson's and Alzheimer's diseases	3 weeks	Right inguinal	Bulky, single (C)	No		Lower limb cellulitis	NP	<i>L monocytogenes</i> CC2	Infiltration with gyn-ecological carcinomatous cells	Amoxicillin-clavulanate (10 days); persistence. Lymph node removal. Amoxicillin 6 g/d.	Discovery of a metastatic gynecological carcinoma. Death (2.5 months)
This study	4	82-year-old woman, uncontrolled type II diabetes, obesity	3 months	Left cervical	Bulky (5cm, C), single (US)	No (US)	No (US)	No	NP	<i>L monocytogenes</i> CC9	NP	Surgical drainage. No antibiotic.	Death (1 day)
This study	5	55-year-old woman, type II diabetes, tobacco smoking	NA	Left inguinal	Bulky, single (C)	No		NA	NP	<i>L monocytogenes</i> CC5	Nonnecrotic epithelioid granuloma	Lymph node removal.	Cure. Death (5 years, metastatic gallbladder carcinoma)
This study	6	60-year-old man, HBV and alcoholic cirrhosis, tobacco smoking, alcoholism	1 month	Median cervical	Bulky, single (CT scan, US)	Fistulization		No	NP	<i>L monocytogenes</i> CC20	Nonnecrotic epithelioid granuloma	Surgical drainage. Amoxicillin (21 days)	Cure (2 months)
This study	7	72-year-old woman, untreated and unbalanced type II diabetes	3 weeks	Left submaxillar	Bulky (7 x 4 x 7 cm; CT scan), single	Yes (CT scan)		Asthenia	NP	<i>L monocytogenes</i> CC199	Necrotic epithelioid granuloma, pus	Lymph node removal. Perifloxacin (10 days)	Cure (6 months)
This study	8	61-year-old man, carcinoma of the esophagus diagnosed 1 month earlier	NA	Right supraclavicular	Bulky, multiple (CT scan)	No		Weight loss	NP	<i>L monocytogenes</i> CC21	NP	Puncture. Amoxicillin 8 g/day (15 days)	Cure (5 months). Metastatic carcinoma of the esophagus (right latero-esophageal and supraclavicular bundle of lymph node).
This study	9	80-year-old woman, type II diabetes	NA	Inguinal	NA	NA	NA	NA	NA	<i>L monocytogenes</i> CC7	NA	NA	Cure (17 years)

**Table 1. Continued**

Reference	Patient No.	Age at Diagnosis, Sex, Underlying Diseases	Clinical and Imaging Features									
			Symptoms Duration Before Diagnosis	Localization	Size, Number	Signs of Abscesses	General and Associated Signs	Blood Cultures	Species and MLST Clonal Complex	Histopathology	Treatment, Surgery, Antibiotics	Outcome
This study	10	11-year-old boy	6 days	Right iliac	Large (16 × 11 mm), multiple (US).	No	Fever, anorexia, abdominal pain	NP	<i>Listeria ivanovii</i> CC883	Chronic lymphadenitis without sign of malignancy	Surgical lymph node removal. Amoxicillin 50 mg/kg per day (15 days)	Cure (1 month)
This study	11	66-year-old man	1.5 months	Right cervical	Large (3cm), multiple (CT scan)	No	Asthenia, weight loss, fever	Negative	<i>L monocytogenes</i> CC20	NP	Puncture. Cotrimoxazole (3 weeks)	Cure (2 months), Discovery of a chronic lymphocytic leukemia.
Larsson S [13]	12	81-year-old woman, type II diabetes	3 weeks	Cervical	NA	Yes	No	NA	NA	NP	Surgical drainage. No antibiotic	Cure
Bojsen-Moller [7]	13	NA	NA	Cervical	Single	NA	NA	NA	NA	NA	NA	NA
Blanche et al [8]	14	55-year-old woman, type II diabetes, HIV infection (CD4 <sup>+</sup> T lymphocyte count 242/mm <sup>3</sup> )	NA	Cervical	Multiple	No	Fever	Negative	NA	Nonnecrotic epithelioid granuloma, pus	Surgical drainage. Amoxicillin (6 weeks)	Cure
Ferrer et al [9]	15	81-year-old man, diabetes mellitus	NA	Cervical	Single	Yes	NA	NA	NA	NA	NA	NA
Goulet et al [14]	16	56-year-old man, type II diabetes, alcoholism	NA	Cervical	NA	NA	NA	NP	NA	NP	Puncture	Cure
Goulet et al [14]	17	60-year-old man	NA	Cervical	NA	NA	NA	NP	NA	NP	Puncture	Cure
Rosenthal et al [10]	18	75-year-old man, type II diabetes, obesity, tobacco smoke, alcoholism	1 month	Cervical	Bulky (4cm), multiple	Fistulization	No	NP	NA	Necrotic epithelioid granuloma, pus	Lymph node removal. Amoxicillin-clavulanate (4 weeks)	Death (4 week); autops: myocardial ischemia, metastatic carcinoma of the bladder, residual neck abscess
Bertru et al [5]	19	52-year-old woman, uncontrolled type II diabetes	NA	Left cervical	Bulky (8 × 3 × 3; CT scan), single	Inflammatory mass (C), collection (CT scan, US)	Fever	Negative	NA	Epithelioid granuloma	Surgical drainage. Amoxicillin (1 month) + Gentamicin (3 days)	Stable (2 months)

Abbreviations: CT, computed tomography; HBV, hepatitis B virus; HIV, human immunodeficiency virus; MLST, multilocus sequence type; NA, not applicable; NP, not practiced.



**Figure 1.** Detection of *Listeria monocytogenes* (*Lm*), which appear in red, in adenitis tissue samples collected from 2 patients with immunoperoxidase staining (A, B, E, and F) and direct immunofluorescence with wheat germ agglutinin (in green) and Hoechst (in blue) staining (C, D, G, and H). The *Lm* are localized (1) within cells evocative of monocytes and macrophages, (2) but also in polymorphonuclear cells in close vicinity with the capsule of the abscessed lymph node in the section available for case 6 (A–D), and (3) within appendix lumen in the section available for case 10 (E–H). Scale bars: 100  $\mu$ m (A and E), 20  $\mu$ m (C), 50  $\mu$ m (G), 5  $\mu$ m (B and F), and 2  $\mu$ m (D and H).

response at the tumor site [16]. It indicates that patients with *Lm*-associated lymphadenitis should be evaluated for neoplasia, mainly in the affected lymph node or locoregionally.

Seeding from a locoregional portal of entry is a possibility, considering the negativity of blood cultures, the absence of neuroinfection, and the involvement of a single lymph node, even though one cannot exclude that it may also result from bacteremia. The predominant cervical involvement may follow translocation in the mucosa-associated lymphoid tissue (MALT) of the pharyngo-oral region. *Listeria* can indeed be detected in tonsils of wild animals [17, 18] and also in humans [19]. The case with appendicitis likely results from *Listeria* translocation across the MALT of the appendix (Peyer's patches).

Management should include (1) drainage of suppurative lesions or excisional biopsy and (2) *Listeria*-targeting amoxicillin-based antimicrobial therapy, which is otherwise not recommended in suppurative lymphadenitis, where *Staphylococcus aureus* should be covered. Because the review of literature was not performed according to PRISMA guidelines, some cases may have been overlooked and the total number of published cases may be higher.

## CONCLUSIONS

In conclusion, *Lm*-associated lymphadenitis is rare but associated with a mortality as high as for invasive listeriosis. Patients with *Lm*-associated lymphadenitis should be evaluated for concomitant neoplasia.

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