**Author’s response to reviews**

**Title:** Left thigh phlegmon caused by Nocardia farcinica identified by 16SrRNA sequencing in a patient with Leprosy: a case report

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**Version:** 3 **Date:** 21 February 2013

**Author’s response to reviews:** see over
Dear Editor,
as requested, I write to You in order to reply to reviewers’ suggestions regarding the manuscript entitled “Left thigh phlegmon caused by Nocardia farcinica identified by 16S rRNA sequencing in a patient with Leprosy: a case report” already submitted to Your journal.

Reviewer's report (1)
Title: Left thigh phlegmon caused by Nocardia farcinica identified by 16SrRNA sequencing in a patient with Leprosy: a case report
Version: 1 Date: 14 December 2012
Reviewer: Bernard Naafs
Reviewer's report:
I think it is an interesting paper. It confirms data and shows some new ones, especially the activity of Daptomycin in vivo.

1. I would like the dosis of the medications given.
The doses of drugs were reported in the paper.

2. Can you explain why you used Rifampicin, minocycline, and mofloxacin to treat the leprosy patient. I think it is only marginal better antibacteriological than the WHO MDT. But it lacks the antireactivity of Lamprene (ENL) and dapsone (Reversal Reaction). It may be that you problems started there.

Two out of the three (rifampicin, dapsone and clofazimine) first line anti-leprosy drugs were changed. Dapsone was suspended due to the elevation of the liver function tests; clofazimine was refused by the patient due to the discolorations of his skin.

3. ENL is a problem, there are no good guidelines to treat it. Did you give all year high dose of steroids or intermittent? Did you know that low dose of steroids and thalidomide together may interfere?

The patient suffered of recurrent ENL reaction; steroids (prednisolone) were administered intermittently and in decreasing doses. At certain stages while decreasing prednisolone and introducing thalidomide the two drugs were used together indeed.

Reviewer's report (2)
- Major Compulsory Revisions

1. More details need to be provided regarding the use of 16S rRNA sequencing to confirm the diagnosis of N. farcinica. What was the source of the specimen that was analyzed? Since the organism was not cultured, how was this assay performed. Please include a summary figure of the molecular data. Since molecular analysis lead to the presumptive diagnosis, this feature of the clinical work-up and evaluations deserves more description in the manuscript. Otherwise there is no data to confirm this diagnosis leading one to wonder if another organism could have been responsible for the infection (although this may be unlikely given the clinical response to therapy directed against N. farcinica).
In order to identify the etiologic agent, phlegmon was also submitted to total DNA extraction and specific biomolecular investigations. After cell lysis with lysozyme and proteinase K, DNA was purified with QIAamp® DNA mini kit (QIAGEN GmbH, Germany). Bacterial universal primers (16S-F 5’-AGAGTTTGATCATGGCTCAG-3’ and 16S-R 5’-GGACTACCAGGGTATCTAAT-3’) were used for polymerase chain reaction (PCR) amplification of a region (798 bp) of the 16S rRNA gene (Richardson DC, Louie L, Louie M, Simor AE. Evaluation of a rapid PCR assay for diagnosis of meningococcal meningitis. J Clin Microbiol. 2003 Aug;41(8):3851-3). Amplified PCR products (Figure 2) were purified by using QIAquick PCR purification kit (QIAGEN GmbH, Germany) and directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Calif., U.S.A.) in an Applied Biosystems (Model 3100) automatic DNA sequencer. Related DNA sequences were searched in GenBank by using the BLAST (Basic Local Alignment Search Tool) server (http://www.ncbi.nlm.nih.gov/BLAST/). The most closely related sequences belonged to Nocardia farcinica species. Very significant matches (100%) were found with strain PCH16S-037 (accession number JN562386.1), strain IFM 11135 (accession number AB634920.1) and strain IFM 11108 (accession number AB631047.1). On the basis of what above, the identification of pathogen was made as Nocardia farcinica. The further identity of the pathogen was also confirmed by sequencing a region of 16S rRNA gene of Nocardia with a PCR that used genus-specific primers. (Laurent FJ, Provost F, Boiron P. Rapid identification of clinically relevant Nocardia species to genus level by 16S rRNA gene PCR. J Clin Microbiol. 1999 Jan;37(1):99-102). Sequence data of the amplified PCR products showed great identity (99%) to the species Nocardia farcinica (GenBank accession number AB634920.1).

- Minor Essential Revisions
NOTE: The lines of the manuscript are not numbered making editorial comments less precise.
1. Page 2, (abstract) line 9: minocycline and moxifloxacin were administered daily? Please indicate the dosing regimen.

Dosing regimen of drugs was reported in the text.

2. 16SrRNA is most commonly written as ‘16S rRNA’ with a space between the S and r.

Change made in the manuscript.

3. Page 4, line 23, please describe reference ranges for all clinical lab values, particularly bacteriological index and morphological index. Some readers will not know what these indices are.

The bacteriological index (BI) gives an indication about the total bacterial load of the patient. It is expressed by a logarithmic scale that may vary from “0” to “6+”. Zero means no bacilli like seen by the slit-skin smear examination; this is the result in normal people or in tuberculous (TT) leprosy. Six plus “6+” is the highest bacterial load seen by slit-skin smear examination in lepromatous (LL) patients.
The morphological index (MI) represents the percentage of bacilli uniformly stained (they are called “solids”) by the Ziehl-Neelsen staining. The “solids” are considered alive bacilli. There are no reference ranges for the MI. Often MI is comprised between 0.5 and 15%. Rarely I have seen borderline lepromatous (BL) and LL patients with a MI of 20% or more.

4. Page 5, line 2: a comma is needed after ‘thigh’ Change made in the manuscript.
5. Page 5, line 4: a comma is needed after ‘admission’ Change made in the manuscript.

6. Page 5, lines 7-10: reference ranges are needed for clinical lab values. Change made in the manuscript.

7. Page 5, line 24: “the patient WAS shifted” (add ‘was’) Change made in the manuscript.

8. Page 6, line 6: change conditions to condition. Change made in the manuscript.

Best regards

Pasquale De nardo