PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Characteristics of patients included and enrolled in studies on the prognostic value of serum biomarkers for prediction of post-concussion symptoms following a mild traumatic brain injury: A systematic review
AUTHORS	Mercier, Eric; Tardif, Pier-Alexandre; Emond, Marcel; Ouellet, Marie-Christine; De Guise, Élaine; Mitra, Biswadev; Cameron, Peter; Le Sage, Natalie

VERSION 1 - REVIEW

REVIEWER	Eric Thelin University of Cambridge, UK. Karolinska Institutet, Sweden.
REVIEW RETURNED	01-Jun-2017

GENERAL COMMENTS	Language: Adequate English language throughout, some minor comments (see below). Main findings: Systematic review of mTBI papers analyzing PCS. The authors found that the patients included in many studies looking at protein biomarkers are not representative of the general mTBI population. This lack of standardization could pose a problem for the translation of research to care. General comments: In the Introduction, page 5 line 27, you write that “but the translation of biomarker research into clinical practice is still pending.” Do you mean to predict PCS? In that case I think you are right, however there are several centers that use the Scandinavian Guidelines and deciding to CT scan patients based on S100B levels and some centers worldwide that serially sample brain enriched proteins in more severe TBI patients to guide treatment. I really think that the authors are highlighting something important in their study (the discrepancy between the studies and reality). However, I miss summarizing Table in the end where you could highlight specific issues with the normal TBI population (frequency of alcohol abuse, multitrauma, neurologic disorders, age etc.) and compare it with your results. Could it not be a good point to exclude certain patients as a lot of these factors that authors adjusted for clearly affect outcome? The thesis in the manuscript (especially Discussion) is quite over-simplified as it takes a stand against the

	enrolment/inclusion/exclusion criteria in these studies. I miss counter-arguments as to why it could be important to exclude patients with comorbidities that would affect these studies. I assume that basically no study sampled biomarkers when they assessed outcome? Did any of your included studies do that? I would consider that a huge limitation as many of these biomarkers have a very limited effective half-life in serum, thus you would need new samples closer to the assessment to really make the connection (more a comment than a question). Specific comments: Minor: Abstract: I miss a sentence describing why protein biomarkers are important in this condition as you suddenly jump straight to aims in the “Objective”. Moreover, you should probably call it “protein biomarker”, “protein marker of brain tissue fate” or “brain enriched proteins” as a “biomarker” could mean anything, including i.e. radiological pathology etc. You should establish your definition early, and then use it throughout the manuscript. Minor: Abstract: I thought Web of Knowledge changed name to Web of Science in 2012? Minor: Page 5, Line 27: Like previously mentioned, the first time you use biomarker I would have used the term “…brain enriched protein (biomarker)” as to indicate that you are looking at protein biomarkers of brain injury. Minor: Table 4: Consider rephrasing “Delay of outcome assessment after TBI” to “Duration between TBI and outcome assessment”, “delay” seems to imply that they meant to do it but there was an unintentional delay. eTable 1: Minor: Why didn’t you include a search string for “neurofilament light”? It is the protein that has gained quite a lot of interest in the last couple of years in mTBI, especially among the different neurofilament proteins. Summary: This systematic review focuses on the population of protein biomarker studies than the biomarkers per se which is an interesting take on how outcome studies are constructed in the TBI literature. It is definitely a well performed systematic review and I suppose the only thing that could be interpreted as negative is the actual value and interest of their findings, but for someone who has worked a lot with similar studies I think it definitely has merit.

REVIEWER	Andrew Baker St. Michael's Hospital University of Toronto Canada
REVIEW RETURNED	19-Jun-2017

GENERAL COMMENTS	The authors should be congratulated for a carefully executed process aimed at wrestling with the diverse literature in this field. In this regard, their results have good credibility. Their conclusions are in part related to factors which may affect the translation from research to everyday patient care. This is based on the important

	interpretive concept of the link or similarity between subjects in a study, and the intended patient application. In a similar or parallel sense, a concern regarding this manuscript, relates to the potential difference between the diverse nature of the actual value of the included studies, and the assumed intended value that is inherent in deriving the conclusions. For example, some studies claim to evaluate the prognostic value of biomarkers by looking at statistical relationships between early concentrations and delayed symptoms. A strong statistical result is interpreted as evidence of prognostic value. The missing piece is that, despite this association, the biomarker adds little to a ROC curve that includes readily available clinical factors on the model. I am concerned that this is the point that is relevant to your conclusions rather than whether these (inadequate) studies may or may not have been conducted with the inclusion of various important subgroups of patients (elderly, intoxicated, mental health). I am concerned that the studies included have described themselves as "evaluating the prognostic value" and that the authors here have included these studies because they report delayed symptoms, but in fact they do not represent studies that evaluate prognostic value at all. The authors may want to expand on the various roles of biomarkers and what constitutes the actual evaluation of prognostic value. The authors have raised the issue of 'everyday patient care'. This manuscript therefore has a few components: the careful conduct of a systematic review (well done); the interpretation of the included studies beyond the stated inclusion criteria (not really done); a discussion of the implications of this systematic review with reference to 'everyday patient care'. I think that many want to either write or read into a biomarker study something related to its prognostic value. In actual fact, there is plenty of (other)value in these studies that relate early concentrations to delayed symptoms. (For example they may validate the biomarker as a potential mechanistic indicator - validated against the syndrome of concussion-with-delayed-symptoms). And so, I congratulate the authors on the well conducted systematic review. However, I don't think the issue is only "the characteristics of patients included and enrolled", I think the issue is the design and interpretation of studies that have early biomarker and late symptoms. Are those studies really evaluating the prognostic value of a biomarker? Is it possible to re-evaluate the included studies to determine whether they even attempt an adequate evaluation of prognostic value of biomarkers? Without this, my concern is that this excellent systematic review may inadvertently perpetuate the original errors of interpretation and continue the confusion about the need to be very clear regarding the important but distinct multiple roles of biomarkers.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Eric Thelin
Institution and Country: University of Cambridge, UK. Karolinska Institutet, Sweden.
Please state any competing interests or state 'None declared': None Declared

Please leave your comments for the authors below
Title: Characteristics of patients included and enrolled in studies on the prognostic value of serum biomarkers for prediction of post-concussion symptoms following a mild traumatic brain injury: A systematic review

Language: Adequate English language throughout, some minor comments (see below).

Main findings: Systematic review of mTBI papers analyzing PCS. The authors found that the patients included in many studies looking at protein biomarkers are not representative of the general mTBI population. This lack of standardization could pose a problem for the translation of research to care.

General comments:

In the Introduction, page 5 line 27, you write that "but the translation of biomarker research into clinical practice is still pending." Do you mean to predict PCS? In that case I think you are right, however there are several centers that use the Scandinavian Guidelines and deciding to CT scan patients based on S100B levels and some centers worldwide that serially sample brain enriched proteins in more severe TBI patients to guide treatment.

Answer:Thank you for this precision. We are aware of the Scandinavian Guidelines but we meant specifically 'translation of protein biomarker to predict PCS' (Introduction, p. 4, second paragraph, last line [all corrections are highlighted in yellow]).

Reviewer: I really think that the authors are highlighting something important in their study (the discrepancy between the studies and reality). However, I miss summarizing Table in the end where you could highlight specific issues with the normal TBI population (frequency of alcohol abuse, multitrauma, neurologic disorders, age etc.) and compare it with your results.

Answer: Thank you for this comment. Although the epidemiology of patients sustaining a mild TBI is poorly studied and highly heterogeneous, we could have better compared our results with the current literature. In the discussion, we have added a comment regarding patients with mild TBI being concomitantly intoxicated by alcohol or having a psychiatric or neurologic disorders (Discussion, second paragraph p. 8, lines 10-11). Later in the discussion, a comparison regarding the age of the patients included in our study versus other age-related epidemiological data on mild TBI patients (Discussion, , second paragraph p. 8, lines 11-18) is present.

Reviewer:Could it not be a good point to exclude certain patients as a lot of these factors that authors adjusted for clearly affect outcome? The thesis in the manuscript (especially Discussion) is quite over-simplified as it takes a stand against the enrolment/inclusion/exclusion criteria in these studies. I miss counter-arguments as to why it could be important to exclude patients with comorbidities that would affect these studies.

Answer: Thank you for this comment. We acknowledge the challenging balance that the researchers are facing in determining the inclusion/exclusion criteria. This is a complex issue. By wanting to highlight the discrepancy between the populations included and the clinical reality, we have omitted to present counter-arguments. The third paragraph of the discussion was modified in order to present

data required to facilitate the inclusion of subgroups such as intoxicated and older patients or patients with neurologic disorder (Discussion, p.8, third paragraph, lines 7-10).

Reviewer:I assume that basically no study sampled biomarkers when they assessed outcome? Did any of your included studies do that? I would consider that a huge limitation as many of these biomarkers have a very limited effective half-life in serum, thus you would need new samples closer to the assessment to really make the connection (more a comment than a question).

Answer:Indeed, some studies took additional samples at 6, 12, 24, 48 or 72 hours after injury, and one study collected serum samples for up to 21 days after the injury, but none sampled biomarkers when they assessed outcomes.

Reviewer: Specific comments:

Minor: Abstract: I miss a sentence describing why protein biomarkers are important in this condition as you suddenly jump straight to aims in the “Objective”. Moreover, you should probably call it “protein biomarker”, “protein marker of brain tissue fate” or “brain enriched proteins” as a “biomarker” could mean anything, including i.e. radiological pathology etc. You should establish your definition early, and then use it throughout the manuscript.

Answer: A sentence has been added to that effect (Abstract, p. 3, lines 3-5).

Reviewer: Minor: Abstract: I thought Web of Knowledge changed name to Web of Science in 2012?

Answer: Indeed. We corrected the name (Abstract, p. 3, lines 6-7; Methods, Search Strategy, p. 5, line 7).

Reviewer: Minor: Page 5, Line 27: Like previously mentioned, the first time you use biomarker I would have used the term “…brain enriched protein (biomarker)” as to indicate that you are looking at protein biomarkers of brain injury.

Answer: We agree with this suggestion. We made the following changes: Abstract, p. 3, lines 6 and 23; Strengths and limitations of this study, p. 3; Introduction, p. 4, second paragraph, lines 11 and 12; Introduction, p. 4, fourth paragraph, line 2; Methods, p. 5, Search Strategy, lines 1, 4, and 9; Methods, p. 5, Study Selection, second paragraph, line 2; Methods, p. 5, Data Extraction, line 5; Results, p. 6, Characteristics of the included studies, line 9; Discussion, p. 7, first paragraph line 8; Discussion, p. 8, third paragraph, line 4; Discussion, p. 9, fifth paragraph, line 15; Discussion, sixth paragraph p. 9, line 13; Conclusion, p. 9, line 1.

Reviewer: Minor: Table 4: Consider rephrasing “Delay of outcome assessment after TBI” to “Duration between TBI and outcome assessment”, “delay” seems to imply that they meant to do it but there was an unintentional delay.

Answer: Thank you for this suggestion. We substituted “delay” for “duration” throughout the manuscript (Methods, p. 5, Study selection, second paragraph, line 3; Results, p. 7, Outcomes presented in the included studies, line 6; Table 4, p. 14 and Table 6, p. 16).

Reviewer: eTable 1: Minor: Why didn’t you include a search string for “neurofilament light”? It is the protein that has gained quite a lot of interest in the last couple of years in mTBI, especially among the different neurofilament proteins.

Answer: The biomarkers included in our search strategy were chosen more than two years ago

following a group discussion and back then, we were not aware of any study that assessed the association between NF-L and PCS in patients with a mTBI. We believe this is unlikely to affect our results.

Reviewer: Summary: This systematic review focuses on the population of protein biomarker studies than the biomarkers per se which is an interesting take on how outcome studies are constructed in the TBI literature. It is definitely a well performed systematic review and I suppose the only thing that could be interpreted as negative is the actual value and interest of their findings, but for someone who has worked a lot with similar studies I think it definitely has merit.

Reviewer: 2
Reviewer Name: Andrew Baker
Institution and Country: St. Michael's Hospital, University of Toronto, Canada Please state any competing interests or state ‘None declared’: None Declared

Please leave your comments for the authors below The authors should be congratulated for a carefully executed process aimed at wrestling with the diverse literature in this field. In this regard, their results have good credibility. Their conclusions are in part related to factors which may affect the translation from research to everyday patient care. This is based on the important interpretive concept of the link or similarity between subjects in a study, and the intended patient application.

In a similar or parallel sense, a concern regarding this manuscript, relates to the potential difference between the diverse nature of the actual value of the included studies, and the assumed intended value that is inherent in deriving the conclusions.

For example, some studies claim to evaluate the prognostic value of biomarkers by looking at statistical relationships between early concentrations and delayed symptoms. A strong statistical result is interpreted as evidence of prognostic value. The missing piece is that, despite this association, the biomarker adds little to a ROC curve that includes readily available clinical factors on the model. I am concerned that this is the point that is relevant to your conclusions rather than whether these (inadequate) studies may or may not have been conducted with the inclusion of various important subgroups of patients (elderly, intoxicated, mental health). I am concerned that the studies included have described themselves as "evaluating the prognostic value" and that the authors here have included these studies because they report delayed symptoms, but in fact they do not represent studies that evaluate prognostic value at all.

The authors may want to expand on the various roles of biomarkers and what constitutes the actual evaluation of prognostic value. The authors have raised the issue of 'everyday patient care'. This manuscript therefore has a few components: the careful conduct of a systematic review (well done); the interpretation of the included studies beyond the stated inclusion criteria (not really done); a discussion of the implications of this systematic review with reference to 'everyday patient care'.

I think that many want to either write or read into a biomarker study something related to its prognostic value. In actual fact, there is plenty of (other)value in these studies that relate early concentrations to delayed symptoms. (For example they may validate the biomarker as a potential mechanistic indicator - validated against the syndrome of concussion-with-delayed-symptoms).

And so, I congratulate the authors on the well conducted systematic review. However, I don't think the issue is only "the characteristics of patients included and enrolled", I think the issue is the design and interpretation of studies that have early biomarker and late symptoms. Are those studies really

evaluating the prognostic value of a biomarker? Is it possible to re-evaluate the included studies to determine whether they even attempt an adequate evaluation of prognostic value of biomarkers? Without this, my concern is that this excellent systematic review may inadvertently perpetuate the original errors of interpretation and continue the confusion about the need to be very clear regarding the important but distinct multiple roles of biomarkers.

Answer:

We wish to thank the reviewer for his thoughtful suggestions. We agree that there is a clinically significant difference between merely statistically assessing the association between an early protein serum biomarker and post-concussion symptoms and determining the added prognostic value of any such biomarker over and above readily available clinical factors. We are also concerned that this difference is too often ignored in the relevant literature since this could lead to some confusion regarding the role/value(s) of biomarkers. In order to quantify the scope of this problem, two authors independently extracted the following data on all including studies: use of a univariate or multivariate regression model and use of AUROC (Area Under the Receiver Operating Characteristic curve) analyses. A study was considered as assessing the prognostic value of a given biomarker if it reported AUROC-related results. In studies who did, we further evaluated if the AUC of the biomarker was compared to the AUC obtained using clinical factors. The manuscript has been modified accordingly: Methods, p. 5, Data extraction, lines 8-9; Results, p. 7, new paragraph «Assessment of outcomes in the included studies»; Discussion, p. 9, new (sixth) paragraph, lines 1-13.

We thought these data to be sufficient to highlight the issue at hand and avoid adding to the confusion relating to the distinct roles of biomarkers. However, we did not develop further on the multiple roles of biomarkers nor on «what constitutes the actual evaluation of prognostic value» since we believe this goes beyond the initial aim of our study. We are aware that, in clinical practice, the two aspects (populations included vs roles of biomarker) goes hand in hand and should be both carefully addressed. Nevertheless, they can be logically distinguished, as is suggested by the fact that whether or not all studies were assessing the prognostic value of biomarkers (which is not the case), we could still raise the issue relating to the populations included in these studies. If specific populations of patients were excluded from these studies (which is the case), then the actual prognostic value of any given biomarker would still be limited to populations included (who are not totally representative of the populations of patients presenting themselves to emergency departments).

VERSION 2 – REVIEW

REVIEWER	Eric Thelin University of Cambridge, UK Karolinska Institutet, Sweden
REVIEW RETURNED	24-Jul-2017

GENERAL COMMENTS	This is a second review of a manuscript I've previously reviewed. The authors have adequately addressed my concerns in this revised version of the manuscript. This manuscript highlights the problematic issues of confounders seen in many mTBI studies making it difficult to fully translate to a normal patient cohort.