Novel diagnostic procedure

Polymicrobial subdural empyema: involvement of *Streptococcus pneumoniae* revealed by *lytA* PCR and antigen detection

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Summary

The authors report a case of a subdural empyema (SDE) caused by a coinfection with *Streptococcus intermedius* and *Streptococcus pneumoniae*, initially considered a *S intermedius* infection only. An otherwise healthy 11-year-old female was admitted to the hospital after 5 days of illness. Symptoms were consistent with classical SDE symptoms and progressed rapidly with finally somnolence before the first neurosurgical procedure despite relevant antibiotic treatment. Primary MRI showed an interhemispheric SDE and a postoperative control CT scan showed progression of the empyema infratentorially. The empyema was evacuated twice, day 8 and 18, with good results. Primary samples showed growth of *S intermedius* only. The severity of the clinical picture elicited supplementary samples, which were additionally positive for *S pneumoniae* by an in-house specific *lytA* PCR and/or a commercial antigen test.

BACKGROUND

Bacterial sinusitis is very common and resolves without sequelae in the majority of cases. Suppurative complication can occur. They are divided into orbital and intracranial infections of which subdural empyema (SDE) is one. Patients may exhibit both an intracranial and orbital focus of infection. In patients hospitalised with sinusitis, the reported rate of intracranial complications varies from 3.7% to 47.6%, clearly overestimating the rate of complications by the selection of severe cases. Sinus disease is presumed to be the underlying cause of about 10% of intracranial suppuration^{1–3}; however, in SDE it is much more strongly associated.^{3–7} Concomitant intracerebral abscess occurs in 6–22% of cases and epidural abscess in 9–17%.⁸

Most cases of SDE occur in the second decade of life in patients who are otherwise healthy. There is a marked male predisposition, with a male-to-female ratio of 3:1, and as high as 8:1 for sinus-associated cases.⁸

The hypothesis is that bacteria enter the central nervous system through the sinus, ear or pharynx, thereby introducing the microbial flora intracranially, which causes meningitis or empyema.^{9–11}

The most common signs and symptoms in patients with SDE are fever, headache, altered mental status, hemiparesis, nausea and vomiting, seizures, meningismus, periorbital oedema, papilloedema and several other symptoms.⁸

The most common microbial causes are streptococci, staphylococci, anaerobe organisms and Gram-negative organisms.^{8–12} Frequently, a single microbe is found, when polymicrobial usually streptococci and anaerobe bacteria are implicated.⁹ Within the streptococcal species, *Streptococcus intermedius* is often found and *Streptococcus pneumoniae* and *Streptococcus anginosus* are isolated.¹⁰

Microbiological diagnosis of intracranial infections is currently based on the culture of blood and/or material

sampled from an entry point infection, for example, sinusitis, or material sampled after surgical drainage of the empyema. Samples obtained during surgical procedures do not yield growth in 7–53% of cases.⁸

Surgical evacuation of SDE is often indicated and studies suggest that rapid surgical intervention improves the prognosis.¹³

CASE PRESENTATION

An otherwise healthy 11-year-old female was admitted to a primary hospital after 5 days of illness. Symptoms consisted of headache, fever, vomiting, change of personality, paresis of the left foot and classical signs of infection of the right upper palpebra (table 1). There was no recent history of upper respiratory tract infections.

An acute CTC displayed an SDE and sinusitis. MRI was not done primarily and surgical drainage of the maxillary sinus was prioritised and empirical antibiotic treatment with cefuroxime and metronidazole was commenced (table 1). No prior antibiotic treatment had been given. Subsequently an MRI was done and the images were conferred with the Regional Department of Neurosurgery. It was decided to continue antibiotic treatment without surgical intervention due to the risk of this procedure.

The MRI showed an interhemispheric SDE with no radiological signs of increased intracerebral pressure (figure 1A).

Culture performed on blood and pus from the maxillary sinus at the day of admittance (day 1) showed growth of streptococci finally identified as *S intermedius* on the 5th day (table 2). The isolate was susceptible to penicillin, ampicillin and ceftriaxone. The species identification was delayed due to slow growth of the bacterium. The presence of *S pneumoniae* in the primary blood culture was not tested for by pneumococcal antigen detection by direct microscopy

Table 1 Development of symptoms and antibiotic treatment during the admittance, postoperative and after neurorehabilitation

Symptoms	Timeline				
	Time of admittance	Within 24 h	8th day	18th day	3 Months postoperative
Headache	+	+	+	+	–
Change of personality	+	+	+	+	–
Fever	+	+	+	–	–
Periorbital swelling	+	+	+	–	–
Vomiting	+	–	–	–	–
Leg paresis	–	+	+	+	–
Strabismus	–	+	–	–	–
Periorbital abscess	–	–	+	–	–
Hemiparesis	–	–	+	–	–
Seizures	–	–	+	–	–
Biochemical findings					
CRP (Reference: <75 nmol/l)	1.228	1.218	1.541	141	6
WBC (Reference: 4.500–10.500 μ l)	8.600	11.770	14.790	9.820	5.800
Radiological findings		CTC and MRI indicate SDE	Progression of the supratentorial SDE	Progression of the infratentorial SDE	Complete regression
Antibiotic treatment	None - intravenous cefuroxime and metronidazol commenced	Intravenous ceftriaxone, ampicillin and metronidazole	Intravenous ceftriaxone and metronidazole	Intravenous meropenem and oral metronidazole	Oral penicillin discontinued

(OMNI Serum test – Statens Serum Institut, Copenhagen, Denmark).

The patient was transferred to the Regional University Hospital at day 3.

Clinically, a periorbital abscess had been developing during admittance and on the 7th day, the progressing orbital abscess was incised after CTC confirmation.

Despite relevant antibiotic treatment, a rapid progression of the left leg paresis developed. An elective MRI scan done on the 7th day showed progression and this was confirmed by an acute CTC on the 8th day when the girl turned somnolent. Therefore, an acute right-sided craniectomy over motor cortex crossing sagittal sinus was performed and the parasagittally located abscess evacuated, and biopsies were taken (table 1). Pneumococcal antigens were found in the periorbital abscess and in the empyema. A specific *lytA* PCR later confirmed the presence of *S pneumoniae* and supported the finding of a coinfection (table 2).

On the 18th day, a control CT scan showed progression of the empyema: supra- and infratentorial (figure 1B). Clinical status, however, had improved, as the girl was walking although her left foot was still paralysed.

By means of a stealth CT scan, two burr holes were made (one supratentorial on the right side and one left-sided in posterior fossa guided by the neuronavigation) with a resulting evacuation of the empyema.

Ten samples from four locations from different time points were examined. Four samples were negative by all tests. Two blood culture samples were examined in the laboratory at the hospital of the primary admission, but unfortunately only the isolates were referred to us. These isolates were tested by Binax NOW, *lytA* PCR and 16S rDNA PCR, the latter assay confirming the isolation of a *S intermedius* strain. The two other assays were negative. The last four samples obtained during admission at the University Hospital, and the isolates from the primary admission are listed in table 2. Blood was negative by culture 2 days after antibiotic treatment was initiated; how-

ever, the *lytA* PCR and the antigen detection were positive for *S pneumoniae*.

The only culture positive sample came from the incised periorbital abscess and was taken 5 days into antibiotic treatment. Growth was sparse and the single colony found on a 5% blood agar plate was consistent by phenotype with non-haemolytic streptococci but not subcultured and further characterised. The sample material, however, was tested positive for *S pneumoniae* using the Binax NOW and that initiated the supplementary PCR tests.

Various combinations of antibiotics were continued for 86 days (table 1). Ceftriaxone was replaced by meropenem because of a rash. Oral penicillin was tolerated though for the last 26 days.

Three months postoperatively, a MRI scan revealed no remaining empyema. The girl was completely neurologically restored after intensive rehabilitation. The craniectomy seemed to close spontaneously.

METHODS

Conventional diagnostic methods

Species identification was done using the VITEK 2 (bioMérieux, Durham, North Carolina, USA).

DNA extraction and amplification

Sample material was centrifuged and divided into supernatant and precipitate. DNA was extracted from the supernatant by the use of the MagnaPure Compact system (Roche Diagnostics, Basel, Switzerland), and from the precipitate by the use of DNeasy, Blood and Tissue Kit (QIAGEN, Hamburg, Germany). The NucliSENS easyMag 2.0 (bioMérieux) was used for DNA extraction from the blood culture bottles. Amplification of bacterial 16S rDNA and sequencing of the amplicon were performed as described by Voldstedlund *et al*¹⁴ and Muyzer *et al*.¹⁵ Specific autolysin (*lytA*) PCR was performed on every sample fraction in accordance with the method of Sheppard *et al*.¹⁶

Figure 1 (A) MRI scan (T2 axial) from the day of admittance. The subdural empyema along falx cerebri. (B) CT scan including gadolinium. The supratentorial and infratentorial empyema showed before the second operation.

Pneumococcal antigen detection

Several sample fractions were tested in accordance with the manufacturer's recommendations for urine or cerebrospinal fluid (CSF), using the immunochromatographic membrane assay, Binax NOW *S pneumoniae* (Inverness Medical, Bedford, UK).¹⁷

DISCUSSION

The severe clinical presentation of this SDE case and the seemingly lacking effect of the antibiotic treatment questioned that the *S intermedius* isolated from the blood was the only microbial cause. Therefore, the Binax NOW *S pneumoniae* was applied on the periorbital abscess material with a positive result. However, the Binax NOW test has only been validated for urine and CSF.^{17 18} Therefore, the positive predictive value (PPV) for Binax NOW *S pneumoniae* is unknown for SDE. A study on pleural empyema suggests that the PPV is 95%; the sensitivity is more than 90%, and the specificity 95% with culture and 16S rDNA PCR as a reference.¹⁹ On the one hand, it may be assumed that these data also are applicable for blood, SDE and periorbital abscess material. It is therefore probable that both *S pneumoniae* and *S intermedius* were present in the blood, the periorbital abscess and the SDE (table 2). On the other hand, the Binax NOW detection kit has been known to produce false-positive tests with viridans streptococci primarily from the mitis group.²⁰ To rule out a false-positive Binax NOW test, the primary isolate of *S intermedius* was tested and found Binax-negative.

To confirm the antigen finding, both 16S rDNA PCR and a *lytA*-specific PCR were done thrice on every sample fraction (table 2). PCR confirmed the presence of *S pneumoniae* in the blood culture from day 3, in the empyema supernatant, but by the *lytA*-specific PCR only.

It is a limitation of the 16S rDNA PCR that mixed infections are difficult to detect and interpret in clinical sample material. Concerning the periorbital abscess, the diagnosis of *S pneumoniae* could not be confirmed by any of the applied PCR methods. This sample is therefore less conclusive, but a strong indication of pneumococcal coinfection was found in the positive Binax NOW test result, remembering, though, that the test is not validated for pus. The detection of *S pneumoniae* by the use of the *lytA*-specific PCR and the Binax NOW test on the negative blood

Table 2 Key diagnostic samples and findings

Material /origin	Time Day of sampling	Days of antibiotic treatment before sampling	Method of analysis			
			Culture	PCR		Antigen detection
				16S rDNA	Specific <i>lytA</i>	Binax NOW <i>Streptococcus pneumoniae</i>
Isolates from blood culture (primary hospital)*	1	0	<i>S intermedius</i>	<i>S intermedius</i>	Negative	Negative
Blood culture (Day 3)	3	2	Negative	Negative	Positive	Positive
Pus from periorbital abscess	7	6	†	<i>S intermedius</i>	Negative	Positive
Empyema material	8	7	Negative	<i>S Intermedius</i>	Positive	Positive
Cerebral biopsy	8	7	Negative	<i>S intermedius</i>	Negative	Not done

Analysis by PCR was done three times on each sample.

*The original blood sample was discarded before referral to our department, only isolates were available.

†Identification based on one single colony found on the 5% blood agar plate was consistent by phenotype with non-haemolytic streptococci.

culture, 2 days into antibiotic treatment, confirmed the presence of (live/dead/phagocytised) *S pneumoniae* in the blood (table 2). We cannot determine whether this actually represents a bacteraemic phase or carriage in the blood of components of *S pneumoniae* from a site of colonisation/normal flora. This sample also underlines another limitation of the 16S rDNA PCR, which requires a relatively higher target concentration compared to the *lytA*-specific PCR. If the 16S rDNA PCR method had been the only non-culture method used, none of the samples examined would have been found positive for *S pneumoniae*. It is generally accepted that it is important to know the limitations of any test used to make a species identification. Morphological and biochemical methods are, for many species, defined as the gold standard. But the introduction of highly sensitive and specific PCR-based tests focusing on a single microbe, for example, the AccuProbe, has challenged the classical definition of a species.²¹ Therefore, neither the classical culture-based nor the DNA/RNA-based methods can stand alone. Most laboratories handle this uncertainty of species identification by accepting a margin of error.²² Because the *lytA*-gene is not exclusive to *S pneumoniae* and also found in *S pseudopneumoniae*, *S mitis*, *S oralis* and *S infantis*, the specific autolysin (*lytA*) PCR was tested by Sheppard *et al*¹⁶ on two *S mitis* known to have the *lytA*-gene present. They were both found negative by the test indicating that the *lytA*-gene is different in *S mitis* compared to *S pneumoniae*. We have further studied the *lytA*-gene in a number of streptococcal species including various serotypes of *S pneumoniae* and found that the present *lytA* PCR of Sheppard *et al* is sensitive and specific for *S pneumoniae* (data to be published).

Learning points

- ▶ This SDE was only classified as a polymicrobial infection because of the supplementary use of an in-house specific PCR and a commercial antigen test.
- ▶ The diagnosis of a coinfection could definitely have been available on the 3rd day of admittance and maybe even earlier had these assays been included in the examination of the initial samples from the patient.
- ▶ High-dose penicillin treatment only would consequently have been enforced in our hospital.
- ▶ Early detection of pneumococci might also have advanced the neurosurgical procedures.
- ▶ In SDE and other serious infections, it is therefore important to consider rapid diagnostic modalities independent of the viability of the pathogenic microorganisms.

Acknowledgements The authors thank Niels Nørskov-Lauritsen for critical reading of the manuscript.

Competing interests None.

Patient consent Obtained.

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Greve T, Clemmensen D, Ridderberg W, Pedersen LN, Møller JK. Polymicrobial subdural empyema: involvement of *Streptococcus pneumoniae* revealed by *lytA* PCR and antigen detection. *BMJ Case Reports* 2011;10.1136/bcr.09.2010.3344, date of publication

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