Reviewer's report

Title: Significant difference between three observers in the assessment of intraepidermal nerve fiber density in skin biopsy

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Reviewer: Giuseppe Lauria

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The Authors aimed at evaluating the inter-observer agreement in the quantification of IENF density. Due to the growing number of labs working in this fields, this is an important topic. However, there are several major concerns to be addressed.

Major Compulsory Revisions

Methods:

1. It is not clear how a “control skin area” was chosen in patients with a generalised disease such a polyneuropathy.

2. It is reported that biopsies were taken “bilaterally from foot or thigh for nerve injury patients”. It should be clarified what nerve was injured and since what time.

3. Although it is stated that “the procedure of skin biopsy followed the Guidelines of the EFNS”, it does not seem the case. In fact, the protocol used is different from that proposed by the EFNS guidelines and used in most laboratories. The authors used the same protocol by Vlckova-Moravcova (Muscle Nerve 37: 50–60, 2008).

4. How did the authors perform the training for immunostaining and nerve counting?

5. It should be clarified that the study was performed to investigate the interobserver agreement using indirect immunofluorescence technique.

6. Normally, 3 sections are quantified and it has been even suggested that 4 would be better. Why did they count just 2 sections?

7. It is reported that “samples were excluded if they were judged to be of bad quality for counting by at least one observer”, but the rules for including one section should be provided. Moreover, what happened if a section was excluded by the quantification? Did the authors immunostain other sections? Did they used as final result the count from the single “good” section?

8. Comparison of results with the normative reference values provided by McArthur et al. is not appropriate because of the different technique used, whereas the cut-off used by Vlckova-Moravcova is 8.8 fibers/mm. Normative data are available from the distal calf (10 cm above lateral malleolus), not from the
foot. It should be specified where the biopsies were taken in the foot (and in the other sites examined). One further major lack is the absence of healthy subject population for comparison with disease subjects.

9. I wonder why the author did not include the analysis of intra-observer agreement that is a further very important issue for diagnostic purposes.

Results
1. Most of this section reports the differences among the 3 observer, which are not so important since by definition they have been trained using the same protocol.

2. On how many section at each site did the authors carried out the comparative study?

Discussion
1. The problem of basal membrane identification has been frequently raised in previous works, mainly when bright field immunohistochemistry is used. Conversely, immunofluorescence techniques allows the use of anti-collagen IV antibodies which properly stain the dermal-epidermal junction. This possibility should be considered.

2. Conclusions are quite obvious, but the findings of the work do not address the issue raised.

Minor Essential Revisions
1. Text should refer to figures (fig 4 and 5 are not cited).

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.