Author's response to reviews

Title: Impact of the clinical context on the 14-3-3 test for the diagnosis of sporadic CJD

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Author's response to reviews: see over
June 7th, 2006

Melissa Norton, M.D.
Medical Editor, BMC Neurology

Dear BMC Neurology Editor,

Enclosed is the revised version of the manuscript with ID number MS: 5536193059899525, entitled: “Impact of the clinical context on the 14-3-3 test for the diagnosis of sporadic CJD”, which previous title was “Impact of the clinical context on the 14-3-3 test, based on the analysis of a Spanish cohort of suspected sporadic CJD patients”.

We have addressed point-by-point the comments of the Editor and reviewers and revised our manuscript accordingly. Following, please find our reply.

Editor:

1. Bioethics statements have been included in the text (page 4, paragraph 1).
2. The manuscript has been formatted according to the Manuscript formatting checklist
3. Language has been reviewed by a native English-speaking colleague.
Reviewer: Inga Zerr

Minor essential revisions:

1. Title has been shortened and focused at the aim of the manuscript, according to the suggestion of the reviewer.

2. Work by Blennow and collaborators (2005) is discussed (page 8, paragraph 3) and cited (new reference #14), according to the suggestion of the reviewer.

3. Misspelling of author’s name Geschwind in page 8 (paragraph 3) has been corrected.

4. Additional data on codon 129 of PRNP gene and presence of PSWCs on EEG for CJD patients with a 14-3-3 positive and false negative results are included on table 3. Other parameters requested by the reviewer such as MRI, PrPSc isoform or CSF tau values are not available.

5. Language has been reviewed by a native English-speaking colleague.
Major compulsory revisions:

1. From an epidemiological standpoint, a probable case (clinically compatible plus PSWCs in EEG, see WHO’s criteria) is classified as a CJD case. However, at initial examination and until the final diagnosis is either confirmed or discarded by neuropathological analysis, for the clinician a probable case is a suspected case for which he/she needs all the relevant information to ascertain the diagnosis. The aim of this paper is to determine whether the 14-3-3 test provides additional information for the differential diagnosis of sCJD in different clinical contexts (patients fulfilling epidemiological criteria for possible or probable CJD, or patients not fulfilling these criteria but that are still suspected cases for a clinician). Therefore, in our understanding, the question whether the 14-3-3 provides useful information for the diagnosis of cases initially classified as probable CJD is relevant. In fact, our data indicate that a positive 14-3-3 test for a case initially fulfilling criteria of probable sCJD results in an increases of the diagnostic certainty of sCJD (as indicated by the Bayesian statistics and the PPV after the test compared to the prevalence of the disease in the probable sCJD subgroup). On the other hand, a negative result is an attention call for the clinician, since this result significantly increases the probability (from 7.0% to 26.1%, table 4) that the patient is not an sCJD case.

Moreover, one of the strengths of this work resides in that it is a prospective analysis of all clinically suspected sCJD patients and represents a real practice situation. Therefore, it allows the estimation of predictive values of relevance for clinical decision-making. By eliminating the cases classified as probable sCJD, we are artificially altering the prevalence of disease in our cohort, and consequently making unfeasible the calculation of predictive values for the whole cohort.

Finally, we believe that by removing the data on probable cases we are losing information that may be of interest for clinicians and people working in the field. As the data for probable sCJD are already segregated from the other two groups in table 4, in case that these data are not relevant for a given situation, they can be simply obviated.
If the reviewer agrees with these comments, our intention is not to modify the manuscript by removing the data of patients initially classified as probable cases. In order to avoid misleading interpretations, we have deleted the word “suspected” from the title of the manuscript.

Minor essential revisions:

1. Additional data on the 6 non-sCJD patients who were initially classified as probable sCJD and had a negative 14-3-3 test had been included in the text (page 6, paragraph 4), according to the suggestions of the reviewer.

2. As correctly pointed by the reviewer, table 3 shows that 5 patients initially classified as possible sCJD with a negative 14-3-3 result (false negatives) were finally classified as sCJD. One of them had postmortem analysis and the other 4 were classified as probable sCJD, because of the development of PSWCs on the EEG later during the course of the disease. Additional data (presence of PSWCs in EEG and genotype of codon 129 of the PRNP gene) on patients who had a 14-3-3 false negative result (17 probable + 5 possible cases at initial classification) had been included in the text (page 6, paragraph 2) and in table 3, according to the suggestions of the reviewer. Other parameters such as MRI scan were not available.

For all calculations, sCJD cases include both probable (WHO’s criteria without the inclusion of the 14-3-3 test results) and definite cases at final diagnosis. This has been clarified in the notes under the tables 2 and 4, according to the suggestion of the reviewer.
Reviewer: Pierluigi Gambetti

Major compulsory revisions:

1. We totally agree with the reviewer’s comment. It is clear that the parameters described depend entirely on the cutoff point used to distinguish positive from negative samples. As any semiquantitative technique, the cutoff point in Western blot maybe subjective to the observers’ interpretation and the selection of reference standard samples. In our experimental setup, we include two reference standards: a positive control from a sCJD patient and a weakly positive control sample from a non-CJD patient. For the purpose of this analysis, we have followed the same strategy that we used for communicating results to the clinicians. Trace samples with immunoreactivity lower than the weakly positive control sample were considered negative. This information that was omitted in the previous version has been included in the revised manuscript in the Methods section (page 5, paragraph 4). The rationale for considering trace result as negative was already discussed in the Discussion section (page 8, paragraph 4).

2. In any case, changing the cutoff point of our data analysis by considering trace results as positive samples (instead of negative) does not alter the main conclusions of the work. In this situation, the sensitivity of the test increases, while the specificity decreases; however, the main message of the work is identical, as it is still clear that the 14-3-3 test provides useful information added to the clinical data for differential diagnosis of sCJD.

We also agree with the reviewer that predictive values are highly dependent on the prevalence of disease in a particular cohort. Thus, for a given diagnostic test, the PPV increases with the prevalence of disease, while the NPV decreases with prevalence. However, there are no reports of large prospective analysis of all sCJD suspected patients in a real practice situation that allow the estimation of predictive values of relevance for clinical decision-making. By the analysis of data from a large cohort and during a long period of observation, we have been able to estimate predictive values with confidence. These predictive values per se (but not the sensitivity and specificity of the test) are informative to the clinician confronted with a particular clinical case and a 14-3-3 test result. Additionally, the analysis of the performance parameters in
the different clinical contexts allowed us to explore the controversial issue of whether the 14-3-3 test provided useful information added to the clinical data.

We consider that this is a major point of interest in the manuscript, as suggested by its title. If the reviewer agrees with our comments, we do not intend to reduce the weight of the “influence of the clinical context on the 14-3-3 test” subheading in the Results section.

3. We agree that genetic data of polymorphism at codon 129 of the \textit{PRNP} gene are not essential to this work. They could be obviated without a significant loss of information. However, these genetic data may be of interest for particular cases, and in fact, one of the reviewers has requested additional genetic information. These additional data on codon 129 have been included in table 3.

We agree that there is some repetition as some information given in figure 1 and figure 2 are also mentioned in the Methods section. Although, these figures do not show relevant results for the message of the paper, they are key to understand the classification algorithm and to allow the interpretation of the tables. For the sake of clarity, the information stated in the figures is also commented in the text.

Thank you for reconsidering this revised manuscript for publication, and I look forward to hearing from you.

Sincerely,

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