Reviewer's report

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

Version: 2 Date: 10 November 2008

Reviewer: Giuseppe Lauria

Reviewer's report:

The manuscript is improved. However, several concerns remain.
• The authors changed the methods and, in the revised version, now report that they referred to the protocol by Vlckova-Moravcova and colleagues. How can it be changed after the study was performed?
• Counting 2 or more sections. The point is not that in the authors' lab the protocol has been standardized counting 2 sections, but that the reliability of IENF density is acceptable only when at least 3 sections are counted. This should be mentioned.
• Point 7: "good sections" - the rules are clear, but the authors should be discussed that in 16 cases only 1 section was quantified, making the quantification of IENF density possibly not reliable, and anyway not in keeping with the suggestions of guidelines and textbook chapter.
• Comparison with previous normative data remains not appropriate. The work by Walk and colleagues was performed using confocal microscope and neurolucida technique. Moreover, they report that, despite a significant correlation between IENF density at foot and calf, there was considerable variation in the calf ENF density and among patients with 0 IENF in the foot, calf IENF density ranged between 0 and 18.
• Intra-observer analysis remains a mandatory step preceding the inter-observer analysis. It should be mentioned.
• Conclusions remain partly obvious whereas the final message of the study should be better explained. The point is that, despite training and protocol were available, this study showed no inter-observer agreement. How do the authors judge the results obtained? External quality control of a skin biopsy lab is mandatory, but it must follow a reliable intra-lab assessment.

Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I have no competing interests