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Manifestations of cutaneous mycobacterial infections in patients with inborn errors of IL-12/IL-23-IFNγ immunity

Background: Inborn errors of IL-12/IL-23-IFNγ immunity underlie Mendelian susceptibility to mycobacterial diseases (MSMD), a group of immunodeficiencies characterized by a highly selective susceptibility to weakly virulent strains of mycobacteria, such as non-tuberculous mycobacteria (NTM) and *bacillus Calmette-Guérin* (BCG). Cutaneous mycobacterial infections are common in MSMD and may represent a red flag for this immunodeficiency. *Objectives:* We present a case series of four paediatric patients with MSMD, specifically with IFNγR1 and STAT1 deficiencies, and cutaneous NTM/BCG infections to increase awareness of this immunodeficiency, which may, in some cases, be intercepted by the dermatologist and thus timely referred to the immunologist. *Materials & Methods*: Clinical, laboratory and genetic investigations of the four paediatric patients with MSMD are presented. *Results:* All four presented patients experienced early complications after BCG vaccination. Two patients suffered recurrent mycobacteriosis, one patient experienced delayed BCG reactivation, and one patient died of disseminated avian mycobacteriosis. The dermatological manifestation in these patients included destructive nasal ulcerations, scrofuloderma of various sites and lupus vulgaris. All patients had a normal basic immune phenotype. *Conclusion:* The presented cases demonstrate that NTM/BCG infections in otherwise seemingly immunocompetent patients should raise suspicion of MSMD. This is of utmost importance as specific therapeutic approaches, such as IFNγ treatment or haematopoietic stem cell transplantation, may be employed to improve the disease outcome.

Keywords: MSMD, mendelian susceptibility to mycobacterial diseases, IFNγR1, STAT1, inborn error of immunity, non-tuberculous mycobacteria, BCG, necrotizing granulomas, antituberculotics

Mendelian susceptibility to mycobacterial disease (MSMD) is an inborn error of immunity due to various monogenic defects in interleukin-(IL) 12/IL-23 - interferon gamma (IFNγ)-mediated communication pathway between mononuclear phagocytes and type 1 helper T cells. To date, several hundreds of patients have been described worldwide to carry one of the 17 known mutations in genes involved in IFNγ production (*e.g. IL12RB1, IL12RB2, IL23R, ISG15, RORC*), responsive to IFN*γ* (*e.g. IFNGR1, IFNGR2, STAT1, JAK1, CYBB*), or both (*IRF8, NEMO*) [1, 2]. Patients typically suffer from selectively increased susceptibility to mycobacteria, particularly to weakly virulent non-tuberculous mycobacteria (NTM) and attenuated vaccination strains of bacillus *Calmette-Guérin* (BCG), and some also display increased susceptibility to non-typhoid salmonellae or viruses, particularly *Herpesviridae* family. Other antimicrobial defences, however, remain undisturbed and basic parameters of humoral and cellular immune functions are usually normal. MSMD typically manifests in childhood, particularly in infants who receive the BCG vaccine, but may present later in adolescence/adulthood. The clinical phenotypes range from mild adverse reactions to the BCG vaccine (*i.e.*, vaccination site-limited BCGitis with regional lymphadenopathy), to recurrent NTM lymphadenitis, osteomyelitis, parenchymatous organs and skin infections, such as in patients with partial signal transducer and activator of transcription (STAT1) or partial IFNγ receptor 1 (IFNγR1) deficiency, to treatment-resistant, life-threatening disseminated mycobacteriosis, such as in complete IFNγR1 deficiency or complete IL-12 receptor beta 1 (IL12Rβ1) deficiency [2, 3]. The diagnosis of MSMD is established by genetic testing and genetic counselling is available to the affected families.

NTM are ubiquitous, opportunistic organisms found globally in soil, water reservoirs and vegetation. In general, the weakly virulent NTM may cause localized or disseminated infections, including skin and soft tissue

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infections, taking an acute or chronic course. The most frequent NTM are rapidly growing mycobacteria, such as *Mycobacterium fortuitum, Mycobacterium marinum, Mycobacterium abscessus, Mycobacterium chelonae* and *Mycobacterium ulcerans*, and slowly growing NMT, such as *Mycobacterium avium complex*, comprising *Mycobacterium avium* and *Mycobacterium intracellulare*. The typical underlying predispositions in immunocompetent patients are traumatic or surgical wounds which become soiled with contaminated materials [4]. The cutaneous manifestations of NTM and BCG are heterogeneous, including papular lesions, subcutaneous nodules, cellulitis, abscesses, draining sinuses, ulcerations, scrofuloderma or lupus vulgaris [5, 6]. For an early diagnosis of NTM infection, it is necessary to maintain a high degree of suspicion in patients with chronic cutaneous diseases with a history of trauma, risk exposure, and negative results from conventional microbiological studies, as well as those with a history of adverse events after BCG vaccination. Laboratory findings are usually non-specific with just mildly elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and often normal full blood count; more profound disturbances accompany more severe or disseminated infections. Approaches based on recall immune response, such as IFNγ release assays (IGRA) and intradermal skin testing are also employed for their differential diagnostic value. Specific histochemical acid-fast staining (AFS) methods (Ziehl-Neelsen, auramine-rhodamine fluorescent stain), immunohistochemistry and real-time polymerase chain reaction (PCR) are used for early detection and differentiation of mycobacteria from biopsy or smear specimens. Histopathology is characterized by the presence of necrotising epithelioid granuloma formed by activated macrophages, multinucleated giant cells (predominantly of Langhans type), and CD4+ T cells under the influence of various cytokines, mainly IL-12 and IFNγ [7]. Nevertheless, the mainstay of NTM diagnosis is based on culture studies. Cultivation of mycobacteria requires long-term incubation in special rich media (*e.g.*, Löwenstein-Jensen, Middlebrook 7h10, Ogawa MB/BACT), often as long as six to nine weeks, and inactivation of rapidly growing microorganisms, whose growth impedes the observation of mycobacterial colonies [8, 9]. Treatment of NTM/BCG includes a combination of various antimicrobial agents, second-line antituberculotics are often used due to the natural antibiotic resistance of NTM [10].

In the presented case series, four patients with MSMD and cutaneous mycobacterial infections are portrayed, highlighting the tell-tale signs of the disease. Two patients harboured distinct mutations in genes encoding IFNγ receptor and two in genes encoding STAT1 protein. STAT1 is a cytoplasmic transcription factor that becomes activated in response to interferons, including IFNγ, inducing the expression of multiple genes involved in antimycobacterial immunity [2, 3]. The complex data of the cohort are summarized in *table 1*.

Materials and methods

The data were collected from retrospective analysis of patients' documentation and from interviews with patients/guardians and attending physicians.

Case series

Patient 1: Destructive nasal lesion due to Mycobacterium marinum in partial STAT1 deficiency

The first patient was a 16-year-old Caucasian girl with a history of poor vaccination site healing and axillary lymphadenitis after a BCG vaccine. Since childhood, she has been suffering from cutaneous herpetic reactivations, typically affecting the periocular area, but otherwise she was healthy and thriving. At 14 years of age, a serosanguinous nasal discharge and mucosal crusts obturating the nasal cavity started appearing. Based on a suspected bacterial infection, despite repeatedly negative cultures, topical antibiotics were applied, which were all ineffective. Three months after initial symptoms, an intercurrent herpes simplex infection exacerbated the local disease, with crusts and ulcers expanding further outwards on the tip of the nose (*figure 1A*) and worsening over time (*figure 1B*). Treatment with orally administered acyclovir and antibiotics was ineffective. A comprehensive laboratory workup, including haematological and immunological investigations and oncologic screening, were normal. The intralesional skin biopsy showed a pattern of specific inflammation, *i.e.*, centrally necrotizing granulomas with multinucleated giant cells (*figure 2B*). Suspecting an NTM infection, Ogawa medium was used to culture the samples, yielding *M. marinum* (*figure 2A, C*). The patient disclosed being a keen aquarist, keeping fish in a home aquarium. The combined antituberculous regimen resulted in complete healing after three months (*figure 1C*). Genetic testing revealed a novel, heterozygous mutation in *STAT1* (c.2071A > G; p.Met691Val). Functional assays revealed decreased, but not abolished IFNγ-induced STAT1 phosphorylation, confirming the partial loss-of-function effect of the mutation (data available from authors upon request). The father, who carries the same mutation, suffers only from frequent, yet mild viral respiratory tract infections.

Patient 2: Lupus vulgaris at the site of BCG vaccination due to partial STAT1 deficiency

The second patient was a 12-year-old Caucasian boy, who experienced complications at the site of BCG inoculation at three months of age, requiring a surgical drainage of the colliquated axillary lymph node (samples were AFS-positive and PCR-negative for NTM). Two nodular lesions developed on the shoulder and regressed after six months of treatment with isoniazid alone. Afterwards, the patient was lost to follow-up, supposedly healthy until six years of age when he acquired *varicella zoster virus* (VZV). The otherwise uncomplicated VZV infection (in the VZV unvaccinated child) coincided with a culture positive reactivation for *M. bovis BCG* at the vaccination site, which presented initially as several papulonodular eruptions that merged into a large lupus vulgaris-like elevated erythemato-squamous annular plaque with a well demarcated serpiginous border (*figure 3A*). The lesion biopsy specimen was AFS-negative and PCR-negative for NTM, however, granuloma formation with multinucleated giant cells was found (*figure 3C*). A combined antituber-

Table 1. The characteristics of MSMD patients.

Pt number	Mutation	Disease	Affected family members	Consanquinity	Age at first manifestation	Age at diagnosis of MSMD/year	Clinical manifestation of NTM	Aetiology	Other infections	Histology	Cellular immunity	Humoral immunity	Therapy	Outcome
1	Heterozygous *STAT1* (c.2071A>G; p.M691V)*	Autosomal dominant partial STAT1 deficiency	Father (48 years) carrier of c.2071A>G, increased viral susceptibility, no mycobacterial infections	No	3 months	16 years/2021	BCGitis, destructive lesion of the nose	*M. bovis BCG, M. marinum*	HSV	Cutaneous biopsy from nose: centrally necrotizing granulomas with multinucleated giant cells	Normal	Normal	Moxifloxacin, rifabutin, clarithromycin	Alive
2	Heterozygous *STAT1* (c.1921G>A; p.A641T)	Autosomal dominant partial STAT1 deficiency	Father (48 years) and two patients female siblings (adults) - healthy carriers of c.1921G>A; paternal grandfather with suspected BCGitis	No	3 months	6 years/2016	BCGitis, lupus vulgaris	*M. bovis BCG*	VZV	Cutaneous biopsy from shoulder: granulomas with multinucleated giant cells and partial central necrosis, scarification, mixed inflammatory cellularization; AFS and NTM PCR-negative	Normal	Normal	Isoniasid, rifampicin, ethambutol, local ointments with streptomycin	Alive
3	Heterozygous *IFNGR1* (microdeletion 818del4)	Autosomal dominant partial IFNγR1 deficiency	None	No	3 months	4 years/2013	Disseminted BCGitis, multifocal osteomyelitis with scrofuloderma, recurrent lymphadenitis, multiorgan NTM dissemination	*M. bovis BCG, M. avium, M. abscessus*	Rotavirus, SARS-CoV-2	Biopsy from the skull: specific granulomatous inflammation; AFS and NTM PCR-positive	Normal	Normal	Isoniazid, rifampicin, cycloserin, clarithromycin, amicacin, azithromycin, interferon gamma	Alive
4	Homozygous *IFNGR1* (c.523del; p.Tyr175fs)	Autosomal recessive complete IFNγR1 deficiency	Several unexplained deaths of siblings in infancy	Yes	1 month	3 years/2010	Disseminated BCGitis, multifocal osteomyelitis with scrofuloderma, recurrent lymphadenitis, multiorgan NTM dissemination	*M. bovis BCG, M. avium - intercellulare*	VZV	Biopsy from spleen: non-specific inflammatory process of red pulp, suppurative and fibroproliferative changes in the splenic hilum, no granuloma formation	Normal	Elevated IgG (IgG1, IgG2), otherwise normal	Isoniasid, rifampicin, ethambutol, pyrazinamid, streptomycin, moxifloxacin, amicacin, cycloserin, clofazimin, linesolid	Died at 13 years

**Mutation previously not reported.Cellular immunity = peripheral blood total neutrophil, eosinophil and lymphocyte count; enumeration of lymphocytes subsets CD3+, CD3+4+, CD3+CD8+, CD19+, CD3-CD16+56+; dihydrorhodamine or nitroblue tetrazolium test for oxidative burst.Humoral immunity = IgG, IgG1-4, IgA, IgM, classic/alternative complement pathway activation.AFS: acid-fast stain; HSV: herpes simplex virus; NTM: non-tuberculous mycobacteria; RT-PCR: real-time polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome-related coronavirus 2; VZV: varicella-zoster virus.*

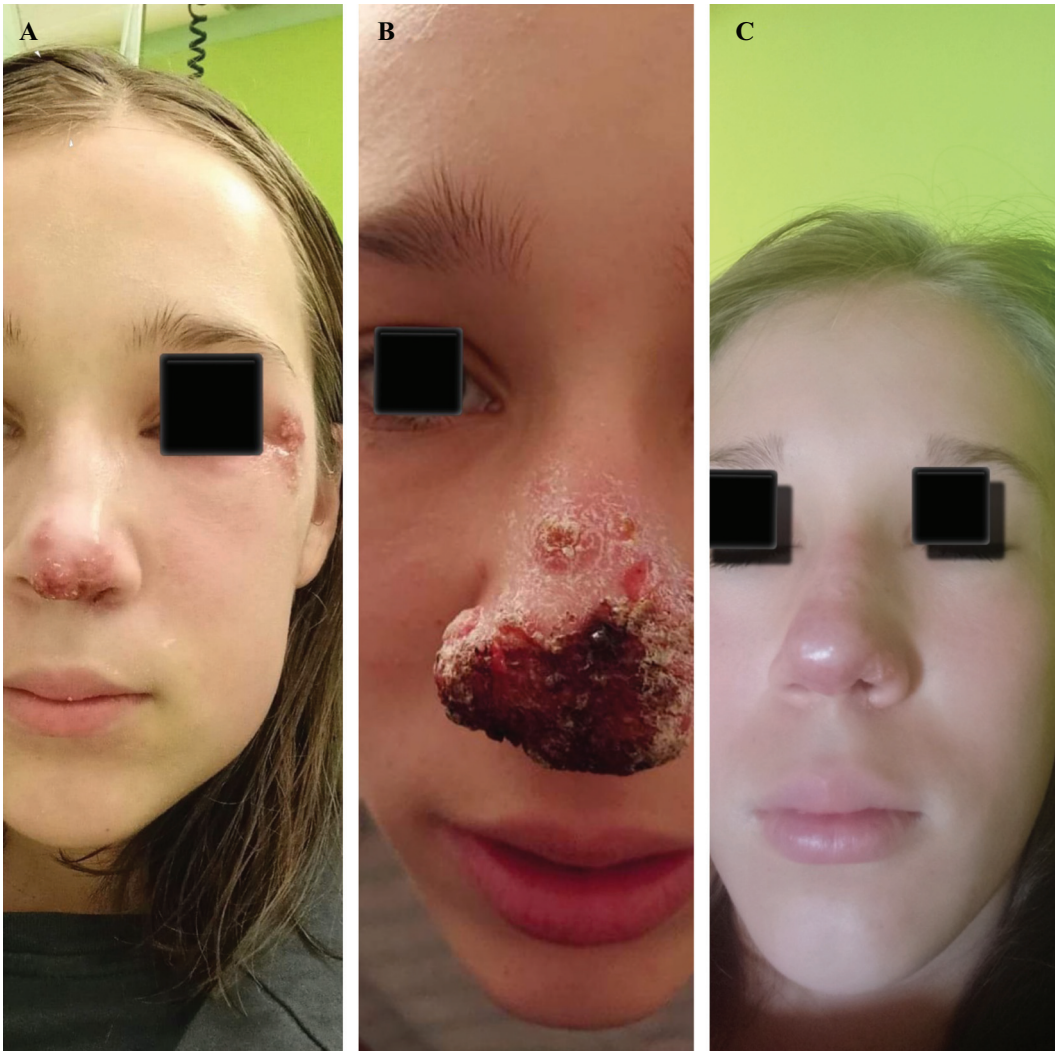

Figure 1. A 16-year-old female with *Mycobacterium marinum* infection due to partial STAT1 deficiency. **A**) Periocular herpes simplex infection and the incipient nasal lesion. **B**) Swelling and ulcerations of the tip of the nose with haemorrhagic crust. **C**) Healing after three months of combined antituberculous therapy.

culous regimen and local antituberculous ointment with streptomycin was administered for 14 months; the lesion eventually healed with an atrophic scar (*figure 3B*). Since then, the child has been healthy. Genetic testing confirmed a heterozygous mutation in *STAT1* (c.1921G > A; p.Ala641Thr). The mutation was also found in the patient's father and the patient's two adult sisters, all of whom received a BCG vaccine without any complications and remain healthy. The patient's paternal grandfather, unavailable for testing, reportedly suffered with severe BCGitis in infancy.

Patient 3: Multifocal NTM mycobacteriosis with scrofuloderma due to partial IFNγ receptor 1 deficiency

The third patient was a 12-year-old Caucasian girl presenting in infancy with suppurative inflammation at the BCG inoculation site, followed by axillary lymphadenitis and scrofuloderma as a contiguous extension of the infection from the lymph node into the overlying skin (*figure 4A, B*). Further investigations revealed lesions in the lungs and a markedly positive tuberculin skin test (35 mm/72 hours; normal range for a BCG-vaccinated person: 5-10 mm). The lymph node biopsy showed specific granulomatous inflammation with positivity for AFS and PCR-positive NTM, and cultures were positive for *M. bovis BCG*. The total 21 months of combined antituberculous therapy achieved slow but complete remission. At four years of age, the patient returned with non-tender cervical lymphadenitis, multifocal osteomyelitis of the skull (*figure 4C*) (extending per continuitatem to the cutaneous structures) and femur (*figure 4E*), and lesions in the spleen and lungs, as diagnosed by whole-body PET/CT (*figure 4D*). *M. avium* was cultured from the lesion on the skull, but only poorly formed granulomas with incipient central necrosis were presented in the biopsy specimen, despite the PCR-positive NTM and the presence of AFS bacilli. All immunological, haematological and oncological investigations were normal. The family reported keeping a parrot in the household. Suspecting a disturbed IL-12/IL-23-IFNγ axis, the diagnosis

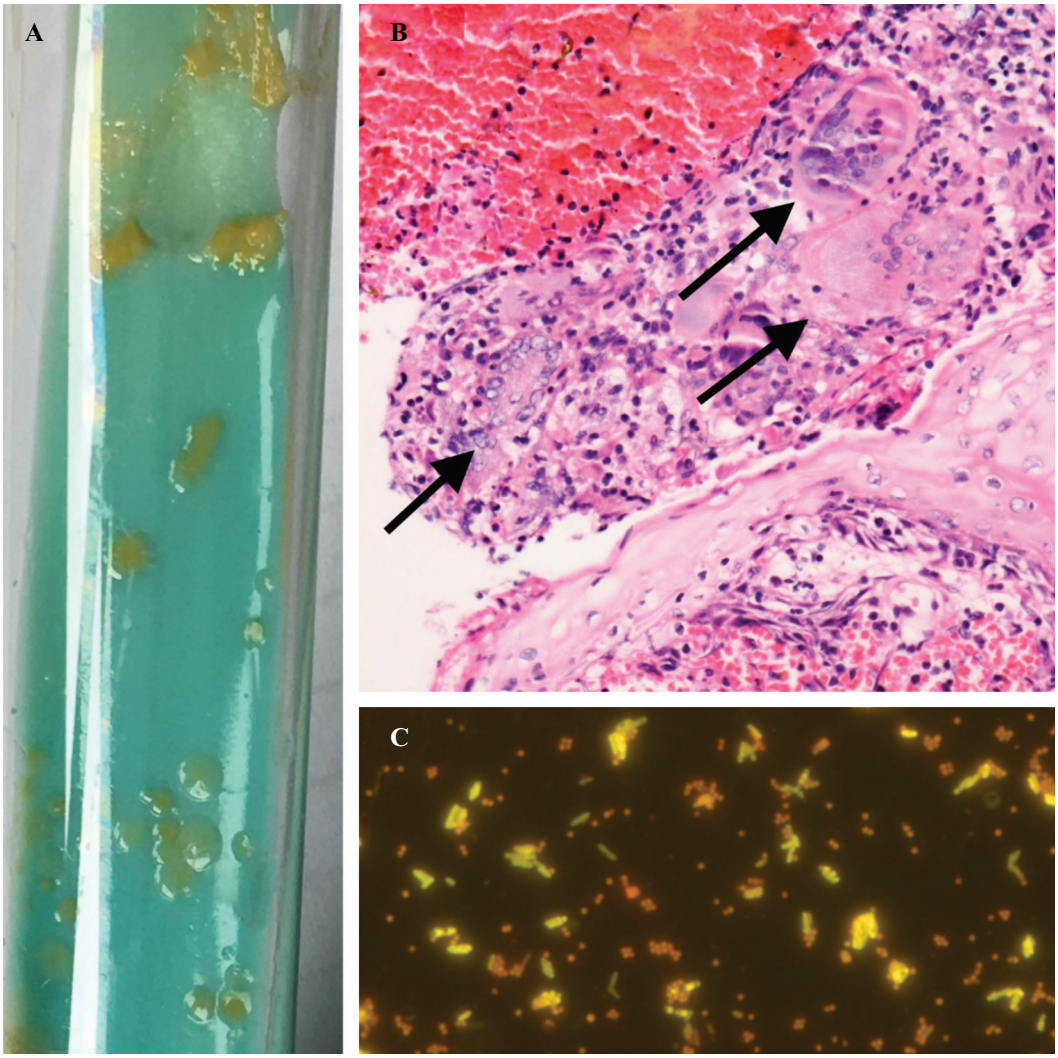

Figure 2. Laboratory evidence of *M. marinum* infection in a patient with partial STAT1 deficiency. **A**) Culture *of M. marinum* on Ogawa medium (image courtesy of Marie Mikulasova MD, Laboratory for Clinical Microbiology and Parasitology, Hospital Ceske Budejovice, Czech Republic). **B**) Granuloma formation with multinucleated giant cells (arrows) from a cutaneous nasal biopsy (H&E staining; 400x magnification) (image courtesy of Marek Grega MD, Department of Pathology and Molecular Medicine, 2nd Faculty of Medicine, University Hospital Motol, Prague, Czech Republic). **C**) Visualization of *M. marinum* by fluorescence microscopy using auramine-rhodamine staining (1000x magnification) showing the presence of contaminating staphylococci (image courtesy of Marie Mikulasova MD, Laboratory for Clinical Microbiology and Parasitology, Hospital Ceske Budejovice, Czech Republic).

of MSMD was established upon the detection of a *de novo* heterozygous microdeletion, 818del4, in *IFNGR1*; a small deletion hotspot region causing a partial defect of the R1 subunit of IFNγ receptor (IFNγR1). Along with multiple antituberculotics, recombinant IFNγ was initiated, allowing slow but complete healing (*figure 4C*). Three years later, at seven years of age, shortly after IFNγ withdrawal, *M. abscessus-immunogenum* cervical lymphadenitis was diagnosed. Another combined regimen with second-line antituberculotics and adjuvant IFNγ was started, again with a slow but favourable outcome after three years of treatment. At 12 years of age, the patient contracted severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which manifested with low-grade fever and mild, self-limited respiratory symptoms.

Patient 4: Scrofuloderma in the thorax and fatal disseminated NTM due to complete IFNyR1 deficiency

The fourth patient was a 13-year-old boy from healthy, consanguineous Roma parents. Within the first weeks of life, he developed BCGitis at the vaccination site and axillary lymphadenopathy, which spontaneously drained externally, forming a large scrofuloderma. Despite three months of isoniazid treatment, the lymph node had to be eventually surgically removed. *M. bovis BCG* was cultured from the tissue sample. Two weeks after the discontinuation of antituberculous therapy, fevers and generalized lymphadenopathy appeared and multiple abscesses were detected in the enlarged spleen. The splenic tissue displayed signs of a

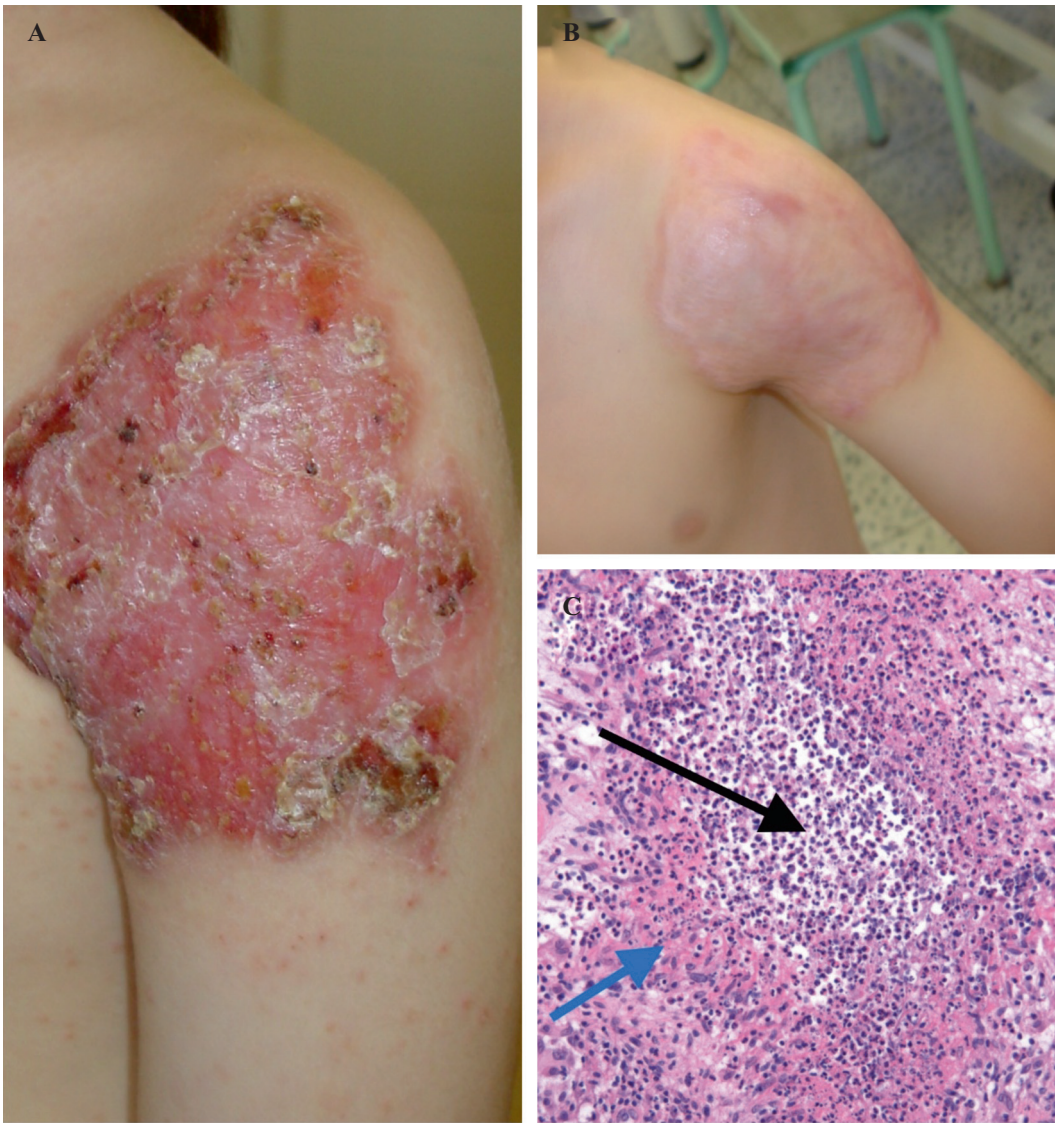

Figure 3. A 12-year-old male with *Mycobacterium bovis BCG* reactivation due to partial STAT1 deficiency. **A**) Lupus vulgaris at the site of BCG vaccination presenting as well-demarcated plastic annular erythemato-squamous plaques with serpiginous edges (aged six years). **B**) Healing with an atrophic scar after 14 months of antituberculous treatment. **C**) Histology of the lesion showing necrotizing granuloma formation; the blue arrow indicates epitheloid macrophages and the black arrow indicates central necrosis (H&E staining; 200x magnification).

non-specific inflammatory process of the red pulp and suppurative and fibroproliferative changes in the splenic hilum, but a surprising absence of granuloma formation. Since the microbiological findings were negative, the tentative diagnosis of disseminated BCG infection was established and a four-drug regimen was continued for 18 months. Again, shortly after therapy cessation, the patient suffered with *M. bovis BCG* osteomyelitis and multifocal suppurative lymphadenopathy. He received continuous antimicrobial treatment, consisting of as many as seven antituberculotics at a time. Despite this, multiple osteolytic lesions of the knee, vertebrae and ribs, and multifocal lymphadenopathy associated with spikes of fever and increased inflammatory markers (particularly ESR, CRP, leukocytosis and thrombocytosis) kept appearing. Subsequently, the infection progressed in a flare-up/regress manner, affecting, per continuitatem, the adjacent pleura and soft tissues of the thorax, eventually draining through the skin, forming a well-demarcated *M. avium-intracellulare*-positive scrofuloderma (*figure 5 A,B*). At 13 years of age, the VZV unvaccinated patient acquired VZV, complicated with severe immune thrombocytopenia requiring high-dose intravenous immunoglobulin treatment. Six months later, he died due to overwhelming multiorgan dissemination of *M. avium.* Haematopoietic stem cell transplantation was refused by the parents. The consanguinity, absence of susceptibility to other infectious, failure of granuloma formation, as well as negative results of extensive immune phenotyping (excepting the elevated serum IgG) suggested MSMD. At three years of age, a homozygous mutation in the gene encoding IFNy receptor subunit 1 (c.523del;p.Tyr175fs) was found, establishing the diagnosis of autosomal recessive complete IFNyR1 deficiency. The parents are healthy heterozygous carriers of the mutation. Additionally, several unexplained infant deaths

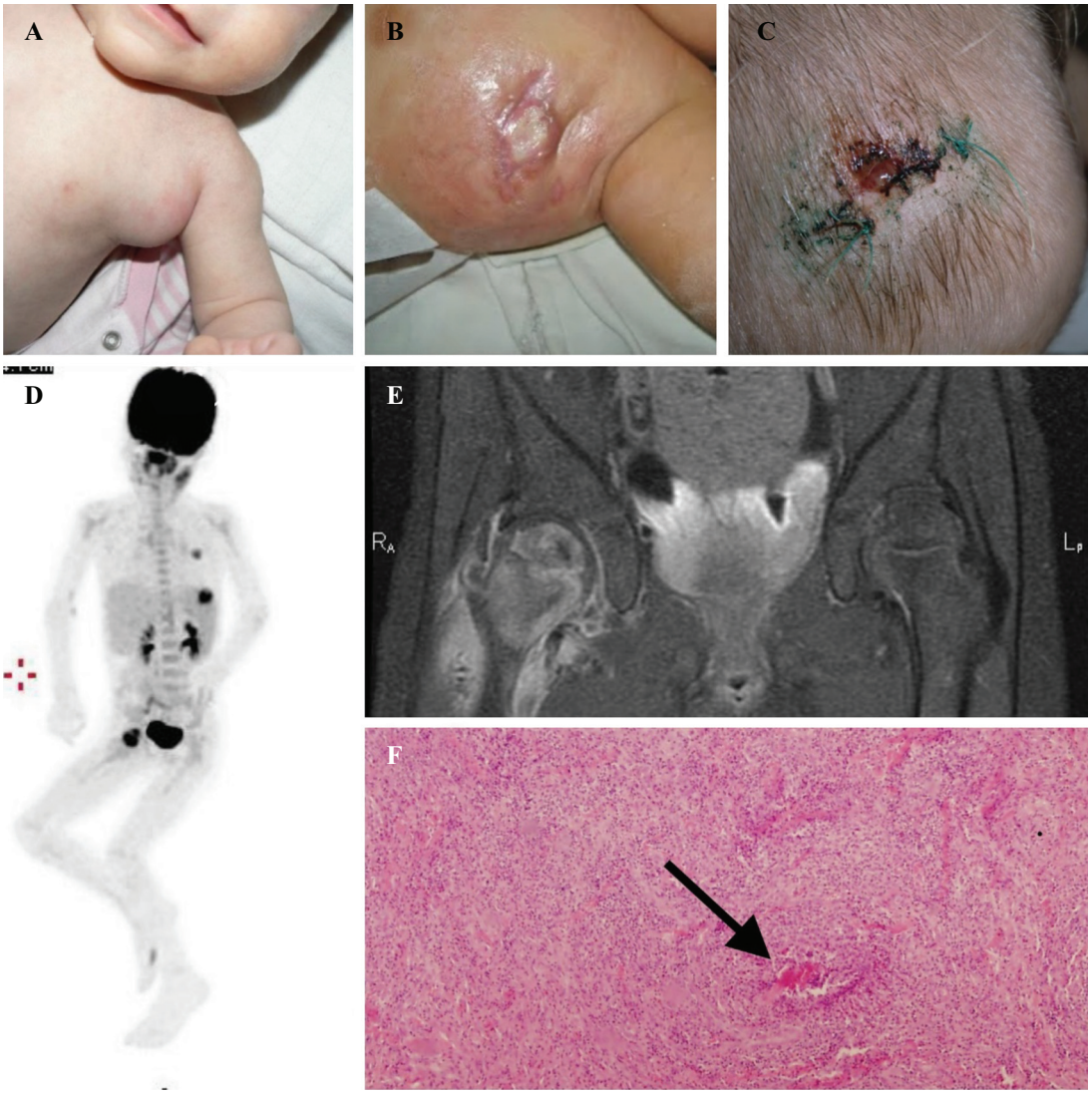

Figure 4. A 12-year-old female with recurrent NTM infections due to partial IFNγR1 deficiency. **A**) Axillary BCG lymphadenitis (aged three months). **B**) Scrofuloderma of the axillary region (aged four months). **C**) Delayed postoperative wound healing of the skull (aged four years). **D**) Whole-body PET/CT with F-18 fludeoxyglucose (FDG) showing anterior projection of increased FDG activity in the cervical lymph nodes, spleen, left lung and right hip (aged four years). **E**) MRI of the pelvis showing osteomyelitis of the right proximal femur, abscess formation in the femoral head, and adjacent soft tissue oedema (aged four years). **F**) Poorly formed granuloma with incipient central necrosis (H&E staining; 200x magnification) (sample was taken from a lesion on the skull; image courtesy of Blanka Rosova MD, Department of Pathology and Molecular Medicine, 3rd Faculty of Medicine, Thomayer University Hospital, Prague, Czech Republic).

within this family were reported, suggestive of disease penetrance in those affected.

Discussion

The presented case series portrays the heterogeneity of cutaneous manifestations of infections with weakly virulent mycobacteria in children with disturbed antimycobacterial defences. These may, in general, arise from both acquired immunodeficiencies (*e.g.*, HIV infection, iatrogenic immunosuppression, treatment with biological agents such as tumour necrosis factor alpha blockers and anti-IL-12/23 monoclonal antibodies, presence of anti-IFNγ autoantibodies) and inborn immunodeficiencies [11, 12]. The latter include defects in various aspects of cellular immunity, for example, severe combined immunodeficiency (SCID), combined or predominantly T cell, NK cell and phagocytic defects [13]. However, in addition to mycobacteria, these entities convey susceptibility to a wider range of pathogens. Contrastingly, MSMD renders patients selectively susceptible to weakly virulent mycobacteria. All four presented MSMD patients suffered early complications of BCG vaccination and consecutive NTM/BCG infections, yet, with the exception of recurrent or complicated herpetic infections in Patient 1 and 4, no clinical signs of disturbed antimicrobial defences were detectable. All patients had normal results of basic immune investigations, except for Patient 4, who had elevated serum IgG, likely as a result of chronic inflammation. Such clinical settings should alert the physician to

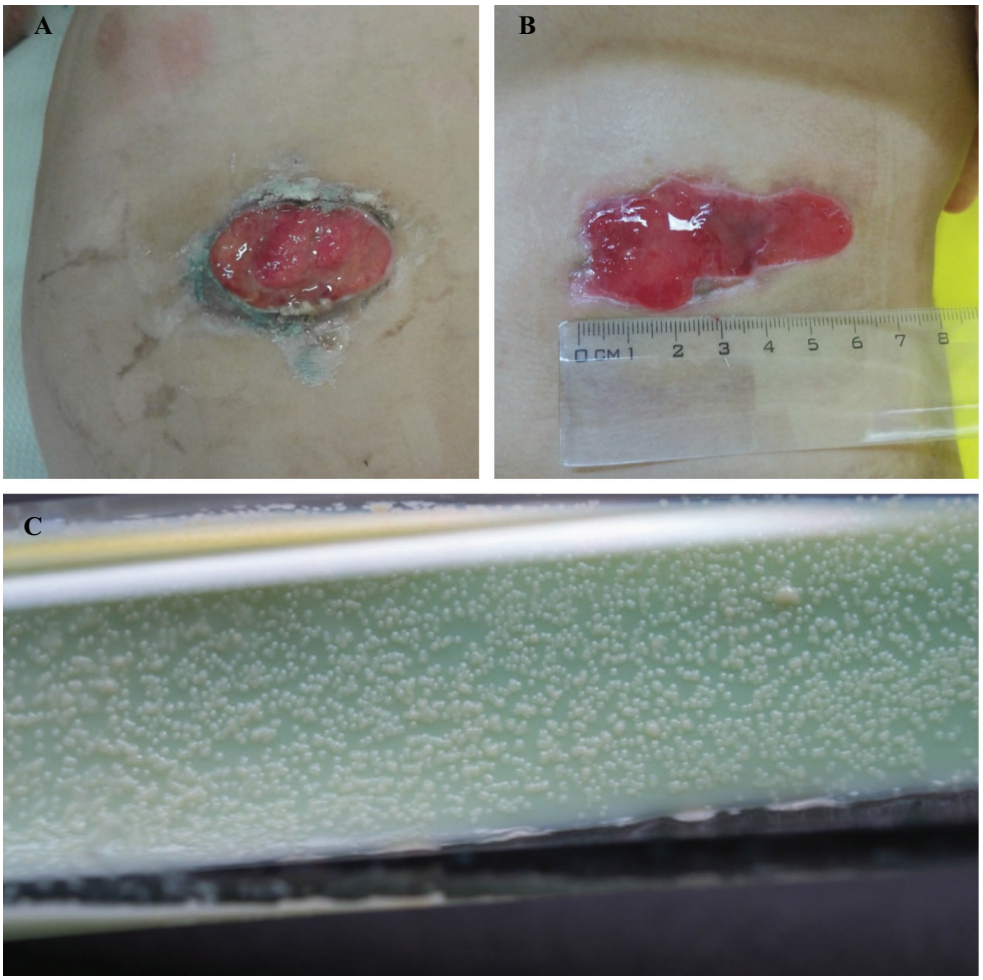

Figure 5. A 13-year-old male with scrofuloderma in the thorax and fatal disseminated NTM due to complete IFNyR1 deficiency **A**, **B**) *M. avium-intracellulare* scrofuloderma due to IFNyR1 deficiency. **C**) Growth of *M. avium* on solid culture.

MSMD. Other warning signs of MSMD may include consanguinity (such as in Patient 4), a history of post-BCG vaccine complications/NTM infections in family members (such as in Patient 2), poorly formed or absence of granulomas in histopathological specimens (such as in Patient 3 and 4), or failure to respond to stimulation in IFNγ-release assays [14]. MSMD may arise from *de novo* mutation or follow autosomal dominant, recessive or X-linked inheritance traits [1, 2]. Given the relatively well-established genotype-phenotype correlation, genetic counselling is an important management tool, yet may be somewhat challenging due to the phenomena of incomplete penetrance and variable expressivity [1, 15] (as seen in the families of Patients 1 and 2). An early diagnosis is of utmost importance, as specific therapeutic approaches may be offered. In patients with MSMD, treatment with antituberculotics is prolonged and may be, in some cases, augmented by subcutaneous administration of human recombinant IFNγ (such as in Patient 3) [2]. In severe patients with a complete lack of signalling, hematopoietic stem cell transplantation was shown to be a curative option, alas with a high mortality and graft rejection rate [16]. Mycobacteria, with over 170 species identified, represent frequently encountered human pathogens [17]. While the classic tuberculosis, caused by *M. tuberculosis*, is still a globally important infection, its incidence in developed countries is decreasing. Conversely, infections with NTM are on the rise. According to Wenworth *et al.,* the incidence of cutaneous NTM infections increased nearly three-fold during the period 1980-2009 in Minnesota [18]. As such, NTM infection should be considered in the case of any unexplained indolent or suppurative inflammatory process with negative routine bacterial cultures. As NTM often present with cutaneous and soft tissue manifestations, the dermatologist may play a critical role in the diagnosis. The most common clinical manifestation of NTM in childhood is unilateral cervical lymphadenitis caused by *M. avium* [19]. This condition usually affects immunocompetent infants, who have not received the BCG vaccine. In most cases, surgical extirpation of the inflamed lymph node alone is therapeutically sufficient. In contrast, *M. avium* infections in patients with advanced immunosuppression or specific immune defects, such as MSMD, may take on a severe or even life-threatening course, with disseminated disease and systemic symptoms [2, 13]. *M. marinum* infections are typically associated with exposure to water from fish tanks, swimming pools, or brackish water, and may arise even in immunocompetent persons. They typically present as nodular lymphangitis affecting the upper extremities, while nasal localization is scarce. The lesions are non-tender but may erode or ulcerate. They usually respond

well to combined antituberculotic regimens [20]. The extent and atypical localisation of the lesion, as well as the poor healing at the BCG vaccination site, were the key indicators of underlying immunodeficiency in Patient 1. Infections are due to rapidly growing NTM, *i.e*., *M. fortuitum* affects mostly immunocompetent patients, and are usually associated with plastic surgery and cosmetic procedures. The common presentation is a solitary painful lesion, such as an erythematous nodule, ulcer or abscess, or cellulitis, which appears four to six weeks after inoculation. Similarly, *M. chelonae* and *M. abscessus* present as localized cellulitis or abscesses, typically affecting the extremities at surgical or catheter sites, or as multiple erythematous draining nodules in immunocompromised patients [4, 5].

The diagnosis and targeted treatment of NTM infection relies mostly on culture results. Good communication between the clinician and the microbiologist is therefore essential for the selection of suitable culture media. Moreover, six to nine weeks must be allowed for the incubation time of mycobacterial culture. Histopathological assessment would typically show the formation of specific necrotizing epithelioid granulomas with either caseous or necrobiotic types of necrosis and the presence of tissue-resident macrophages; multinucleated giant cells [21]. Traditional staining for acid-fast bacilli and auramine-rhodamine fluorescent methods may ascertain the presence of mycobacteria, however, they cannot distinguish between individual species. While immunohistochemical staining and real-time PCR-based methods would differentiate between *Mycobacterium tuberculosis* and NTM infections, these methods show limited sensitivity. For specimens obtained by fine needle aspiration biopsy from lymph nodes, the sensitivity is approximately 70% [22], for paraffin-embedded tissue, this is even lower [23]. IFNγ release assays performed on peripheral blood, widely utilized for *M. tuberculosis* infections, have shown good specificity for distinguishing *M. tuberculosis* from NTM with no cross-reactivity with BCG and most NTM. Mycobacterial skin testing for antigens specific to *M. avium*, *M. kansasii* and *M. scrofulaceum,* if available*,* may also indirectly indicate the presence of NTM infection, with sensitivity and specificity as high as 93%, and 97%, respectively, for *M. avium* cervical lymphadenitis [24].

The treatment of NTM infection consists of a combination of first-line and second-line antituberculous drugs, antibiotics and/or surgical removal of the affected tissue [10]. The selection of antimicrobial drugs should be governed by national guidelines and individual antibiotic sensitivity to the offending pathogen, accounting for the naturally broad multi-drug resistance of NTM.

Conclusion

The diagnosis of weakly virulent mycobacterial infection requires a high level of clinical suspicion and specific microbiological approaches. The cutaneous manifestations of infections with these organisms in otherwise seemingly immunocompetent patients, localisation other than distal extremities, multi-site affections and repeated occurrence should raise a suspicion of Mendelian susceptibility to mycobacterial diseases and the patient should consult an immunologist. The dermatologist may thus facilitate early diagnosis and improved disease outcome, allowing specific therapeutic approaches to be considered, such as IFNγ treatment or hematopoietic stem cell transplantation. ■

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