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CASE REPORT - HISTORY OF NEUROSURGERY

Biofilm-associated infection: the hidden face of cerebrospinal fluid shunt malfunction

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Abstract Diagnosis of cerebrospinal fluid (CSF) shunt infection is difficult. Growing evidence links this pattern to biofilm-associated infections (BAI). Biofilm may explain the indolent development of the infection, and the poor efficiency of traditional microbiologic methods. We report the case of a patient admitted for hydrocephalus associated to CSF shunt malfunction. None of the clinical, serum, or CSF laboratory findings were in favor of an infectious process. Only scanning electron microscopy (SEM) revealed the presence of biofilm. Hence, despite a broad CSF shunt infection definition, some infections could remain undiagnosed by the traditional approach. This study is the first to provide some direct evidence for bacterial biofilm-associated CSF shunt infection.

Keywords Biofilm · CSF shunt infection · Diagnosis · Sterile culture · Shunt malfunction · Scanning electron microscopy

Introduction

Diagnosis of CSF shunt infection is difficult, because of its pleomorphic pattern. It lacks sometimes the presence of any clinical symptom, and laboratory results may be normal. The most obvious clinical pattern usually is device malfunction. The ultimate proof for infection is a positive CSF and/or catheter culture; nevertheless, these can lack sensitivity. Thus, the initial definition from the Center for Disease Control (CDC) was expanded in order to improve our diagnostic performances [7].

Growing evidence links this pattern (device-related infection and chronic infections with few infectious symptoms) to biofilm-associated infections (BAI). The importance of biofilm in human pathology has been described recently and it is becoming increasingly clear that it plays a pivotal role in post-operative infections involving neurosurgical devices. Literature on this subject remains scant, but BAI have been better understood and described in orthopedics [3]. Biofilm lifestyle allows the development of an indolent infection, mostly by escaping from the immune system [5, 6]. Although potentially harmful to the host by itself, biofilm is often not as pathogenic as the host's own inflammatory response generated by its presence (frustrated phagocytosis). Planktonic bacteria shed from the biofilm, however, can cause acute systemic illness. Infectious symptoms can therefore range from absent to obvious, including all intermediate states. BAI detection is seriously hampered by the general failure of culture methods to recover and grow biofilm cells from infected tissues [3]. The characterization of an infection linked to biofilm is based on the unequivocal detection, by scanning electron microscopy (SEM), of matrix-enclosed microbial communities within or upon the infected tissues or prosthesis [3, 6]. Diagnostic performances are not well assessed in this context, and the Food and Drug Administration approved new molecular methods only for the detection of a small number of pathogens (difficult to culture) [3].

Some authors report that most, and perhaps all, shunt malfunctions are related to an infectious process, despite the absence of any clinical symptom or a any evidence for a

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growing bacteria. We describe the first clinical case for which all CDC-definition criteria were negative despite an obvious BAI diagnosed by scanning electron microscopy. Informed consent was obtained.

Case report

A 21-year-old man was admitted to our intensive care unit (ICU) for a CSF shunt obstruction. Three years earlier, he had presented to the ER with an acute hydrocephalus for which an MRI revealed a pituitary tumor. An emergent CSF diversion was completed, via a ventriculo-atrial shunt. Hormonal evaluation was normal. After a multidisciplinary consultation, surgical indication was approved. It was performed 3 weeks later, and histology revealed a pilocytic astrocytoma. Unfortunately, the extraction was incomplete and was followed by a subsequent panhypopituitarism. Two years later, MRI monitoring revealed the growth of the residual tumor, leading to an adjunctive treatment with radiotherapy. No new complication occurred before the patient's current admission.

No septic episode was described in this case history. Neither fever nor any neurologic disorders were reported. The patient did not receive any antibiotics during the previous 6 months. Clinical examination did not reveal any meningeal inflammation. Body temperature was 37.0 °C. The only anomaly was an itch of the skin surrounding the shunt and the ventricular catheter, the week before admission. Skin examination did not reveal any abnormality. Laboratory findings did not highlight any inflammatory syndrome. Cerebral CT scan revealed active hydrocephalus. On admission, white blood count (WBC) was 12.5 G/l, including 9.9 G/l neutrophils. Serum C-reactive protein (CRP) was 2.5 mg/l; blood cultures were sterile. In the absence of any local signs of infection, the ventricular catheter and the shunt valve were exchanged during the same emergent surgery, and the distal extremity of the catheter was removed for culture. No prophylactic antibiotic treatment was performed before removal of the catheter and the valve, and sampling of CSF. CSF biochemistry identified a protein concentration of 150 mg/l, a glucose concentration of 3.8 mmol/l, <1 leukocytes/ml, a red blood count of 85/ml, and standard culture of both the CSF and catheter tip were sterile (with vortexing but without sonication). Culture was incubated for 10 days to avoid any false-negative due to anaerobic germs. Outcome was good at 3 months.

Case history could end here, but our team is currently working on biofilm. Ventricular catheter was therefore observed through SEM. Immediately after being removed, a section of the ventricular catheter was fixed in a mixture of 4 % paraformaldehyde-2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 1 h at room temperature, and then maintained at 4 °C. After washing and dehydrating with crescent ethanol concentrations, and drying with liquid CO_2 (without critical-point drying), the sample was coated with gold in Jeol FJC-1200 sputter coater. Surprisingly, examination under a Hitachi SU 3500 revealed a confluent abundant biofilm matrix surrounded by cocci (Fig. 1). Unfortunately, no polymerase chain reaction (PCR) was realized on the sample. Thus, we provide the first objective report of bacterial biofilm colonization responsible for shunt malfunction, despite negative standard culture and no infectious symptom.

Discussion

CSF shunt is a common procedure in neurosurgery [8]. It persists life-long. Patients face many potential long-term complications.

Shunt malfunction requiring secondary intervention occurs in 40 % of children within the first 2 years after original placement [9].

The rate of shunt infection is about 5–9 % per procedure [8] and occurs mostly within 3 months of surgery, presenting itself as the combination of infectious symptoms and underlying neurosurgical condition. Sometimes, no infectious symptoms are retrieved. Moreover, the biological diagnosis of a shunt infection can be difficult and cultures lack sensitivity [2, 8, 11, 12]. An infection can be considered to be associated with a CSF shunt if at least one of two criteria are fulfilled: (1) a positive CSF culture, (2) a clinical symptom AND at least one of the following: (a) increased white cells, elevated protein, and/or decreased glucose in CSF, (b) organisms seen on Gram's stain of CSF, (c) organisms cultured from blood, (d) positive antigen test from CSF, blood, or urine [7]. In a large retrospective series, clinical symptoms were sometimes absent, C-reactive protein (CRP) levels were over 3 mg/dl in 98 % of cases, and diagnosis of shunt infection was made without any microbiological documentation in 40 % of cases [12]. Another cohort ($n = 78$), this time prospective, also using the CDC definition, reported no local signs or symptoms in 40 % of cases, a CSF leukocyte count $>5.10^6$ cells/l in 80 % of cases, a total CSF protein level > 0.45 g/l in 58 % of cases, and an infecting pathogen in 91 % of cases [2].

Can radiological exams help? Von der Brelie et al. obtained a computed tomography (CT) scan of the head in 60 % of all episodes of infection, and hydrocephalus was diagnosed in 30 % of all patients ($n = 92$) [12]. Conen et al. reported that a cranial CT scan showed signs of shunt-associated infection in eight (12 %) of 66 episodes, with meningeal enhancement in five cases and brain abscess in three episodes [2].

Thus, diagnosis is difficult, and despite a nonspecific definition, there are probably a lot of false negatives. On the other hand, an important number of diagnoses are suspected despite a negative culture. It has been proposed that shunt obstruction

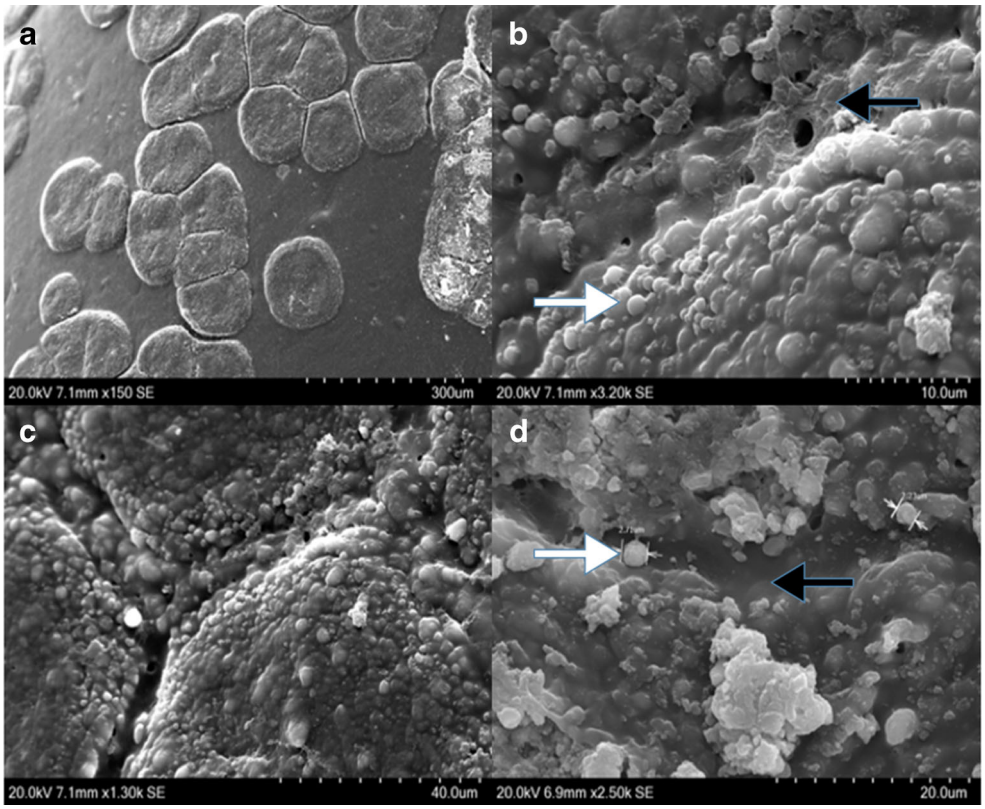

Fig. 1 Scanning electron microscopic (SEM) images of biofilms growing on the ventricular catheter. **a** Lower-power image showing adherent mature confluent biofilm to the smooth catheter surface, magnification ×150. **b**, **c** and **d** Higher-power image showing the extracellular polymeric slime matrix (*black arrows*), with some cocci (*white arrows*), mostly in the cracks. Cocci are spherical images measuring around 2 μm. We observed the same images evocative of small-colony variants, because ~10 times smaller than those of the normal phenotype (not shown). Magnification of **b**, **c**, and **d**: ×3200, ×1300, ×2500, respectively

or malfunction might be caused by an undetectable infection [1, 5, 6]. Some studies report the presence of biofilm in animal models, but no evidence was found in the literature that BAI leads to shunt malfunction.

A growing body of studies focused on device-associated infections. Biofilm is currently defined by a morphologic criteria, as "an aggregate of microbial cells adherent to a living or nonliving surface, embedded within a matrix of extracellular polysaccharides" [5]. Clinical findings are characterized by an insidious or asymptomatic presentation [6]. CSF's poor immune response capacity could hide the development of symptoms [10]. Altogether, shunt BAI can be asymptomatic. Traditionally, clinical microbiology laboratories have focused on culturing under planktonic growth conditions, and are not used to evaluate bacteria living in biofilm, leading to a lot of false-negatives exclusively related to culture diagnosis method [1, 5]. Relying on culture as the "gold standard" of medical microbiology for the identification of bacterial pathogens is not such a clear-cut considering biofilm associated-infection. Other culture-independent methods have been developed and are currently being tested. SEM cannot be used routinely in medical laboratories [5, 6]. Molecular methods (such as PCR) seem promising but lack study assessment, compared to SEM or traditional culture. They appear more sensitive than culture, but the exact diagnostic performance is unknown [5]. Unfortunately, we were not combining universal 16s-RNA to SEM investigation. We just know that our BAI involved cocci.

Few studies have brought to light direct evidence of biofilm formation on ventriculoperitoneal shunt; all but one were evaluated in vitro or investigated a rodent model [1]. The only clinical work reported was a fungal biofilm-associated shunt infection [1, 4]. Our report is the first direct evidence for bacterial biofilm-associated CSF shunt infection; it is particularly educational because absolutely no inflammatory clinical or biological marker was reported, and this without the prior administration of any antibiotics. Some authors consider that probably all CSF shunts are colonized by biofilm [1]. Considering that more than 120,000 ventriculostomy catheters are placed each year, additional studies are necessary to assess the impact of 16s-RNA gene detection for BAI in light of clinical symptoms' traditional culture and SEM.

Biofilm is an ancestral lifestyle that allows bacteria to survive even in a hostile environment. A long-lasting antibiotherapy does not guarantee its eradication. Therefore, today's recommendations identify prevention and removal of the device as the two key strategies against biofilm [6].

This report highlights the role of biofilm in shunt malfunction. Despite the broad and complex CDC definition, diagnosis of infection is complicated, as is exposed in this case report. BAI are becoming a major public health issue, underdiagnosed by traditional microbiologic diagnostic methods. We can assume that the role of SEM to diagnose BAI in shunt malfunction will increase with time. Further studies are necessary to assess new methods, such as 16s-

RNA, in order to improve the diagnostic performance of BAI and/or shunt malfunction.

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The patient has consented to the submission of this case report to this journal.

Compliance with ethical standards

Conflicts of interest None.

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Comments

Mounier and colleagues present a case report about cerebrospinal fluid shunt malfunction due to bacterial biofilm shunt infection demonstrated by scanning electron microscopy. Traditional clinical or biological signs and symptoms of an infection had been absent. In my opinion, this is an interesting and important report, increasing our knowledge of and attention to biofilm-associated infections.

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