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Axillary abscess complicated by venous thrombosis, identification of *Streptococcus pyogenes* by 16S PCR.

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Running head: *S. pyogenes* axillary abscess

Abstract

We report a case of an axillary abscess with *Streptococcus pyogenes* complicated by venous thrombosis. Bacterial etiology and typing was obtained by PCR and sequencing of the 16S rRNA and the M-protein genes from abscess material. The bacterium was of M41 serotype and serology indicated that it had expressed pro-coagulant factors.

Case report

A 62 year-old woman presented at our department with a seven-days history of fever, chills, and nausea. She was previously healthy apart from having an atopical eczema, and she worked as a technician in a microbiology department handling bacterial specimens. For some months, she had experienced pain in the left part of her thoracic wall, which she related to repetitive movements. Two days prior to admission she started to feel pain in her left axilla. On the day of admittance she had vomited and suffered from diarrhea. At admission she had a temperature of 39.5ºC. Routine physical examination was normal except for a slight tenderness upon palpation in the left axilla. There were no signs of erysipelas, lymphangitis or enlarged lymph nodes in the axilla. Laboratory investigation revealed a white blood cell count of $18x10^9$/L (neutrophils were $17x10^9$/L), C-reactive protein (CRP) of 53 mg/L, and normal renal and liver function tests. Coagulation tests were within normal limits PT(INR) (Prothrombin time (international normalized ratio) 1.1, aPTT (activated partial thromboplastin time) 36s and platelet count $329x10^9$/L. After obtaining two aerobic and two anaerobic blood cultures (BacT/Alert, Biomérieux, Durham, USA) as well as a urinary culture, the patient was sent home and she was told to return if she got worse. No antibiotics were prescribed. Blood cultures turned out negative.

Seven days later the patient returned with persistent axillary pain and intermittent chills and she was now hospitalized. The temperature fluctuated the following days between 38.0ºC and 39.9ºC. White blood cell count was $21x10^9$/L (neutrophils were $19x10^9$/L) and CRP was 393 mg/L. Upon examination of the axillary region, pain was provoked by palpation but no enlarged lymph nodes or suspected abscesses were felt and no signs of arthritis were noted. Treatment

with cefuroxime and clindamycin was instituted due to suspicion of a soft tissue infection in the axillary region. Plain X-ray of the shoulder showed degenerative changes in the acromio-clavicular joint and ultrasonographic examination of the axilla was normal with no signs of edema in the musculature or in the subcutaneous layer and no signs of abscess. A slight improvement occurred over the following days. Renewed blood cultures taken at the time of admittance turned out negative. On the sixth day after admission a swelling of the left arm developed and venous flebography confirmed the presence of a venous thrombosis in the axillary vein. Coagulation tests were obtained and showed PT(INR) 1.1, aPTT 40s, and platelet count of $430x10^9$/L. Low molecular weight heparin and warfarin treatment was initiated. An MRI-scan revealed a multilobulated lesion of 7x4x7 cm in the left axilla approximately 1.5 cm from the skin enclosing the axillary vein with contrast signal in the periphery and a surrounding edema (Fig 1A). A renewed ultrasonographic examination could visualize the abscess, which was punctured led by a CT-scan. Abscess material was added to an anaerobic blood culture bottle (BacT/Alert). Direct cultures were negative and no growth in the bottle was detected.

Abscess material was also subjected to Polymerase chain reaction (PCR) amplification of the 16S rRNA gene and subsequently of the *emm* gene. DNA was extracted from 200 µl abscess material using Bio Robot EZ-1 with DNA Tissue Kit (Qiagen, Qiagen Strasse 1 407 24 Hilden, Germany) after treatment with Proteinase K according to instructions by the manufacturer. The amplification was carried out in 50 µl of reaction mixture containing 1 x PCR buffer (Qiagen), 3 mM $MgCl_2$, 200 µM of each dNTP, 1.0 U HotStar Taq DNA polymerase (Qiagen,), 10 pmol of each primer and 5 µl template. P515f (5'- TGCCAGCMGCCGCGGTWAT -3' (12)) and P1067r (5'-AACATYTCACRACACGAGCT -3'(this study)) were used as PCR and sequencing primers.

A pre PCR step of 15 min at 95°C, was followed by 40 cycles of 93°C for 50 s, 52°C for 50 s and 72°C for 50 s. A final step of 5 min at 72°C terminated the amplification. Tubes with no target DNA and *E. coli* DNA were included as negative and positive controls, respectively. Both strands of the approximately 520 base pair (bp) PCR product were sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.,850 Lincoln Centre Drive Foster City, CA 94404USA) , and analysed on an ABI PRISM 3100 Genetic Analyser (Applied Biosystems Inc., CA, USA) by BMLabbet (Furulund, Sweden). The sequence was identical (523/523 bp) to the 16S rRNA gene of *Streptococcus pyogenes* available at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

The *emm* gene encoding the *S. pyogenes* M protein was amplified from abscess material, as described above, using primers derived from conserved parts of the *emm1* gene (5'-GCTTAGAAAATTAAAAACAGG-3' (emm for); 5'-GCGTTTTACAACTGCTGC-3'(emm rev)). A 1.2 kbp fragment was generated and sequencing, as described above, with emmfor yielded a sequence, which was highly similar (596/598 bp) to the hypervariable part of the *emm41* gene. These results are strongly suggestive of *S. pyogenes* serotype M41 as the causative agent and treatment with clindamycin was continued for a total of three weeks. The patient had an uncomplicated recovery.

Antibodies directed towards the variable part of the cell wall-attached M protein of *S. pyogenes* are believed to confer serotype specific protection. Stored serum samples obtained several years before the present episode were available from the patient, and Immunoglobulin G (IgG) antibody levels against *S. pyogenes* PAM (plasminogen-binding group A streptococcal M-like

protein), an M-like protein expressed by serotype M41 (19), and other virulence determinants in these samples were compared to IgG antibody levels in convalescence sera. ELISA (enzyme-linked immunosorbent assay) was performed essentially as described previously (2). The following *S. pyogenes* antigens were used for coating; PAM 0.5µg/ml, GRAB (protein G-related α_2M-binding protein) 0.8 µg/mL, IdeS (IgG-degrading enzyme of *S. pyogenes*) 1.1 µg/mL, SpeB (the secreted streptococcal cysteine proteinase) 0.5 µg/mL, SclA and SclB (Streptococcal collagen like protein A and B) (both from serotype M41) 4µg/ml. Antigens were purified as described previously (2, 13, 19). Serum samples were diluted 1:500 (PAM, GRAB, IdeS, SpeB) or 1:50 (SclA, SclB). All antigens gave an absorbance of at least 0.5 when tested with Octagam (human IgG 50mg/ml) (Octapharma) or a positive control serum in the same dilutions as the patient sera. There was a marked increase in IgG antibody levels against PAM as well as against a collagen-like surface protein (SclB), and SpeB, whereas IgG antibody levels against other streptococcal surface proteins remained unchanged (Fig 1B). Anti-streptolysin O and anti-DNAseB antibody levels on day 21 (of the illness) were elevated.

Streptococcus pyogenes, or Group A *Streptococcus*, is an important human pathogen causing a variety of diseases ranging from mild skin infections like impetigo to life-threatening necrotizing fasciitis and toxic shock-like syndrome. Soft tissue infections caused by *S. pyogenes*, such as erysipelas and cellulitis, are characterized by diffuse spreading of the inflammation in the tissue. The bacterium also causes tonsillitis and following this infection, abscess formation in the peritonsillar and pharyngeal tissues is relatively common. Abscess formation at other sites occurs rarely. Cases of abscesses with *S. pyogenes* have been reported in the brain (6, 7, 9, 17), in the

epidural space (10, 16), in the mediastinum (5), in the lung (8), in the spleen (4), in the retroperitoneum (11), in the pericolic tissue (15), in muscular tissue (1, 3, 18), and in periprosthetic breast tissue (14). Considering how common *S. pyogenes* infections are, abscess formation at other sites than around the tonsils is distinctly uncommon. To our knowledge this is the first reported case with an axillary abscess due to *S. pyogenes.* Though no signs of erysipelas or lymphangitis were present we believe that the bacteria entered through the skin and spread to the axillary lymph nodes.

The complicating venous thrombosis, which drew the attention to the abscess, was probably due to compression of the axillary vein by the abscess. A previous report (3) of an abscess with *S. pyogenes* causing venous thrombosis also implicated vein compression as the pathogenetic mechanism behind thrombosis formation. However, *S. pyogenes* binds many components of the coagulation system and the M41 serotype express SclA and SclB protein that recruits Thrombin-activatable Fibrinolysis Inhibitor (TAFI) to the bacterial surface (13). By serology we could show that SclB was expressed during the infection, which could mediate a more pro-coagulatory state at the site of infection. This molecular mechanism may also have contributed to the thrombosis.

The use of 16S PCR and sequencing was invaluable for correct diagnosis in this case as all cultures were negative. This diagnostic procedure should always be considered in cases where antibiotic treatment has already been commenced. Moreover, DNA extraction from the abscess material made molecular typing of the isolate possible demonstrating that also the presence of for example resistance genes can be detected without culturable bacteria.

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Potential conflicts of interest. All authors: no conflicts.

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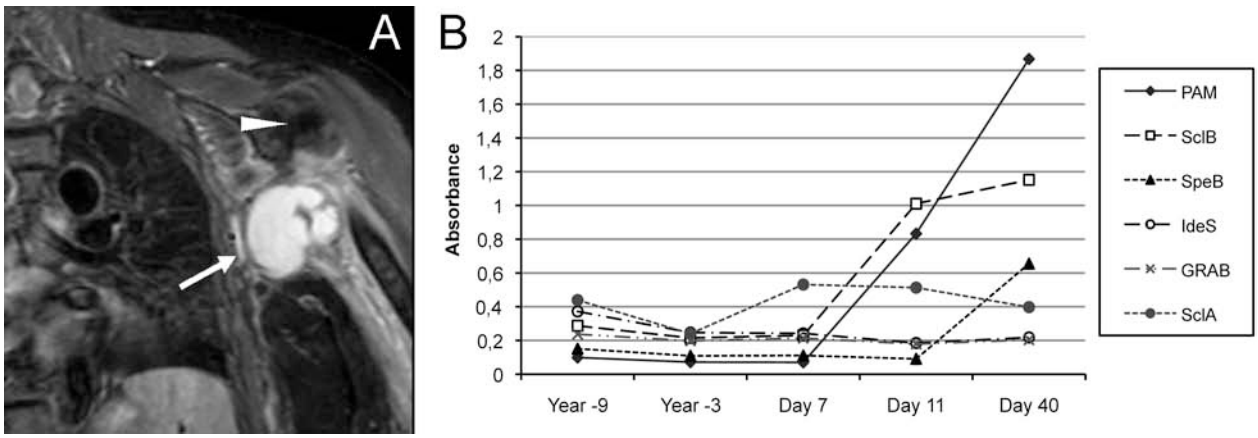
Legends

Figure 1. *S. pyogenes* causing an axillary abscess. **A**. MRI picture, T2 weighted with STIR sequencing, showing the abscess in the left axilla. Arrow indicates the abscess and arrow head indicates caput humeri. **B**. Time course for antibody titers against various streptococcal surface antigens where day 1 is the first day of the illness.

Figure 1.


A
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Absorbance
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Day 40
PAM
SclB
SpeB
IdeS
GRAB
SclA