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A Cold-Blooded Tiptoer: Nonresolving Cellulitis in an Immunocompromised Patient

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Mycobacterium haemophilum is a nontuberculous mycobacteria (NTM) with a predilection for skin and soft tissue infection (SSTI) in the immunocompromised host. We report a case of disseminated *M haemophilum* infection initially presenting as a nonresolving subacute cellulitis of bilateral lower extremities. Genetic sequencing was used for final identification, while a commercially available polymerase chain reaction test returned a false-positive result for *Mycobacterium intracellulare*. Consequently, we highlight the importance of *M haemophilum* as a major differential diagnosis of SSTI in the immunocompromised host and the need for careful interpretation of rapid diagnostic tests.

Keywords. immunocompromised patients; *Mycobacterium haemophilum*; nontuberculous mycobacteria; skin and soft tissue infection.

CASE PRESENTATION

A 53-year-old man with a history of nonischemic cardiomyopathy, end-stage renal disease on hemodialysis, and idiopathic alveolar hemorrhage on 40 mg of prednisolone daily was admitted for heart failure exacerbation. The patient had received prednisolone for 20 months after onset of idiopathic alveolar hemorrhage with a cumulative dose of approximately 17 grams. He had a prolonged hospital course requiring inotrope and continuous renal replacement therapy. Three months after admission, the patient gradually developed left thigh pain, erythema, and swelling without fever. Vancomycin and cefepime were empirically initiated for nosocomial cellulitis. Blood cultures showed no growth. Nonresolving pain, erythema, and swelling extended to the entire left lower extremity and then to the right lower extremity over the course of 2 weeks despite antibiotic therapy (Figure 1). Laboratory findings were only remarkable for slightly increased C-reactive protein level of 0.59 mg/dL (reference range, 0–0.3 mg/dL) and normal white blood cell counts.

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A skin biopsy specimen was subjected to pathological evaluation. Gram stain was negative for bacteria or fungi; however, Ziehl-Neelsen staining of the culture and pathology specimen revealed abundant acid-fast bacilli with Gaffky scale 9 (Figure 2). Polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* (COBAS TaqMan MTB; Roche, Basel, Switzerland) was negative while simultaneous testing for *Mycobacterium intracellulare* (COBAS TaqMan MAI; Roche) yielded weakly positive results (Figure 3). The atypical appearance of the amplification curve prompted the microbiology laboratory and the team to consider the MAC PCR test as a potential false-positive result. The skin sample was sent to a reference laboratory for identification. According to the advice from the reference laboratory, the skin samples were cultured on Ogawa medium (Kyokuto Pharmaceutical Industrial, Tokyo, Japan) and in Mycobacteria Growth Indicator Tube (BD BBL MGIT; Becton Dickinson, Franklin Lakes, New Jersey, USA) at 30°C. A blood sample was submitted for mycobacterial culture (BD BACTEC Myco/F Lytic Culture Vials; Becton Dickinson) in consideration of disseminated nontuberculous mycobacteria (NTM) infection. Subsequently, treatment with amikacin, imipenem, rifampin, ethambutol, and clarithromycin was initiated to encompass both rapid- and slow-growing NTM pending final identification. Because there was no growth on mycobacterial culture after 7 days, we assumed it was a slow glower and discontinued amikacin and imipenem. Subsequent genetic analysis revealed *Mycobacterium haemophilum* with 100% homology based on 16S, hsp65, and rpoB gene sequencing. The antimicrobial treatment was changed to rifampin, ciprofloxacin, and

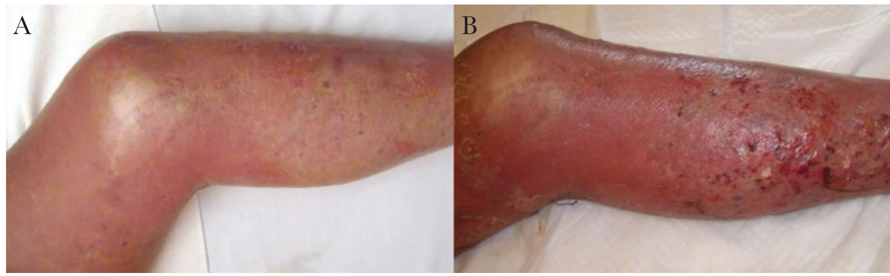

Figure 1. (a) The patient's left lower limb at approximately 2 weeks after the onset of cellulitis symptoms. (b) The left lower limb approximately 2 weeks after (a).

clarithromycin. Despite maximal medical therapy, the hospital course was soon complicated by concomitant *Acinetobacter* bacteremia and worsening cardiac function with multiorgan failure. The patient was eventually transitioned to comfort care and died 1 month after the skin biopsy. The mycobacterial blood culture turned positive after 24 days. In view of *M haemophilum* found in the skin sample, the blood was plated on chocolate agar (Cholate II agar; Beckton Dickson). After 10 days of incubation, the culture showed growth and it was identified as *M haemophilum* using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI Biotyper Version 2.0; Bruker Daltonics, Billerica, Massachusetts, USA) with a score of 1.960. The skin culture completed incubation without heme supplement and showed no growth. The final diagnosis was disseminated *M haemophilum* infection.

Patient Consent

Consent was obtained from the patient's family to publish this case report. The article does not require formal approval by an ethics committee.

DISCUSSION

Nontuberculous mycobacterium skin and soft tissue infections (SSTIs) is a common presentation following respiratory infections [1, 2]. The advent of cosmetic procedures and increased use of immunosuppressive agents have created new risks for acquiring nontuberculosis mycobacteria (NTM) SSTIs [3]. Seven to eighteen percent of non-human immunodeficiency virus (HIV) patients with NTM infection manifest as SSTI [2, 4], and 25% of those were immunocompromised patients receiving systemic glucocorticoids, immunosuppressants, chemotherapeutics, and/or immunomodulators [2]. Several species within NTM, including both rapid-growing mycobacteria and slow-growing mycobacteria, are known to cause SSTIs [5, 6]. The proportion of causative NTM vary depending on previous studies [2, 4, 7, 8]. Common rapid growers in SSTI include the *Mycobacterium fortuitum* group, *Mycobacterium abscessus* group, and *Mycobacterium chelonae*, whereas slow growers include *Mycobacterium marinum*, *Mycobacterium avium* complex, *M haemophilum*, and *Mycobacterium ulcerans* [3].

Mycobacterium haemophilum was the culprit in our patient. First described in 1978, *M haemophilum* was named as a "blood-loving" organism due to its specific requirement for iron or hemin supplementation [9, 10]. Its optimal growth also requires lower temperatures, which explains its predilection to skin and soft tissues of distal body parts [9]. These specific culture requirements make diagnosis difficult [9]. In contrast to cervicofacial lymphadenitis in children, most adult cases involve SSTIs in immunocompromised states including lymphoma, HIV/acquired immune deficiency syndrome (AIDS), and organ transplantation [9]. A case series of *M haemophilum* infections revealed HIV/AIDS as the most common immunocompromising condition, followed by systemic lupus erythematosus [11]. Skin and

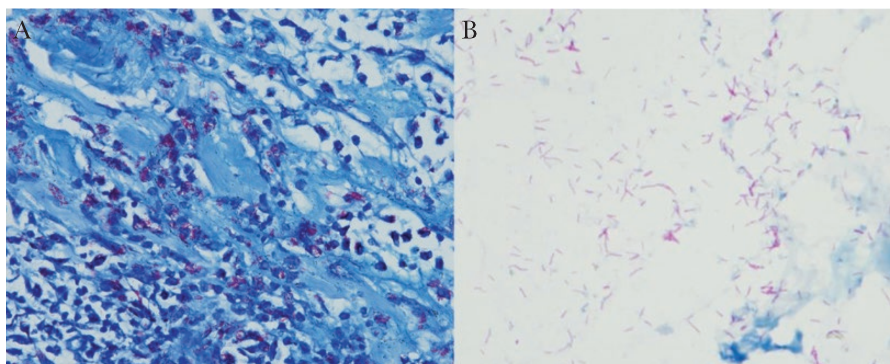

Figure 2. (a) Ziehl-Neelsen stain of skin pathology specimen (objective, ×40). (b) Ziehl-Neelsen stain of pulverized tissue for culture (objective, ×100).

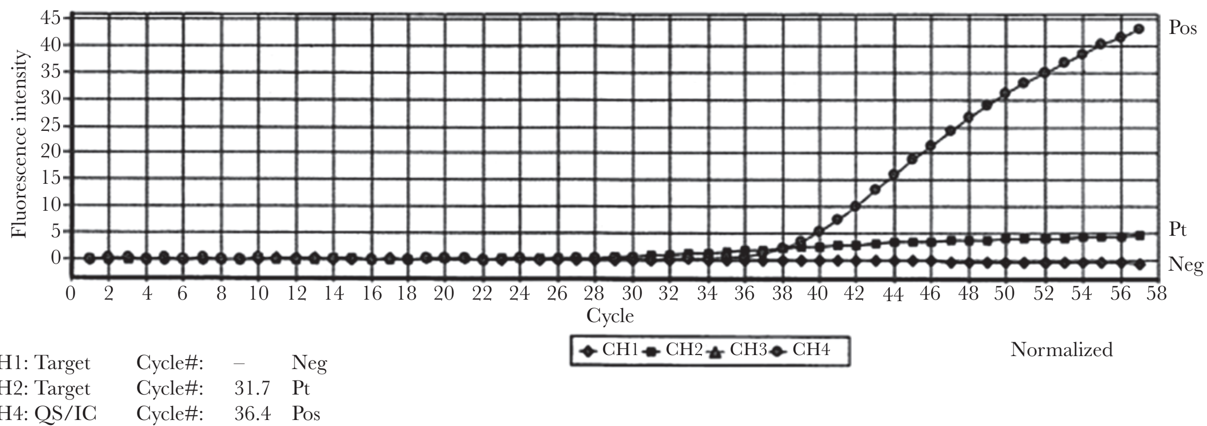

Figure 3. False-positive signals on polymerase chain reaction amplification curves for *Mycobacterium intracellulare* (COBAS TaqMan MAI). Neg, negative control; Pt, patient specimen; Pos, positive control.

soft tissue was the most common site of infection [11, 12]. In particular, erythematous nodules on the extensor surface of elbows, lower extremities, and the auricular region were the most common [11, 12]. Skin biopsy of these regions revealed granulomatous inflammation as the most common pathological finding [11]. Other skin manifestations include erythematous plaques, necrotic abscesses, or chronic ulcers [11, 12]. These lesions tend to develop more frequently on the extremities, particularly over the joints and less commonly on the trunk or face [12]. In addition, past reports have described disseminated infection [11, 12]. Our patient's presentation was concurrent with *M haemophilum* infection including the immunocompromised state and the initial cellulitis presentation.

The optimal diagnostic procedure for *M haemophilum* disease involves acid-fast staining and mycobacterial culturing at 2 temperatures (for instance, 35°C and 30°C), with and without iron supplementation [9, 11]. Preparation of 2 temperatures for mycobacterial culture is also beneficial in isolating *M marinum* and *M ulcerans* because they both exhibit optimal growth at lower temperatures [13]. In addition, concurrent molecular diagnostics, including PCR and sequencing of complete/partial internal transcribed spacer (ITS) regions and 16s rRNA, *rpoB*, and *hsp65*, may be necessary for confirmatory identification [9].

Treatment of *M haemophilum* infections varies across reported cases, and a consensus for interpreting susceptibility patterns is lacking [9]. However, *M haemophilum* is presumed to be resistant to isoniazid and ethambutol, whereas it is most likely susceptible to ciprofloxacin, clarithromycin, rifabutin, and clofazimine, based on in vitro results [9, 14]. In accordance with past literature, experts generally recommend a combination therapy of clarithromycin, ciprofloxacin, and rifamycin [9, 15]. Treatment duration is generally prolonged to several months but is adjusted to clinical response and the degree of underlying immunosuppression [9].

In our case, an initial false-positive result from COBAS TaqMan MAI (Roche) confounded the diagnosis and was a reminder that rapid diagnostics must be interpreted cautiously. Previous reports have identified COBAS AMPLICOR (Roche) and COBAS TaqMan (Roche) showing false-positive tests in cases of *Mycobacterium leprae*, *Mycobacterium lentiflavum*, and *M haemophilum* [16–20]. Although COBAS TaqMan MAI was used in our case, the available rapid diagnostics may vary among countries, and most tests are known to have risk of misidentification [16–30]. For instance, Tortoli [26] et al investigated the specificity of commercially available deoxyribonucleic acid probes and discovered that commonly used AccuProbe (Hologic, Marlborough, Massachusetts, USA) cross-reacted with 9 species, whereas probes targeting *M avium* complex were the most involved. The study further discovered that INNO LiPA Mycobacteria (Innogenetics, Ghent, Belgium) and GenoType Mycobacterium (Hain Lifescience, Nehren, Germany) misidentified 20 and 28 taxa, respectively [26]. Among other commercially available tests, GenoType Mycobacterium-DR version 1.0 (Hain Lifescience) revealed 16 misidentifications as *M intracellulare*, and a recently introduced Speed-oligo assay (Vircell SL, Granada, Spain) have shown that *M marinum*, *Mycobacterium pereprinum*, and *Mycobacterium kansasii* may require more in-depth speciation for accurate identification [27–30].

In contrast to extensive investigations on various commercially available probes, only a handful of misidentification case reports have been described in the literature, as shown in Table 1 [17, 20–25]. Previous case reports revealed that most misidentification cases involved *M avium* complex, and reasons for further identification usually involved discrepancy between rapid diagnostic test and culture results [17, 20, 22–24]. In our case, *M intracellulare* PCR testing was positive, although the validity of the test result was questioned due to an atypical clinical presentation as *M intracellulare* disease and a suboptimal PCR amplification curve (Figure 3). A report on misidentification of *M haemophilum* for *M intracellulare* due to a single base insertion in the bacterial genome also supported our hypothesis for a false-positive test result [20]. The scarcity of case reports on

Table 1. A Literature Review of Case Reports With False-Positive Results From Various Rapid Diagnostic Tests for NTM

Test	Country	Year	Number of Patient/Total	Misidentified as	Confirmed as	Confirmed by	Ref.
COBAS AMPLICOR *Mycobacterium intracellulare* test[a]	Germany	2005	2/2	*M intracellulare*	*Mycobacterium leprae*	16S rRNA *M leprae*-specific proline-rich-antigen gene	[17]
COBAS TaqMan MAI[a]	Japan	2018	1/1	*M intracellulare*	*Mycobacterium haemophilum*	16s rRNA, *rpoB*, *hsp65*	[20]
COBAS TaqMan MAI	Japan	2021	1/1	*M intracellulare*	*M haemophilum*	16s rRNA, *rpoB*, *hsp65*	Our case
COBAS TaqMan MAI + Accuprobe[b]	Japan	2018	1/1	*M intracellulare* *Mycobacterium avium* complex	*Mycobacterium marseillense*	*rpoB*, *hsp65* MALDI-TOF	[21]
Accuprobe	USA	2004	1/1	*M avium complex*	*Mycobacterium saskatchewanense sp. nov.*	16s rRNA/rDNA, ITS, hsp65	[22]
Accuprobe	USA	2017	1/1	*M avium* complex	*Mycobacterium paraense*	16s rDNA, subculture characteristics on solid media	[23]
INNO LiPA Mycobacteria[c]	Netherlands	2019	1/1	*Mycobacterium fortuitum* complex	*Mycobacteriumsmegmatis*	16s rRNA, ITS, hsp65, MALDI-TOF	[24]
Genotype Mycobacterium CM/AS Kit[d]	Italy	2005	1/1	*M intracellulare*	*M marseillense*	*rpoB*, ITS	[25]

Abbreviations: ITS, internal transcribed spacer; MALDI-TOF, matrix-assisted laser desorption/ionization mass spectrometry; NA, no answer; NTM, nontuberculous mycobacteria; rDNA, ribosomal deoxyribonucleic acid; rRNA, ribosomal ribonucleic acid.

[a]Roche.

[b]Hologic.

[c]Innogenetics.

[d]Hain Lifesciences.

misidentification may be a result of overreliance on rapid diagnostics in clinical practice [16–20, 22–24, 26–30].

In summary, NTM SSTI is a significant differential diagnosis especially in immunocompromised patients. In particular, *M haemophilum* is an iron-loving pathogen with a tendency to cause nonresolving skin and soft tissue infections in colder body parts. Diagnosis involves biopsy and culturing at 2 temperatures along with molecular studies as key tools. A good diagnostic strategy is to deploy several modes of identification to prevent misidentification. Hence, good communication among the primary team, dermatology, and infectious disease specialists is crucial in the management of such patients.

Acknowledgments

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CONCLUSIONS

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