CLINICAL CASE

NF-kappa-B essential modulator (NEMO) gene polymorphism in an adult woman with systemic lupus erythematosus and recurrent non-tuberculous mycobacterial disseminated infections

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ABSTRACT
Infections are among the most serious complications in patients with systemic lupus erythematosus (SLE), with bacterial and viral infections being the most common. Non-tuberculous mycobacterial (NTM) infections are quite rare and are typically seen in older patients with SLE with longstanding disease duration treated with corticosteroids. Here, we describe a 39-year-old woman with SLE and an unusual pattern of recurrent NTM disseminated infections. After excluding the presence of autoantibodies against interferon-γ, whole exome sequencing revealed a homozygous polymorphism in the NF-kappa-B essential modulator (NEMO) gene. Primary immunodeficiencies should be included in the differential diagnosis of patients with recurrent opportunistic infections, even in those with iatrogenic immunosuppression.

INTRODUCTION
Almost 25% of patients with systemic lupus erythematosus (SLE) develop infections due to intrinsic immunological abnormalities and iatrogenic immunosuppression.1 Disease activity is an independent risk factor for infections with their pattern being similar to the general population. As such, respiratory, urinary tract and skin and soft tissue (SST) infections are the most common. Moreover, patients with SLE have an increased risk of uncommon yet fatal opportunistic infections.2 Infections from tuberculous and non-tuberculous mycobacteria (NTM) in SLE are quite rare.2 Patients with systemic lupus erythematosus (SLE), however recurrent non-tuberculous mycobacterial infections are quite rare.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Infections are common in patients with systemic lupus erythematosus (SLE), however recurrent non-tuberculous mycobacterial infections are quite rare.

WHAT THIS STUDY ADDS
⇒ Genetic polymorphisms, such as in the NEMO (NF-kappa-B essential modulator) gene, contribute to the susceptibility in non-tuberculous mycobacteria.
⇒ Whole exome sequencing provides a valuable tool in the study of inherited immunodeficiencies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY
⇒ The recognition of atypical infectious patterns with rare pathogens in patients with SLE should prompt thorough work-up for non-iatrogenic causes of susceptibility to infections.

Herein, we present a woman with SLE and recurrent infections by rare NTM pathogens.

Case presentation
A 39-year-old woman presented with 1 month of persistent fever. Her medical history was significant for lupus nephritis and secondary antiphospholipid syndrome. She had received cyclophosphamide and at that time she was on methylprednisolone (16 mg/day), and hydroxychloroquine.

She had episodes of bacteremias and fungemias with various bacteria (Pseudomonas aeruginosa, Nocardia spp, Bacillus cereus, Chryseobacterium indologenes, Sphingomonas paucimobilis) and fungi (Candida parapsilosis, Fusarium chlamydosporum). Despite broad-spectrum antimicrobial and antifungal therapy she...
remained febrile and developed pulmonary nodules in chest CT and nodular skin lesions. Peripheral blood cultures and PCR from skin biopsy returned positive for *Mycobacterium gordoniae*. She was treated with rifampicin 600 mg once a day, clarithromycin 500 mg two times a day, ethambutol 1500 mg once a day and levofloxacin 750 mg once a day, with subsequent improvement and was discharged with a 12-month treatment plan that was completed without complications.

Twenty-one months after the initial presentation, she presented again with fever and cough. Chest CT revealed ground glass opacities with subpleural nodules. Blood cultures were positive for *Mycobacterium neoaurum* and treatment was modified to rifampicin 600 mg once a day, ethambutol 1500 mg once a day,isoniazid 300 mg once a day and levofloxacin 750 mg once a day but, despite initial improvement, she remained febrile. Subsequent blood cultures were positive for *Mycobacterium arupense* (rifampicin-resistant), so, a month later, antimycobacterial treatment was modified again to levofloxacin 750 mg once a day, ethambutol 1500 mg once a day and clarithromycin 500 mg two times a day with consequent fever remission and sterile blood cultures. The aforementioned infections were followed by lupus flares, successfully managed with methylprednisolone pulses, methotrexate, increased hydroxychloroquine doses and the B-cell activating factor (BAFF)-targeted monoclonal antibody, belimumab. CD4 count and immunoglobulin levels were normal.

Twenty-five months after the initial presentation, she presented with fever and nodular skin lesions. Blood cultures were positive for *Mycobacterium gordoniae*, susceptible to the already administered antimycobacterial treatment. All blood samples were cultured in liquid media (BACTEC MGIT 960) and susceptibility testing was performed with broth microdilution according to 2011 Clinical & Laboratory Standards Institute (CLSI) guidance.

Five months after the last admission, she developed acute flaccid paraplegia. After exclusion of infectious causes, SLE myelitis was diagnosed and she received methylprednisolone pulses and cyclophosphamide. However, she deteriorated clinically, was intubated and passed away due to septic shock. Throughout her illness and postmortem, we performed a series of laboratory tests to identify predisposing factors associated with susceptibility to NTM infections.

In order to explain this predisposition to NTM infections, we studied the patient’s plasma interferon (IFN-γ) neutralising activity and the presence of anti-IFN-γ autoantibodies and/or of genetic variants predisposing to NTM. The methods used are described in detail in the online supplemental material 1. First, we assessed the patient’s plasma neutralising activity against IFN-γ. We evaluated CD80+/CD86+ co-expression in peripheral blood mononuclear cells (PBMCs) of healthy donors after stimulation with IFN-γ in the presence of patient’s and healthy donors’ plasma. We found similar co-expression after IFN-γ with and without a healthy donor’s plasma, whereas the co-expression was reduced in the presence of the patient’s plasma (figure 1). A similar pattern of reduction in intracellular phospho-STAT1 expression was noted (figure 1), although neither of these findings were statistically significant. These findings were not confirmed by examining the patient’s plasma for specific anti-cytokine antibodies as no anti-interleukin (IL)-12, anti-IL-23 or anti-IFN-γ autoantibodies were detected (figure 2).

No mutations were identified in genes of the IL-12/IFN-γ signalling axis, known to predispose to NTM infection, using whole exome sequencing (online supplemental material 1). We detected a homozygous polymorphism in the NF-kappa-B essential modulator (NEMO) gene, known as Inhibitor of Nuclear Factor Kappa B Kinase Regulatory Subunit Gamma (IKBKG), which was Sanger sequencing-confirmed. Of note, the patient did not have clinical features consistent with Turner (XO) syndrome. NEMO acts as the regulatory subunit of IκB kinase complex that activates NF-κB resulting in gene activation of inflammation signalling. The variant IKBKG NM_001099857.5 c.169G>A, leading to a p.Glu57Lys (E57K) missense mutation, is reported in NCBI dbSNP (rs148695964) at a frequency of 0.001080 (GnomAD v.2.1.1); 0 homozygotes and 64 hemizygotes are reported. This variant is reported in ClinVar (VCV000068234.7, accessed June 2021) as ‘Benign/Likely benign’, and is classified as ‘Likely benign’ according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) 2015 guidelines (criteria applied: BS1, BP6, PP3, PM1).

**DISCUSSION**

We present a rare case of a patient with SLE with recurrent NTM infections (table 1). NTM are ubiquitous in the environment, mainly in soil, dust and natural, municipal and hospital water sources. NTM infections have raised interest due to increasing incidence among patients previously not considered at risk, the elucidating host-pathogen-environment interplay, and the recognition of novel risk factors. Disseminated NTM infections are rare in patients with SLE, and the majority presents with isolated SST or lung involvement. Unique features of our patient were the recurrent and disseminated infections with different NTMs even in the absence of central venous catheters and severe bloodstream infections caused by fungi (*Candida parapsilosis, Fusarium chlamydosporum*), rarely observed outside of haematological malignancies.

Anti-cytokine autoantibodies are reported in healthy individuals and rheumatic and haematological patients, however their exact pathogenetic role in predisposition to infections is not clear, as these antibodies are not always neutralising. Disseminated NTM infections have been associated with neutralising anti-IFN-γ autoantibodies, occurring primarily in healthy adults of Asian ancestry, with specific HLA genotypes, without known ancestry, with specific HLA genotypes, without known ancestry, with specific HLA genotypes, without known ancestry, with specific HLA genotypes, without known ancestry, with specific HLA genotypes.

This variant is reported in ClinVar (VCV000068234.7, accessed June 2021) as ‘Benign/Likely benign’, and is classified as ‘Likely benign’ according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) 2015 guidelines (criteria applied: BS1, BP6, PP3, PM1).
immunosuppression.\textsuperscript{7} We found a partial inhibition of intracellular IFN-γ-dependent STAT1 phosphorylation in the patient, however no autoantibodies against IL-12, IL-23 and IFN-γ were detected. Type I and II interferons signal in bacterial or viral infections, through NF-κB activation.\textsuperscript{8,9} Furthermore, NF-κB dictates transcriptionally the expression of various interferon stimulated genes (ISGs), as rela−/− mice cannot express a full set of ISGs on induction of various stimuli,\textsuperscript{10} while LMP1-induced tyrosine phosphorylation of STAT1 is almost exclusively due to the NF-κB-dependent secretion of IFNs.\textsuperscript{11} Given the effect of IKKγ to the NF-κB activation and the positive feedback loop of type-I IFN activation of NF-κB and STATs, we believe that there could be an association of NEMO dysregulation and STAT-1 activation, depicted by our experiments.

Whole exome sequencing provides a valuable tool in the study of inherited immunodeficiencies. Patients with pulmonary NTM infections carry variants in genes involved in immune response, variants of cystic fibrosis transmembrane conductance regulator, genes regulating cilia formation and function and genes associated with connective tissue disorders.\textsuperscript{5,12} A growing number of gene mutations implicated in host response against NTM has

Figure 1  Patient derived plasma partially neutralised IFN-γ activity on healthy donor-derived PBMCs. (A) IFN-γ stimulation on PBMCs is assessed by HLA-DR/CD80/CD86 extracellular staining (activation status). Co-expression of CD80/CD86 by flow cytometry, histogram of HLA-DR+ cells and mean percentage of either HLA-DR+ or CD80/CD86+ cells are sequentially shown. (B) IFN-γ stimulation on PBMCs is assessed by phosphorylation levels of STAT1, monitored by intracellular staining of phospho-STAT1. PBMCs derived of healthy volunteers were used (n=3) and stimulated by either 1000 IU IFN-γ only, or 1000 IU IFN-γ only plus healthy derived plasma or 1000 IU IFN-γ only plus patient derived plasma. HLA, human leukocyte antigen; IFN, interferon; PBMCs, peripheral blood mononuclear cells,
been reported, mostly affecting functions of IFN-γ receptors, IL-12, STAT1, GATA2 and IKBKG. These disorders are autosomal dominant or recessive, mainly diagnosed during infancy or childhood. Our patient carried a NEMO variant in homozygosity without other mutations in crucial genes for the immune response against NTMs. NEMO deficiency is a X-linked genetic disorder described in men with anhidrotic ectodermal dysplasia with immunodeficiency. Heterozygotic women can present with incontinentia pigmenti (IP), however hypomorphic phenotypes are being increasingly reported. Of note, our patient did not have clinical features consistent with IP. NEMO is crucial for cytokine regulation, CD40-mediated B-cell activation and IL-12 production, B-cell class-switching and Toll-like receptors function. The cardinal features of NEMO deficiency-associated immunodeficiency include recurrent bacterial, atypical and mycobacterial infections.

Our patient’s NEMO variant has been reported in patients with mild clinical phenotypes and in two studies describing the functional consequences of this hypomorphic variant, however its penetrance may be incomplete and can also found in asymptomatic carriers or carriers with only mild skin involvement that often goes undiagnosed. Interestingly, there are reports of patients carrying NEMO mutations that resulted in immunodeficiency without developmental defects. Others have shown that several missense NEMO mutations can cause susceptibility to mycobacterial infections without developmental defects, in the presence of normal amount of NEMO mutant protein. This susceptibility was mediated by impaired CD40-dependent IL-12 production. Regarding the E57K variant of our patient, although it has been associated with maintained NEMO activity, it has been shown that this specific mutation resulted in reduced NF-κB activation after IL-1 stimulation, mainly because of a defect in TRAF6-regulated ubiquitination of NEMO protein. Keller et al described a male infant with recurrent respiratory tract infections, low CD4 and CD8-memory T-cells counts and vaccine non-responsiveness. Similarly, Frans et al reported specific immune response deficiencies in a male infant with recurrent bacterial infections. To our knowledge, this is the first case of E57K mutation-associated disease.

### Table 1

<table>
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<tr>
<th>#</th>
<th>Date</th>
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<th>Sample</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>31 March 2016</td>
<td><em>Mycobacterium gordonae</em></td>
<td>Blood</td>
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<tr>
<td>2</td>
<td>1 April 2016</td>
<td><em>Mycobacterium gordonae</em></td>
<td>Skin</td>
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<td>3</td>
<td>30 November 2017</td>
<td><em>Mycobacterium neoaurum</em></td>
<td>Blood</td>
</tr>
<tr>
<td>4</td>
<td>18 December 2017</td>
<td><em>Mycobacterium arupense</em></td>
<td>Blood</td>
</tr>
<tr>
<td>5</td>
<td>8 January 2018</td>
<td><em>Mycobacterium arupense</em></td>
<td>Blood</td>
</tr>
<tr>
<td>6</td>
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<td><em>Mycobacterium gordonae</em></td>
<td>Blood</td>
</tr>
<tr>
<td>7</td>
<td>7 March 2018</td>
<td><em>Mycobacterium gordonae</em></td>
<td>Blood</td>
</tr>
<tr>
<td>8</td>
<td>8 March 2018</td>
<td><em>Mycobacterium gordonae</em></td>
<td>Blood</td>
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diagnosed during adulthood. Nevertheless, we believe that neither this specific polymorphism associated with mild phenotypes (as supported by the presence of hemizygous healthy carriers and the absence of opportunistic infections in the patient’s medical history during childhood and before SLE diagnosis), nor iatrogenic immunosuppression for SLE by themselves seem sufficient to explain her clinical course. We support a multifactorial impact of genetics plus immunosuppression on the patient’s unique susceptibility to NTM infections.

Autoimmune features have also been associated with NEMO deficiency as with other immunodeficiencies, such as autoimmune cytopenias, SLE, arthritis, inflammatory bowel disease, Behcet’s disease and other non-typical autoimmune/inflammatory disease phenotypes.22–25 Of note, immunodeficiency and autoimmune or autoinflammatory features coexisted in patients with NEMO mutations.22 25 Interestingly, it has been recently reported that specific NEMO mutations can lead to the overexpression of the protein, increased NF-κB activation with concomitant highly expressed type I IFN signature from T cells, monocytes and macrophages.25 Unfortunately, we could not perform additional mechanistic investigations in order to assess the functionality of NEMO in our patient. These findings, however, indicate that genetic aberrations of NEMO could contribute to various autoimmune phenotypes, such as SLE.

This case offers important clinical and pathophysiological insights. First, it highlights the need for extensive work-up in patients with recurrent atypical infections that could lead to identification of contributors of non-iatrogenic immunodeficiency. Second, it underscores the common pathways and interaction between immunodeficiency and autoimmunity. Finally, it shows the increasingly important role of sequencing technologies in the diagnosis of complex syndromes.

Ethics approval This study involves human participants and was approved by Attikon University Hospital Institutional Board (Reference number: EBA2324/03-05-2017). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES


