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1 ***Listeria*-associated lymphadenitis: a series of 11 consecutive cases**
2 **and review of the literature**

3
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1 **Abstract**

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

1 **Introduction**

2 *Listeria monocytogenes* (*Lm*) is responsible for listeriosis, a severe foodborne infection mostly
3 reported in immunocompromised patients where it mostly presents as septicemia and
4 neurolisteriosis, it is also responsible for maternal-neonatal infection. Focal infections are rare,
5 consecutive to hematogenous seeding or direct inoculation¹. Among them, *Lm*-associated
6 lymphadenitis have not been characterized. We undertook a national retrospective study of this
7 presentation over the last 25 years and identified 11 cases, and reviewed the 8 published cases.

9 **Patients and methods**

10 **Data collection** – French surveillance of human listeriosis relies on mandatory reporting since
11 1999 with strains submission to the French National Reference center for *Listeria* (NRCL)².
12 Reporting exhaustiveness is 87%³. We retrospectively studied culture-proven *Listeria*-
13 associated lymphadenitis cases reported from January 1994 to December 2019. All cases with
14 mention of “lymphadenitis”, “lymph node infection” or “abscess” were reviewed. A confirmed
15 case was defined as a patient from whom *Listeria* was isolated by culture of a lymph node.
16 Eleven cases were identified, their medical charts reviewed, and bacterial isolates sequenced.
17 Ethical evaluation Because of the observational nature of the study with all data collected as a
18 part of French National Surveillance, it did not request patients’ written consent nor formal
19 Institutional Review Board approval, according to the French legislation.

20 **Review of the literature** – We searched the PubMed database for reports between January
21 1966 and December 2019, using the terms “*Listeria*”, “listeriosis”, “lymphadenitis”, “lymph
22 node” without language restriction⁴⁻¹⁰.

23 ***L. monocytogenes* characterization** – Species identification was carried out with API-*Listeria*
24 microgallery (bioMérieux, Marcy l’Etoile, France) before January 2017, and then MALDI-TOF
25 mass spectrometry¹¹. Genome sequencing was performed as described¹². PSR serogrouping,

1 Multilocus sequence types (MLST) and core genome MLST profiles were deduced from
2 genome assemblies using the BIGSdb-*Lm* platform (<https://bigsdb.pasteur.fr/listeria>)¹².

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

10 For immunofluorescence staining, paraffin-embedded tissues were deparaffinized, and antigens
11 retrieved by citric acid buffer. Samples were stained with R12 antibody for *Lm* serotype I/IIa¹
12 or *Listeria* O V/VI for *Listeria ivanovii* and a goat anti-rabbit antibody conjugated with Alexa
13 Fluor© 546 (Invitrogen) together with Hoechst 33342. Images were captured on a LSM710
14 confocal microscope (Zeiss).

15

16 **Results**

17 **Clinical cohort** – Among 7,643 human cases collected between 1994 and 2019 in France, 11
18 were *Lm*-associated lymphadenitis (0.14%). Eight more published cases were identified,
19 including six with some clinical data available. The 19 cases are listed in **Table 1**.

20 **Epidemiological features** – Ten patients (10/18, 56%) were male. Median age [interquartile
21 range] age was 65 years [57-79]. Four patients (4/18, 22%) were ≥ 80-year-old. Sixteen (16/18,
22 89%) presented 1-3 immunosuppressive comorbidities, either preexisting (n=12), or revealed
23 by listeriosis (n=4): diabetes was reported in 12/18 (67%), alcoholism and neoplasia each in
24 4/18 (22%); neoplasia was diagnosed shortly before (case 8), concomitantly (cases 3 and 11),
25 or shortly after (case 18) the diagnosis of lymphadenitis (no patient had received chemotherapy

1 at lymphadenitis onset); myelofibrosis with chronic neutropenia and HIV infection were also
2 reported (n=1, each).

3 **Clinical features** – Median [IQR] time from first symptom to diagnosis was 4 weeks ([3-4]
4 weeks, n=10). Lymphadenitis was isolated (9/15, 60%) or multiple (6/15, 40%). All involved a
5 single unilateral area: cervical (14/19, 74%), inguinal (3/19, 16%), supraclavicular or iliac
6 (1/19, 5% each). Lesions were bulky in 12/15 (80%). Suppuration was evidenced clinically
7 and/or by computed tomography or ultrasound imaging in 7/16 (44%). Five patients (5/13,
8 38%) reported fever. No neurological involvement was reported. One patient with inguinal
9 lymphadenitis exhibited concomitant intertrigo and lower limb cellulitis, another with cervical
10 adenitis had concomitant upper lobe consolidation pneumonia that resolved respectively with
11 amoxicillin/clavulanate and amoxicillin/gentamicin. Another patient with iliac lymphadenitis
12 had confirmed *Listeria*-associated appendicitis.

13 **Laboratory characteristics and microbiological features** – Median leucocyte count [IQR]
14 was 10,150 /mm³ [6,250-10,525] (n=6), median C-reactive protein level [IQR] was 42 mg/L
15 [36-54] (n=5). *Listeria* was isolated from pus or tissue culture in 17/17 cases. In one patient
16 (case 10, iliac lymphadenitis), samples also grew *Streptococcus anginosus* and anaerobic flora.
17 Blood cultures, when performed, were all negative (4/4). *L. monocytogenes* was identified in
18 10/11 (91%) cases, *L. ivanovii* in one. Distribution of *Lm* clonal complexes was as follows: two
19 isolates belonged to CC2 and CC4 hypervirulent clones, two belonged to hypovirulent clones
20 CC9 and 121, others belonged to clones with intermediate virulence (**Table 1**).

21 **Histopathological findings** – Histopathological reports were available for 8 patients, and
22 showed epithelioid granuloma in 6/8 (75%, including 2 with necrosis), chronic lymphadenitis
23 or metastatic gynecological carcinoma without epithelioid granuloma (1/8, 13% each). *Listeria*
24 could be identified in samples of two cases (cases 6 and 10), by immunoperoxidase staining
25 (Figure 1A,B,E,F) and immunofluorescence (Figure 1C,D,G,H). Bacteria were localized

1 mostly in cells having a morphology evocative of monocytes/macrophages and also in
2 polymorphonuclear cells (Figure 1B, F).

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

12
13

14 **Discussion**

15
16 We studied the detailed clinical and microbiological features of *Lm*-associated lymphadenitis
17 in a cohort of 19 patients, including 11 from the French cohort and the 8 previously published
18 cases. Several conclusions can be drawn.

19 First, although rare, *Lm*-associated lymphadenitis is a genuine disease entity, whose mortality
20 is as high as invasive listeriosis¹³. In line with this observation, most patients presented
21 predisposing conditions associated with invasive listeriosis, namely older age and
22 immunosuppressive comorbidities, including diabetes, alcoholism and ongoing neoplasia¹³.

23 *Lm*-associated lymphadenitis and cancer were diagnosed concomitantly in four patients, in the
24 range reported in non-maternal invasive listeriosis (22% versus 20%)¹³. None had received anti-
25 tumoral chemotherapy at lymphadenitis onset, possibly reflecting tumor-associated
26 immunosuppression. Indeed, studies in a BALB/c model of mammary carcinoma have shown
27 that *Lm* survives and multiplies in the tumoral microenvironment, while rapidly killed in healthy

1 tissues¹⁴. This selective survival and growth is linked to the recruitment of myeloid-derived
2 suppressor cells that produce interleukin 10 and TGF- β that could help *Lm* escape the cellular
3 immune response at the tumor site¹⁴. It indicates that patients with *Lm*-associated lymphadenitis
4 should be evaluated for neoplasia, mainly in the affected lymph node or loco-regionally.

5 Seeding from a loco-regional portal of entry is a possibility, considering the negativity of blood
6 cultures, the absence of neurolysteriosis and the involvement of a single lymph node, even
7 though one cannot exclude it may also result from bacteremia. The predominant cervical
8 involvement may follow translocation in the mucosa-associated lymphoid tissue of the
9 pharyngo-oral region. *Listeria* can indeed be detected in tonsils of wild animals^{15,16} and also in
10 humans¹⁷. The case with appendicitis likely results from *Listeria* translocation across the
11 MALT tissue of the appendix (Peyer's patches).

12 Management should include drainage of suppurative lesions or excisional biopsy, and *Listeria*-
13 targeting amoxicillin-based antimicrobial therapy, which is otherwise not recommended in
14 suppurative lymphadenitis, where *Staphylococcus aureus* should be covered.

15 Since the review of literature was not performed according to PRISMA guidelines, some cases
16 may have been overlooked and the total number of published cases may this be higher.

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

20

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4

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11 Sete).

Table 1. Features of 19 patients with *Listeria monocytogenes*-associated lymphadenitis.

Reference	Patient No	Age at Diagnosis, Sex, Underlying Diseases	Clinical and Imaging features					Blood Cultures	Species and MLST Clonal Complex	Histopathology	Treatment, Surgery, Antibiotics	Outcome
			Symptoms duration before diagnosis	Localization	Size, Number	Signs of Abscess	General and Associated Signs					
This study	1	72-year-old man, Essential thrombocythemia, myelofibrosis, chronic neutropenia, unbalanced diabetes, prednisone 7.5mg/d	3w	Right cervical and under the chin	Bulky, Multiple (CT-scan)	No	Pneumonia	Negative	<i>Listeria monocytogenes</i> CC121	NP	Puncture. Amoxicillin (26d) + Gentamicin (5d)	2 recurrences treated with Amoxicillin (3 then 4w). Cure (4m). Death(1y).
This study	2	63-year-old man, Uncontrolled diabetes (glycated hemoglobin 17.4%), tobacco smoke, alcoholism.	1m	Right cervical	Bulky (6.5x8cm; CT-scan), Single	Inflammatory (C) and Necrotic (CT-scan) mass	Weight loss (12kg)	NP	<i>Listeria monocytogenes</i> CC4	NP	Puncture. Amoxicillin + Gentamicin.	Cure. Death (4y, hepatocellular carcinoma)
This study	3	88-year-old woman. Parkinson's and Alzheimer's diseases	3w	Right inguinal	Bulky, Single (C)	No	Lower limb cellulitis	NP	<i>Listeria monocytogenes</i> CC2	Infiltration with gynecological carcinomatous cells	Amoxicillin-clavulanate (10d): persistence. Lymph node removal. Amoxicillin 6g/d.	Discovery of a metastatic gynecological carcinoma. Death (2.5m)
This study	4	82-year-old woman. Uncontrolled type II diabetes, obesity	3m	Left cervical	Bulky (5cm, C), Single (US)	No (US)	No	NP	<i>Listeria monocytogenes</i> CC9	NP	Surgical drainage. No antibiotic.	Death (1d)

This study	5	55-year-old-woman. Type II diabetes. Tobacco smoking	NA	Left inguinal	Bulky, Single (C).	No	NA	NP	<i>Listeria monocytogenes</i> CC5	Non necrotic epithelioid granuloma	Lymph node removal.	Cure. Death (5y, metastatic gallbladder carcinoma)
This study	6	60-year-old-man. HBV and alcoholic cirrhosis. Tobacco smoking, alcoholism	1m	Median cervical	Bulky, Single (CT-scan, US)	Fistulization	No	NP	<i>Listeria monocytogenes</i> CC20	Non necrotic epithelioid granuloma	Surgical drainage. Amoxicillin (21d)	Cure (2m)
This study	7	72-year-old-woman. Untreated and unbalanced type II diabetes	3w	Left submaxillar	Bulky (7x4x7cm; CT-scan), Single	Yes (CT-scan)	Asthenia	NP	<i>Listeria monocytogenes</i> CC199	Necrotic epithelioid granuloma, Pus	Lymph node removal. Pefloxacin (10d)	Cure (6m)
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>Listeria monocytogenes</i> CC21	NP	Puncture. Amoxicillin 8g/d (15d)	Cure (5m). 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	<i>Listeria monocytogenes</i> CC7	NA	NA	Cure (17y)
This study	10	11-year-old man	6d	Right iliac	Large (16x11mm), multiple (US).	No	Fever Anorexia Abdominal pain	NP	<i>Listeria ivanovii</i> CC883	Chronic lymphadenitis without sign of malignancy.	Surgical lymph node removal. Amoxicillin 50mg/kg/d (15d)	Cure (1m)
This study	11	66-year-old-man	1.5m	Right cervical	Large (3cm), multiple (CT-scan).	No	Asthenia Weight loss Fever	Negative	<i>Listeria monocytogenes</i> CC20	NP	Puncture. Cotrimoxazole (3w)	Cure (2m). Discovery of a chronic lymphocytic leukemia.

Larsson S.	12	81-year-old woman. Type II diabetes	3w	Cervical	NA	Yes	No	NA	NA	NP	Surgical drainage. No antibiotic	Cure
Bojsen-Moller J.	13	NA	NA	Cervical	Single	NA	NA	NA	NA	NA	NA	NA
Blanche P.	14	55-year-old woman. Types II diabetes. HIV infection (CD4+ T lymphocytes count 242/mm ³)	NA	Cervical	Multiple	No	Fever	Negative	NA	Non necrotic epithelioid granuloma, Pus	Surgical drainage. Amoxicillin (6 w)	Cure
Ferrer D.	15	81-year-old man. Diabetes mellitus	NA	Cervical	Single	Yes	NA	NA	NA	NA	NA	NA
Goulet V.	16	56-year-old man. Type II Diabetes. Alcoholism	NA	Cervical	NA	NA	NA	NP	NA	NP	Puncture	Cure
Goulet V.	17	60-year-old-man	NA	Cervical	NA	NA	NA	NP	NA	NP	Puncture	Cure
Rosenthal R.	18	75-year-old man. Type II diabetes, Obesity, Tobacco smoke, Alcoholism	1m	Cervical	Bulky (4cm), Multiple	Fistulization	No	NP	NA	Necrotic epithelioid granuloma, Pus	Lymph node removal. Amoxicillin-clavulanate (4w)	Death (4w); autopsy: myocardial ischemia, metastatic carcinoma of the bladder, residual neck abscess
Betriu C.	19	52-year-old woman. Uncontrolled type II diabetes	NA	Left cervical	Bulky (8x3x3; CT-Scan), Single	Inflammatory mass (C), collection (CT-scan, US)	Fever	Negative	NA	Epithelioid granuloma.	Surgical drainage. Amoxicillin (1m) + Gentamicin (3d)	Stable (2m)

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Figure 1.

Detection of *Listeria monocytogenes* (*Lm*), which appear in red, in adenitis tissue samples collected from two patients with immunoperoxidase staining (A,B,E,F) and direct immunofluorescence with Wheat Germ Agglutinin (in green) and Hoechst (in blue) staining (C,D,G,H). *Lm* are localized within cells evocative of monocytes and macrophages, but also in polymorphonuclear cells in close vicinity with the capsule of the abscessed lymph node in the section available for case 6 (A,B,C,D); and within appendix lumen in the section available for case 10 (E,F,G,H). Scale bars: 100 μm (panels A,E); 20 μm (panel C), 50 μm (Panel G), 5 μm (panels B,F) 2 μm (panels D,H).