Meningitis caused by \textit{Porphyromonas endodontalis} detected by PCR amplification and sequencing of 16S rRNA genes direct from cerebrospinal fluid and cerebral tissue

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Introduction: \textit{Porphyromonas endodontalis} (formerly \textit{Bacteroides endontalis}) is a black-pigmented, non-motile, obligate anaerobe. It has an established role in endodontal infections and has rarely been isolated from other sites. To the best of our knowledge, this is the first time that this organism has been detected as the sole cause of meningitis. This was made possible by the use of direct-on-sample 16S rRNA gene amplification and sequencing.

Case presentation: We present a case of meningitis that progressed to a cerebral abscess in an elderly but otherwise immunocompetent male. Despite broad-spectrum antibiotic therapy, the patient died.

Conclusion: Although anaerobic meningitis is rarely described, it should be considered if there are clinical and biochemical features of meningitis but no organism isolated on standard aerobic culture.

Keywords: Anaerobe; anaerobic meningitis; central nervous system (CNS) abscess; meningitis; \textit{Porphyromonas endodontalis}.

Introduction

Meningitis is a common and serious presenting illness to medical services worldwide (Brouwer \textit{et al.}, 2010). In adults, the most common causes of community acquired meningitis are \textit{Streptococcus pneumoniae}, \textit{Neisseria meningitidis} and \textit{Listeria monocytogenes}, the latter of which is particularly prevalent in the elderly (Brouwer \textit{et al.}, 2010). However, since the advent of vaccines targeting \textit{S. pneumoniae}, \textit{N. meningitidis} and \textit{Haemophilus influenzae}, together with the increasing life expectancy of patients with chronic diseases and the number of people with chronic immunosuppression, the proportion of infections due to atypical pathogens is increasing (Pittman \textit{et al.}, 2014). One such example is \textit{Streptococcus suis}, a zoonotic infection associated with pigs, that has caused two large outbreaks in China (Gottschalk \textit{et al.}, 2010; Goyette-Desjardins \textit{et al.}, 2014).

Anaerobic meningitis is a rare but recognized disease entity, reported in children (Law & Aronoff, 1992) and the elderly (Hagiya & Otsuka, 2014). Anaerobic culture is not performed routinely (Baron \textit{et al.}, 2013), and as such the incidence of anaerobic meningitis may be underreported. Thus, the use of novel techniques, such as direct-on-sample 16S rRNA gene analysis, may become increasingly valuable. To the best of our knowledge, this is the first case of meningitis caused by \textit{Porphyromonas endodontalis} described in the literature.

Case report

A 78-year-old Asian male presented with a 3-day history of increasing drowsiness, confusion and fevers. His past medical history included hypothyroidism (treated with 100 mg thyroxine daily) and daily aspirin (100 mg) for primary ischaemic heart disease prophylaxis. The patient had poor dentition and approximately 1 month prior to admission underwent a tooth extraction. Prior to his illness, he was otherwise cognitively intact and lived independently at home with his wife.

On examination, he was febrile (39.9 °C), tachycardic (110 beats min\textsuperscript{-1}) and had a Glasgow Coma Scale score of 9.
He was agitated but was able to follow basic commands. The rest of his physical examination was unremarkable. A diagnosis of sepsis of unknown source was made. Blood cultures were drawn and empirical antibiotics (piperacillin-tazobactam, 4/0.5 g three times daily) were commenced in the emergency department. His initial blood test results are shown in Table 1.

Given his neurological findings, a contrast-enhanced computed tomography (CT) scan was performed on the following day (day 2). No collections or meningeal enhancement were seen. A lumbar puncture was then performed, and the cerebrospinal fluid (CSF) analysis was consistent with bacterial infection: the glucose was 0.6 mmol l$^{-1}$ (2.7–4.4 mmol l$^{-1}$) and the leukocyte count was 1299 $\times$ 10$^6$ l$^{-1}$ with a 95% neutrophil predominance. A Gram stain showed occasional polymorphonuclear cells containing small, Gram-negative organisms (Fig. 1). An Indian ink stain was negative and no other methods of staining were performed. The CSF was incubated on horse blood agar and chocolate agar in 5% CO$_2$. There was no anaerobic culture undertaken, as this is not routinely performed for CSF specimens at our centre. The CSF was also inoculated into an aerobic blood culture bottle (BacTec FX; BD) for enrichment culture but not into an anaerobic bottle.

The antibiotic regimen was changed to ceftriaxone 2 g twice daily and vancomycin (1.5 g loading dose, followed by a 1 g twice daily maintenance dose) on day 2 after review by the Infectious Diseases Department. Two doses of aciclovir (10 mg kg$^{-1}$) were given before the results of human simplex virus DNA PCR were returned as not detected. Other PCRs for enterovirus, varicella-zoster virus, N. meningitis, S. pneumoniae and Mycobacterium tuberculosis complex were all negative. Cryptococcal antigen testing (Cryptococcal Antigen Lateral Flow Assay; IMMY) was not detected.

Following 6 days of no growth on culture, on day 8 of the admission, a clinical decision was made to test the CSF for the presence of bacteria using a universal 16S rRNA gene PCR approach (Weisburg et al., 1991). This method uses universal primers fD1 and rP2 to amplify the entire 16S rRNA gene. The results were not available for a number of days.

Vancomycin was ceased 8 days into the admission and benzylpenicillin (2.4 g every 4 h) was added. The patient was transferred to the intensive care unit on day 12 of admission due to seizure activity. On day 13 of his admission, the presence of an approximately 1500 bp product was confirmed by gel electrophoresis and was sent for sequencing. A contrast-enhanced CT scan was repeated on that same day, which showed changes consistent with abscess formation in the left frontal lobe and leptomeningeal enhancement of the left cerebral hemisphere (Fig. 2). A repeat lumbar puncture and CSF analysis revealed an ongoing neutrophil pleocytosis with a total count of 570 $\times$ 10$^6$ l$^{-1}$ (77% neutrophils), glucose of 1.0 mmol l$^{-1}$ and protein of 1.38 g l$^{-1}$. No organisms were seen on Gram staining.

On day 16 of admission, antibiotics were changed to intravenous metronidazole (500 mg twice daily and

### Table 1. Blood test results

<table>
<thead>
<tr>
<th>Test parameter (normal range)</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (135–180 g l$^{-1}$)</td>
<td>137</td>
<td>121</td>
<td>125</td>
</tr>
<tr>
<td>Platelets (150 $\times$ 10$^9$–400 $\times$ 10$^9$ l$^{-1}$)</td>
<td>326</td>
<td>351</td>
<td>492</td>
</tr>
<tr>
<td>Total white cell count (4.00 $\times$ 10$^9$–11.0 $\times$ 10$^9$ l$^{-1}$)</td>
<td>22.60</td>
<td>21.20</td>
<td>12.8</td>
</tr>
<tr>
<td>Neutrophils absolute (2.00 $\times$ 10$^9$–7.50 $\times$ 10$^9$ l$^{-1}$)</td>
<td>20.79</td>
<td>18.87</td>
<td>10.74</td>
</tr>
<tr>
<td>C-reactive protein ($\leq$5.0 mg l$^{-1}$)</td>
<td>210</td>
<td>210</td>
<td>58</td>
</tr>
<tr>
<td>Creatinine (60–100 $\mu$mol l$^{-1}$)</td>
<td>66</td>
<td>57</td>
<td>49</td>
</tr>
<tr>
<td>Bilirubin ($\leq$20 $\mu$mol l$^{-1}$)</td>
<td>20</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Albumin (35–50 g l$^{-1}$)</td>
<td>36</td>
<td>27</td>
<td>25</td>
</tr>
</tbody>
</table>
meropenem 1 g three times daily). There was minimal clinical change. On day 22, the patient was transferred to the state neurosurgical centre for cerebral biopsy. Tissue samples were sent for bacterial culture, mycobacterial culture, histopathology and 16S rRNA gene PCR and sequencing. No organisms were seen on Gram staining. After 3 months, no organisms (including *Mycobacteria* spp.) were cultured. The lack of growth from the biopsy samples is most likely explained by the prolonged (22 days) prior antibiotic usage.

On day 24, the patient was transferred back to our institution. On the same day, the results of the 16S rRNA gene sequencing were available on the initial CSF. The delay in the result was due to excessive signal strengths in an initial sequencing result, which required repeat sequencing to resolve.

The repeat sequence was edited using SeqScape (Applied Biosystems) and compared with the NCBI RefSeq nucleotide database using nucleotide–nucleotide BLAST. The sequence was also submitted to the RDP Classifier and Seqmatch (RDP, Centre for Microbial Ecology, Michigan State University, USA) for analysis. The 16S rRNA gene sequence generated shared 99.6 % sequence similarity (1416/1422 bp, 0/1422 gaps) with *P. endodontalis* JCM 8526 (GenBank accession no. NR_113085.1) and 99.6 % sequence similarity (1414/1420 bp, 0/1422 gaps) with *P. endodontalis* ATCC 35406 (GenBank accession no. NR_113087.1). There were no intervening sequences between these records indicating any other *Porphyromonas* sp. Analysis of the RDP Seqmatch reported an S_ab score of 0.971 using 1377 nt with *P. endodontalis* ATCC 35406. *P. endodontalis* ATCC 35406 is the type strain of this species. The 16S rRNA gene sequencing results for the cerebral biopsy followed a few days later and generated identical results. Following the sequencing results and a review of the phylogeny of *Porphyromonas* spp. (Finegold et al., 2004; Paster et al., 1994), we concluded that the 16S rRNA gene sequence identified could be ascribed to *P. endodontalis* based on less than 0.5 % sequence difference from *P. endodontalis* ATCC 35406.

Although the causative organism has been discovered, the patient had not made any clinical improvement, despite broad-spectrum antibiotics that included anaerobic cover. A review by the maxilla-facial surgeons did not find any surgically amendable source, but it was noted that the patient had poor dentition. On day 27, the patient was palliated and died on day 44 after admission.

**Conclusions**

*P. endodontalis* (formerly *Bacteroides endodontalis*) is a black-pigmented, non-motile, obligate anaerobe (Shah & Collins, 1988). It has an established role in endodontal infections (Gomes et al., 2005; van Winkelhoff et al., 1992).
Anaerobic infections of the central nervous system (CNS) are rare but are typically associated with intraparenchymal abscess formation (Brouwer et al., 2014). Anaerobic meningitis has been described in the literature (Korman et al., 1997; Law & Aronoff, 1992; Wexler, 2007); however, most cases are associated with CNS devices, head and neck infections (e.g. otitis media), trauma or recent surgery (Pittman et al., 2014).

This case is significant as it appears to have occurred without the presence of known classical risk factors and, presumably due to a lack of initial anaerobic antibiotic coverage, progressed to the formation of a brain abscess. His poor dentition and recent tooth extraction were probably contributing factors.

Australia’s current recommended empirical regimen for meningitis is intravenous ceftriaxone and vancomycin, which have little or no anaerobic cover (Antibiotic Expert Group, 2014). Furthermore, to the best of our knowledge, this is the only case report in the literature of P. endodontalis causing meningitis. A case report of a mixed infection with P. endodontalis in a brain abscess has been described (Keller et al., 2010).

Our centre does not currently routinely process CSF specimens anaerobically, unless there is a documentation of CNS prosthetic material, brain abscess or recent CNS surgery. As demonstrated in the study by Pittman et al. (2014), there may be a case for initiating the practice routinely.

The use of 16S rRNA gene PCR and sequencing to identify bacteria directly from clinical samples and from cultured bacteria is firmly established in the literature (Rampini et al., 2011). In the absence of successful culture, 16S rRNA gene PCR and sequencing is a useful diagnostic approach.

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**References**


