中文題目：神秘的隱藏者──多次培養陰性的非分枝結核桿菌 *Mycobacterium haemophilum* 的皮膚軟組織感染

英文題目：Extensively Culture-negative Skin and Soft Tissue Infection Caused by *Mycobacterium haemophilum*—A Case report

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Introduction: Skin and soft tissue infections (SSTI) are common medical problems either in the outpatient department or admission. Patients with SSTI may present with fever, local erythema, tenderess, heatness and swollen. Pyogenic bacteria are the common pathogens. In immunocompromised patients, nontuberculous mycobacterium (NTM) may become pathogens of SSTI. NTM can be divided into rapid growth and slow growth. About cutaneous mycobacterium infection, *M. marinum* and *M. abscessus* were most commonly reported. We reported a rare cutaneous infection caused by *M. haemophilum*.

Case Presentation: A 93-year-old male with the underlying diseases of, 1. chronic kidney disease stage IV, 2. coronary artery disease status post percutaneous coronary intervention with stents implantation, 3. hypertensive cardiovascular disease, 4. bullous pemphigoid was referred from dermatology outpatient department due to multiple progressive erythematous nodules and pus formation at his left lower limb for two months.

Initially, the patient was under regular oral prednisolone treatment for his bullous pemphigoid. Three months later, some erythematous, tender nodules appeared at his left lower limb, accompanied with bloody pus and peripheral edema. He did not have fever or other systemic symptoms, and there was neither insect bites or trauma over skin lesions. Dermatologic examination revealed vesicles, ulcers, and subcutaneous nodules on his left lower limb, particularly on the dorsal side. Twice skin biopsy revealed no specific finding about bacterial, fungal or mycobacterila culture. Panniculitis and inflamed granulation tissue were found. Gram Stain showed abundant polymorphonuclear leukocyte and some Gram-positive bacillus (GPB). Mycobacterium infection was impressed. Rifampicin, azithromycin and ciprofloxacin were prescribed. However, deterioration of his skin lesions were noted over the course of 4 weeks anti-NTM treatment. Repeated Ziehl-Neelsen's staining of left leg pus showed Acid-fast stain (AFS) 1+, but mycobacterium culture were negative for two times. Due to poor progression of his left leg, he was admitted to our infection ward.

After admission, rifampicin was switched to tigecycline, azithromycin and ciprofloxacin were maintained for infection control. Laboratory findings were only remarkable for predominant neutrophilic white blood cell counts (85.3%) and elevated E.S.R: 77/1 hr (reference range, 0-15mm/1hr), high sensitive C-reactive protein 3.71 (reference range, <0.1mg/dL). Bradycardia and

ECG with prolonged QTc ever occurred in the hospitalization, we held Azithromycin and Ciprofloxacin and added Moxifloxacin. However, one acute decompensated heart failure episode forced us to hold Moxifloxacin. Only Tigecycline was maintained until improved condition of cardiac wheezing. In the third week, oral Baktar was prescribed plus Tigecycline. We carefully held tigecycline, baktar and resumed azithromycin, rifampicin, and added doxycycline step by step. The patient was discharged with oral azithromycin, rifampicin, and doxycycline on the 28th hospital day. His left foot wounds improved a lot in hospitalization after antibiotic treatment and wound care.

Repeated subcutaneous abscess still devloped at OPD and AFS of pus were positive, even up to 4+ and 2+ but mycobacterial cultures were still negative. Leprosy was excluded by PCR. We kept antibiotic treatment. Finally, NTM-PCR from large volume of subcutaneous abscess revealed *M. haemophilum*. After that, we adjusted regimen to rifabutin, azithromycin and doxycycline. His leg lesion gradually improved without further abscess formation.

Discussion: Cutaneous mycobacterial infection has variant clinical manifestations including, cellulitis, nonhealing ulcers, nodular lesions, abscesses, superficial lymphadenitis, verrucous lesions, and other different findings. Skin biopsies of cutaneous lesions are the cornerstone of diagnosis to identify acid-fast staining bacilli and culture. For immunocompromised patients, *M. kansasii*, *M. avium-intracellulare* complex, and *M. haemophilum* may cause cutaneous or disseminated disease. Recent databases of NTM infection, seven to eighteen of non-human immunodeficiency virus (HIV) infected patients present with SSTI, and 25% of those were immunocompromised patients, receiving systemic glucocorticoids and immunosuppressants. Most adult cases with *M. haemophilum* involved SSTIs and had immunocompromised states including lymphoma, HIV/acquired immune deficiency syndrome (AIDS), and organ transplantation.

Mycobacterium haemophilum requires fastidious growth conditions including lower temperatures and iron or hemin supplemented agar. Without appropiate culture media and temperature, it is difficult to identify. Clinicians need to communicate with lab staffs when suspecting *M. haemophilum* infection. Due to slow growth and favoring low temperatures, it may explain its predilection to skin and soft tissues of distal body parts.

The diagnostic procedures for *M. haemophilum* diseases involve acid-fast staining and mycobacterial culturing at 2 temperatures (35°C and 30°C), with and without iron supplementation. It is beneficial for us to distinguish *M. marinum* and *M. ulcerans* because these two NTM do not grow under low temperature, compared to *M. haemophilum.* 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.

Conclusion: NTM SSTI is a significant differential diagnosis especially in immunocompromised patients. Therefore, if AFS is positive but no finding of extensive general Mycobacterium cultures, some other ubiquitous NTM organisms with specific growth criterias must need to be considered. In

particular, *M. haemophilum* is an iron-loving pathogen to cause skin and soft tissue infections in colder body parts. This cold-blooded lover involves biopsy and culturing at 2 temperatures (for example, 30℃ and 35℃), special agar medium and molecular studies as key tools for diagnosis. Several approachs of identification are to prevent misidentification or delay diagnosis.