ORIGINAL ARTICLE INFECTIOUS DISEASES

Which tissues are best for microbiological diagnosis in patients with pyogenic vertebral osteomyelitis undergoing needle biopsy?

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Abstract

Identification of the causative microorganism is important in the management of pyogenic vertebral osteomyelitis (PVO). The aim of this study was to investigate whether culture positive rates differ between needle biopsy sites in patients with PVO, and which tissues are best for microbiological diagnosis. Between January 2005 and December 2013, we conducted a retrospective cohort study of PVO patients who had soft-tissue abscesses (paraspinal or psoas abscesses) and who received needle biopsy for microbiological diagnosis. Needle biopsy sites were classified into two anatomical categories: vertebral bodies, or soft tissues (intervertebral discs, paraspinal abscesses, or psoas abscesses). A generalized estimating equation model was developed to identify factors associated with tissue-culture positivity. During the study period a total of 136 tissues were obtained by needle biopsy from 128 PVO patients with soft-tissue abscesses. The culture positive rates of vertebral bodies and soft tissues were 39.7% (29/73), and 63.5% (40/63), respectively ($p < 0.05$). In a multivariate analysis, male gender (adjusted odds ratio (aOR) 2.24, 95% CI 1.00–5.02), higher C-reactive protein (aOR 1.07, 95% CI 1.01–1.15), positive blood culture (aOR 2.57, 95% CI 1.01–6.59), and soft tissues as biopsy site compared with vertebral bodies (aOR 2.28, 95% CI 1.08–4.78) were independent factors associated with tissue culture positivity. Soft tissues were the best sites for microbiological diagnosis in PVO patients undergoing needle biopsy.

Keywords: Biopsy, culture, psoas abscess, spondylitis, tissue
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Introduction

Pyogenic vertebral osteomyelitis (PVO) is an infectious disease of the vertebrae or paravertebral structures caused by a variety of bacteria. Because long-term antibiotic treatment is essential, and antibiotic choice depends on susceptibility tests, it is important to identify the causative microorganism. Blood cultures and/or tissue cultures are recommended for this purpose. Several factors influence culture positivity rates [1–5]. Paravertebral abscesses, elevated C-reactive protein (CRP), and elevated erythrocyte sedimentation rate were associated with higher culture positivity, whereas previous antibiotic exposure was associated with negative results of culture in some studies [2,4–8].

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Tissue culture specimens can be obtained by needle or surgical biopsy. The microbiological yield of surgical biopsy is higher than that of needle biopsy [3,9,10]. However surgical biopsy requires anaesthesia and incision of the skin, and percutaneous needle biopsy is known to be safe [11–13]. For these reasons, needle biopsy is generally preferred to surgical biopsy as the initial diagnostic approach.

Tissues for microbiological diagnosis can be obtained by needle biopsy from vertebral bodies, intervertebral discs and soft-tissue abscesses (paraspinal abscesses or psoas abscesses). As tissue-culture positivity rates could differ between biopsy sites it would be better to choose biopsy sites which have higher culture positivity rates when performing needle biopsies in patients with PVO. Little is known about which tissues are the best biopsy sites for microbiological diagnosis in PVO [4,14–16].

The primary objective of this study was to investigate factors associated with culture positivity of tissues obtained by needle biopsy in patients with PVO. We also compared the rates of culture positivity of tissue specimens obtained from vertebral bodies and soft tissues, as a secondary objective, to determine which tissues are most suitable for microbiological diagnosis.

Methods

We conducted a retrospective cohort study at three university-affiliated teaching hospitals from January 2005 through December 2013. All of the study hospitals were tertiary referral centres and had 900–1700 beds.

Patients with PVO who had soft-tissue abscesses (paraspinal abscesses and/or psoas abscesses) and had undergone needle biopsy for tissue culture were investigated. PVO was diagnosed when the causative microorganism was isolated from spinal or paraspinal tissues, or if there were compatible clinical signs or symptoms and radiological evidence of vertebral infection. Compatible signs or symptoms were defined as pain, fever, or neurological manifestations. Characteristic radiographic changes included decreased signal intensity in the vertebral body and disc and loss of end plate on T1-weighted images, and increased signal intensity of the disc and vertebral body on T2-weighted images on magnetic resonance imaging.

Needle biopsy sites were classified into two anatomical categories: vertebral bodies, or soft tissues (intervertebral discs, paraspinal abscesses or psoas abscesses). Patients <18 years, or who had undergone surgical biopsy for tissue culture were excluded, as were patients with vertebral osteomyelitis caused by *Mycobacterium tuberculosis* or fungi. Patients who had been exposed to antibiotics in the 2 weeks before obtaining tissue by needle biopsy were also excluded. When coagulase-negative staphylococci grew in tissue culture they were considered the true pathogen if one or both of the following criteria was met: the same organism grew in blood culture, or the same organism grew in two or more spinal tissue specimens.

We collected data about baseline characteristics (age, gender), underlying diseases, spinal surgery and procedures within the year before diagnosis of pyogenic spondylitis, clinical characteristics (pain, fever, neurological deficit), laboratory data (white blood cell counts, CRP levels) and radiographic data (vertebral region involved, presence and location of abscess).

Statistical analysis

The Mann–Whitney *U* test was used to compare continuous variables, and the chi-squared test was used to compare categorical variables. If the expected number of instances of a given outcome was <5, Fisher's exact test was used. Multivariate analysis was performed to investigate the factors influencing tissue culture positivity in patients with PVO. Because needle biopsies were performed at two anatomical sites in a proportion of patients, a generalized estimating equation model was used to take account of a possible clustering effect of multiple specimens from the same patient. We entered all variables that were statistically significant in univariate analysis into the generalized estimating equation model. All *P* values were two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS program (version 20.0, SPSS Inc., Chicago, IL, USA).

Results

During the study period 567 patients >18 years were diagnosed as having pyogenic vertebral osteomyelitis. Of these, 439 were excluded for the following reasons: antibiotic exposure before obtaining a tissue specimen (n = 201), percutaneous needle biopsy not performed (n = 179) and absence of abscess (n = 59). Ultimately a total of 136 tissues from 128 PVO patients with soft-tissue abscesses were included in the analysis. In 120 of the patients, tissues were obtained from one anatomical category, and in eight patients they were obtained from two anatomical categories. Of the tissues used for culture, 53.7% (73/136) and 46.3% (63/136) were obtained from vertebral bodies and from soft tissues, respectively. Intervertebral discs, paraspinal abscess and psoas abscess comprised 27.0%, 54.0% and 19.0% of the 63 soft tissues, respectively.

The culture positive rates of vertebral bodies and soft tissues were 39.7% (29/73) and 63.5% (40/63), respectively (p 0.006). Among soft tissues, the culture positive rates of intervertebral discs, paraspinal abscesses and psoas abscesses were 52.9% (9/17), 70.6% (24/34) and 58.3% (7/12), respectively (for disc versus abscess p 0.290). Of the tissues obtained from two

anatomical categories in the eight patients, concordant results were observed in seven patients and discordant ones in one. In the patient whose culture results were discordant, a negative result was obtained from a vertebral body and a positive result was obtained from soft tissue. The most common causative organism was *Staphylococcus aureus* (*n* = 34), of which 22 were methicillin susceptible, followed by coagulase-negative staphylococci (*n* = 12), streptococci (*n* = 8), *Escherichia coli* (*n* = 11) and *Klebsiella pneumoniae* (*n* = 3), and some of the above were cultured only from blood.

The demographic, clinical, laboratory and radiographic characteristics of patients according to the sites of the tissues obtained are summarized in Table 1. Diabetes mellitus and chronic kidney disease were more common and CRP levels were significantly higher in those patients whose biopsies were obtained from soft tissues. In a multivariate logistic regression analysis to identify factors associated with tissue culture positivity, male gender (adjusted odds ratio (aOR) 2.24, 95% CI 1.00–5.02), higher CRP (aOR 1.07, 95% CI 1.01–1.15), positive blood culture (aOR 2.57, 95% CI 1.01–6.59) and soft tissues as biopsy site compared with vertebral body (aOR 2.28, 95% CI 1.08–4.78) were independent factors identified (Table 2).

TABLE 1. Demographic, clinical, laboratory and radiographic characteristics of patients with pyogenic vertebral osteomyelitis according to biopsy site

Variable	Biopsy site: Vertebral body (*n* = 73)	Biopsy site: Soft tissue (*n* = 63)	p
Age (years)[a]	68 (56–76)	65 (53–73)	0.195
Sex (male)	44 (60.3%)	41 (65.1%)	0.564
Underlying Disease			
Diabetes mellitus	11 (15.1%)	20 (31.7%)	0.021
Liver cirrhosis	7 (9.6%)	4 (6.3%)	0.490
Chronic kidney disease	2 (2.7%)	8 (12.7%)	0.044[b]
Solid tumour	5 (6.8%)	6 (9.5%)	0.568
Haematological disease	2 (2.7%)	1 (1.6%)	1.000[b]
Predisposing condition			
Epidural block	15 (20.5%)	10 (15.9%)	0.483
Spine surgery or vertebroplasty	14 (19.2%)	15 (23.8%)	0.511
Presentation			
Fever	22 (30.1%)	28 (44.4%)	0.084
Duration of spine pain (days)[a]	23 (7-56)	15 (5-30)	0.156
Spine area pain	72 (98.6%)	62 (98.4%)	1.000[b]
Radiating pain	41 (56.2%)	35 (55.6%)	0.943
Tingling sensation	8 (11.0%)	8 (12.7%)	0.754
Motor dysfunction, sensory deficit, paralysis	7 (9.6%)	6 (9.5%)	0.990
Sepsis	18 (24.7%)	20 (31.7%)	0.358
White blood cells count ($\times 10^3/\mu L$)	9,000 (7,050–12,950)	10,300 (7,800–13,600)	0.235
C-reactive protein (mg/dL)	5.3 (2.3-10.9)	7.1 (3.7-17.5)	0.032
Involved spine			
Cervical spine	1 (1.4%)	5 (7.9%)	
Cervicothoracic spine	1 (1.4%)	0	
Thoracic spine	12 (16.4%)	9 (14.3%)	
Thoracolumbar spine	6 (8.2%)	3 (4.8%)	
Lumbar spine	48 (65.8%)	42 (66.7%)	
Lumbosacral spine	5 (6.8%)	4 (6.3%)	
No. of vertebrae involved			
≤2	61 (83.6%)	45 (71.4%)	0.089
≥3	12 (16.4%)	18 (28.6%)	
Presence of abscess			
Epidural abscess	46 (63.0%)	47 (74.6%)	0.147
Paravertebral abscess	64 (87.7%)	51 (81.0%)	0.280
Psoas abscess	21 (28.8%)	23 (36.5%)	0.336
Blood culture positivity[c]	24 (42.9%)	28 (52.8%)	0.323
Tissue culture positive rate	29 (39.7%)	40 (63.5%)	0.006
Microorganism obtained by tissue culture			
Methicillin susceptible *Staphylococcus aureus*	9 (31.0%)	13 (32.5%)	
Methicillin-resistant *Staphylococcus aureus*	4 (13.8%)	4 (10.0%)	
Coagulase-negative *Staphylococcus*	2 (6.9%)	7 (17.5%)	
Streptococcus	3 (10.3%)	3 (7.5%)	
Escherichia coli	4 (13.8%)	4 (10.0%)	
Others	7 (24.1%)	9 (22.5%)	

[a]Median (interquartile range).
[b]Fischer's exact test.
[c]Blood culture was performed in 56 patients in the vertebral body group, and 53 patients in the soft tissue group.

Discussion

The independent factors that we have found by multivariate analysis to be associated with culture positivity of tissues obtained by needle biopsy in patients with PVO were male gender, higher CRP, positive blood culture and soft tissues as biopsy site. Culture positive rates were significantly higher when specimens were obtained from soft tissues (intervertebral discs, paravertebral abscesses or psoas abscesses) than when they were obtained from vertebral bodies.

Several factors have been reported to influence culture positivity of percutaneous needle biopsies in patients with PVO. Culture positive rates were higher with surgical sampling, even when minimally invasive techniques were used [17]. They were also higher in patients with paravertebral abscesses, or higher levels of inflammatory markers such as CRP and erythrocyte sedimentation rate [2]. In contrast, biopsy yields were found to be reduced by previous antibiotic exposure [2,4,5]. Three reports investigating the culture positivity of biopsies at different tissue sites found that rates were highest for paravertebral abscesses. In one study, 29 tissue cultures were evaluated and the culture positive rate from paravertebral abscesses was 43%; this was higher than the 10% rate from other tissues, but the difference was not statistically significant [14]. In the second study, the culture positive rate was 80% (8/10) in abscesses and 26.7% (8/30) in lumbar tissues [15]. In the third study, the rate was 61% (8/13) in spinal/epidural abscesses and 44% (12/27) in vertebral bodies [16]. All three studies had the limitations that less than 50 tissue cultures were investigated, that tissues were obtained either by needle or surgical biopsy, and that no statistical analysis was performed to control for confounding variables.

In the present study white blood cell counts and CRP levels were significantly higher in patients whose culture specimens were obtained from soft tissues than in those from vertebral bodies. We used multivariate analysis to control for other factors influencing tissue culture positivity, and the multivariate

TABLE 2. Multivariate logistic regression analysis of factors associated with tissue culture positivity in patients with pyogenic vertebral osteomyelitis

	Culture negative	Culture positive	Odds ratio	p	Adjusted odds ratio	p
Age (years), median (interquartile range)	67 (55–76)	65 (55–73)	0.99 (0.96–1.01)	0.314		
Sex						
Female	29 (47.5%)	19 (28.4%)	1.00	0.026	1.00	0.049
Male	32 (52.5%)	48 (71.6%)	2.29 (1.10–4.76)		2.24 (1.00–5.02)	
Diabetes mellitus	11 (18.0%)	20 (29.9%)	1.93 (0.84–4.47)	0.119		
Liver cirrhosis	6 (9.8%)	5 (7.5%)	0.74 (0.21–2.56)	0.632		
Chronic kidney disease	6 (9.8%)	4 (6.0%)	0.58 (0.16–2.17)	0.517		
Solid tumour	3 (4.9%)	8 (11.9%)	2.62 (0.66–10.37)	0.157		
Haematological disease	1 (1.6%)	2 (3.0%)	1.85 (0.16–20.89)	1.000		
Epidural block	15 (24.6%)	9 (13.4%)	0.48 (0.19–1.19)	0.106		
Spinal surgery	15 (24.6%)	12 (17.9%)	0.67 (0.29–1.57)	0.355		
Fever	19 (31.1%)	28 (41.8%)	1.59 (0.77–3.29)	0.212		
Spine pain	61 (100%)	65 (97.0%)	—	0.497		
Motor dysfunction	8 (13.1%)	5 (7.5%)	0.53 (0.17–1.73)	0.290		
Sepsis	14 (23.0%)	23 (34.3%)	1.76 (0.80–3.83)	0.156		
White blood cell count (× 10^6/μL)	9.10 (6.40–12.75)	10.30 (7.30–14.55)	1.06 (0.99–1.14)	0.082	0.99 (0.92–1.07)	0.771
C-reactive protein (mg/dL)	4.7 (2.0–10.4)	9.5 (4.5–18.5)	1.08 (1.03–1.14)	0.002	1.07 (1.01–1.15)	0.032
Presence of abscesses						
Epidural	45 (73.8%)	42 (62.7%)	0.60 (0.28–1.27)	0.179		
Paravertebral	55 (90.2%)	53 (79.1%)	0.41 (0.14–1.16)	0.085	0.95 (0.32–2.78)	0.921
Psoas	17 (27.8%)	24 (35.8%)	1.45 (0.68–3.06)	0.336		
Blood culture positivity	13 (27.1%)	35 (63.6%)	4.71 (2.03–10.9)	<0.001	2.57 (1.01–6.59)	0.049
Biopsy site						
Vertebral body	44 (65.7%)	29 (42.0%)	1.00		1.00	
Soft tissue	23 (34.3%)	40 (58.0%)	2.64 (1.33–5.24)	0.006	2.28 (1.08–4.78)	0.030

analysis revealed that obtaining specimens from soft tissues was an independent factor associated with culture positivity. We suggest that this reflects the higher burden of bacteria in abscesses, the burden of bacteria in vertebral bodies being considerably lower. We conclude that soft tissues are the best sites for microbiological diagnosis in patients with PVO, and recommend using these sites when needle biopsy is performed.

It is well known that the level of CRP is higher in patients with culture positive PVO [1,2,14]. In contrast male gender has not previously been reported to be an independent factor associated with tissue-culture positivity in patients with PVO. The culture positive rates for males and females were 60.0% (48/80) and 39.6% (19/48), respectively. We are unable to explain why male gender is an independent factor associated with tissue-culture positivity.

Histological studies of specimens can be helpful in diagnosing vertebral osteomyelitis, especially when it is caused by *M. tuberculosis*, or *Brucella*, because the presence of granulomas points to the diagnosis [11,18,19]. Pathological findings of bone biopsy specimens are also important in differentiating metastatic cancer [20]. Specimens for histology can be obtained from vertebral bodies but are not easily obtained from soft tissues. This point should be considered when choosing a biopsy site, especially when there is suspicion of spondylitis caused by *M. tuberculosis* or *Brucella*.

Our study had several limitations. Although it involved the largest number of tissues of different anatomical categories from patients with PVO so far, the number of cases in which tissues were obtained from two anatomical categories was relatively small. Obtaining tissues from different anatomical categories in the same patients would make it easier to determine which sites are best for microbiological diagnosis. Second, it is a retrospective study and there may be unrecognized confounding factors associated with tissue-culture positivity.

In conclusion, male gender, higher CRP, positive blood culture and soft tissues as biopsy sites are independent factors associated with culture positivity of tissues obtained by needle biopsy in patients with PVO. Soft tissues appear to be the best sites for obtaining tissue for microbiological diagnosis by needle biopsy in these patients.

Transparency Declaration

The authors have no conflicts of interest to report.

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