Author's response to reviews

Title: Cutaneous infection by Mycobacterium haemophilum and kansasii in an IgA-deficient man

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Author's response to reviews: see over
Dear editor,

Please find enclosed our answers to the raised questions of the referee's item by item and the accordingly revised manuscript. We omitted to highlight the changes. We apologize for this, but several things changed and we think it would not really be helpful to mark all of them in this brief report.

In this case we present a cutaneous co-infection by *M. haemophilum* and *M. kansasii* proven by culture in a patient with IgA-deficiency. From published data or the current knowledge about immunopathology of infections by nontubeculous mycobacteria we cannot draw a clear pathogenic link between IgA-deficiency and NTM-infection. Nevertheless, we addressed this point in the revised discussion, as suggested by reviewer 1. We are convinced that the co-infection by *M. haemophilum* and *M. kansasii* as well as the co-incidence of NTM-infection and IgA-deficiency is a striking result and of great interest for the readership of BMC dermatology.

**Reviewer 1:**

1(a). The link between the described NTM infection and IgA deficiency requires strengthening. Provide a pathomechanism for skin-based disease in the setting of IgA deficiency.

In fact, both selective IgA-deficiency and cutaneous NTM infection described in this report are well documented and in general not under any doubt. In the patient's history, IgA deficiency has been reported and verified since years, but without any known extraordinary susceptibility to infections. The disease was diagnosed in childhood due to maternal IgA-deficiency (familial clustering is well known). Since that time human immunoglobulin (Beriglobin®) was administered, although therapy in selective IgA-deficiency is uncommon and the amount of IgA in these preparations is thought to be low. Over time and especially due to the travel activities of the patient the substitution was realized only irregularly in the last years. By the authors the patient was therefore advised to visit an immunodeficiency ward to reconsider the necessity of a substitution but so far without success.

Strikingly, we present in this case an association of NTM skin infection in a patient with IgA-deficiency and we cannot draw a clear causality between NTM infection and underlying IgA-deficiency. Selective IgA deficiency is usually a mild form of immunodeficiency. Many individuals are asymptomatic, while others suffer from infections, allergies, gastrointestinal disorders or autoimmune disease. Obviously, this biological diversity has both genetic and environmental components.

In literature, an association with dermatitis herpetiformis and pyoderma gangraenosum and IgA deficiency is described. It is known that IgA is secreted with sweat (sIgA) assuming an immunological function on skin. Moreover, lower cutaneous sIgA levels and increased skin infections has been reported in patients with atopic dermatitis, while these patients of course often present also an impairment of the normal skin barrier. In conclusion, there are some links between skin infections and IgA-deficiency although the exact pathomechanism is unclear.

Thus, we can anticipate a contribution of IgA-deficiency but anything more. Most likely, in our patient NTM's are transmitted from a contaminated environmental source by a direct cutaneous route. The exact source and the infection dose remain unclear. Even in a healthy individual an appropriate exposition (e.g. skin injury, “high” bacterial burden) may lead to infections as described for *M. marinum*. In IgA-deficiency, beside reduced serum IgA levels often also other immune deficiencies e.g. in cytokine levels and B-cell function but also associated T cell defects have been described. Thus, we cannot exclude that IgA-deficiency may be a surrogate for another undetected less marked immune dysfunction.

Finally, to provide a pathomechanism is clearly beyond the scope of this case report and it is not the intention of the authors to make wild speculations about this issue. This should be the
aim of an experimental work. Nevertheless, we addressed the reviewer’s comment to some extend in the revised version.

- (b) provide information on the presence or absence of IgA-associated mucosal-based diseases

IgA-associated mucosal-based diseases (e.g. celiac disease, respiratory infections, lupus erythematoses) are not reported in this patient (this information was added to manuscript as requested)

- (c) We are informed that “interestingly our patient’s history revealed an IgA-deficiency”. Perhaps it would be of value to know how / why the IgA studies were undertaken?

In the patient’s mother, IgA deficiency was diagnosed decades ago. Obviously, due to known genetic factors immunoglobulin status was checked and IgA-deficiency was detected in the patient (information was added to manuscript as suggested).

- (d) unless a good pathogenetic link to IgA deficiency is formulated, the association, at most, appears fortuitous. One needs to make an informed statement to this effect.

According to literature and our knowledge no clear pathogenetic link may be given for mycobacterial disease and IgA-deficiency. In the revised version this was stated more clearly (see also comment to question 1a).

2. Although there is synchronous evidence of Mycobacterial disease, there is no dual infection in a single lesion, and no clarity that the co-cultures were from lesions on the right side, to indicate sporotrichoid co-involvement. Please attend to this.

Mycobacterial cutaneous disease was diagnosed by microbiological cultivation, while M. haemophilum was recovered twice from independent biopsies (three months apart) from the lesions of the right hand. In contrast, M. kansasii was recovered from a lesion at the right elbow. These facts were given in the revised version. Culture positive lesions were both from the right body site, but a co-infection of a single lesion was not found. The therapeutic response to anti-mycobacterial treatment indicates infective causality of skin lesions.

3. The sporotrichoid nature of the clinical lesions is not imaged to optimal effect in the submitted figures. There are many randomly placed flat and nodular lesions. Please demonstrate an unequivocal sporotrichoid pattern of spread in the limb/s.

Since the cutaneous lesions presented herein probably do not absolutely fit to the classical presentation of a sporotrichoid infection of the skin, in the revised version we used for the description of lesions the term cutaneous or sporotrichoid-like lesions instead of sporotrichoid (see also comment 4).

4. My greatest difficulty is with the clinical presentation: the skin lesions are not “sporotrichoid” exclusively. There are disseminated cutaneous lesions. This does not therefore specifically mean that the disease was a primary focus in an acral extremity with spread along the lymphatics. The pattern of cutaneous involvement favours disseminated disease, as seen with blood spread. a. If the lesions did not contain acid fast bacilli, they are “older”; with lymphangitic spread, ipsilateral lymphadenopathy is a usual finding (in my experience: reactive/dermatopathic or infective). There was NO lymphadenopathy. We therefore require a mode of spread that will allow dissemination of infection other than by local lymphatics.
Sporotrichoid lymphocutaneous infection is a syndrome characterized by the development of superficial cutaneous lesions that progress along dermal and subcutaneous lymphatics. This syndrome includes a variety of common and uncommon microorganisms including NTM.

Of course, on the one hand not all foci fit to the most likely lymphatic transmission, while on the other hand we have no evidence for a transmission via blood and no evidence for lymphadenopathy. Nevertheless, we cannot exclude neither systemic blood transmission nor the combined systemic and lymphatic transmission especially if two pathogens were detected. First, the distribution of skin lesions is a fact and second there is no doubt about the mycobacterial origin of skin signs although not each area was verified by a biopsy.

The mycobacterial nature of the lesions at the right hand (dorsum digit IV, dorsum of the hand, both *M. haemophilum* and the right elbow (*M. kansasii*) are proven by culture. Second, the histopathological findings granulomas from biopsies taken from the same regions and from his back support this diagnosis. Third, the regressions of the entire skin lesions under anti-mycobacterial therapy argue for their infective/mycobacterial origin at all. This is a clear meassage.

b. We do not have a record of pulmonary or chest X-ray findings. This is a crucial omission given that *M. kansasii* usually targets the lung.

No systemic manifestations including lymhadenopathy have been found neither by abdominal ultrasound, chest X-ray, abdominal and thoracic computer tomography) nor signs of lung involvement by *M. kansasii* were detected (information was added to manuscript as requested)

c. The clinical background should at least include occupational or hobby details that may strengthen or negate a primary cutaneous source of infection.

Some more details about hobbies of occupational expositions were added.

d. The authors state that there was no “clinical” evidence of mycobacterial systemic infection. This requires explanation. We need to be informed how systemic disease was excluded. Were blood and sputum cultures undertaken?

Data about undertaken clinical investigations were added to manuscript as requested.

Minor Revisions:
1. Spelling errors": mycobacterium, Changed as requested

2. Other corrections: culture instead of cultivation, circumscribed instead of sharp Changed as requested

3. Clinical findings: Ziehl Neelsen not included as special stain used in histopathological assessment of skin biopsy.

Both Ziehl-Neelson and Auramin stain for the detection of acid fast bacilli were done and negative (information added as requested)

4. The Mycobacterial profiling are part of the “Results” and should not be included under “Methods”. As all the results are included under “Clinical findings”, perhaps the best fit would be as a clinical finding.

In the revised version we skipped paragraph about methods and combined data about mycobacterial profiling and clinical findings within the section case presentation.
1. Were the lesions that were biopsied for histopathological assessment the same that were biopsied for culture purposes?

Histopathologic (dorsum right digi IV, dorsum of the right hand, the right elbow, the patient's back) and microbiological (dorsum right digi IV, dorsum of the right hand, the right elbow) were performed in parallel. This was stated more clearly in the revised manuscript.

I would undertake Mycobacterial PCR testing (nested, if necessary) or cloning/sequence studies to confirm a Mycobacterial origin and subtype in the histopathological paraffin sections.

The detection of viable mycobacteria by culture is the gold standard and much more valuable than the detection of DNA only. Unfortunately, in our microbiological laboratory the detection of DNA from nontuberculous mycobacteria from clinical specimens is not established. Moreover, the detection of DNA from paraffin sections is critical. Beside some promising reports in literature according to our experience the risk of contamination during embedding in a pathological laboratory is a great problem. Further, due to problems in the extraction of DNA from acid fast bacilli from tissue section the sensitivity is below that of culture. The authors also do not really understand why positive culture combined with molecular species identification should be verified by PCR.

2. Post treatment images demonstrating disease resolution will enhance the submission.

After treatment full resolution of skin manifestations were reached. A photophraph of the patients back was added below and if accepted by the editor simply for reviewing purposes. The colour figure does not add significant information to the manuscript where the complete remission is clearly stated.

**Reviewer 2:**

For example, although the authors described that the patient had a history of history of IgA-deficiency, no detection of immunoglobulin has been done. The immunoglobulin (IgG, IgM, IgA) should be described in this paper.

IgA data were included. IgG and IgM data were unfortunately not available.
Since the patient was diagnosed to have IgA-deficiency, so it is necessary to describe if the patient has repeated infections.

Information was added. See comment question 1 of reviewer 1.