Axillary Abscess Complicated by Venous Thrombosis: Identification of Streptococcus pyogenes by 16S PCR

Fredrik Kahn,1* Adam Linder,1 Ann Cathrine Petersson,2 Bertil Christensson,1 and Magnus Rasmussen1

Division of Infection Medicine, Department of Clinical Sciences, Lund University, Lund, Sweden,1 and Clinical Microbiology, University and Regional Laboratories, Lund, Sweden2

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

CASE REPORT

A 62-year-old woman presented at our department with a 7-day history of fever, chills, and nausea. She was previously healthy, apart from having atopic 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 admission, she had vomited and suffered from diarrhea. At admission, she had a temperature of 39.5°C. The routine physical examination was normal, except for a slight tenderness upon palpation of 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 21 × 109/liter (her neutrophil count was 19 × 109/liter), a C-reactive protein (CRP) level of 53 mg/liter, and normal renal and liver function test results. Coagulation test results were within normal limits, with a PT(INR) [prothrombin time (international normalized ratio)] of 1.1, an aPTT (activated partial thromboplastin time) of 36 s, and a platelet count of 329 × 109/liter. After two aerobic and two anaerobic blood cultures (BacT/Alert; bioMérieux, Durham, NC) and a urinary culture were obtained, the patient was sent home and 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 was hospitalized. Her body temperature fluctuated between 38.0°C and 39.9°C in the following days. Her white blood cell count was 21 × 109/liter (her neutrophil count was 19 × 109/liter), and her CRP level was 393 mg/liter. 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. A 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 muscular or in the subcutaneous layer and no signs of abscess. A slight improvement occurred over the following days. Repeated blood cultures taken at the time of admission turned out negative. On the 6th 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 done and showed a PT(INR) of 1.1, an aPTT of 40 s, and a platelet count of 430 × 109/liter. Low-molecular-weight heparin and warfarin treatment was initiated. A magnetic resonance imaging (MRI) scan revealed a multilobulated lesion of 7 by 4 by 7 cm in the left axilla approximately 1.5 cm from the skin enclosing the axillary vein with a contrast signal in the periphery and surrounding edema (Fig. 1A). A renewed ultrasonographic examination could visualize the abscess, which was punctured led by a computed tomography 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 PCR amplification of the 16S rRNA gene and subsequently of the emm gene. DNA was extracted from 200 μl of abscess material using Bio Robot EZ-1 with a DNA Tissue kit (Qiagen, Hilden, Germany) after treatment with proteinase K according to the instructions of the manufacturer. Amplification was carried out in a 50-μl reaction mixture containing 1× PCR buffer (Qiagen), 3 mM MgCl2, 200 μM each deoxynucleoside triphosphate, 1.0 U of HotStarTag DNA polymerase (Qiagen), 10 pmol of each primer, and 5 μl of template. P515f (5′-TGGCAGCAGMCGCG CGGTWAT-3′ [12]) and P1067r (5′-AACATYTGCAACRA CA CGAGCT-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 Escherichia coli DNA were included as negative and positive controls, respectively. Both strands of the approximately 520-bp PCR product were sequenced using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems Inc., Foster City, CA) and analyzed on an ABI PRISM 3100
Antibodies directed toward the variable part of the cell wall–attached M protein of *S. pyogenes* are believed to confer serotype-specific protection. Stored serum samples obtained from the patient several years before the present episode were 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′-GCTTAGAAAAATTAAAAACAGG-3′ [*emm for*] and 5′-GC GTTTACAACCTGCTGC-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 3 weeks. The patient had an uncomplicated recovery.

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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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REFERENCES