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ORIGINAL ARTICLE

How is the microbial diagnosis of bacterial vertebral osteomyelitis performed? An 11-year retrospective study

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Abstract

Vertebral osteomyelitis (VOM) is often diagnosed with delays, resulting in poorer outcomes. Microbial documentation is particularly challenging and obtained using blood cultures (BCs) and vertebral biopsies (VBs; CT-guided or surgical). We retrospectively analysed VOM cases in a tertiary reference centre between 2004 and 2015, focusing on how and how quickly microbiological diagnosis was performed. Among 220 VOM, 88.2% had documentation, including Gram-positive cocci (GPC) (70.6%), Gram-negative rods (GNR) (9.3%), anaerobes (3.6%), polybacterial infections (6.7%) and tuberculosis (9.8%). BCs were performed in 98.2% and positive in 59.3%, identifying most GPC (80.3%) and half of GNR (54.6%). VBs were performed in fewer cases (37.7%), but were more frequently positive (68.8% for CT-guided and 81.0% for surgical biopsies). They documented all anaerobes (100.0%), most *M. tuberculosis* (84.2%) and polybacterial infections (76.9%), and GNR (45.4%). Extra-vertebral samples highly contributed to tuberculosis diagnosis (52.6%, and 15.8% as the only positive sample). Documentations most often followed radiological diagnosis (53.4%). They were obtained earlier by BCs than by VB after first clinical symptoms (median of 14 versus 51 days). Antibiotic treatments were mostly initiated after samplings (88.0%). BCs allow the documentation of most VOM and should be performed without delay in case of clinical or radiological suspicion; however, they may miss 1 out of 5 GPC and 1 out of 2 GNR. VBs have a higher positivity rate and should be rapidly performed if negative BCs. It is likely that delayed and missed diagnoses result from the insufficient use of VB.

Keywords Vertebral osteomyelitis · Microbiological diagnosis · Blood cultures · Vertebral biopsy · Diagnosis delay

Highlights
- Blood cultures (BCs) and vertebral biopsies (VBs) are main VOM diagnosis techniques.
- Both must be performed simultaneously, with systematic BCs and second-line VBs.
- Microbiological samplings must not be delayed.
- BCs allowed most pyogenic documentation, but missed some bacteria identified by VBs.
- VBs and extravertebral samples are essential for documentation of *M. tuberculosis*.

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Introduction

Vertebral osteomyelitis (VOM) is a heterogeneous group of diseases that can be caused by diverse pathogens of various virulence (pyogenic aerobic and anaerobic bacteria, mycobacteria or fungi). It can present as an acute, subacute or chronic infection. Pathogens can reach the spine by hematogenous dissemination, contiguous spread from an extra-vertebral site of infection or direct inoculation due to trauma or surgery [1–3].

A significant increase in VOM incidence has been observed over the last few decades [1, 4, 5]; it was recently estimated to be 47, 58 and 74 cases per 1,000,000 per year in three industrialized countries (USA, Denmark and Japan, respectively) [5–7]. This may be due to an increase in susceptible populations (e.g. elderly individuals and/or patients undergoing vertebral surgery [4, 6–10]) as well as improved diagnosis thanks to both better clinical awareness [2, 7, 11, 12] and broader use of magnetic resonance imaging [11, 13].

The diagnosis of VOM is based on clinical suspicion, then radiological and microbiological confirmations, and has long been challenging [14, 15]. An extended duration from the first clinical symptoms to diagnosis is frequent [9, 16–18] and represents a major risk factor for poor outcome [2, 8, 19, 20] such as vertebral, neurological [11, 19, 21] or systemic complications, and even death [22–24].

Prompt and accurate microbial diagnosis is crucial to identify the causative agent and its antibiotic susceptibility profile, both being required before prescribing prolonged antibiotic treatment [14, 24, 25].

Microbiological diagnosis can be performed by several methods [1, 12, 14, 26]: it may be readily obtained by blood cultures (BCs), or may require direct samples from the infected site using computerized tomography (CT)-guided percutaneous (CtB) or surgical vertebral biopsy (SuB) [14, 15, 26–28]. Finally, positive samplings taken from other sites may be useful in VOM associated with disseminated infections [11, 17, 27, 29].

We aimed to determine how the microbiological diagnosis of VOM has been obtained in our centre in recent years.

Materials and methods

Study design

We conducted a retrospective observational study including all bacterial VOM diagnosed in inpatients aged over 16 years from February 2004 to May 2015 in the departments of Infectious Diseases, Internal Medicine and Rheumatology in the Grenoble-Alpes University Hospital, Grenoble, France, a tertiary referral centre for complex osteoarticular infections.

Definitions

During this period, 257 patients received a diagnosis of VOM. We retained all cases with radiological findings consistent with VOM (spondylodiscitis, discitis, spondylitis, vertebral osteomyelitis and/or epiduritis) and microbiological explorations. We excluded differential diagnosis ($n = 23$), cases without microbiological exploration ($n = 5$) and cases occurring within the first month or the first year if locally implanted hardware ($n = 9$), after spinal surgery. We finally analysed 220 cases.

VOM were considered healthcare related if they occurred within the first month after any extra-spinal surgery, or within the first year after any implantation of extra-spinal hardware or in association with documented infections of intravascular device.

Data collection

Data were retrospectively collected from electronic medical records and paper charts, and included demographics, underlying morbid conditions, VOM characteristics (clinical presentation, imaging data, method(s) for microbiological documentation, antibiotic treatment) and key dates (first clinical spine-related symptoms, imaging confirmation, microbial documentation, and antibiotic treatment).

First clinical spine-related symptoms included spinal (e.g. lumbar pain), radicular (e.g. radicular pain) or medullary (e.g. sensitive or motor deficit) symptoms, occurring in association with other suggestive symptoms of infection (asthenia, fever, thrills) and/or de novo.

Methods for microbiological documentation involved blood cultures (BCs), vertebral biopsies (VBs) and other samplings. VBs were performed under Ct-guidance (16 to 20 gauge needles) or by surgical approach, in accordance with the practices of our institution. Cultures, pathology and some PCR were performed on the vertebral samples.

Imaging data included all diagnostic radiological findings.

Confirmed bacterial cases

VOM were considered microbiologically documented according to the following definitions.

1. The diagnosis of pyogenic VOM was confirmed in each of the following cases:

(a) Positive cultures or positive polymerase chain reaction (PCR) on VB, by CtB or SuB
(b) AND/OR, positive BCs

For the case of coagulase-negative *Staphylococcus*-positive BC, bacteraemia was only retained if at least one second significant sample (e.g. second BC, urine, pacemaker lead)

was also positive for the same bacteria; one positive BC was not sufficient to retain the diagnosis (contamination).

(c) AND/OR, positive cultures or positive PCR on others significant samples (e.g. psoas abscess, articular or lumbar punctures).

2. The diagnosis of mycobacterial VOM was confirmed by:

(a) Positive Ziehl-Neelsen stain, positive cultures on Lowenstein media
(b) AND/OR, positive specific PCR for *Mycobacterium* of the complex *tuberculosis* on VB
(c) AND/OR, other significant samples (e.g. sputum, bronchial aspiration, urine, abscess, lymph node, bone biopsy)
(d) AND/OR, pathology evocative of tuberculosis on VB (granulomatosis with caseous necrosis)

Patients whose samples did not meet these definitions were considered as non-documented cases.

Indirect microbiological diagnosis techniques (e.g. *S. pneumonia* antigenuria, or *Brucella* serology), unspecific pathology results on VB (e.g. unspecific chronic inflammation) and/or mycological findings (e.g. fungal cultures or *Aspergillus* or *Candida* antigenemia) were not included in our documented cases.

Timing analysis

We established duration between key management dates: first clinical symptoms, date of microbiological documentation by BCs or VBs (in cases of multiple positive methods, the date of the first technique was considered), radiological diagnosis and treatment.

The date of microbiological confirmation was the date of the sampling.

Statistical analysis

Data management and statistical analyses were performed using Stata 13.1 software (College Station, Texas, USA). Parametric variables were compared with the Student *t* test, non-parametric variables with the Mann-Whitney *U* test and associations between categorical variables with the chi-square test. We fitted stepwise logistic regression models to determine the variables independently associated with main positive diagnosis techniques; variables with a $p < 0.2$ in univariate analysis were included in the model.

We estimated the mean time between first clinical symptoms and microbial diagnosis according to the diagnosis techniques using the Kaplan-Meier method, and we compared the two survival curves using the log-rank test.

Results

During the 2004–2015 period, 220 VOM met the inclusion criteria in our centre; 194 (88.2%) had microbiological documentation.

Population and infections

Main characteristics of the patients and the infections are reported in the Table 1 (and in Supp. Figures 1a and 1b).

Procedures for bacterial diagnosis

Microbiological documentation was obtained mostly by BCs, CtB and SuB ($n = 187$, 85.0% of all VOM and 96.4% of documented VOM) (Fig. 1; Table 2). BCs were almost always performed ($n = 216$, 98.2% of all VOM); they were positive in 128 cases (59.3%; 58.2% of all VOM). A CtB was performed in 80 cases (36.4% of all VOM); it was positive in 55 patients (68.8%; 25.0% of all VOM). A SuB was performed in 21 cases (9.5% of all VOM): 13 cases for decompression, 4 cases for paravertebral or spinal samplings and 4 cases without specification. It was positive in 17 cases (81.0%; 7.7% of all VOM).

In 12 cases (5.5% of all VOM and 6.2% of documented cases), both BCs and VBs (CtB or SuB) were positive for the same pathogen. In 7 cases (3.2% of all VOM and 3.6% of documented cases), bacterial documentation was obtained through samples from other sites.

Efficacy of each procedure: proportion of positive samples for each procedure performed (Supp. Table 1)

BCs were positive in 59.3% when performed ($n = 128$ positive for $n = 216$ performed BCs, accounting for 66.0% of the documented VOM), CtB in 68.8% ($n = 55$ positive for $n = 80$, accounting for 28.4% of the documented VOM) and SuB in 81.0% ($n = 17$ positive for $n = 21$, accounting for 8.8% of the documented VOM).

BCs have been performed in all non-documented VOM ($n = 26$), and most of them benefited VBs, including 80.8% of CtB ($n = 21$) and 3.8% of SuB ($n = 1$).

Identified bacteria ($n = 194$)

Pyogenic bacteria were the most commonly involved pathogens ($n = 175$, 90.9%), with a majority of Gram-positive cocci (GPC, $n = 137$, 78.3%) followed by Gram-negative rods (GNR, $n = 18$, 10.3%) and anaerobes ($n = 7$, 4.0%). The infection was polymicrobial in 13 cases (7.4%).

Figure 2 shows the identification method according to pathogen:

Table 1 Main characteristics of the patients and infections ($n = 220$)

Demographics	
Age, median (IQR), range, years	67 (IQR, 64–77), 15.5–95
Male sex, *n* (%)	155 (70.4%)
Immunocompromised status	30 (13.6%)
HIV with T CD4 count < 500/mm^3	3 (1.4%)
Immunosuppressive therapies: [Steroid therapy > 20 mg/day, immunomodulators (e.g. monoclonal antibodies) or immunosuppressors (e.g. antimetabolites, chemotherapy or post-transplantation regimen)]	27 (12.2%)
Cancer	50 (22.7%)
Diabetes mellitus	38 (17.3%)
IV drug users	7 (3.2%)
Past spinal surgery	11 (5.0%)
Without hardware after > 1 month	9 (4.1%)
With hardware after > 1 year	2 (0.9%)
Migrants	59 (26.8%)
No previous history of disease	30 (13.6%)
Inpatient wards	
Infectious diseases department	142 (64.8%)
Internal medicine	51 (23.3%)
Rheumatology	26 (11.9%)
Clinical findings	
Fever	134 (60.9%)
Spinal pain	193 (87.7%)
Neurological complications (e.g., radicular pain, sensitive or motor deficit)	53 (24.1%)
Radiological findings	
Magnetic resonance imaging	193 (87.7%)
CT scan	61 (27.7%)
Bone scintigraphy	28 (12.7%)
Spondylitis	213 (96.8%)
Discitis	199 (90.4%)
Epiduritis (e.g. epidural signal changes)	114 (51.8%)
Cervical VOM	37 (16.8%)
Thoracic VOM	73 (33.2%)
Lumbar VOM	121 (55.0%)
Sacral VOM	21 (9.6 %)
Unifocal VOM	183 (83.2%)
Loco-regional complications	
Medullar compression	40 (18.2%)
Paravertebral abscess	57 (25.9%)
Psoas abscess	35 (15.9%)
Other localizations	
Infectious endocarditis	30 (13.6%)
Endovascular infections (e.g. arterial prosthesis)	20 (9.1%)
Extra-vertebral bone and joint infections	18 (8.2%)
Other sites: pneumonia, pleurisy, hepatic abscess, meningitis, urinary tract infections	93 (42.3%)
Healthcare-related VOM	
Certain	21 (9.6%)
Post-operative (1 cataract surgery, 2 joint prosthesis implantations, 2 vascular bypass surgeries, 2 digestive surgeries)	7 (3.2%)

Table 1 (continued)

Related to vascular devices infections (4 pacemakers, 7 central venous catheter ports, 1 peripherally inserted central catheter line, 1 dialysis catheter, 1 peripheral venous catheter)	14 (6.4%)
Possible, after spinal infiltration	5 (2.3%)
Certain microbial diagnosis	194 (88.2%)
Pyogenic bacteria	175 (79.5%)
Gram-positive Cocci	137 (70.6%)
S. aureus	79 (57.7%)
SCN	17 (12.4%)
Streptococci	37 (27%)
Enterococci	4 (2.1%)
Gram-negative rod	18 (9.3%)
Enterobacteria	14 (87.5%)
Fastidious GNR (e.g. *Haemophilus sp.*)	2 (11.1%)
Non-fermentative GNR (e.g. *Pseudomonas* sp.)	2 (11.1%)
Anaerobes	7 (3.6%)
Multiple	13 (6.7%)
Mycobacteria	19 (8.6%)

IQR interquartile range, *IV drug* intravenous drug, *VOM* vertebral osteomyelitis, *CT* computerized tomography, *SCN Staphylococcus* coagulase negative

– Staphylococci were the most frequent pathogens ($n = 96$, 49.5%) with 79 *S. aureus* (SA) and 17 coagulase-negative staphylococci (CNS) (including 12 *S. epidermidis* and 2 *S. capitis*). SA were mostly documented by BCs (75.9%, with 19.5% confirmed by another positive sample: joint fluid, urine, abscess and/or vertebral biopsies), followed by VBs (13.9%), both (7.6%) or others sites (2.5%; articular puncture or cerebral spinal fluid). CNS were mostly documented by BCs (64.7%, with 17.7% associated with a second positive sample: pacemaker lead, urine, peripherally inserted central catheter), followed by CtB (29.4%) and non-vertebral abscess sample (5.9%).
– Streptococci ($n = 37$, 19.1%) were the second most frequent pathogens, including 6 *S. agalactiae*, 6 *S. gallolyticus*, *4 S. pneumoniae*, 3 *S. anginosus*, 2 *S. gallolyticus*, 2 *S. gordonii*, 2 *S. constellatus*, 2 *S. oralis*, and 2 *S. sanguinis*. Streptococci were mostly documented by BCs (78.4%, associated with another pos-

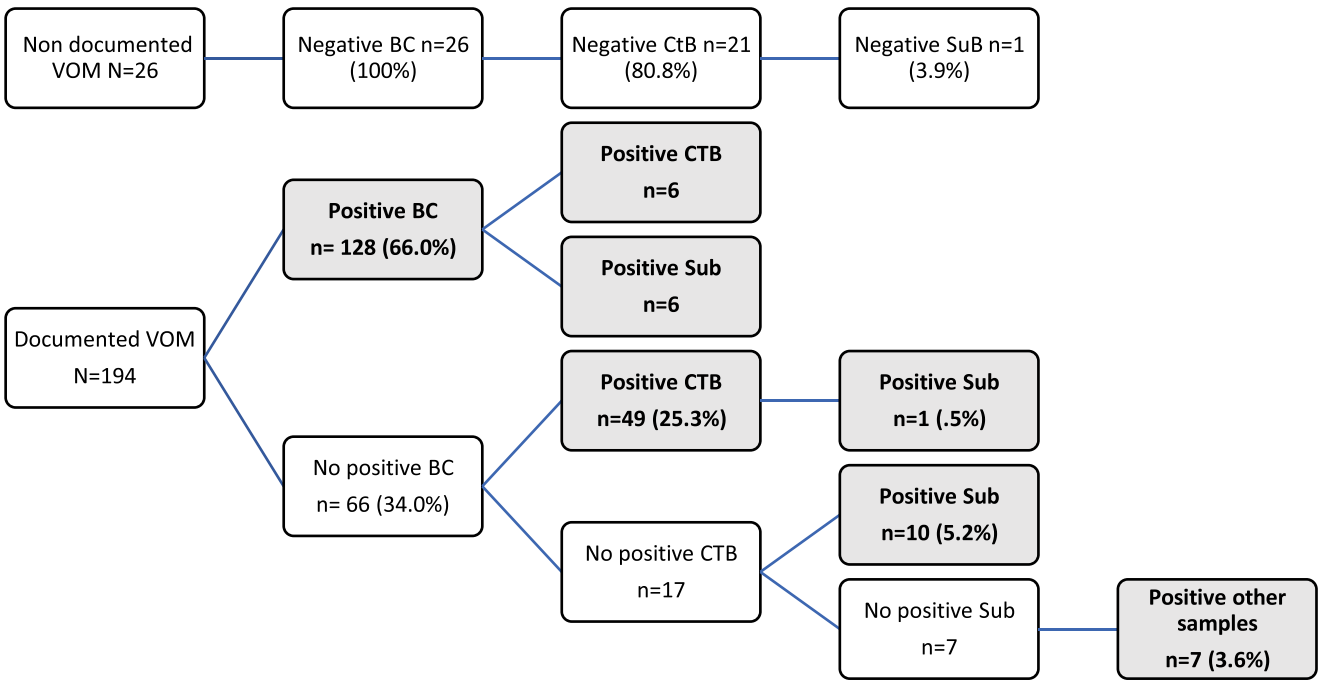

Fig. 1 Methods of microbial documentation

Table 2 Distribution by reference diagnostic modes (n = 220)

	Vertebral biopsies not performed (*n* = 121)	Positive vertebral biopsies (*n* = 71)	Negative vertebral biopsies (*n* = 28)
Positive blood cultures (*n* = 128)	112	12	4
Negative blood cultures (*n* = 88)	9	55	24
Blood cultures not performed (*n* = 4)	0	4	0

itive sample in 2 cases: non-vertebral abscess, cardiac valve), followed by CtB (16.2%) and SuB (2.7%). One S. pneumoniae was documented from lumbar puncture.

- *Enterococcus faecalis* (*n* = 4, 2.1%) were all documented by BC. In one case, surgical sampling of a vascular bypass was also positive.
- GNR (*n* = 18, 9.3%), featuring 8 *Escherichia coli*, 2 *Pseudomonas aeruginosa* and 2 *Klebsiella pneumoniae* were documented by BC (55.5%), CtB (38.9%) and SuB (5.6%). In 2 cases, positive BCs were associated with positive cultures from psoas abscess biopsies. One 16s PCR was positive.
- Polymicrobial VOM (*n* = 13, 7.3%) including 3 bacteria in 2 cases and 2 bacteria in 11 cases were diagnosed using VB (*n* = 10, 46.2%, including 8 CtB), BCs (*n* = 3, 23.1%) or both (*n* = 4, 30.8%).
- Anaerobes (*n* = 7, 3.6%), featuring 3 *Propionibacterium acnes*, were all documented by VBs (6 CtB and 1 Sub) associated with positive BC in one case. One 16s PCR was positive.
- *Mycobacteria* of the *tuberculosis* complex were involved in 19 cases (9.8%), featuring 16 *Mycobacteria tuberculosis*, 1 *Mycobacterium africanum* and 1 *Mycobacterium bovis* (in 1 more case, the diagnosis relied on pathology alone). Diagnosis was based on VB in 16 cases (84.2%). CtB was positive in 11 cases (including 1 positive Ziehl-Neelsen stain, 4 positive cultures, 4 positive PCR and 3 positive pathologies); in 5 of these cases, a sample from another site (e.g. iliac or lymph node biopsies, sputum, bronchoalveolar lavage, gastric tubing) was also positive in cultures. SuB was positive in 5 cases (3 positive cultures, 1 positive PCR and 1 positive pathology); in 2 of these cases, a sample from another site (cervical lymph node or abscess biopsy) was also positive. In 3 cases (15.8%), cultures from extra-vertebral sites only were positive (tracheal aspiration, sputum or urine).

Among the VOM occurring after spinal surgery (*n* = 11; including 2 cases on hardware), 2 occurred in the first year (after 4 and 9 months) and 9 later (median of 5 years, range 2–20). We collected 7 documented cases with 1 positive BC and 6 VBs (4 CtB for 2 SuB). They accounted for 2 SCN, 2 anaerobes, 1 SA, 1 GNR and 1 polybacterial VOM.

Differences in diagnostic procedures

We determined whether the patient characteristics varied according to the mode of microbial identification (Table 3).

By univariate analysis, identification by positive BCs was significantly associated with older age, past mellitus diabetes, healthcare-related VOM, fever, secondary infectious foci, associated infectious endocarditis, higher levels of blood inflammatory markers and CGP infection (SA or *Streptococci)*. Conversely, documentation by VBs was associated with younger age, past spinal surgery, local neurological symptoms, paravertebral abscesses and anaerobes, polybacterial and mycobacteria infections.

By multivariate analysis, fever (adjusted odd ratio, aOR, 3.4; 95% CI, [1.2, 9.5]), other infectious localizations (3.3; [1.1, 9.9]), infectious endocarditis (12.0, [1.1, 133.5]), CRP higher than median of 100 (2.8; [1.0, 7.7]), context of healthcare-related infection (123.5; [5.9, 2569.8]), *Staphylococcus aureus* (35.8, [8.6–148.9]) and *Streptococci* (5, [1.3–18.8]) were also independently associated with documentation by BCs. Conversely, documentation by VBs was independently associated with spinal pain (11.4; [1.8, 73.6]), neurological complications (16.4; [3.9, 69.0]), paravertebral abscesses (4.3; [1.4, 13.3]) and polybacterial documentation (13.4; [1.2, 154.3].

Timing analysis

Median delay between radiological and microbiological diagnosis was 0 day (IQR, − 7; 4 and range, − 76; 44 days for *N* = 178), reflecting that the samplings for bacterial documentation might either precede or follow the radiological diagnosis (Supp. Figure 2). Positive BCs were most frequently drawn before the radiological diagnosis (*n* = 83; median of 7 days, IQR, 4; 15 and range, 1; 76) or immediately after (*n* = 41; median of 1 day, IQR, 0; 5 and range, 0; 42). Meanwhile, microbial diagnoses relying on VBs (*n* = 54) occurred after the radiological diagnosis, with a median of 5 days (IQR, 2; 10 and range, 0; 44).

Median durations from first clinical symptoms to microbial diagnosis varied according to the diagnostic method (Supp. Table 2; *N* = 160).

- When the diagnosis relies on BCs, the median duration was 12 days (IQR, 3; 41, and range, 0; 188) (i.e. 5 days in median when BCs were sampled before radiology (IQR,

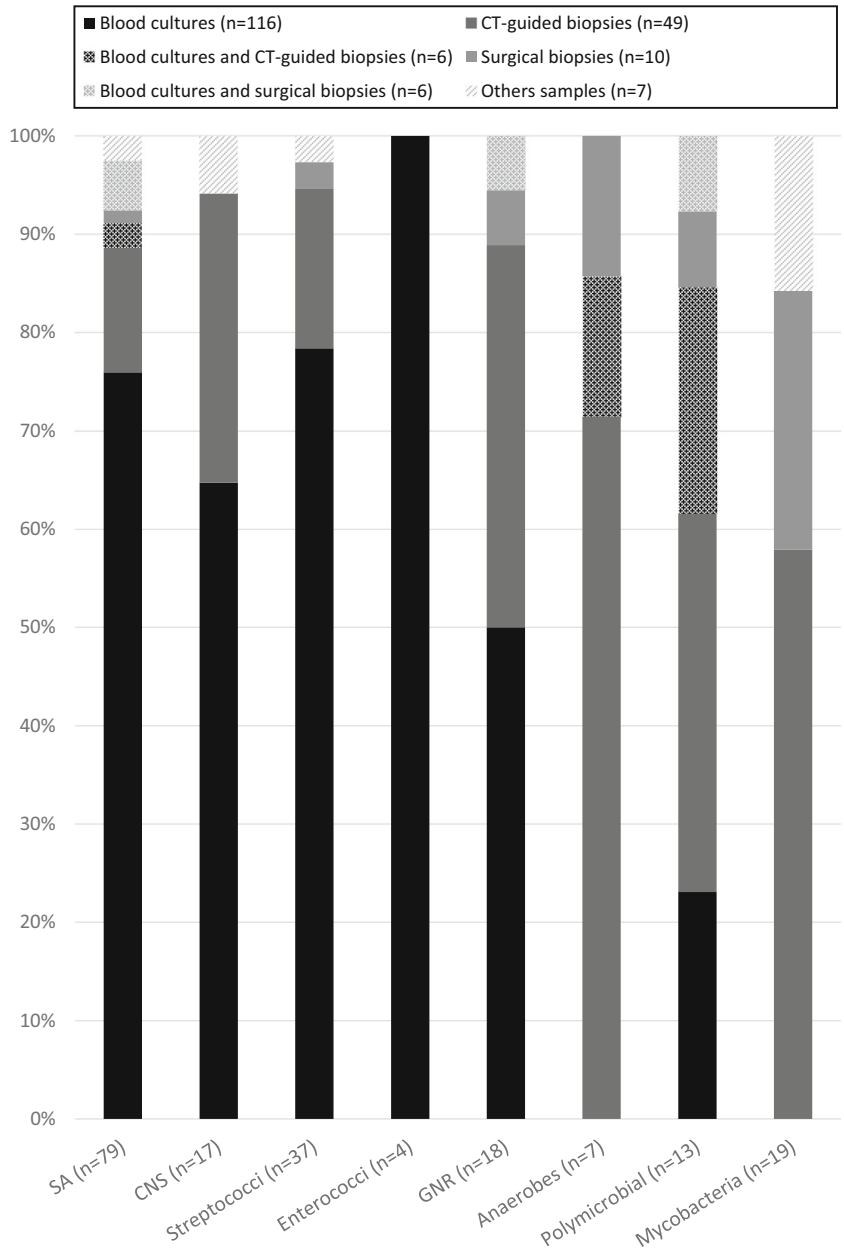

Fig. 2 Documentation method by pathogens ($n = 194$)

1;30 and range, 0;188) and 15 days in median when BCs were sampled after (IQR, 6;43 and range, 0;103).

– When the diagnosis relies on VBs, the median duration was 51 days (IQR, 27; 97 and range, 0; 209) (i.e., 52 days in median by CtB and 36 by SuB). When excluding tuberculosis cases, the median duration was 47 days.

As represented in the Fig. 3a, Kaplan-Meier estimates showed significantly different average durations according to the technique, with 28 days (Std. Dev. 37) by BCs and 81 days (Std. Dev. 70) by VBs.

Median delays between first clinical symptoms and microbial diagnosis varied also by genus, as described in Fig. 3b, with:

– 15 days (IQR, 5; 43, range, 0; 187) for CGP

- 11 days (IQR, 1; 39 and Range, 0; 187) for SA
- 28 days (IQR, 12; 41 and Range, 8; 62) for CNS
- 19 days (IQR, 7; 70, Range, 1; 126) for *Streptococci*
- 22 days (Range, 5; 24) for *Enterococci*

– 52 days (IQR, 6; 113, range, 0; 188) for GNR
– 104 days (IQR, 43; 162, range, 27; 180) for anaerobes
– 27 days (IQR, 0; 92, range, 0; 181) in polymicrobial infection
– 73 days (IQR, 46; 143, range, 18; 209) for mycobacteria

Table 3 Case characteristics according to the method of bacteria identification (blood cultures or vertebral biopsy) (diagnoses by both features were not included)

	Positive blood cultures ($n = 116$)	Positive vertebral biopsies ($n = 59$)	p
Age, mean (IQR), years	68 (65–70)	56 (51–61)	*< 0.001*
Male sex, *n* (%)	83 (71.6%)	53 (74.6%)	0.7
Immunocompromised status[a]	19 (16.4%)	7 (11.9%)	0.4
Cancer	28 (24.1%)	15 (25.4%)	0.8
Diabetes mellitus	25 (21.6%)	5 (8.5%)	*0.03*
IV drug users	4 (3.5%)	1 (1.7%)	0.5
After spinal surgery[b]	1 (0.9%)	6 (10.2%)	*0.003*
Healthcare-related VOM[c]	19 (16.4%)	0 (0%)	*0.001*
Clinical symptoms			
Fever	88 (75.9%)	26 (44.1%)	*< 0.001*
Back pain	97 (83.6%)	54 (91.5%)	0.1
Neurological symptoms	20 (17.2%)	15 (25.4%)	0.2
Biological results			
C-reactive protein, median (IQR) mg/L	144 (125–163)	82 (60–103)	*< 0.001*
Leukocyte count, median (IQR), cells/mm^3	10,535 (9700–13,365)	8875 (7989–9760)	*0.006*
Radiological findings			
Spondylitis	111 (95.7%)	59 (100%)	0.1
Discitis	106 (91.4%)	51 (86.4%)	0.3
Epiduritis	59 (50.9%)	34 (57.6%)	0.4
Cervical VOM	24 (20.7%)	7 (11.9%)	0.1
Thoracic VOM	37 (31.9%)	21 (35.6%)	0.6
Lumbar VOM	60 (51.7%)	36 (61.0%)	0.2
Sacral VOM	7 (6.0 %)	9 (15.2%)	*0.05*
Multifocal VOM	17 (14.7%)	11 (18.6%)	0.5
Compression	20 (17.2%)	12 (20.3%)	0.6
Paravertebral abscess	23 (19.8%)	22 (37.3%)	*0.01*
Psoas abscess	15 (12.9%)	13 (22.0%)	0.1
Other localizations	63 (54.3%)	15 (25.4%)	*< 0.001*
Associated infectious endocarditis	27 (23.3%)	1 (1.7%)	*< 0.001*
Microbial documentation			
Pyogenic bacteria	*116 (100%)*	*43 (72.9%)*	*< 0.001*
Gram-positive Cocci	104 (89.7%)	23 (39.0%)	*< 0.001*
S. Aureus	60 (51.7%)	11 (18.6%)	*< 0.001*
CNS	11 (9.5%)	5 (8.5%)	0.8
Streptococci	29 (25%)	7 (11.9%)	*0.04*
Enterococci	4 (3.4%)	0	0.1
Gram-negative rod	9 (7.8%)	8 (13.6%)	0.2
Enterobacteria	9 (100%)	4 (44.4%)	*0.06*
Fastidious GNR (*Haemophilus sp.*)	0	2 (25%)	0.1
Non fermentative GNR (*Pseudomonas sp.*)	0	2 (25%)	0.1
Anaerobes	0	6 (10.2%)	*< 0.001*
Polybacterial	3 (2.6%)	6 (10.2%)	*0.03*
Mycobacteria	*0*	*16 (27.1%)*	*< 0.001*

Median duration from first clinical symptoms to antibiotic treatment was 15 days (IQR, 5; 44 and range, 0; 188 for $N = 164$) by BCs and 68 days (IQR, 39; 113 and range, 9; 369) by VBs (with 68 days by CtB and 51 days by SuB).

CT computerized tomography, *IQR* interquartile range, *IV drug* intravenous drug, *VOM* vertebral osteomyelitis

Significant p-values are in italic (i.e., when $p<.05$)

[a] Including patients with HIV with T CD4 count < 500/mm^3 and patients with immunosuppressive therapies (e.g. steroid therapy > 20 mg/day, chemotherapy or post-transplantation regimen)

[b] After spinal surgery (e.g. at least 1 month after surgery or after 1 year after surgery if hardware)

[c] Certain healthcare-related VOM (e.g. after extra-spinal surgery, or associated with vascular infections)

Median delay from microbial samplings to beginning of treatment lasted for 1 day (IQR, 0; 2, range, −28; 158 for $N = 184$ cases). Among documented cases (n = 172), treatment was mostly initiated after the documentation in a median of 1 day (IQR, 0; 3 and range, 0; 158 days) for 87.8%. Meanwhile, it was begun before the samples in 12.2% of the cases, in 2 days in median (IQR, 1; 4 and range, 1; 28 days).In cases without documentation (n = 12), antibiotic treatment was mostly introduced after samplings (91.7%).

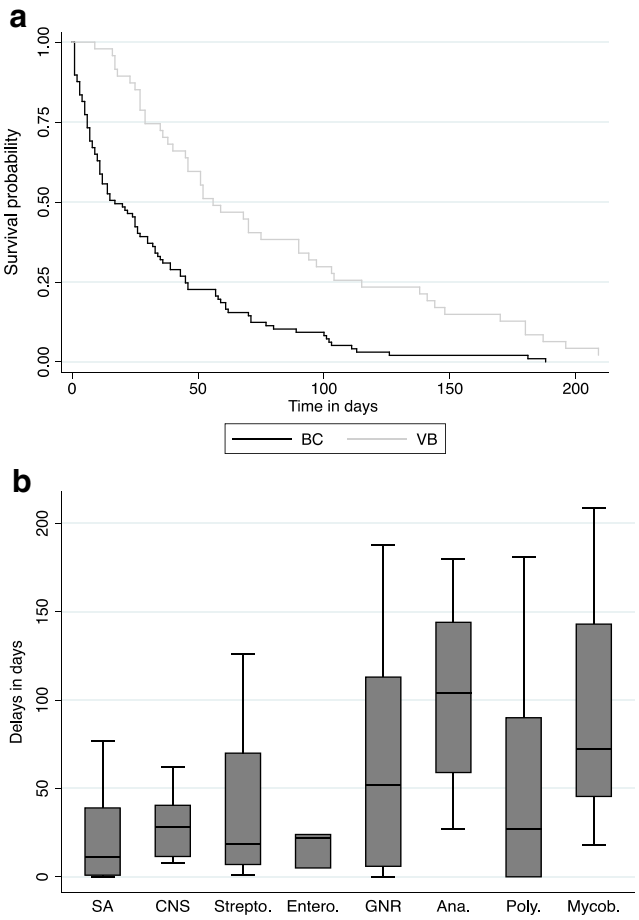

Fig. 3 **a** Kaplan-Meier survival estimates of duration between first clinical symptoms and microbial diagnostic, by BCs and VBs, and log-rank test with $p < 0.001$. **b** Duration (days) between first clinical symptoms and microbial diagnosis per genus

Discussion

Through the analysis of our recent 11-year cohort of VOM, we aimed to determine how the bacterial identification was performed in our centre. We also defined the time to microbial documentation from other key diagnostic steps (i.e., first clinical symptoms and radiological diagnostic).

Our cohort had similar epidemiological characteristics of patients (median age of 67 years, range (15.5–95) and 70.4% of men) and of infections than previous studies [2, 4, 8–11].

Our work confirmed that BCs as simple routine samples and VBs as their second-line complement are both efficient techniques in the microbiological documentation of VOM, as previously reported [12, 14, 15, 26, 30–32].

Both provided most of the identifications, including 59.8% by BCs and 36.6% by VB, the remaining cases being diagnosed by extra-vertebral samples. Positive BCs were the only conclusive sample in half of all cases (52.7%), VBs documented 89.4% of VOM without positive BCs (e.g. negative or not done) and both positive BCs and VBs were rare (5.5% of all VOM). At least one of the two techniques was performed in all cases (98.2% of BCs among all cases and 90.2% of BCs in cases with negative BCs).

As a result, microbial documentation was largely reached in our work ($n = 194$, 88.2%, for 76.7% on average, and range, 56–92% in the literature [7, 8, 16, 19–23, 32–37]). We counted 9.8% of mycobacterial (n=19) for 90.2% of pyogenic VOM (n = 175), with 78.2% of CGP, 10.3% of GNR, 7.4% polymicrobial and 4% of anaerobic VOM). This distribution was similar to other cohorts [2, 4, 8, 9, 10, 11].

An association between genus and reference techniques was confirmed, BCs and VBs being complementary for the documentation of all different pathogens. As expected, most pyogenic bacteria (72.7%) were identified by BCs, and the majority of mycobacterial (84.2%) by VBs [4, 19, 20]. But, we also noticed that one third of pyogenic bacteria (31.4%) was diagnosed by VB. GNR infections occurred almost equally with (55.6%, including 71.4% of Enterobacteria) or without positive BCs (44.4%, including all fastidious and non-fermentative GNR), whereas GPC appeared less likely if BCs are negative (19.7% of all GPC cases, including 35.3% of CNS, 21.6% of Streptococci, and 16.5% of SA cases). The VBs were important in documenting anaerobic (100%) and polymicrobial VOM (76.9%).

Regarding the diagnosis of mycobacteria, particular features were noted, in accordance with current recommendations [17, 30, 38].

A notable contribution of VB was found, by CtB (59.9% vs 25.1% in pyogenic VOM) and by SuB (26.3% vs 6.9%). Wider use of SuB was not related here to higher share of decompressive surgery than in other VOM (20.6% vs 21.1%). SuB has been previously described as particularly beneficial for rare pathogens like tuberculosis and non-tuberculosis mycobacteria [1 ,7,30, 38], brucellosis [17] or even fungi [27, 30]. In our work, SuB also allowed the identification of SA (31.3%), and non-fermentative GNR (12.5%).

Second, extra-vertebral samples (e.g. lymph nodes, lungs, urines) proved to be a good alternative to VB, contributing to the identification of 7 out of 10 mycobacteria (15.8% as single positive sample and 52.6% with VB). In contrast, their proportions for confirmation of pyogenic documentation were low (2.3%; including, 2 CSF, 1 psoas abscess and 1 other joint punctures). Subsequently, other extra-vertebral samples were associated with hematogenous dissemination confirmed by BCs, in one-quarter of the pyogenic VOM (including, one third of positive cytobacteriological examinations of urine).

Thirdly, molecular biology on VB frequently enabled mycobacterial diagnosis (31.3% of positive *M. tuberculosis*-specific PCR), while it rarely contributed to pyogenic documentation (1.2% of positive 16S PCR). Specific PCR allows a more rapid confirmation of the diagnosis of mycobacteria, whereas 16S-PCR is a second-line technic, useful if standard cultures are negative [3, 21, 30, 33]. In addition, molecular biology was not available for a large part of the study (e.g. before 2012).

Based on those aforementioned results, we advocate that BCs should be systematic as would confirm the diagnosis in 6 out of 10 suspicions of VOM. If negative, VBs should be performed as they are efficient (positivity rate of 71.7%,vs 59.3%). Ct-guided procedure will be considered first, as it is the least invasive VB technique, and with a 68.8% positivity rate. But, if this procedure is in turn negative, a surgical biopsy may be considered given their highest yield (positivity rate of 81.0%), although most authors proposed a second CtB [9, 24, 29, 30, 32, 36, 39] with few exceptions [1–3, 11]. Following this rationale, microbiological samples should be systematic in all spinal surgery for other primary purpose (e.g. decompression).

Extra-vertebral samplings should be recommended in disseminated infections such as tuberculosis.

However and in comparison with previous studies, our positivity rates were similar or higher for SuB (81% in our study vs 77.9% for Mc Henry et al., 64.4% for Aagaard et al., and 76.0% for McNamara et al.) and for CtB (68.8% vs 69.4% for Mc Henry et al., 74.4% for Perronne et al., 46.2% for Aagaard et al. and 48% in McNamara et al.) [2, 16, 35, 39]. But, it was similar or lower for BCs (59.3% vs 59.5% for Carragee et al., 61.2% for Mc Henry et al., 62.5% for Torda et al. and 69.8% for Aagaard et al. [2, 10, 35, 39]). Those differences could be explained by the inclusion of mycobacterial or post-operative VOM depending on the cohorts.

Additional data were provided by our timing analysis. Concerning the diagnostic timeline, most documentations (53.4%) occurred after the radiological diagnosis of VOM, either simultaneously by positive BCs (after 1 day in median; 33.1% of BCs) or later by positive VBs (after 5 days in median; all VBs). Or, VOM were confirmed by radiology after most bacteraemia (66.9%; after 7 days in median).

This reminds that the challenging diagnosis of VOM is multifactorial and based on clinical, radiological and microbiological findings. For this reason, it is crucial to largely suspect the infection since the symptoms are often neither sensitive nor specific and to proceed in careful spinal examination in all bacteraemia. VOM should be confirmed by accurate radiological explorations and prompt microbial documentation should be obtained by immediate BCs at any stage of diagnosis (e.g. clinical suspicion or radiological confirmation), then VB (after radiological confirmation).

We also observed heterogeneity in VOM course, with a duration between first clinical symptoms and conclusive microbial samples being about 1 month and half in infections diagnosed by VB (51 days in median) while it lasted for 2 weeks when positive BCs (median of 14 days in median). It was also verified when considering pyogenic infections only (e.g. without tuberculosis cases; 47 days in median). This delay could be explained by the invasive procedure of VB itself (e.g. invasive technique, limiting feasibility), causing retardation in its implementation. But, it was also surely influenced by the virulence of the involved pathogens, resulting in variations in clinical symptomatology and consequently, variation in latencies prior to diagnosis sampling (from 11 days in median for SA to 104 days for anaerobes).

In our work, two profiles of infections may be indeed highlighted depending on the techniques.

- Bacteraemia were associated with symptomatic and acute infection, including fever and high inflammatory parameters. VOM occurred as a secondary localization in systemic dissemination. Those infections were more frequent in older patients with more comorbidities or in health-related context. Bacteraemia were here significantly associated with GPC (89.7% of bacteraemia and 82.7% of GPC), SA (51.7% and 75.9%) and *Streptococci* (25% and 67.6%), CNS accounting for 9.5% and *Enterococci* for 3.4%.
- VOM diagnosed by VB were associated with focal picture with back pain, in younger patients. They were often associated with neurological complications as compressions. The course of infection was subacute to chronic (36 days by SuB, 52 days by CtB). Those more torpid

pictures were significantly associated with mycobacteria (27.1% of focal VOM and 84.2% of TB), but also anaerobes (10.2% and 85.7%) and polybacterial (10.2% and 69.2%) documentations.

At last, antibiotic treatment was mostly initiated after samplings in our documented cases (88.0%). Treatment should be based on microbiological results, and empirical therapy reserved for most severe infections (sepsis, neutropenia, compression) [30, 39]. Noteworthy, physician should not refrain to perform VB if needed in patients who already received antibiotics, as still positive after the first days [30, 34].

Our study suffers from several imitations due to its observational, retrospective and monocentric design, which accounts for potential selection bias.

The moderate size of our cohort may be explained by the fact that VOM is a rare and probably under-diagnosed disease. As in clinical practice, Our selection of cases was confirmed by a body of criteria following medical reasoning (anamnestic, clinical, biological, radiological, microbiological and/or pathological findings). We thus considered all bacterial diagnosis defined by cultures, including tuberculosis and "non-hematogenous." This point may differ from other cohorts but may be one of the strengths of our work

Due to the retrospective data collection, some VOM may also have been missed (e.g. misclassification in severe sepsis) and some inaccuracies may have been possible (e.g. dates of onset of symptoms). Retrospective inclusion of clinical symptoms may also be mildly imprecise, especially since spinal pain is a very common symptom in medicine.

Due to monocentric feature, the generalization of our results may remain limited (e.g. the use of CtB or SuB may be more common in our centre). In addition, regional aspects (i.e., social, economic or related to access to the health system) may complicate the translation of our results in other area of the country (e.g. poorer medical environs with less medical support services). Nor our results are reproducible on a global scale; relative education, wealth and universal healthcare availability in France may have an impact on all outcomes.

In conclusion, our work confirms that BCs and VBs are both essential, efficient and complementary techniques for microbial documentation of all potentially involved pathogens, in focal and systemic VOM.

Microbial documentation must be obtained as accurately and quickly as possible. BCs must be systematically sampled in all cases. If negative, VBs should be performed, with Ct-guided biopsy in first intention, then SuB.Although an invasive technique, VBs are important for VOM documentation, being particularly contributory for the diagnosis of least virulent pathogens (e.g. M. tuberculosis or anaerobes) and focal VOM. In tuberculosis suspicion, extra-vertebral sites should also be sampled.

Delayed and missed diagnosis may be partly related to the insufficient and inaccurate use of the different diagnosis techniques and especially VBs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Ethical approval from an ethics committee was not needed according to the French legislation.

Informed consent Formal consents were not required for this retrospective study.

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