Infections by \textit{Nocardia} spp. are generally regarded as opportunistic diseases in immunocompromised patients, but can also affect immunocompetent subjects. Such infections represent an important diagnostic challenge for clinicians and microbiologists, and diagnosis is frequently delayed or even conducted post mortem. A 54 year-old man was admitted to our hospital because of ventriculitis and relapsing brain abscess. Five months prior, this patient had undergone external ventricular drain and surgery for a cerebellar abscess. Histopathology demonstrated pyogenic inflammatory reaction, microbiologic investigations proved negative and empiric antimicrobial therapy was administered for a total of eight weeks. Six weeks later, the patient developed relapsing neurologic manifestations. On reviewing the patient’s clinical history it emerged that the patient had suffered from pneumonia two months prior to neurosurgery, treated with amoxicillin/clavulanate 3g a day and levofloxacin 500 mg a day for three weeks. On the central nervous system (CNS) relapsing manifestations, nocardiosis was suspected and DNA sequencing from the formalin-fixed paraffin-embedded cerebellar tissue collected during neurosurgery allowed diagnosis of \textit{Nocardia paucivorans} infection. The patient received medical therapy for 11 months. At follow-up, eight months after treatment was discontinued, the patient was asymptomatic. \textit{Nocardia} spp. infections need to be suspected not only in immunocompromised, but also in immunocompetent patients. Proper samples need to be collected for proper microbiologic investigations. Paraffin-embedded tissue genomic sequencing can be a useful tool for diagnosis of nocardiosis.

\textbf{Keywords}: cerebral nocardiosis, DNA sequencing, paraffin embedding.

\section*{INTRODUCTION}

Infections by \textit{Nocardia} spp. are generally regarded as opportunistic diseases in immunocompromised patients. Nevertheless, these microorganisms can rarely cause serious disease also in immunocompetent subjects [1].

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Approximately 80 species of \textit{Nocardia} have been described, the most frequent responsible for human disease being \textit{Nocardia asteroides} group, \textit{Nocardia farcinica}, \textit{Nocardia brasiliensis}, and \textit{Nocardia otitidiscaviarum} [1-4]. \textit{Nocardia paucivorans}, previously included among the \textit{N. asteroides} group, was firstly identified in 2000 [5]. Subsequently, it has been associated with central nervous system (CNS) infections, disseminated diseases, and endocarditis, in both immunocompromised and immunocompetent subjects [6-9]. Diagnosis of nocardiosis may be missed by rou-
tine laboratory methods. Direct smears from clinical specimens show branching, filamentous Gram-positive bacteria [4, 10]. Most of Nocardia spp. are acid-fast, but this characteristic may vary with strain and culture media used [2, 10]. Growth of Nocardia spp. may require from 48h to several weeks [1, 5, 11]. Molecular methods have expanded the spectrum of pathogenic Nocardia spp. [3, 4, 11, 12].

The authors report on a case of disseminated nocardiosis diagnosed by DNA sequencing on paraffin-embedded cerebellar tissue.

**CASE REPORT**

A 54 year-old Caucasian man was admitted to our ward because of ventriculitis and relapsing brain abscess. Seven months before, the patient had been diagnosed a right pneumonia for which he had received oral amoxicillin/clavulanic acid 3g a day and levofloxacin 500 mg a day for a total of 3 weeks with slow resolution of his pneumonia. Two months later, the patient had been hospitalized because of a right cerebellar abscess (4.5×4 cm) plus hydrocephalus (Figure 1a). The abscess was drained and an external temporary ventricular drainage was positioned. The cerebrospinal fluid (CSF) biochemistry and cytology were normal. Microbiological investigations for bacteria and fungi were negative. Histological findings showed acute pyogenic inflammatory reaction, consistent with brain abscess. Ceftriaxone 2g twice a day and metronidazole 500 mg four times a day were administered intravenously (iv). At admission, the chest radiography and computerized tomography (CT) scan did not show lung infiltrates. Two weeks after surgery, ceftriaxone and metronidazole were replaced by meropenem 6g a day. The patient’s clinical course was complicated by pulmonary embolism and methicillin-resistant Staphylococcus aureus ventilator associated pneumonia. Linezolid 600 mg twice a day iv was added to meropenem. One week later, meropenem was replaced by imipenem 500 mg four times a day and linezolid was continued for a total of 11 days. Overall, antimicrobials were administered for eight weeks. When the treatment was discontinued white blood cell count was normal, C-reactive protein (C-RP) was 0.7
mg/dl (normal value ≤0.5 mg/dl) and cerebral magnetic resonance imaging (MRI) findings indicated post neurosurgical alterations (Figure 1b). Six weeks later, the patient developed headache, drowsiness, fever and was re-admitted. The patient was confused (Glasgow Coma Scale 15), leucocyte and neutrophil counts, C-RP, and erythrocyte sedimentation rate were normal. Brain CT did not evidence pathological findings, while MRI depicted a right lateral ventriculitis with a developing abscess of the septum pellucidum [Figure 1c]. CSF leucocytes were 144 mm$^3$, proteins 324 mg/dl, and CSF/blood glucose ratio 0.40. CSF cultures for bacteria and fungi were negative. The patient was administered ceftriaxone 2g twice a day, metronidazole 500 mg four times a day and meropenem 6g a day, but he remained febrile. A repeated CSF examination showed: leucocytes 96/mm$^3$, proteins 340 mg/dl, and CSF/blood glucose ratio 0.44. Culture for bacteria evidenced a few colonies of Staphylococcus capitis and Actinomyces odontolyticus, culture for fungi was negative. Real-time PCR (SeptiFast, Roche Diagnostics, Mannheim, Germany) detected coagulase negative staphylococci DNA, below the cut-off of 100 bacterial cells/mL, set for the diagnosis of sepsis [13]. Vancomycin 1g three times a day resulting in trough blood levels not greater than 10 mg/L and rifampin 600 mg a day were introduced, while ceftriaxone and metronidazole were discontinued. Sections of paraffin-embedded cerebellar abscess were deparaffinized by extraction with xylene and stained with Gram and modified Ziehl-Neelsen stains. Microscopic examination showed numerous Gram-positive, branching bacteria (Figure 2), non acid fast. After paraffin removal another slide was subjected to isolation of DNA according to the QIAamp DNA Mini Tissue Protocol (QIAGEN GmbH-Hilden, Germany) and Hsp65 gene amplification by PCR with primers TB11 (5'-ACCAACCGATGGTGTCATT-3') and TB12 (5'-CTTGTCGAACCGCATACCT-3') [11]. The 441 bp amplification product was purified and sequenced using ABI PRISM 3130 xl Genetic Analyzer (Applied Biosystems). The species N. paucivorans was identified by database searching with the BLAST sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/). Ceftriaxone 4 g a day and co-trimoxazole 240/1.200 mg four times a day iv were added to vancomycin and rifampin. After 12 days, fever subsided and clinical findings improved. After a further two weeks, the patient developed hydrocephalus, needing the placement of a ventricular-peritoneal shunt. The day before surgery, antimicrobial therapy was further modified: ceftriaxone 4g a day, co-trimoxazole iv 240/1.200 mg four times a day and linezolid 600 mg twice a day. Vancomycin and rifampin were discontinued. After a

Figure 2 - Gram-positive branching bacilli in deparaffinized brain abscess section.
<table>
<thead>
<tr>
<th>Time</th>
<th>Clinical or Instrumental findings</th>
<th>Blood WBCs x 10^3 (N%)</th>
<th>CSF findings</th>
<th>CNS tissue findings</th>
<th>Invasive procedure</th>
<th>Antimicrobial therapy</th>
<th>Time of treatment administration</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pneumonia</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td>amoxicillin /clavulanic 1 g q8h + levofoxacin 500 mg q24h</td>
<td>3 weeks</td>
<td>Resolution pneumonia</td>
</tr>
<tr>
<td>Day 60</td>
<td>Cerebellar abscess plus hydrocephalus</td>
<td>22.5 (85.3)</td>
<td>WBCs: 325 mm^3</td>
<td>Pyogenic infection</td>
<td>Swabs cultures negative</td>
<td>ceftriaxone 2 g q12h + metronidazole 500 mg q6h</td>
<td>2 weeks</td>
<td>Chest radiography and CT scan negative</td>
</tr>
<tr>
<td>Day 74</td>
<td>Pulmonary embolism, MRSA ventilator associated pneumonia</td>
<td>7.4 (67.2)</td>
<td>WBCs: 144 mm^3</td>
<td></td>
<td></td>
<td>linezolid 600 mg q12h + meropenem 2 g q8h for 1 week, then imipenem 500 mg q6h</td>
<td>8 weeks</td>
<td>Improvement</td>
</tr>
<tr>
<td>Day 93</td>
<td>Ventriculitis, and septum pellucidum abscess</td>
<td>7.4 (67.2)</td>
<td>WBCs: 96 mm^3</td>
<td></td>
<td></td>
<td>ceftriaxone 2 g q12h + metronidazole 500 mg q6h + meropenem 2g q8h</td>
<td>2 weeks</td>
<td>Persistence clinical and laboratories findings</td>
</tr>
<tr>
<td>Day 108</td>
<td>Ventriculitis, and septum pellucidum abscess</td>
<td>7.4 (67.2)</td>
<td>WBCs: 340 mm^3</td>
<td></td>
<td></td>
<td>vancomycin 1g q8h + rifampin 600 mg q24h + meropenem 2g q8h</td>
<td>2 weeks</td>
<td>Persistence clinical and laboratories findings</td>
</tr>
<tr>
<td>Day 122</td>
<td>Ventriculitis, and septum pellucidum abscess</td>
<td>7.4 (67.2)</td>
<td>Gram staining: Gram+branching bacteria</td>
<td></td>
<td></td>
<td>vancomycin 1g q8h + rifampin 600 mg q24h + ceftriaxone 2g q12h + co-trimoxazole 240/1 1200mg q6h</td>
<td>2 weeks</td>
<td>Improvement</td>
</tr>
<tr>
<td>Day 134</td>
<td>Hydrocephalus</td>
<td>WBCs: 4 mm^3</td>
<td>WBCs: 77 mm^3</td>
<td></td>
<td>Ventricular-peritoneal shunt and surgery</td>
<td>ceftriaxone 2g q12h + co-trimoxazole 240/1 200mg q6h + linezolid 600 mg q12h</td>
<td>2 months</td>
<td>Patients discharge</td>
</tr>
</tbody>
</table>

Legend: WBCs = white blood cells; CSF = cerebrospinal fluid; MRSA = methicillin resistant Staphylococcus aureus; S. capitis = Staphylococcus capitis; A. odotolyticus = Actinomyces odotolyticus; N. paucivorans = Nocardia paucivorans
total of two months of therapy, the patient was discharged on ceftriaxone 4g a day, oral co-trimoxazole DS 3 pills a day and rifampin 600 mg a day. This therapy was continued for five weeks, then changed with oral co-trimoxazole DS 3 pills a day and linezolid 600 mg twice a day, for other four weeks, then only co-trimoxazole was prescribed. MRI performed on the seventh month of therapy, did not show altered signals of the subependymal areas next to the lateral ventricle and disappearance of contrast enhancement (Figure 1d). Treatment was continued for a total of 11 months. At the last follow up, eight months after antimicrobials were discontinued, the patient was asymptomatic.

**DISCUSSION**

Overall, it is very likely that this patient had relapsing cerebellar abscess secondary to *N. paucivorans* disseminated disease with lung infection. In this patient it was very difficult to prove nocardial aetiology for several reasons. First of all the patient did not have known cause of immunodeficiency, also he was HIV negative, and his CD4+ lymphocyte count was normal. The initial lung infection was not suggestive of nocardiosis and antimicrobials administered to treat pneumonia were possibly active also against *Nocardia* spp. and led to clinical improvement [1]. Nevertheless, this therapy did prevent systemic infection and CNS dissemination [1, 4, 14, 15]. Two months later, when our patient was admitted because of hydrocephalus and cerebellar abscess, lung disease was not anymore evident, based on clinical and radiological findings. Microbiological and histopathological investigations of CNS samples showed acute pyogenic inflammatory reaction, however, specific studies to diagnose *Nocardia* spp. and led to clinical improvement [1]. Nevertheless, this therapy did prevent systemic infection and CNS dissemination [1, 4, 14, 15]. Two months later, when our patient was admitted because of hydrocephalus and cerebellar abscess, lung disease was not anymore evident, based on clinical and radiological findings. Microbiological and histopathological investigations of CNS samples showed acute pyogenic inflammatory reaction, however, specific studies to diagnose *Nocardia* spp. infection were not performed, thus leading to false-negative results. In addition, incorrect specimens were collected for microbiologic investigation and specific microbiological procedures for *Nocardia* spp. isolation and identification were not performed [4, 10]. Given the missed diagnosis of CNS nocardiosis, treatment was discontinued too early. At this regard, most of the authors recommend six months of therapy in HIV negative, immunosuppressed patients with pulmonary infection and 6-12 months in those with CNS involvement [4, 14, 15]. No recommendations are available for immunocompetent patients. During the second relapse, Gram staining of deparaffinized cerebellar tissue showed branching Gram-positive bacteria suggesting the diagnosis of *Nocardia* infection.

However, there was a further delay to the correct diagnosis due the fact that *S. capitatus* and *A. odontolyticus* were grown from the second CSF sample. Both these microorganisms can cause CNS infections in selected patients [16]. Also, *A. odontolyticus* like *Nocardia* spp. has a branching Gram-positive appearance but unlike *Nocardia* spp. does not stain acid fast with the modified Ziehl-Neelsen [2, 10]. Finally, only DNA sequencing from deparaffinized tissue led to the definitive aetiological diagnosis at the species-level, allowing the correct treatment.

**CONCLUSION**

*Nocardia* spp. infections need to be suspected not only in immunocompromised, but also in immunocompetent patients. Proper samples need to be collected for microbiologic investigations. DNA sequencing from paraffin-embedded tissue can be a useful tool for diagnosis of nocardiosis.

No conflict of interest.

**REFERENCES**


