Author's response to reviews

Title: Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Prospective survey of patients seen in general practice

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Version: 2 Date: 6 April 2010

Author's response to reviews: see over
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Title of Manuscript:
Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Survey of patients seen in general practice

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Version: 2
Date:   April 6th, 2010

To the editor.

Hereby we return the manuscript revised according to the points raised by the reviewers. We thank both reviewers for the thorough and very useful comments to our manuscript.

Please note that there is some discrepancy in the overall assessment between the two referees

Referee 2 has raised some very relevant and interesting points. Some of these issues may, however, not be answered by the data available in our study. Instead we have made a few additions and changes to the discussion.

Two versions of the manuscript are re-submitted
  1. The new version
  2. The old version compared to new one with marking of all changes.

Author's response to reviews: see over
Referee 1:
Reviewer's report
Title: Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Prospective survey of patients seen in general practice
Version: 1 Date: 22 February 2010
Reviewer: Mary Petzke
Reviewer's report:
Early diagnosis of Lyme disease is mainly based on clinical manifestations including a characteristic skin lesion, erythema migrans (EM), which is present at the site of the tick bite in up to 80% of patients. Unlike most bacterial diseases that can be diagnosed by direct microscopic observation, cultivation or PCR, clinical diagnosis of Lyme disease is supported by serological assays, primarily ELISA and Western immunoblot. However, these assays suffer from a number of limitations, including inter- and intra-laboratory variability, subjective interpretation and lack of both high sensitivity and specificity. The flagellin-based ELISA assay used for serodiagnosis in the present study has a false positive rate of 2% (98% specificity). In areas highly endemic for Lyme disease, the false positive rate is compensated for by the high incidence of Lyme borreliosis cases. However, in areas in which the incidence of Lyme disease approaches the limit of sensitivity of the test (low pretest probability), the demand for serological testing can lead to incorrect diagnosis and unnecessary treatment. In this study by Dessau et al, the authors assessed the utility of serological testing for Lyme disease in three counties of Denmark, where the background seropositivity is 1.6%. Serological testing was performed by three laboratories using the same commercial ELISA assay. The results (IgM and IgG titers) from 2,647 patients, performed over one year, were compared with both the presence of clinical indicators of Lyme disease and the decision to treat. Rigorous statistical and experimental methods were employed. The authors determined that age (0-15 years) and suspected EM, or suspected acrodermatitis, were significant predictors of IgM or IgG seropositivity, respectively. Due to the low pretest probability, serological testing was not recommended for the diagnosis of Lyme arthritis. This study concluded that EM is a distinct clinical entity and an indicator for treatment, without the need for laboratory testing. While the authors were careful to note that the results of this study may not be extrapolated to areas where the epidemiology of Lyme disease and diagnostic methods may differ, they concluded that the use of serological testing is of limited utility in certain patient groups and in areas with low rates of pretest probability.

We thank the reviewer for the comments.

Only a few minor modifications to this excellent and well-written manuscript are suggested:

Discretionary Revisions:
1. Page 9, paragraph 2, sentence 7; Page 9 paragraph 3, first sentence: both sentences should be rewritten for clarity.
The sentences have been rewritten in the manuscript.

Minor Essential Revisions:
1. In Table 1, footnotes should be added to explain the following:
   a) items in bold font

   A footnote has been added just below the table explaining:
   **Statistically significant odds ratios (OR) with 95% Confidence Intervals (CI) not including “one” are highlighted with bold**

   b) the reference population used for determination of the odds ratio in the lower half of the table

   The footnote to table 1 (now numbered 4) has been revised and expanded.

**Level of interest:** An article of importance in its field

**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.

**Declaration of competing interests:**
I declare that I have no competing interests.
Referee 2:

Reviewer’s report
Title: Utilization of serology for the diagnosis of suspected Lyme borreliosis in Denmark: Prospective survey of patients seen in general practice
Version: 1 Date: 27 February 2010
Reviewer: Hermann Girschick

Reviewer’s report:
Major Compulsory Revisions
1. Is the question posed by the authors well defined?
yes
2. Are the methods appropriate and well described?
in part, the reader would like to have a comparative analysis using other modes of serology typing, like ELISA including more epitopes than the flagellin prone for cross reactivity, or western blotting. Especially the high IGM rates found in children are unexplained and might reflect cross reactivity to other flagella bearing bacterial pathogens, not only EBV or CMV as suspected by the authors but not proven.
In addition, the non significant variations of the serotyping IgM and IgG over the months of the year indicate a low specificity for borrelia but a high sensitivity to other pathogens of the test, finally resulting in an underestimation of the actual infection rates/seropositivity in borrelia specific clinical pictures like lymphocytoma or chronic neuroborreliosis, but an overestimation of seropositivity in clinical pictures like rash (17% IgG positive). IgM?
The authors state this in part themselves with regard to the seropositivity of arthritis patients. Here the test does not help at all to decide who has an infection or not, or one would state that decision on treatment in 8.4 % of individuals can not be based on the ELISA performed (IgM 2.3 % and IgG 6.6%). It is not clear wether the treated individuals were actually the ones who were seropositive.
Whether 8.4 % percent ment that this is the fraction of treated individuals out of the whole group or whether this is the seropositivity ot the treated ones.
On the other hand, the surprisingly low incidence rates for IgG in acrodermatitis, chronic neuroborreliosis realy question the clinical value of the serological test, because here one would at least consider the seropositivity to be much higher. this remains unexplained by the authors.

This is an observational study limited by the procedures performed in the daily routine. Please see the more general comments about advantages/limitations of the study design in our comment on the point “Level of interest” below.
We agree that our study raises a number of scientific questions, which cannot be answered from the data in this study.

- Including ELISA with more epitopes in the study would be interesting. However this would be prohibitively expensive on more than 4000 samples. Please see the discussion below on the advantages and limitations of the study design.
- The flagellin is prone to some unexplained cross reactivity (especially IgM). In fact the present study may be used to give a crude assessment of the magnitude of this problem in routine clinical practice (See the discussion page 11-12 of the manuscript).

- Concerning western blot we have motivated the choice of strategy for the antibody testing (See discussion page 11). The specificity (98% for IgG and IgM respectively) of the flagella assay used in our study is comparable to various two-tier combinations. Different specificities for IB are cited in European studies, for example 89-98% specificity (independent assessment) depending on method increasing in various two-tier combinations to 94-100% (e.g. Goossens HA, van den Bogaard AE, Nohlmans MK. Evaluation of fifteen commercially available serological tests for diagnosis of Lyme borreliosis. Eur J Clin Microbiol Infect Dis 1999;18:551-560, Marangoni A, Sparacino M, Cavrini F, Storni E, Mondardini V, Sambri V et al. Comparative evaluation of three different ELISA methods for the diagnosis of early culture-confirmed Lyme disease in Italy. J Med Microbiol 2005;54:361-367).

- Concerning the 8.4% see below concerning the 6th column.

- We agree with the reviewer that the reason for variations in rates of seropositivity in the different subgroups is essentially unexplained. This could be an interesting focus for further studies. The rates of seropositivity depend much on the choice of patients tested. For example from our clinical experiences patients with chronic venous insufficiency are sometimes tested for suspected acrodermatitis. The most simple explanation for the high IgM rate in younger children could be that they are more exposed (outdoor playing habits) and have better clinical selection before testing (unnecessary venipunctures are rarely performed in children). Patient selection may also explain the very modest seasonal variation in the rate of seropositivity.

We have revised the discussion (page 10-11).

It is not really clear in the table 1 which patient was treated, whether the percentage shown in the 6th column is the seropositivity or the mere percentage of patients treated.

The legend for table 1 has been revised accordingly.

3. Are the data sound?
questions remain on the clinical usefulness of the test

Please see the discussion in point 2.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
the reviewer would consider the test much more questionable than the authors
do

6. Are limitations of the work clearly stated?
Not to the extent, as stated above

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
yes

8. Do the title and abstract accurately convey what has been found?
In part the survey has been retrospective, because the reports on diagnosis were done after serology was obtained.

The word “Prospective” has been removed from the title.

Level of interest: An article of limited interest

The objectives of our study were to highlight the extent of consecutive routine borrelia serology, the relationships between clinical manifestations of suspected borreliosis and seropositive rates together with the extent of antibiotic treatment. This information is important for the development of support for the general practitioners’ interpretation of borrelia serology.

Advantages and limitations
This study is population-based and observational. This design offers several advantages:
- a possibility to include large numbers of patients
- it provides information on rates of seropositivity in patients when suspected of borreliosis for the first time (i.e. when borrelia serology is requested for the first time)
- patients are representative for those seen in the general practitioner’s office at least in this country (and not merely a subset of patients as defined by strict inclusion criteria)
- to some extent they are patients in whom general practitioners wanted to rule out the diagnosis of borreliosis rather than to confirm it
- and not least, we were able to include the seronegative patients. This provided us with an opportunity to give some hints on the rate of true and false seropositivity as indicated in the discussion.

The limitations are among others:
- that details in excess of the normal routine are not readily available
- accordingly, it was not feasible to conduct further serological studies (neither more extensive serological testing including viral agents nor supplementary Immunoblotting. - conclusions about causality must be drawn with utmost care

Our study is a supplement to existing studies conducted on selected subpopulations (which are inherently non-representative of the routinely tested population). As opposed to many other studies our study provides information on rates of seropositivity in patients tested on more or less sustained suspicion of Lyme boreliosis. The issue of overuse and risk of low predictive value of borrelia serology have frequently been addressed in publications and at international meetings.

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**
nothing to report
'I declare that I have no competing interests'