Reviewer's report

Title: Analysis of the clinical relevance of antimitochondrial antibodies to the beta- and gamma-subunits of the F1F0-ATPase in patients with primary biliary cirrhosis

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Reviewer: Lars Komorowski

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The authors describe the prevalence and clinical relevance of autoantibodies against subunits of mitochondrial F1F0-ATPase in primary biliary cirrhosis. For this purpose, they employ two ELISA systems based on proteins expressed in E. coli and purified under denaturing conditions. They proclaim an additional value for the initial serological diagnosis of the disease but neglect the usefulness of the autoantibodies for activity monitoring or prognosis of disease outcome.

1) Anti-M2 determination
The authors describe the use of a preparation of the 2-oxoacid dehydrogenase complex (ODC) from beef heart mitochondria but its use – probably as an antigen in an immunoassay for the determination of anti-M2 – and the actual immunoassay that was conducted for the determination of anti-M2 are obscure. Moreover, the classical protocol for the preparation of ODC mainly enriches the pyruvate dehydrogenase complex (PDC) but the proportions of the branched-chain 2-oxo-acid dehydrogenase complex (BCOADC) and the 2-oxo-glutarate dehydrogenase complex (OGDC) vary. Taken together with the importance of the E2 subunits of BCOADC and OGDC for a competent determination of anti-M2 – as has been shown in several studies – this results in a reference assay that can not be considered state-of-the-art. This probably explains the lower clinical sensitivity of this assay compared to anti-mitochondrial autoantibodies (AMA) determined by indirect immunofluorescence. The authors should employ either the three E2 subunits separately or the fusion protein consisting of them (MIT3) as the basis of the reference assay. Otherwise, samples must not be deemed anti-M2 negative. Therefore, the statement that the determination of anti-beta- and/or gamma-antibodies increases the sensitivity of the serological diagnosis is not valid.

2) Relation of immunofluorescence pattern and positivity in the ELISA
In the introduction part, the authors state that a considerable proportion of sera generating the anti-mitochondrial autoantibody (AMA) pattern in indirect immunofluorescence are negative for anti-M2 in their reference assay (s. #1). They indicate that in these cases the beta- and gamma-subunits of the F1F0-ATPase – located in mitochondrial membrane – are targeted, resulting in the AMA staining pattern. However, in their experiments AMA negative sera are also
positive but the interpretation is never corrected.

3) Data of PBC patients vs. healthy controls

In Figure 1 the authors show the consolidated data of their ELISA experiments employing sera from 59 PBC patients and 41 healthy controls. In order to let the reader get a better impression the individual data of each samples should be given in a dot plot. Although the given comparison of the mean values demonstrates statistical significant differences the data – even in this consolidated format – indicate a highly impractical narrow margin between the two groups. Moreover, disease controls with high probability of “false” reactivities are missing which obscures the true value of the autantibodies for serological diagnosis.

In conclusion the manuscript can not be considered fit for publication.

**Level of interest:** An article of limited interest

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

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