

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Is postoperative decrease of serum albumin an early predictor of complications after major abdominal surgery? A prospective cohort study in a European centre
<b>AUTHORS</b>	Labgaa, Ismail; Joliat, Gaëtan-Romain; Kefleyesus, Amanuel; Mantziari, Styliani; Schäfer, Markus; Demartines, Nicolas; HUBNER, Martin

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Åke Norberg, MD, PhD, Ass Prof Karolinska Institutet, Inst CLINTEC, Dept. of Anesthesia at Dept of Perioperative Medicine and Intensive Care, Karolinska University Hospital, Huddinge, Sweden
<b>REVIEW RETURNED</b>	28-Sep-2016

<b>GENERAL COMMENTS</b>	<p>Thank you for the opportunity to review this interesting manuscript. The authors have prospectively evaluated perioperative plasma albumin drop as a marker of surgical stress and as an early predictor of surgical outcome. Although clearly written and concise, some issues need clarifications and my concerns are listed below.</p> <p>1. Study size and power. Sample size is stated to be "similar to comparable studies in the field" (Page 5 line mark, LM 25-26) whereas statistics section contains no information regarding study size or power. This aspect can be improved. Please provide some information on how study size was determined and what power was anticipated with n=150.</p> <p>2. Perioperative fluid management. There is no information at all regarding the perioperative fluid management. Many factors, other than the surgical trauma as such, might influence perioperative albumin drop. If colloids or large amounts of chrystalloids are provided, this can dilute plasma volume. Epidural blocks can promote vasoplegia and thus contribute to decreased albumin levels by dilution. Postoperative unit guidelines for fluid management are important to understand volume status of the patients. The authors should provide some information on unit routines regarding restrictive/generous or other unit guidance of fluids administration during surgery i.e. amount and types of fluids given, the use of epidural blocks, bleeding substitution (according to Fig 2B at least 10 patients had more than 1000 ml bleeding), and routines for postoperative fluid management. How many patients received albumin or plasma? Were other colloids used?</p> <p>3. Patient selection.</p>
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	<p>Patients are stated to be consecutive (page 7 LM 7). However, some information to help the reader to understand the representativeness of the sample is missing. How many patients were excluded because of immunosuppression and preoperative antibiotics, respectively? This should be stated in the text or as a flow chart. Furthermore, "antibiotics treatment before surgery" is a vague parameter (page 5 LM 18-19), not stated in the NCT registration. Please clarify.</p> <p>4. Surgery time. One inclusion criteria was "...operative procedure with duration &gt; 2 h" (page 2 LM 27, page 5 LM 16). According to figure 1C about 15 patients had shorter duration of surgery, but were not excluded. I suggest to change text to "... with anticipated duration &gt; 2 hrs".</p> <p>5. Statistics. 5.1 In Table 2 HR (Hazards ratio?) is used (incorrectly?) whereas on page 8 last paragraph LM 40-45, OR is used (odds ratio?), and CI is spelled IC (LM40). This is incongruent, and furthermore not stated nor explained in statistical analysis. Please correct. 5.2 I suggest inserting (r) and (ρ) on page 6 LM 47 "...after categorical (ρ)and continuous variables (r), respectively." to increase clarity, if ρ is your chosen symbol for Spearman's rank correlation. 5.3 Shouldn't multivariate analysis in the logistic regression model be performed either backwards or forwards, and non-significant parameters be eliminated from the final model? 5.4 Likely the absence of significance of several parameters in the multivariate analysis is caused by the strong interrelationship between different indices of surgical stress. In discussion this can be commented on (table 2 and discussion).</p> <p>6. Introduction Page 4 LM 12-14. I think patient suffering is at least as important as costs, and deserves being mentioned.</p> <p>7. Figures. Figures 1, 2 and supplementary figure 2 are difficult to read. Please improve resolution and increase fonts until readable</p>
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<b>REVIEWER</b>	Sean Goh Gold Coast University Hospital 1 Hospital Blvd Southport QLD 4215 Australia
<b>REVIEW RETURNED</b>	03-Oct-2016

<b>GENERAL COMMENTS</b>	This study examined the use of serum albumin as a marker of post-operative complications in patients undergoing elective general surgery. Given the nature of surgery and the stress placed on patients, it is imperative to identify "at risk" populations and optimise their post-operative recovery. Apart from albumin levels, it would be useful to use nutritional status (pre- and post-op) to monitor these patients, as albumin levels are a surrogate marker of nutritional status, amongst others. In summary, while an albumin drop of >10g/L is a useful marker to detect patients at risk of post-op
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	complications, one would then have to identify potential complications and how best to optimise patient's post-op status to avoid them.
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<b>REVIEWER</b>	Richard Hall Dalhousie University Canada
<b>REVIEW RETURNED</b>	14-Oct-2016

<b>GENERAL COMMENTS</b>	<p><b>GENERAL COMMENT.</b></p> <p>The role of albumin as a marker of adverse outcomes has been previously examined in other populations including the critically ill. In those populations it has been determined to be a prognostic marker and this has been confirmed in the current study. However, several limitations are evident from a review of the manuscript as currently presented. The absence of a validation cohort is a significant limitation which is acknowledged by the investigators. Moreover, the correlations between the outcomes measured and albumin are rather poor, even though statistically significant. There are many outliers and the predictive capacity of albumin under these circumstances would be difficult to ascertain. It is suggested that a validation study be performed which would more appropriately delineate the role that albumin may play as an investigative marker for outcomes. Not examined, but potentially of use, would be the role that combinations of biomarkers might play in further increasing the sensitivity and specificity, in particular the measurement of lactate. It was a significant marker in the current study, although it was transient in nature, but it might, when combined with albumin, provide a more sensitive marker of adverse outcome. It too is readily measured within the first several hours following surgery and has many of the features, as acknowledged by the investigators, as albumin has as a useful predictive biomarker.</p> <p><b>SPECIFIC COMMENTS</b></p> <p>Page 4, LM6, suggest inserting the word “have” between “improvements” and “reduced”.LM11, the phrase “while the current ... expenditures” is somewhat awkward English. It is suggested that perhaps one could consider the phrasing “in the context of a desire to reduce health care expenditures”.</p> <p>LM22, delete “overshooting”.</p> <p>LM27, delete “a” in the phrase so as to read “limitation of slow kinetics”.</p> <p>LM29, albumin is not considered to be an acute phase protein in a traditional way. It is usually considered to be a maintenance protein which is downregulated in response to inflammation. It is suggested that this phrasing be reconsidered.</p> <p>Page 5, it would seem that a better estimate of an appropriate sample size could be determined given the number of studies reporting on the role of albumin in various types of surgery as reported above and cited by the authors. The reference citation for this sample size (Ref 18) is incomplete as currently provided.</p> <p>Page 9, LM51 “calculate”, not “calculated”.</p> <p>Page 10, LM6, the sentence beginning “Notwithstanding ... complexity” is rather poor English. Do you mean “Integrating more complex and costly markers would unlikely to be more informative given their poor reproducibility, cost and assay measurement</p>
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	<p>complexity”?</p> <p>LM17, the phrase “potential variability from the protocol cannot be excluded” is somewhat concerning. The measurement of the markers was protocolized, therefore the timing of the blood draw should have been recorded and would be known, and the protocol deviations should be measurable. Therefore, the degree to which the variability from the protocol occurred should be possible to calculate.</p> <p>LM31 “discerned”, not “discriminated”.</p> <p>Page 11, LM6, the phrase “even more performant” might be better phrased as “more sensitive”.</p> <p>LM11, the discussion related to IL-6 could potentially be deleted. It was not measured in the current study and, although of some information as it relates to other types of surgery, its relevance to the current findings is somewhat difficult to put into context given that it was not measured in the current study.</p> <p>Under References, Ref 3 is not complete. Ref 8, Ref 9, Ref 14, Ref 15, Ref 18</p> <p>Table 1 – the title of the column is Complications and then the number of patients is provided in brackets. It is not certain, therefore, what the numbers in brackets under, for example, Type, represent – this needs clarification.</p> <p>Table 2 – Postoperative complications – how was the univariate variables determined and what led to their inclusion in the multivariable analysis?</p> <p>Under Legends, Figure 1 – the correlations appear to be somewhat modest and perhaps should receive some comment on this in the Discussion.</p> <p>Supplementary Table 2 – what do the numbers in brackets represent?</p>
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<b>REVIEWER</b>	Domagoj Drenjancevic University Josip Juraj Strossmayer of Osijek Faculty of medicine, Croatia Osijek University Hospital Center, Croatia
<b>REVIEW RETURNED</b>	28-Oct-2016

<b>GENERAL COMMENTS</b>	<p>I find this article very interesting, although, in my opinion, an albumin decline can not be the only early marker that can be used in the prediction of the outcome for major surgical procedures, it can be used in the conjunction with other well established biomarkers.</p> <p>Overall, the article is written in clear language and is very interesting, hypotheses and objectives are substantiated strongly and comprehensively with very good statistical analysis. The paper is well written and easy to understand, the arguments are logical and not internally contradictory.</p> <p>I recommend the publication of the paper after minor revision. Minor objections that may need clarification:</p> <p>1. In section Statistical analysis (page 6, line 42): it is stated that the parameter age was dichotomized: (&gt;60 years) but in the Table 1. (page 16, line 12) it is written Age ≥ 70 years. And also, in same</p>
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	<p>section same are stated for the other parameters (BMI, operative time and blood loss) sign &gt; but in the same table (Table 1) it is written ≥. Same is in Table 2. (page 18)</p> <p>2. Table 2. (page 18): there is no explanation for abbreviation HR.</p>
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## VERSION 1 – AUTHOR RESPONSE

### Peer reviewer 1 comments

#### 1. Study size and power.

Sample size is stated to be “similar to comparable studies in the field” (Page 5 line mark, LM 25-26) whereas statistics section contains no information regarding study size or power. This aspect can be improved. Please provide some information on how study size was determined and what power was anticipated with n=150.

#### **Author response:**

By performing a two-sample t-test sample size calculation to detect a size effect of 0.8, with a degree of confidence defined by a 0.99 probability to find a true effect (power), with a significance level of 0.05, the number of required patients per group (i.e. with complication vs. without complication) was n=49.98. Anticipating a complication rate of 40%, in this setting of major abdominal surgery, the final sample size for this study was n=125 patients. Conversely, a sample size of n=150 would have yielded a power of 0.9994. This point was added in the methods:

A two-sample t-test was used to calculate sample size, with size effect of 0.8, power of 0.99 and significance level of 0.05. This determined a required number of 50 patients per group (i.e. with complication vs. without complication). Anticipating a complication rate of 40%, the sample size for this study was n=125 patients. In order to adjust for 10% drop-out or missing data, final sample size resulted in n=138.

#### 2. Perioperative fluid management.

There is no information at all regarding the perioperative fluid management. Many factors, other than the surgical trauma as such, might influence perioperative albumin drop. If colloids or large amounts of crystalloids are provided, this can dilute plasma volume. Epidural blocks can promote vasoplegia

and thus contribute to decreased albumin levels by dilution. Postoperative unit guidelines for fluid management are important to understand volume status of the patients.

The authors should provide some information on unit routines regarding restrictive/generous or other unit guidance of fluids administration during surgery i.e. amount and types of fluids given, the use of epidural blocks, bleeding substitution (according to Fig 2B at least 10 patients had more than 1000 ml bleeding), and routines for postoperative fluid management. How many patients received albumin or plasma? Were other colloids used?

**Author response:**

Prof. Norberg raised an important point. Fluid management might indeed influence postoperative albuminemia. Underlying mechanisms of postoperative albumin drop were detailed in a previous paper of our group (Hübner, Gastroenterology, Research & Practice 2016). Findings indicated that redistribution, through capillary leak, is the driving mechanism (77%) of albumin decrease whereas, blood loss and catabolism were the underlying cause of postoperative albumin decrease in only 17% and 6%, respectively. This was further confirmed by hematocrit measurements. The increased capillary leak was suggested to be triggered by the systemic inflammatory response (Smeets International Surgery 1994, Fleck Lancet 1985).

Nonetheless, perioperative fluid management is critical for the understanding of the data. Our department, closely adheres to enhanced recovery guidelines (e.g. Gustafsson World J Surg 2013, <http://erassociety.org.loopiadns.com/guidelines/list-of-guidelines/>). As a result, we pursue a stringent fluid regimen following specific clinical pathways for both intra-operative and postoperative fluid administration. Furthermore, advanced hemodynamic monitoring (LiDCO, CarioQ, etc.) is used to guide fluid administration. Ringer's lactate is the default crystalloid solution, while physiogel is given as colloid solution if needed. Intravenous albumin administration is exceptionally used in our practice. Epidurals are no longer recommended for laparoscopic procedures (Hübner Ann Surg 2015) but also for open surgeries. EDA-induced hypotension is typically counter-acted with low-dose vasopressor treatment and rarely requires generous fluid administration.

We expanded on these important elements in methods and discussion sections and we are ready to provide more details if needed:

*Methods (page 5):* Perioperative care closely adhered to recently published enhanced recovery guidelines (<http://erassociety.org.loopiadns.com/guidelines/list-of-guidelines>). Standardized fluid administration was followed by advanced hemodynamic monitoring to avoid intraoperative fluid overload. According to the clinical care pathway, intravenous fluid was typically discontinued the morning after surgery.

*Discussion (page 11):* The mechanisms of early postoperative albumin decrease combine altered metabolism, blood loss/dilution and most importantly redistribution into the third space, due to capillary leakage. The latter accounts for >75% of albumin decrease in the early postoperative phase and appears to be related to the magnitude of systemic inflammatory response<sup>10, 25, 26</sup>. Therefore, albumin decrease is certainly influenced by perioperative fluid management (liberal vs. restrictive) but it mainly reflects the extent of postsurgical stress response.

### 3. Patient selection.

Patients are stated to be consecutive (page 7 LM 7). However, some information to help the reader to understand the representativeness of the sample is missing. How many patients were excluded because of immunosuppression and preoperative antibiotics, respectively? This should be stated in the text or as a flow chart. Furthermore, “antibiotics treatment before surgery” is a vague parameter (page 5 LM 18-19), not stated in the NCT registration. Please clarify.

#### **Author response:**

Overall, 155 patients were identified as candidates for the study, but 17 patients refused to consent. No patient was excluded because of immunosuppression or preoperative antibiotics. Hence, these “exclusion” criteria were removed from the methods, as they did not impact patients’ selection.

### 4. Surgery time.

One inclusion criteria was "...operative procedure with duration > 2 h" (page 2 LM 27, page 5 LM 16). According to figure 1C about 15 patients had shorter duration of surgery, but were not excluded. I suggest to change text to ".... with anticipated duration > 2 hrs".

**Author response:**

The text was modified following the reviewer's suggestion:

- Abstract (page 2): [...] with **anticipated** duration  $\geq 2h$
- Methods (page 5): operative procedure with **anticipated** duration duration  $\geq 2h$

5. Statistics.

5.1 In Table 2 HR (Hazards ratio?) is used (incorrectly?) whereas on page 8 last paragraph LM 40-45, OR is used (odds ratio?), and CI is spelled IC (LM40). This is incongruent, and furthermore not stated nor explained in statistical analysis. Please correct.

**Author response:**

This was an error and HR was replaced by OR (odds ratio) in table 2, whereas the misspelling IC was corrected by CI.

5.2 I suggest inserting (r) and ( $\rho$ ) on page 6 LM 47 "...after categorical ( $\rho$ ) and continuous variables (r), respectively." to increase clarity, if  $\rho$  is your chosen symbol for Spearman's rank correlation.

**Author response:**

To increase clarity, ( $\rho$ ) and (r) were inserted after "categorical" and "continuous", respectively.

5.3 Shouldn't multivariate analysis in the logistic regression model be performed either backwards or forwards, and non-significant parameters be eliminated from the final model?

**Author response:**



For the logistic regression, variables with a p-value <0.1 in univariable analysis were subsequently considered in the multivariable analysis. In the final model (multivariable analysis), statistical significance was defined as a p-value <0.05.

This point was mentioned in the methods section, but we modified it for more clarity: “. Logistic regression was applied to identify independent predictors; variables with significance < 0.1 in univariable analyses were further included in multivariable analyses. A p value <0.05 was considered to be statistically significant in all tests”.

5.4 Likely the absence of significance of several parameters in the multivariate analysis is caused by the strong interrelationship between different indices of surgical stress. In discussion this can be commented on (table 2 and discussion).

**Author response:**

The reviewer made a good point. On one hand, the impact of several parameters on surgical stress is likely to overlap and this may, in part, explain the results obtained in multivariable analyses. On the other hand, it may suggest that albumin drop recapitulates these different surrogates of surgical stress. This was elaborated in the discussion.

The overlap of certain parameters of surgical stress may, in part, explain why they were not identified as independent predictor of complication. It may also suggest that serum albumin drop recapitulates these different parameters.

6. Introduction

Page 4 LM 12-14. I think patient suffering is at least as important as costs, and deserves being mentioned.

**Author response:**

This important point was added:

In addition to being troublesome experiences for patients, postoperative complications cause a substantial financial burden, [...].

## 7. Figures.

Figures 1, 2 and supplementary figure 2 are difficult to read. Please improve resolution and increase fonts until readable

### **Author response**

Figures 1, 2 and supplementary figure 2 were modified to enhance readability.

### **Peer reviewer 2 comments**

1. This study examined the use of serum albumin as a marker of post-operative complications in patients undergoing elective general surgery. Given the nature of surgery and the stress placed on patients, it is imperative to identify "at risk" populations and optimize their post-operative recovery. Apart from albumin levels, it would be useful to use nutritional status (pre- and post-op) to monitor these patients, as albumin levels are a surrogate marker of nutritional status, amongst others. In summary, while an albumin drop of  $>10\text{g/L}$  is a useful marker to detect patients at risk of post-op complications, one would then have to identify potential complications and how best to optimize patient's post-op status to avoid them.

### **Author response**

This nicely summarizes some challenges in major surgery, such as the integration and the optimization of nutritional status. Preoperative measurement of albumin to assess nutritional status and predict outcomes is widely acknowledged (discussion, page 10):

[...] or from low preoperative level, which is an acknowledged predictor of increased postoperative complication.

The second point made by the reviewer is also critical and appeared in the discussion:

“How the monitoring of Alb in surgical patients can lead to better outcomes is key question. Measures to preoperatively attenuate the stress response to surgery have been extensively explored. Interestingly, successful attempts were reported with immunonutrition, enhanced recovery programs (ERAS), or high-dose glucocorticoids. Whether these options would be able to restrain the stress response, once triggered, in the early postoperative phase remains to be investigated. In this setting, albumin drop may facilitate to test whether these measures may also be beneficial in the early postoperative phase, by permitting to design clinical trials enriched for patients at higher risk”.

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### Peer reviewer 3 comments

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1. The absence of a validation cohort is a significant limitation which is acknowledged by the investigators. Moreover, the correlations between the outcomes measured and albumin are rather poor, even though statistically significant. There are many outliers and the predictive capacity of albumin under these circumstances would be difficult to ascertain. It is suggested that a validation study be performed which would more appropriately delineate the role that albumin may play as an investigative marker for outcomes.

### Author response

The absence of a validation cohort is indeed a drawback of the study, as underscored in the section Strength and Limitations: “This study involved a single center and included a training cohort, without validation cohort”.

Regarding correlations, Prof. Hall made a fair point. Although being statistically significant, some correlations were not striking. This point was addressed in the discussion:

**Although correlation coefficients were modest**, the decrease in serum albumin significantly correlated with (I) the extent of surgery (mE-PASS, blood loss, duration of surgery, and surgical approach), (II) the maximal amplitude of other stress markers, such as CRP, PCT and LCT and

(III) was consistently associated with adverse outcomes (according to both Clavien classification, CCI, and LoS).

2. Not examined, but potentially of use, would be the role that combinations of biomarkers might play in further increasing the sensitivity and specificity, in particular the measurement of lactate. It was a significant marker in the current study, although it was transient in nature, but it might, when combined with albumin, provide a more sensitive marker of adverse outcome. It too is readily measured within the first several hours following surgery and has many of the features, as acknowledged by the investigators, as albumin has as a useful predictive biomarker.

#### **Author response**

The approach of combining biomarkers, proposed by Prof. Hall, is indeed insightful. On one hand, combining biomarkers (and/or clinical variables) is likely to improve sensitivity and specificity but on the other hand, this may also complicate the model, which could ultimately preclude its implementation in clinical practice. The present study primarily aimed to decipher the predictive value of single biomarkers that could easily be used in clinical practice, and to determine their ideal cut-off. That being said, we definitely agree with the relevance to develop a score integrating combined biomarkers (and/or clinical variables) and we may explore this strategy in the future.

This point was added in the “Strength and Limitations” and also addressed in the discussion:

- The predictive value of combined biomarkers was not assessed in the present study.
- Likewise, this study did not assess the predictive value of albumin drop combined with other biomarker and/or clinical variables. Although such a classifier may presumably improve sensitivity and specificity, it will also be more complex which could ultimately preclude its implementation in clinical practice.

3. Page 4, LM6, suggest inserting the word “have” between “improvements” and “reduced”.LM11, the phrase “while the current ... expenditures” is somewhat awkward English. It is suggested that perhaps one could consider the phrasing “in the context of a desire to reduce health care expenditures”.

### **Author response**

- “have” was inserted between “improvements” and “reduced”.
- The sentence on health costs was rephrased:  
[...] postoperative complications cause a substantial financial burden, **while important efforts are currently pursued to reduce health care expenditures.**

4. LM22, delete “overshooting”.

### **Author response**

“Overshooting” was deleted in page 4 and page 11.

5. LM27, delete “a” in the phrase so as to read “limitation of slow kinetics”.

### **Author response**

“Limitation of a slow kinetics” was replaced by “limitation of slow kinetics”.

6. LM29, albumin is not considered to be an acute phase protein in a traditional way. It is usually considered to be a maintenance protein which is downregulated in response to inflammation. It is suggested that this phrasing be reconsidered.

### **Author response**

This point was clarified and the previous sentence was rephrased as followed:

**Conversely, serum albumin is a maintenance protein that is rapidly downregulated by inflammatory signals.**

7. Page 5, it would seem that a better estimate of an appropriate sample size could be determined given the number of studies reporting on the role of albumin in various types of surgery as reported

above and cited by the authors. The reference citation for this sample size (Ref 18) is incomplete as currently provided.

#### **Author response**

By performing a two-sample t-test sample size calculation to detect a size effect of 0.8, with a degree of confidence defined by a 0.99 probability to find a true effect (power), with a significance level of 0.05, the number of required patients per group (i.e. with complication vs. without complication) was  $n=49.98$ . Anticipating a complication rate of 40%, in this setting of major abdominal surgery, the sample size was  $n=125$  patients. Conversely, a sample size of  $n=150$  would have yielded a power of 0.999. This point was added in the methods and reference 18 was removed:

A two-sample t-test was used to calculate sample size, with size effect of 0.8, power of 0.99 and significance level of 0.05. This determined a required number of 50 patients per group (i.e. with complication vs. without complication). Anticipating a complication rate of 40%, the sample size for this study was  $n=125$  patients. In order to adjust for 10% drop-out or missing data, final sample size resulted in  $n=138$ .

8. Page 9, LM51 “calculate”, not “calculated”.

#### **Author response**

This was corrected.

9. Page 10, LM6, the sentence beginning “Notwithstanding ... complexity” is rather poor English. Do you mean “Integrating more complex and costly markers would unlikely to be more informative given their poor reproducibility, cost and assay measurement complexity”?

#### **Author response**

The sentence was modified according to the reviewer’s suggestion:

Notwithstanding, integrating more complex and costly markers would unlikely to be more informative given their poor reproducibility, cost and assay measurement complexity.

10. LM17, the phrase “potential variability from the protocol cannot be excluded” is somewhat concerning. The measurement of the markers was protocolized, therefore the timing of the blood draw should have been recorded and would be known, and the protocol deviations should be measurable. Therefore, the degree to which the variability from the protocol occurred should be possible to calculate.

**Author response**

This phrase is indeed confusing. On POD0, blood was drawn 4-6h after surgery and we wondered whether this intrinsic variation (i.e. 4h vs. 6h. after surgery) could have had any impact on blood tests results, although there was no deviation from the protocol. To avoid confusion, this sentence was removed.

11. LM31 “discerned”, not “discriminated”.

**Author response**

This was changed.

12. Page 11, LM6, the phrase “even more performant” might be better phrased as “more sensitive”.

**Author response**

This was modified, following reviewer’s suggestion.

13. LM11, the discussion related to IL-6 could potentially be deleted. It was not measured in the current study and, although of some information as it relates to other types of surgery, its relevance to

the current findings is somewhat difficult to put into context given that it was not measured in the current study.

**Author response**

Since IL-6 was not assessed in the present study, this paragraph was removed from the discussion section.

14. Under References, Ref 3 is not complete. Ref 8, Ref 9, Ref 14, Ref 15, Ref 18

**Author response**

Reference 18 was removed whereas references 8, 9, 14 and 15 were completed.

15. Table 1 – the title of the column is Complications and then the number of patients is provided in brackets. It is not certain, therefore, what the numbers in brackets under, for example, Type, represent – this needs clarification.

**Author response**

In Table 1, the numbers in brackets represent percentages. However, for median values, such as age, BMI, they represent interquartile range. To clarify this point. “n (%)” was added in the title of the columns, and a footnote related to median values was also inserted: \* Median values (IQR)

16. Table 2 – Postoperative complications – how was the univariate variables determined and what led to their inclusion in the multivariable analysis?

**Author response**

For the logistic regression, variables with a p-value <0.1 in univariable analysis were subsequently considered in the multivariable analysis. In the final model (multivariable analysis), statistical significance was defined as a p-value <0.05.



This point was mentioned in the methods section, but we modified it for more clarity: “. Logistic regression was applied to identify independent predictors; variables with significance < 0.1 in univariable analyses were further included in multivariable analyses. A p value <0.05 was considered to be statistically significant in all tests”.

17. Under Legends, Figure 1 – the correlations appear to be somewhat modest and perhaps should receive some comment on this in the Discussion.

**Author response**

The reviewer made a fair point. Although being statistically significant, some correlations were not striking. This point was addressed in the discussion:

Although correlation coefficients were modest, the decrease in serum albumin significantly correlated with (I) the extent of surgery (mE-PASS, blood loss, duration of surgery, and surgical approach), (II) the maximal amplitude of other stress markers, such as CRP, PCT and LCT and (III) was consistently associated with adverse outcomes (according to both Clavien classification, CCI, and LoS).

18. Supplementary Table 2 – what do the numbers in brackets represent?

**Author response**

The numbers in brackets represent percentages. This was clarified.

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**Peer reviewer 4 comments**

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1. In section Statistical analysis (page 6, line 42): it is stated that the parameter age was dichotomized: (>60 years) but in the Table 1. (page 16, line 12) it is written Age  $\geq$  70 years. And also, in same section same are stated for the other parameters (BMI, operative time and blood loss) sign > but in the same table (Table 1) it is written  $\geq$ . Same is in Table 2. (page 18)

**Author response**

We modified these errors. In the methods, age  $\geq 70$  years was used for dichotomization. In methods and in table 1, the sign ">" was replaced by " $\geq$ " for duration, blood loss and BMI.

2. Table 2. (page 18): there is no explanation for abbreviation HR.

### Author response

This was corrected and replaced by "OR", standing for odds ratio. A footnote was also added.

### VERSION 2 – REVIEW

<b>REVIEWER</b>	Åke Norberg, MD, PhD, Ass Prof Karolinska Institutet, Inst CLINTEC, Dept of Anaesthesia at Department of Perioperative Medicine and Intensive Care, Karolinska University Hospital, Huddinge, Sweden
<b>REVIEW RETURNED</b>	04-Dec-2016

<b>GENERAL COMMENTS</b>	The manuscript is much improved, and the authors have addressed all my concerns and queries adequately.
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<b>REVIEWER</b>	Richard Hall Dalhousie University Canada
<b>REVIEW RETURNED</b>	28-Nov-2016

<b>GENERAL COMMENTS</b>	<p>A review of BMJ Open manuscript <b>Assessing postoperative decrease of serum albumin as an early predictor of complications after major abdominal surgery: a prospective cohort study.</b></p> <p>Authors: Labгаа I, Joliat G-R, Kefleyesus A et al.</p> <p><b>Manuscript # BMJOPEN-2016-013966.R1</b></p> <p><b>GENERAL OVERVIEW.</b></p> <p>This is a revision to a manuscript which I had the pleasure of reviewing. It examines the changes in albumin concentration as a marker of stress response and its prediction of early adverse clinical outcomes. They have determined that the early postoperative decrease of serum albumin correlated with 1) the extent of surgery, 2) its metabolic response, and 3) adverse outcomes such as complications and length of hospital stay. A decreased concentration</p>
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of serum albumin >10 g/L on POD 1 was associated with a 3-fold increased risk of overall postoperative complications.

**GENERAL COMMENT.**

The investigators have addressed many of the issues which I and other reviewers identified in the review of the first manuscript. The current version provides additional clarity to the question which was addressed. The major outstanding issue which this reviewer identifies is that when examining Figure 2 the investigators have examined the plots in a linear fashion. One wonders if a curvilinear fit would not have been a better representation of the data and whether this was examined. Particularly for length of stay (LOS) it looks as if there is a significant curvilinear relationship and, if so, this would address many of the concerns I previously had with respect to the number of outliers for these figures. If a curvilinear was a better fit then this would alter the conclusions to some extent, particularly with reference to the sensitivity and specificity and the interpretation of the relationship between the fall in serum albumin and its relationship to complications and LOS.

**SPECIFIC COMMENTS**

In the Introduction, page 4, LM9, the sentence beginning “In addition to being troublesome ... expenditures” reads somewhat awkwardly to me. Suggest perhaps the wording “In addition to the morbidity which patients are exposed to, postoperative complications pose a significant financial burden”. [Ref2]

LM16, rather than “recapitulates” perhaps the word “mirror” might be more representative.

Page 6, LM37, instead of “accounting” the word “counting” should be used.

For the Comprehensive Complication Index (CCI), LM42, suggest that some wording be provided to identify what the two anchors represent a) what does 0 mean in ref to 100?

Page 10, LM50 “used in the clinical setting”.

LM53 “which allowed us to capture”.

Page 11, LM19 “independent predictors of complications”.

LM22 “mirrors” rather than “recapitulates”.

LM24, suggest the word “biomarkers” rather than “markers”.

LM26 “in the clinical setting”.

LM31 “unlikely be” and delete “to”.

LM37, the sentence beginning “Although such a classifier ... practice” – while this reviewer agrees with this sentiment, the degree to which this argument applies will depend upon the degree of improvement in sensitivity and specificity obtained by the addition of the other marker. If, for example, a lactate measured at 6 hour postoperatively in addition to alteration in albumin improves the sensitivity to 90%, this might be well worth consideration and would be relatively easy to do.

Comment has already been made about the suggestion of a curvilinear analysis as a better representation of the data.

Page 12, LM33 “outcomes is a key question”.

LM44, the sentence beginning “In this setting ... risk” is awkward. Would suggest “In this setting, albumin drop may indicate whether these measures may be beneficial in the perioperative period by being incorporated into the design of clinical trials as a marker for patients at higher risk of perioperative complications”.

Page 13, LM2, delete comma between “surgery” and “remains” so as to read “after surgery and remains stable for several days”.

Table 1 – there are two definitions for the asterisk (\*) median mE-PASS and Median values (IQR) range. Please clarify.

#### **COMMENT TO THE EDITOR**

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	Recommendation: ?  Score: ?  Submitted: ?/10/16
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<b>REVIEWER</b>	Domagoj Drenjancevic Osijek University Hospital Centre Josip Juraj Strossmayer University of Osijek, Faculty of Medicine Croatia
<b>REVIEW RETURNED</b>	16-Dec-2016

<b>GENERAL COMMENTS</b>	I have no additional comments or suggestions after the revision of the manuscript. The authors have responded clearly and unambiguously to the questions.
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## VERSION 2 – AUTHOR RESPONSE

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### Peer reviewer 3 comments

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#### ***A. General comment***

The investigators have addressed many of the issues which I and other reviewers identified in the review of the first manuscript. The current version provides additional clarity to the question which was addressed. The major outstanding issue which this reviewer identifies is that when examining Figure 2 the investigators have examined the plots in a linear fashion. One wonders if a curvilinear fit would not have been a better representation of the data and whether this was examined. Particularly for length of stay (LOS) it looks as if there is a significant curvilinear relationship and, if so, this would address many of the concerns I previously had with respect to the number of outliers for these figures. If a curvilinear was a better fit then this would alter the conclusions to some extent, particularly with reference to the sensitivity and specificity and the interpretation of the relationship between the fall in serum albumin and its relationship to

complications and LOS.

**Author response:**

Figure 2 displays two graphs that illustrate the correlation between the postoperative decrease of serum albumin and (A) the global morbidity, with the CCI score and (B) the length of stay. Both correlations were tested with a Pearson's test ( $r$ ). This is a standard approach where the correlation between the two tested variables is *de facto* linear.

**B. Specific comments**

1. In the Introduction, page 4, LM9, the sentence beginning "In addition to being troublesome ... expenditures" reads somewhat awkwardly to me. Suggest perhaps the wording "In addition to the morbidity which patients are exposed to, postoperative complications pose a significant financial burden". [Ref2]

**Author response:**

This was modified according to the reviewer's suggestion.

1. LM16, rather than "recapitulates" perhaps the word "mirror" might be more representative.

**Author response:**

This was modified according to the reviewer's suggestion.

2. Page 6, LM37, instead of "accounting" the word "counting" should be used.

**Author response:**

This was modified according to the reviewer's suggestion.

3. For the Comprehensive Complication Index (CCI), LM42, suggest that some wording be provided to identify what the two anchors represent a) what does 0 mean in ref to 100?

**Author response:**

This was clarified as followed: "Global morbidity for each patient was quantified by the Comprehensive Complication Index (CCI) on a scale from 0 to 100<sup>20</sup>, representing respectively no complication and postoperative death"

4. Page 10, LM50 "used in the clinical setting".

**Author response:**

This was modified according to the reviewer's suggestion.

5. LM53 "which allowed us to capture".

**Author response:**

This was modified according to the reviewer's suggestion.

6. Page 11, LM19 "independent predictors of complications".

**Author response:**

This was modified according to the reviewer's suggestion.

7. LM22 "mirrors" rather than "recapitulates".

**Author response:**

This was modified according to the reviewer's suggestion.

8. LM24, suggest the word "biomarkers" rather than "markers".

**Author response:**

This was modified according to the reviewer's suggestion.

9. LM26 "in the clinical setting".

**Author response:**

This was modified according to the reviewer's suggestion.

10. LM31 "unlikely be" and delete "to".

**Author response:**

This was modified according to the reviewer's suggestion.

11. LM37, the sentence beginning "Although such a classifier ... practice" – while this reviewer agrees with this sentiment, the degree to which this argument applies will depend upon the degree of improvement in sensitivity and specificity obtained by the addition of the other marker. If, for example, a lactate measured at 6 hour postoperatively in addition to alteration in albumin improves the sensitivity to 90%, this might be well worth consideration and would be relatively easy to do.

**Author response:**

The reviewer made a fair point. The value of such a classifier will obviously depend on its ability to improve the overall performance (sensitivity and specificity) to predict postoperative complications. We also think it is worth considering this option and are aiming to dedicate a separate study to thoroughly build an accurate score that would be easy to use in clinical practice.

12. Page 12, LM33 "outcomes is a key question".

**Author response:**

This was modified according to the reviewer's suggestion.

13. LM44, the sentence beginning "In this setting ... risk" is awkward. Would suggest "In this setting, albumin drop may indicate whether these measures may be beneficial in the perioperative period by being incorporated into the design of clinical trials as a marker for patients at higher risk of perioperative complications".

**Author response:**



This was modified according to the reviewer's suggestion.

14. Page 13, LM2, delete comma between "surgery" and "remains" so as to read "after surgery and remains stable for several days".

**Author response:**

This was modified according to the reviewer's suggestion.

15. Table 1 – there are two definitions for the asterisk (\*) median mE-PASS and Median values (IQR) range. Please clarify.

**Author response:**

The asterisk after median mE-PASS was removed

**VERSION 3 – REVIEW**

<b>REVIEWER</b>	Richard Hall Dalhousie University Canada
<b>REVIEW RETURNED</b>	27-Jan-2017

<b>GENERAL COMMENTS</b>	<p>A Review of the Article :</p> <p>Assessing Postoperative Decrease of Serum Albumin as an Early Predictor of Complications After Major Abdominal Surgery: A Prospective Cohort Study in a Western Center</p> <p>BmjOpen-2016-013966.R2</p> <p>Authors: Labгаа I et al</p> <p>General comment: This is the second revision of an article which I have previously had the privilege to review. The current revision has addressed many of the previous concerns. However, the increased clarity has identified some issues which I think need further consideration.</p> <p>The Abstract:: The conclusion lacks punch – the results are much better summarized in the first pg of the conclusions and again in the last paragraph of the conclusion.</p> <p>Strengths and Limitations: The rather poor correlations are major limitations which should be acknowledged</p> <p>Main Manuscript:</p>
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	<p>Pg 5 LM 31 – your description of the power analysis – as currently worded it reads awkwardly and the definition of what the metric you are trying to investigate poorly described. What exactly were you trying to examine – identify the two groups under consideration (? Complications vs no complications), the variability that is known to exist in the outcome of interest, and the difference that you would accept as meaningful so that others could replicate the result (or argue about the input values) and then the derived numbers for each group of interest.</p> <p>Pg 8 LM 31 – why not include the data for all the biomarkers in this figure – would require some creativity so as to include the measured values but %change from baseline might be one way</p> <p>Pg 9 LM 22 - Would add supplementary Table for all other biomarkers with this data provided - would serve to highlight the degree to which any of these biomarkers have utility in predicting Post op complications and serve to better position the current findings for albumin Some indication of the amount of fluid administered would be helpful in putting this statement in context - particularly if different between those with vs without complications</p> <p>Pg 11 LM 10 - Some indication of the amount of fluid administered would be helpful in putting this statement in context - particularly if different between those with vs without complications</p> <p>Pg 12 LM 33 - Suggest this paragraph be deleted as you have not determined in a validated way what the role of albumin may play and it is a long stretch to suggest it may play a role in management of the surgical stress response</p> <p>Conclusion – Have already suggested that the Abstract be amended to reflect the concepts outlined here in last pg</p>
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### VERSION 3 – AUTHOR RESPONSE

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#### Peer reviewer 3 comments

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##### ***B. General comment***

1. The Abstract: The conclusion lacks punch – the results are much better summarized in the first pg of the conclusions and again in the last paragraph of the conclusion.

##### **Author response:**

Following the reviewer’s suggestion, the conclusion of the abstract was modified as followed:

Early postoperative decrease of serum albumin correlated with the extent of surgery, its metabolic response, and with adverse outcomes such as complications and length of stay. A decreased concentration of serum albumin  $\geq 10\text{g/l}$  on POD 1 was associated with a 3-fold

increased risk of overall postoperative complications, and may thus be used to identify patients at risk.

2. Strengths and Limitations: The rather poor correlations are major limitations which should be acknowledged

**Author response:**

This point was added in the strengths and limitations:

- Albumin decrease modestly correlated with several markers of surgical stress.

3. Pg 5 LM 31 – Your description of the power analysis – as currently worded it reads awkwardly and the definition of what the metric you are trying to investigate poorly described. What exactly were you trying to examine – identify the two groups under consideration (? Complications vs no complications), the variability that is known to exist in the outcome of interest, and the difference that you would accept as meaningful so that others could replicate the result (or argue about the input values) and then the derived numbers for each group of interest.

**Author response:**

The sample size calculation was already addressed during the 1<sup>st</sup> round of revisions. We proposed the following modifications that were approved by the reviewers in the 2<sup>nd</sup> version of the manuscript:

By performing a two-sample t-test sample size calculation to detect a size effect of 0.8, with a degree of confidence defined by a 0.99 probability to find a true effect (power), with a significance level of 0.05, the number of required patients per group (i.e. with complication vs. without complication) was n=49.98. Anticipating a complication rate of 40%, in this setting of major abdominal surgery, the final sample size for this study was n=125 patients. Conversely, a sample size of n=150 would have yielded a power of 0.9994. This point was also added in the methods:

A two-sample t-test was used to calculate sample size, with size effect of 0.8, power of 0.99 and significance level of 0.05. This determined a required number of 50 patients per group (i.e. with

complication vs. without complication). Anticipating a complication rate of 40%, the sample size for this study was n=125 patients. In order to adjust for 10% drop-out or missing data, final sample size resulted in n=138.

4. Pg 8 LM 31 – Why not include the data for all the biomarkers in this figure – would require some creativity so as to include the measured values but %change from baseline might be one way

**Author response:**

We aimed to avoid generating an overwhelming figure. CRP and PCT being widely known and used, integrating their profiles in this figure would not add substantial information for the readers.

5. Pg 9 LM 22 - Would add supplementary Table for all other biomarkers with this data provided - would serve to highlight the degree to which any of these biomarkers have utility in predicting Post op complications and serve to better position the current findings for albumin

**Author response:**

The performance of each biomarker to predict postoperative complications is provided in supplementary figure 2. The results precisely enable to position albumin as an accurate biomarker to predict complications. Using ROC curves, its AUC at POD1 was 0.73, compared to 0.63 and 0.64 for PCT and LCT, respectively. CRP had a similar AUC of 0.75 but only at POD4. As you know, the relative slow kinetic of CRP is widely described in the literature. These results reinforce the interest to measure albumin in surgical patients.

6. Pg 11 LM 10 - Some indication of the amount of fluid administered would be helpful in putting this statement in context - particularly if different between those with vs without complications

**Author response:**

This was added in Table 1.

7. Pg 12 LM 33 - Suggest this paragraph be deleted as you have not determined in a validated way what the role of albumin may play and it is a long stretch to suggest it may play a role in management of the surgical stress response

**Author response:**

Although not validated yet, the present findings support the potential role of  $\Delta$ Alb POD1 for the identification of patients at risk. Whether this may be translated into better outcomes, through certain measures, is indeed a crucial question. This paragraph aims to suggest strategies to integrate  $\Delta$ Alb POD1 in clinical practice, with the ultimate objective to improve patients' outcomes.

Therefore, we estimated that this paragraph is of interest for surgeons or clinical investigators and that it should be maintained in the discussion.

**VERSION 4 – REVIEW**

<b>REVIEWER</b>	Richard Hall Dalhousie University Halifax NS Canada
<b>REVIEW RETURNED</b>	13-Feb-2017
<b>GENERAL COMMENTS</b>	The authors have addressed my concerns