PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prospective analysis of neutralising antibody titres in secondary non-responders under continuous treatment with a botulinumtoxin type A preparation free of complexing proteins – a single cohort 4-year follow-up study
AUTHORS	Hefter, Harald ; Hartmann, Christian; Kahlen, Ulrike; Moll, Marek; Bigalke, Hans

VERSION 1 - REVIEW

REVIEWER	Prof. Dr. med. R. Benecke Chairman of the Department od Neurology University of Rostock Gehlsheimer Str. 20 D-18147 Rostock Germany There are NO conflicts of interest.
REVIEW RETURNED	25-Nov-2011 - Reviewer completed the checklist but made no further comments.

REVIEWER	Prof. Dr. Wolfgang Jost Chief neurologist Deutsche Klinik für Diagnostik, Wiesbaden No competing interests
REVIEW RETURNED	23-Jan-2012

GENERAL COMMENTS	-the manuscript needs correction by a native speaker; some mistakes, not serious -some references are superfluous, do you really need Ref. 5 and 9? -fig. 2: please delete patients published by Dressler et al. -please illustrate your hypothesis in detail

REVIEWER	Dirk Dressler, md, phd movement disorders section department of neurology hannover medical school
REVIEW RETURNED	03-Feb-2012

THE STUDY	titer quantification confusing
GENERAL COMMENTS	The authors studied antibodies against botulinum toxin (bt, bt-ab) under bt cessation and under continued application of incobotulinumtoxinA (X). They found that in both groups bt-ab titers declined. Decline under cessation has been described before, description of decline under X is novel. The authors touched upon an important issue. However, there are some major problems: 1) For prolonged periods of time X is obviously given to numerous patients with bt-ab titers which should produce complete therapy failure. How can it be justified to apply X to patients who do not benefit from this procedure? How can it be justified to apply X, if those patients would see their bt-ab titers dropping faster under cessation? There seems to be a major logical flaw in the treatment strategy. 2) The graphics are just awful and totally uninstructive. 3) The core of the work was performed by a contractor who is not co-author. BMJ Open needs documentation that the contractor is aware of the publication of these data and that he agrees. Otherwise major IP infringements are at stake. 4) The role of the complexing proteins is uncritically overestimated. Cited publications (by X's manufacturer) do not provide sufficient evidence.

VERSION 1 – AUTHOR RESPONSE

Prof. Benecke
It's confusing to use the term "open", as that usually means "unblinded"
Answer:
Prof. Benecke is correct; the antibody titres were determined by a contractor blinded to the clinical data. We therefore changed the word ‘open’ to ‘blinded’

Prof. Jost
1.-the manuscript needs correction by a native speaker; some mistakes, not serious
Answer:
The manuscript has been corrected by a native speaker

2. -some references are superfluous, do you really need Ref. 5 and 9?
Answer:
Prof. Jost is correct that ref. 9 can be omitted, because we also included the more relevant paper by Prof. Benecke et al. as ref. 8. We prefer to keep ref. 5, because it best describes the current understanding.

3.-fig. 2: please delete patients published by Dressler et al.
Answer:
The patients presented by Dressler and Bigalke in 2008 are different from our patients. Their patients had had extremely high titres. The decline of the titres was documented after cessation of therapy and when the titres had dropped down to the upper limit of the standard range of titres being analysed in the laboratory. This time point varied from patient to patient. To visualize and compare the steepness of the decline we have synchronized the data to the timing point when the titres touched into this standard range. Thus the data of Dressler are presented differently from their presentation in 2008. Furthermore, in several of our patients the initial titre was much lower than in the patients by Dressler and Bigalke when therapy was stopped. As Dressler´s data are the only existing ones so far, we think that the comparison with our data is necessary. Prof. Dressler did not object to the presentation of

these data.

4. -please illustrate your hypothesis in detail
Answer:
This point is well-taken. The manuscript has been changed accordingly.

Prof. Dressler
1. titer quantification confusing
Answer:
Titres result from dilution series and therefore grow exponentially. The logarithm is the inverse function to the exponential function and the appropriate mathematical tool to transform titres into a range so that decrease and increase of titres can be visualized.

2. a. For prolonged periods of time X is obviously given to numerous patients with bt-ab titers which should produce complete therapy failure.
Answer:
Why should X produce complete therapy failure when applied to patients with NAB-titres? So far it had not been investigated if X would produce complete therapy failure; we present here some evidence that this is not the case.

b.How can it be justified to apply X to patients who do not benefit from this procedure?
Answer:
Without any adequate investigation the response behaviour of a secondary non-responder can not be predicted in advance.

c. How can it be justified to apply X, if those patients would see their bt-ab titers dropping faster under cessation?
Answer:
If nobody analyses the development of antibodies under BTX-A treatment, nobody will see a decline and develop an alternative to treatment nihilism.

d.There seems to be a major logical flaw in the treatment strategy.
Answer:
The results of our study demonstrate that our treatment strategy seems to work.

3. The graphics are just awful and totally uninstructive.
Answer:
We are open to any suggestion improving the graphs. We have tried to present raw titres, but then the relevant range of titres is compressed to such a small band in the figures that they really become uninstructive. For the sake of comparison we decided to use the logarithmic ordinate scale. Furthermore, for the sake of transparency we think that it is necessary to present the titre changes for each single patient instead of mean values or medians. So far, this is the only investigation evaluating titre changes in such detail over up to 50 months.

4. The core of the work was performed by a contractor who is not co-author. BMJ Open needs documentation that the contractor is aware of the publication of these data and that he agrees. Otherwise major IP infringements are at stake.
Answer:
We do not share Prof. Dressler's opinion that the determination of NAB-titres of coded probes is the "core of the work". However, because we have a good ongoing collaboration with Prof. Bigalke under whose guidance the NAB-titres were determined as mentioned in the acknowledgement section, we asked him to become co-author of the present paper to which he agreed.

5. The role of the complexing proteins is uncritically overestimated. Cited publications (by X's manufacturer) do not provide sufficient evidence.
Answer:
Prof. Dressler is correct that we need to be careful about our statement of the antigenic role of the complexing proteins and we have rephrased statements in the text more cautiously to reflect the issue.

VERSION 2 – REVIEW

REVIEWER	Dirk Dressler, md, phd movement disorders section department of neurology hannover medical school
REVIEW RETURNED	27-Apr-2012

GENERAL COMMENTS	I raised four points: 1) None of the questions raised were answered. Instead, the authors produce further unsubstantivated claims on lack of cross-reactivity of bt-a antibodies against X. As now outlined in their reply, they based their study on the assumption that X application could produce a faster bt-ab decline than cessation, i.e. that antigen presentation rather than antigen cessation induces bt-ab decline. This is an odd hypothesis which they - consequently - can not prove. Prolonged application of X in patients with complete therapy failure is not 'therpeutic nihilism', but good clinical practise. Their therapeutic strategy, therefore, doesn't work, at least as long they expand it to patients with complete therapy failure. Avoiding the idiosyncratic log-calibration of their bt-ab titers would have greatly helped to compare them to those in the literature. 2) The graphics are still unchanged. This is not the obligation of the reviewer, but that of the authors. We still do not know whether bt-ab decline in the X group is identical, faster or slower than in the control group. There is no supporting statistics to clarify. 3) This issue was solved. Obviously, the contractor was not aware of the publication and their intellectual rights are now acknowledged. 4) This issue was solved. The authors missed a great opportunity. They could have improved our understanding and our therapeutic strategies, if they had 1) clarified the relationship of the bt-ab decline in the X group and in the control group 2) focussed on patients with partial rather than with complete therapy failure 2) compared the bt-ab decline of X to that of conventional bt drugs (without this, claims of 'low' X-antigenicity are speculative) 3) demonstrated cross reactivity of bt-ab between conventional drugs and X (as now claimed in their reply)

VERSION 2 – AUTHOR RESPONSE

1) None of the questions raised were answered.
Answer: We have tried to respond to these questions in detail.

Instead, the authors produce further unsubstantivated claims on lack of cross-reactivity of bt-a

antibodies against X.
Answer: Nowhere in the manuscript we talk about lack of cross-reactivity of bt-antibodies against X. We only observe that NABTs which occurred after Botox or Dysport decrease after some years of Xeomin treatment when patients were switched to Xeomin.

As now outlined in their reply, they based their study on the assumption that X application could produce a faster bt-ab decline than cessation, i.e. that antigen presentation rather than antigen cessation induces bt-ab decline. This is an odd hypothesis which they – consequently can not prove.
Answer: The reviewer should take into account how statistical testing works:
We demonstrated that the hypothesis that Xeomin treatment leads to a further increase of preexisting NABTs had to be rejected. This is equivalent to say that preexisting NABTs do not increase under NABTs after several years of Xeomin treatment.
This is the key message of our manuscript. We do not have any idea where we have mentioned the assumption cited by the reviewer.

Prolonged application of X in patients with complete therapy failure is not “therapeutic nihilism”, but good clinical practise.
Answer: We are glad to read that the reviewer thinks that prolonged application of X in patients with therapy failure is good clinical practice. However, complete therapy failure means that X therapy also failed in these patients. Obviously the reviewer means “complete therapy failure after Botox or Dysport treatment”.

Their therapeutic strategy, therefore, doesn´t work, at least as long they expand it to patients with complete therapy failure.
Answer: Our therapeutic strategy, to switch therapy to Xeomin in patients who developed NABTs under Botox or Dysport obviously works. Complete therapy failure can only be diagnosed when patients have been treated not only with Botox and/or Dysport but also with Xeomin.

Avoiding the idiosyncratic log-calibration of their bt-ab titers would have helped to compare them to those in the literature.
Answer: The log-scale has nothing to do with titer calibration. It is simply a mathematical transformation of titers to compress the (uninteresting) higher titer range and to expand the interesting lower titer range.
To allow detailed comparison with the titers described in the literature we present the transformed titers of 8 patients described by Dressler et al.

2. The graphics are still unchanged. This is not the obligation of the reviewer, but that of the authors.
Answer: We have discussed this point in detail with our department of Biomathematics and with members of the Institute of Biomechanics of the University of Duisburg. They strongly advised us not to use original titers since otherwise we would lose information and compress the interesting range of titer changes to less than 10% of a figure.
Furthermore, they supported the statistical approach described now in the methods.

3. This issue was solved. Obviously, the contractor was not aware of the publication and their intellectual rights are now acknowledged.
Answer: Co-authorship of Prof. Bigalke is a matter of good scientific cooperation and not a matter of debate about intellectual rights. Such a debate would probably yield different results than suggested by the reviewer.

In his response to our changes in the manuscript the reviewer raises further points:
They could have improved our understanding and our therapeutic strategies, if hey had…
Answer: The previous manuscript version already improved our understanding of antibody induction.

The study presented was not designed to answer all the interesting questions Prof. Dressler raises now.

1) clarified the relationship of the bt-ab decline in the X group and in the control group
Answer: Following the advice of our mathematical colleagues we have used a special mathematical approach to present evidence that there were no group statistical differences between patients with X treatment and patients not receiving BTX therapy. Whether this is sufficient for “clarification” seems to depend on the understanding of statistical models.

2) focussed on patients with partial rather than with complete therapy failure
Answer: That is what we have done. We have focused on patients with partial therapy failure. Patients with complete therapy failure as described by Prof. Dressler were only included for the sake of comparison and to respect his previous work. These patients can easily be deleted from the present manuscript without any major loss of information.

2) compared the bt-ab decline of X to that of conventional bt drugs without this, claims of 'low' X-antigenicity are speculative)
Answer: This suggests a complete research program. We agree with the reviewer and have recommended further studies in the discussion of the present manuscript.

3) demonstrated cross reactivity of bt-ab between conventional drugs and X (as now claimed in their reply)
Answer: We do not understand this point since under 1) the reviewer writes that “the authors produce further unsubstantiated claims on lack of cross-reactivity of bt-a antibodies against X.