

[CASE REPORT]

Disseminated Cutaneous Infection of *Mycobacterium colombiense* in a Patient with Myelodysplastic Syndrome

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Abstract:

Mycobacterium colombiense (*M. colombiense*) is a member of the *Mycobacterium avium* complex (MAC). To our knowledge, this is the third case report of an *M. colombiense* infection. An 80-year-old man, immunocompromised by myelodysplastic syndrome (MDS), developed a skin rash with exfoliation and eruption on his face and scalp. Mycobacteria were detected in pus samples. Broad-range polymerase chain reaction (PCR) revealed the mycobacteria to be *M. colombiense*. The lesions resolved after daily administration of rifampicin, ethambutol, and clarithromycin. In conclusion, broad-range PCR identified this rare mycobacterium, allowing for the administration of appropriate combination antibiotic therapy.

Key words: broad-range polymerase chain reaction, immunocompromised host, *Mycobacterium avium* complex, nontuberculous mycobacteria

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Introduction

Nontuberculous mycobacteria (NTM) exist in various environments, including soil and water, and as commensals on the skin (1). They can cause opportunistic infections in immunocompromised patients. The progression of NTM infection depends on many factors, including the mycobacterial species, the degree and route of exposure, and the immune status of the patient. NTM accidentally transmitted by percutaneous penetration can lead to infection of other organs, such as lymph nodes and the genitourinary system. *Mycobacterium colombiense* (*M. colombiense*) is an NTM that forms a part of the *Mycobacterium avium* complex (MAC) (2). Infection with *M. colombiense* is very rare. We herein report the third known case of *M. colombiense* infection in an 80-year-old man with myelodysplastic syndrome (MDS).

Case Report

In June 2006, an 80-year-old man on medication for hypertension and diabetes mellitus visited our hospital with a complaint of a systemic skin rash. His white blood cell

count was 14.9×10^9 cells/L, comprising 80% neutrophils and 10% eosinophils. Allergic dermatitis was suspected. Two months later, a pruritic rash developed on his limbs and trunk, sparing his face. He was diagnosed with eczematous dermatitis and was treated with a nonsteroidal ointment. After a short admission to our hospital for the treatment of *Enterobacter* pleuropneumonia, a skin biopsy was performed because the rash had worsened. The pathologic diagnosis was superficial and deep perivascular dermatitis with multinucleated giant cells. The findings resembled tuberculosis, but no mycobacteria were detected. After a recurrent episode of mild pneumonia, laboratory signs of inflammation persisted.

In December 2006, he developed rubbery, hard, axillary and inguinal lymphadenopathy, although no specific findings other than reactive changes were detected on a needle biopsy specimen. In January 2007, an open biopsy of an axillary lymph node revealed pyogenic lymphadenopathy. The patient experienced progressive exhaustion and anorexia. During this period, neutrophil-dominant leukocytosis and normocytic anemia were detected. The patient's white blood cell count and hemoglobin level were 19.2×10^9 cells/L and 106 g/L, respectively, but his platelet count was normal. The examination of a bone marrow aspirate showed normal kary-

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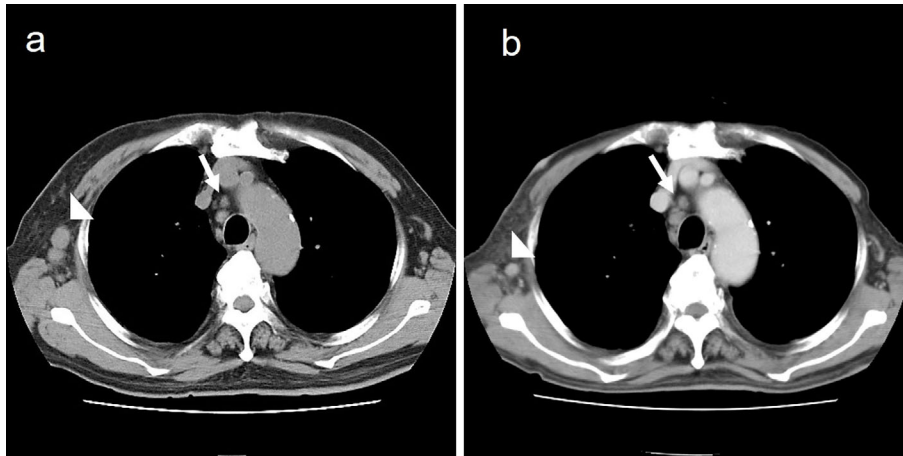


Figure 1. The right axillary lymph nodes decreased in size after treatment with combination therapy (a, b) (arrow heads), but the mediastinal lymph nodes enlarged (a, b) (arrows).

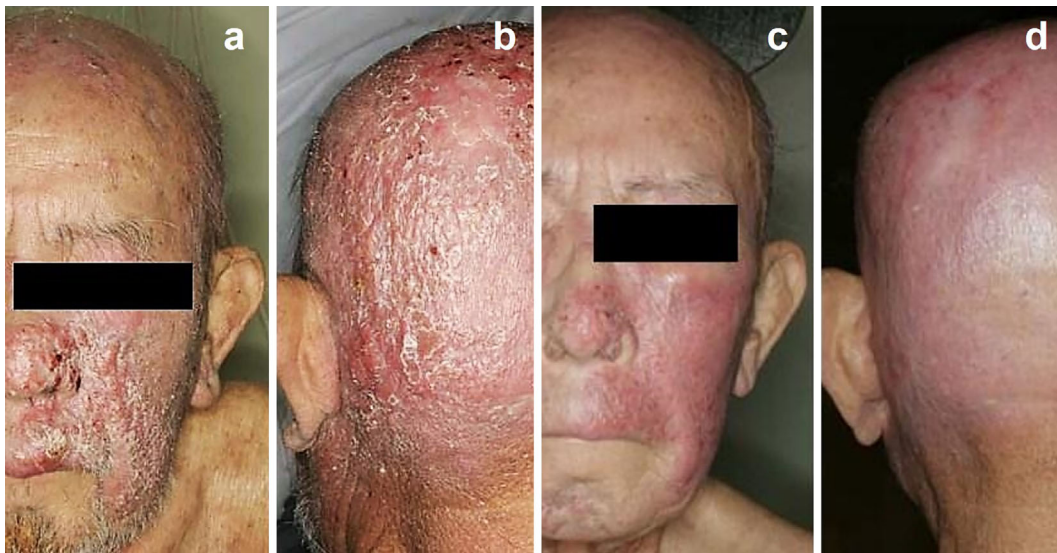


Figure 2. The skin lesion on the patient's face and scalp before (a, b) and after (c, d) treatment with combination therapy; the improvement after treatment is demonstrated.

otypes with no specific findings. These results led to the suspicion of chronic granulocytosis and recurrent opportunistic infections due to a functional deficiency of neutrophils.

In March 2007, a repeat biopsy of the patient's right inguinal lymph nodes showed dermatopathic lymphadenopathy. Prednisolone (10 mg/day) was prescribed as anti-inflammatory therapy. Repeated skin biopsies showed nodular dermatitis with a predominance of neutrophils. At this time, no mycobacteria were detected on sputum culture; only commensal oral bacteria, *Haemophilus parahaemolyticus* and *Haemophilus parainfluenzae*, were cultured. His superficial lymphadenopathy slowly subsided. Subsequently, the recurrent opportunistic infections, including pneumonia and dermatitis, again strongly suggested a functional deficiency of neutrophils. The examination of a repeat bone marrow aspirate revealed hypocellular marrow and mature hypersegmented granulocytes, indicative of differentiation arrest of neutrophils due to MDS; the patient's MDS was

classified as refractory anemia with a normal karyotype.

In July 2008, cyclosporine (50 mg/day) was prescribed as immunosuppressive therapy for hematopoietic deficiency secondary to MDS. Computed tomography (CT) of the chest demonstrated pleural thickening and enlargement of the right axillary and mediastinal lymph nodes (Fig. 1a).

In October 2008, a skin biopsy was repeated because of a symptomatic skin rash. The results suggested acute generalized exanthematous pustulosis. The administration of clarithromycin and minocycline was effective.

In July 2009, an impetiginous rash with hard exfoliation and eruption appeared on his scalp and face (Fig. 2a, b). In addition, the granulocytosis and anemia had worsened. Repeat CT demonstrated that the size of the right axillary lymphadenopathy had decreased to 10 mm in diameter, while the multiple mediastinal lymph nodes had enlarged (Fig. 1b). Throughout the patient's clinical course, no specific bacteria, including mycobacteria, were detected in any

Table. Comparison of Characteristics of the Members of *Mycobacterium avium* Complex.

Characteristic	<i>Mycobacterium colombiense</i>	<i>Mycobacterium avium</i>	<i>Mycobacterium intracellulare</i>	<i>Mycobacterium chimaera</i>
AccuProbe test:				
<i>M. avium</i>	(+) / (-)	(+)	(-)	(-)
<i>M. intracellulare</i>	(-)	(-)	(+)	(+)
Catalase activity:				
Room temperature	(+)	unknown	(-)	unknown
70°C	(+)	(+)	(-)	(+)
Urease	(+)	(-)	(-)	(-)
Growth temperature:				
Room temperature	(+)	(+) / (-)	(+)	(+)
45°C	(-)	(-)	(+) / (-)	(-)

samples collected from the patient, including blood, sputum, and cutaneous abscess pus samples. However, in July 2009, mycobacteria were detected in a pus sample from a right axillary lymph node. They appeared as red bacilli without villi on Ziehl-Neelsen staining. On Ogawa agar, wet yellow colonies formed within approximately two weeks. Nontuberculous mycobacterial infection was diagnosed. In an attempt to obtain a specific diagnosis, broad-range polymerase chain reaction (PCR) was performed on the *hsp65* gene and the 16S rRNA gene at the Department of Microbiology Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine. The 398 bp sequence of the *hsp65* gene was a 99% match to that of *M. colombiense* (394/398 bp) according to the GeneBank database CIP 108962. The 16S rRNA gene was also compared, and the 1,473 bp sequence was a 99.8% (1,470/1,473) match to that of the strain. We therefore identified the organism as *M. colombiense*.

The patient was prescribed combination antibiotic therapy consisting of 450 mg rifampicin, 750 mg ethambutol, and 800 mg clarithromycin; his skin lesions improved dramatically with this treatment (Fig. 2c, d). Although the mediastinal lymph nodes did not shrink, they did display a normal hilar lymph node structure. We presumed that the mediastinal lymphadenopathy was either infectious or a combination of infectious and reactive lymphadenopathy. The pathogenesis of the mediastinal and axillary lymphadenopathy was most likely different, thereby explaining the difference in the efficacy of anti-mycobacterial therapy between these two regions. The combination therapy was continued for 11 months.

After 11 months, the patient developed a drug rash caused by rifampicin; the administration of rifampicin was therefore stopped. In August 2010, *M. colombiense* was detected in a skin biopsy specimen taken from his left digitus annularis; the skin lesion was thus recognized as being a symptom of disseminated *M. colombiense* infection. The impetiginous skin rash and eruption followed a fluctuating course of improvement and deterioration. Clarithromycin and ethambutol were therefore prescribed for about six years. In March

2013, the prescription of ethambutol was cancelled because of optic neuritis; thereafter, the exanthematous pustulosis relapsed. Ethambutol was thus re-prescribed, with consequent improvement in the exanthematous pustulosis. The patient had repeated episodes of bronchopneumonia as opportunistic infections due to the functional neutrophil deficiency. Antibacterial therapy, including ceftriaxone or levofloxacin, was prescribed for these episodes, with consequent clinical improvement. However, in September 2015 the patient developed lobar pneumonia with progressive respiratory failure and died.

Discussion

NTM are aerobic bacteria that grow at approximately 37°C. They are categorized into Groups I-IV according to Runyon's classification (3). *M. colombiense* belongs to group III (nonchromogens). MAC is another group of non-chromogens. It is divided into *Mycobacterium avium* subspecies, such as *Mycobacterium paratuberculosis*, *Mycobacterium silvaticum*, and *Mycobacterium hominissuis*. These subspecies are now identified by the molecular approach of DNA typing, as it is difficult to identify the subspecies of NTM using classical biologic methods that involve culture or the histochemical Ziehl-Neelsen stain.

M. colombiense is a slow-growing mycobacterium. Morphologically, it resembles an acid-fast rod without villi. It forms non-pigmented rough colonies within 3 weeks when cultured between 20 and 37°C on Ogawa-Kudoh agar, Sauton agar, or Lowenstein-Jensen agar. It does not grow on MacConkey agar. It is negative for niacin production, acid-phosphatase activity, and Tween hydrolysis and positive for catalase activity at 20°C. This strain can be differentiated from other MAC species by its ability to produce urease, as shown in Table (3). High-performance liquid chromatography is also useful for differentiating this strain from other species of MAC (4). In addition, PCR is useful for the identification of MAC species. *M. colombiense* and *M. avium* both show a positive result on the AccuProbe MAC identification test; thus, a more specific identification method is

needed. The *hsp65* gene polymorphism analysis (PRA) pattern and the 16S rRNA gene are unique to *M. colombiense*; the latter is considered the gold standard molecular marker. DNA hybridization is also available for differentiating *M. colombiense* from other species of MAC (4, 5).

M. colombiense was first isolated and identified in 2006 from the blood and sputum samples of a patient in Colombia who was infected with human immunodeficiency virus (HIV) (2). The second case was identified by the examination of the lymph nodes of a three-year-old girl in Spain who developed cervical lymphadenopathy (6). The present case is the third reported case of symptomatic infection with this strain. Immunocompromised patients often develop NTM infection as an opportunistic infection (7). In this case, the patient had functional deficiency of neutrophils secondary to MDS. The patient in the first case had developed acquired immunodeficiency syndrome (AIDS) (2). However, the second case, the three-year-old child, had no indications of immunodeficiency (6). The possibility of NTM infection must be borne in mind in patients presenting with an opportunistic infection. In immunocompromised patients, the inhalation of aerosolized MAC is the major route of infection, and systemic infection through pneumonia tends to progress to severe disease. However, in healthy patients, ingestion or direct invasion through skin injury can be the route of infection, with development of local lymphadenopathy (6, 8, 9). The patient presented in this case developed disseminated cutaneous infection without a proven lung infection. The functional deficiency of neutrophils secondary to MDS probably underlay the complicated infection. The prognosis of patients with local infection, such as lymphadenopathy or cutaneous infection, is good (6, 8, 9). In contrast, the prognosis of immunocompromised patients with systemic infection is reported to be very poor (2, 10).

A pathologic examination of the cutaneous lesions of *M. colombiense* infection reveals rings of epithelial cells, giant cells, and caseous necrosis; altered leukocytes are not seen. Ring-like enhancement is the typical radiographic appearance of the lymphadenopathy caused by NTM infection on contrast-enhanced CT. In the present case, such enhancement was absent, and a pathologic examination of the lymph node biopsy specimens did not detect any specific findings of giant cells or caseous necrosis. We therefore hypothesize that the mediastinal lymphadenopathy might have been due to reactive lymphadenitis.

Cases of NTM infection must be treated intensively. Combination therapy, including clarithromycin, ethambutol, rifampicin, isoniazid, and azithromycin, is reported to be effective (7). Monotherapy using single antibiotics is not recommended for the treatment of *Mycobacterium avium-intracellulare*, including *M. colombiense*, because drug resistance can develop rapidly. Macrolides are specifically recommended against as monotherapy for this reason (11). A multidrug antibiotic chemotherapy regimen of 250-500 mg clarithromycin twice daily, 15 mg/kg ethambutol daily, and 600 mg rifampicin daily is recommended (7). However,

there is no consensus about the anti-mycobacterial regimen for *M. colombiense* infection nor the optimum duration of therapy. We followed the guideline for current systemic MAC infection that recommends continuing anti-mycobacterial therapy for at least six months. Isoniazid, rifampicin, and ethambutol were prescribed based on the use of this combination to treat the first case of systemic *M. colombiense* infection (2). Clarithromycin, rifampicin, and pyrazinamide were prescribed for the second case, but the antibiotic therapy was suspended due to digestive tract intolerance. Surgical excision of the pathologic lymph node, including the affected skin, was therefore performed in that case (6).

In the case report of systemic *M. colombiense* infection in a patient with acquired immunodeficiency (10), *M. colombiense* was revealed to be susceptible to clarithromycin [minimum inhibitory concentration (MIC), 0.25 mg/L], rifampin (MIC, 4 mg/L), and ethambutol (MIC, >16 mg/L). The report suggested that clarithromycin and rifampin were expected to be effective against *M. colombiense*. In another case of disseminated *M. colombiense* skin infection in an immunocompetent patient, the patient was treated with clarithromycin 500 mg/day, rifabutin 750 mg/day, and moxifloxacin 300 mg/day for 3 months (9). The skin lesions improved greatly on this regimen. As stated above, combination therapy that includes clarithromycin, rifampicin, and ethambutol is expected to be effective.

In the present case, we examined the drug sensitivity using the micro-titer method. The strain showed resistance to isoniazid and ethambutol and sensitivity to streptomycin, rifampicin, and kanamycin. Drug sensitivity testing for MAC is not established, except for clarithromycin (11). Other drugs are not effective against MAC as monotherapy, so drug sensitivity testing is not recommended in clinical practice; we performed drug sensitivity testing for reference purposes. We prescribed a combination of 450 mg rifampicin, 750 mg ethambutol, and 800 mg clarithromycin per day, according to the standard recommended therapy for MAC (7); this treatment was effective.

In summary, we diagnosed and managed a case of a *M. colombiense* disseminated cutaneous infection in an immunocompromised host with functional deficiency of neutrophils due to MDS. Infection with NTM frequently results in pneumonia and/or pulmonary disease; however, in this case, it resulted in multiple cutaneous lesions. A skin biopsy was performed several times, but the definitive diagnosis was difficult to establish. A repeated biopsy of the lesions is usually required to confirm the diagnosis of cutaneous NTM infection.

Correct identification of the mycobacterial strain acting as the infectious pathogen is important for deciding on an effective treatment. An analysis of the 16S rRNA gene and the *hsp65* gene by broad-range PCR is a more effective and rapid means of identifying *M. colombiense* than the AccuProbe test.

Conclusion

In conclusion, we herein reported the third known case of *M. colombiense* infection, a recently described species of mycobacterium. Molecular diagnostic methods can aid in the identification of the less-common NTM species that may cause persistent infection.

The authors state that they have no Conflict of Interest (COI).

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