PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Is high-sensitivity troponin, alone or in combination with copeptin, sensitive enough for ruling out NSTEMI in very early presenters at admission ? A post-hoc analysis performed in emergency departments.
AUTHORS	Chenevier-Gobeaux, Camille; Sebbane, Mustapha; Meune, Christophe; Lefebvre, Sophie; Dupuy, Anne-Marie; Lefevre, Guillaume; Peschanski, Nicolas; Ray, Patrick

VERSION 1 - REVIEW

REVIEWER	Jasper Boeddinghaus University Hospital Basel Cardiovascular Research Institute Basel (CRIB) Basel, Switzerland
REVIEW RETURNED	25-Jun-2018

GENERAL COMMENTS	I appreciate the opportunity to review the article by Chenevier-Gobeaux et al. entitled "Is high-sensitivity troponin (alone or in combination with copeptin) enough for ruling out NSTEMI in very early presenters at admission?". The authors question whether hs-cTnT and copeptin (alone or in combination) allow a safe rule-out of NSTEMI in the vulnerable subgroup of early presenters. The manuscript is well written, and the analyses appropriately assessed. However, there are major concerns that need to be addressed.
	Major:
	1.) Previous studies have shown that an early rule-out of NSTEMI using hs-cTn alone, also in the vulnerable subgroup of early presenters, is safe (with high sensitivities and negative predictive values). The conclusion of the authors of the presents study is that both biomarkers are NOT safe for early rule-out of NSTEMI in patients presenting early. The major concern of the reviewer is that gold standard diagnoses were adjudicated by the use of a conventional but not a hs-cTn assay and that different assays where used. This may have led to the underdiagnoses of patients who were then later falsely ruled-out when using hs-cTn instead of conventional cTn concentrations. This point needs upfront discussion, as it limits the generalizability of the findings and explains why sensitivities and NPVs were much lower as compared to previous studies.
	2.) As mentioned by the authors, the number of early presenters (cpo<2h) is relatively small, although data from three cohorts was used. This explains why false rule-out of 3 patients results in a

significant drop in sensitivity and NPV. Many previous studies investigated the rule-out performance (e.g., in subgroup analyses) in early presenters using e.g. the LoD of hs-cTnT and hs-cTnI and found much higher sensitivities and NPVs. This can be explained by differences of final adjudication (see point 1) or due to a larger number of patients presenting early. Please discuss in more detail.

3.) Methods: What is the median time from chest pain onset to first study blood draw? These data are highly important to guarantee that the investigated early presenters are "real early presenters" who were correctly triaged towards rule-out by the hs-cTn concentrations used.

4.) Is there an increase in safety when adding a non-ischemic ECG on top of hs-cTnT concentrations, as suggested by Pickering et al. (Ann Intern Med)?

Minor:

1.) This reviewer would suggest deleting the "()" from the title.

2.) This reviewer would suggest using the term "high-sensitivity cardiac troponin assay" instead of "highly-sensitive".

3.) Please provide p-values for comparisons between rule-out sensitivities, NPVs (e.g., cTnI alone vs. cTnI+copeptin). Not mandatory, as 95%CI shown.

4.) Please discuss in more detail that previous studies have shown that there is no (or only a marginal) benefit when adding copeptin to hs-cTn assays.

5.) The quite low AUC of 0.85 for hs-cTnT in early presenters (but also patients presenting within 4h after CPO) may also be explained by the fact that NSTEMI presence was not adjudicated by the use of a hs-cTn assay. This should be discussed.

6.) The current ESC guidelines incorporate an additional criterion for direct rule-out of patients (rule-out only possible if cpo>3h). Therefore, the recommendations are in line with the conclusions of the authors. The use of a 1h-delta (0/1h-algorithms) seems necessary for safe rule-out. This point should be highlighted and discussed.

REVIEWER	Prof Paul Collinson
	St George's University Hospitals NHS Foundation Trust, London,UK
REVIEW RETURNED	03-Sep-2018

| GENERAL COMMENTS | The authors have undertaken a pooled analysis of three previous studies to address the slightly vexed question of the problem of early presentation as well, as the additional value of adding copeptin to troponin estimation. Their most interesting conclusion is that very early presentation with pain, less than two hours, requires further evaluation. That they are able to demonstrate this in a relatively small study is very important. |

Please would the authors answer the following points.

1. Abstract. The first line of the results section is not very clear. Do the authors mean that diagnostic accuracy with reduced as time to onset of chest pain gets less?

2. Page 3 gold standard diagnosis. The authors state that they have used the universal definition of myocardial infarction. Assuming that they used the methods stated in the troponin measurements section, both of these methods are contemporary sensitive troponin assays with a 99th percentile above the 10% CV for the assay. The authors then go on to state that they use a rise/fall pattern above the 10% CV. Since the universal definition uses the 99th percentile and even for assays with a 99th percentile in the 10 to 20% CV range, the 99th percentile can be used, the choice of a 10% CV seems rather odd. Especially as both of the assays meet acceptable diagnostic criteria. Please will the authors clarify exactly what they mean. Are they using the 99th percentile (in accordance with universal definition) or are they using a diagnostic threshold above the 99th percentile. If this is the case, this is highly significant as the diagnostic sensitivity for myocardial infarction will be reduced as the decision threshold is being set at a higher value. Conversely, if they are using a 10% CV which is below the 99th percentile, they will be understating the case.

3. Laboratory methods (troponin and copeptin). This section is most unclear and the authors are inconsistent and incorrect in their use of analytical terminology. For all of the assays, the authors need to clearly state the following information, the limit of detection of the assay and the limit of quantitation of the assay (using the conventionally accepted definition of the clinical laboratory standards Institute, the 20% imprecision point is an FDA construct), the 20% imprecision point on the imprecision curve, the 10% imprecision point for the assay, the 99th percentile for the assay, the measuring range of the assay and the expected imprecision values across the measuring range. As they are mixing contemporary sensitive and high sensitive assays, I would suggest that they provide units for comparison and provide units in parentheses as nanograms/L for the conventional sensitive assays. For example 0.04 µg/L (40 ng/L).

4. Statistical analysis. The authors are mixing two methodologically different troponin I (cTnI) assays. Whilst I have no trouble with the use of appropriate decision thresholds for diagnosis of myocardial infarction (subject to the caveats mentioned above) and to the use of diagnostic tabulation (as shown in table 4), I have some concerns about combining the data for ROC analysis. Certainly, the data from those sites using the same cTnI method can be combined, but the lack of standardisation between methodologies does not really support pooling all cTnI data regardless of method without some form of data transformation. If the data was normalised by factorising to the 99th percentile, then log transformed prior to ROC analysis that would likely remove any bias due to methodological differences. I would wish to see some statistical methodological justification for simple pooling of all cTnI data. Clearly, this does not apply to the troponin T (cTnT) and copeptin data. In addition, will the authors clearly state in this section the diagnostic thresholds being used for classification of the data. This

| | information (which is crucial to the analysis) is scattered across the laboratory methodology and the tables and not clearly gathered together in one place.

5. A diagnostic discriminant of 3 ng/L has also been used in the literature as a rule out threshold for cTnT and I am surprised the authors have not included in this analysis.

6. The areas under the ROC curves should be included in the figures or the figure legends. |
|---|---|

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Jasper Boeddinghaus

Institution and Country: University Hospital Basel, Cardiovascular Research Institute Basel (CRIB), Basel, Switzerland

I appreciate the opportunity to review the article by Chenevier-Gobeaux et al. entitled "Is high-sensitivity troponin (alone or in combination with copeptin) enough for ruling out NSTEMI in very early presenters at admission?". The authors question whether hs-cTnT and copeptin (alone or in combination) allow a safe rule-out of NSTEMI in the vulnerable subgroup of early presenters. The manuscript is well written, and the analyses appropriately assessed. However, there are major concerns that need to be addressed.

Answer to Reviewer #1: We would like to thank reviewer #1 for his comments. We have revised our manuscript so as to take account of all these comments. We have no rebuttal. All changes are in red script in the manuscript.

Major:

1.) Previous studies have shown that an early rule-out of NSTEMI using hs-cTn alone, also in the vulnerable subgroup of early presenters, is safe (with high sensitivities and negative predictive values). The conclusion of the authors of the presents study is that both biomarkers are NOT safe for early rule-out of NSTEMI in patients presenting early. The major concern of the reviewer is that gold standard diagnoses were adjudicated by the use of a conventional but not a hs-cTn assay and that different assays where used. This may have led to the underdiagnoses of patients who were then later falsely ruled-out when using hs-cTn instead of conventional cTn concentrations. This point needs upfront discussion, as it limits the generalizability of the findings and explains why sensitivities and NPVs were much lower as compared to previous studies.

We up fronted this limit of our study (see Limitation section) in our revised manuscript.

2.) As mentioned by the authors, the number of early presenters (cpo<2h) is relatively small, although data from three cohorts was used. This explains why false rule-out of 3 patients results in a significant drop in sensitivity and NPV. Many previous studies investigated the rule-out performance (e.g., in subgroup analyses) in early presenters using e.g. the LoD of hs-cTnT and hs-cTnI and found much higher sensitivities and NPVs. This can be explained by

differences of final adjudication (see point 1) or due to a larger number of patients presenting early. Please discuss in more detail.

We discussed in more details this limit of our study (see Limitation section).

3.) Methods: What is the median time from chest pain onset to first study blood draw? These data are highly important to guarantee that the investigated early presenters are "real early presenters" who were correctly triaged towards rule-out by the hs-cTn concentrations used.

We defined the onset of chest pain (CPO), defined as the delay from symptom onset to the ED presentation.

The median time from chest pain onset to first study blood draw was not reported for all patients; patients from [ref. 12] indicated a median time at 21 minutes (IQR 16-30 minutes). Knowing that the mean time of patients with CPO<2h is 60 minutes (IQR 28-90 minutes), we presume that all patients had similar timelaps. We consider this value as low and thus we can consider that early presenters are "real early presenters".

4.) Is there an increase in safety when adding a non-ischemic ECG on top of hs-cTnT concentrations, as suggested by Pickering et al. (Ann Intern Med)?

In this post-hoc analysis, we did not collect the ischemic criteria of the ECG for all patients. This criterion was collected in the sub group from [ref 12]. Considering this sub-group, we could not confirm that there is an increase in safety when adding a non-ischemic ECG on top of HS-cTnT concentrations, as suggested by Pickering et al. [Ann Intern Med. 2017;166: 715-724]. Indeed, in the 136 patients with CPO <2h analyzed in this sub-group analysis, there was 11 patients with NSTEMI, and we found that an HS-cTnT had a sensitivity of 100%, a specificity of 57%, a NPV of 100% and a PPV of 17%. An ischemic ECG had lower diagnostic than HS-cTnT, as we found a sensitivity of 73%, a specificity of 70%, a NPV of 97% and a PPV of 17% (3 NSTEMI missed).

Thus, in the context of very early presenters, there is no increase in safety when adding a non-ischemic ECG on the top of HS-cTnT.

Minor:

1.) This reviewer would suggest deleting the "()" from the title.

The "()" were deleted from the revised title as suggested.

2.) This reviewer would suggest using the term "high-sensitivity cardiac troponin assay" instead of "highly-sensitive".

The term "highly-sensitive" was replaced with "high-sensitivity" in the revised manuscript, as suggested.

3.) Please provide p-values for comparisons between rule-out sensitivities, NPVs (e.g., cTnI alone vs. cTnI+copeptin). Not mandatory, as 95%CI shown.

We provided p-values for comparisons in Table 4. Only significative p-values were reported using superscript signs (ε, ζ, ψ, μ) and corresponding footnotes.

4.) Please discuss in more detail that previous studies have shown that there is no (or only a marginal) benefit when adding copeptin to hs-cTn assays.

The revised version of our manuscript contains, in the discussion section, more details on previous studies that have shown that there is no (or only a marginal) benefit when adding copeptin to hs-cTn assays. For this purpose, a new reference was added (ref. 28).

5.) The quite low AUC of 0.85 for hs-cTnT in early presenters (but also patients presenting within 4h after CPO) may also be explained by the fact that NSTEMI presence was not adjudicated by the use of a hs-cTn assay. This should be discussed.

We discuss this limit of our study in our revised manuscript (see Limitation section).

6.) The current ESC guidelines incorporate an additional criterion for direct rule-out of patients (rule-out only possible if cpo>3h). Therefore, the recommendations are in line with the conclusions of the authors. The use of a 1h-delta (0/1h-algorithms) seems necessary for safe rule-out. This point should be highlighted and discussed.

We highlighted and discussed this point in our revised manuscript at the end of the discussion section, as recommended by the reviewer.

Reviewer: 2

Reviewer Name: Prof Paul Collinson

Institution and Country: St George's University Hospitals NHS Foundation Trust, London,UK

Please state any competing interests or state 'None declared': None declared

The authors have undertaken a pooled analysis of three previous studies to address the slightly vexed question of the problem of early presentation as well, as the additional value of adding copeptin to troponin estimation. Their most interesting conclusion is that very early presentation with pain, less than two hours, requires further evaluation. That they are able to demonstrate this in a relatively small study is very important.

Please would the authors answer the following points.

Answer to Reviewer #2: We would like to thank reviewer #2 for his/her comments. We have revised our manuscript so as to take account of all these comments. We have no rebuttal. All changes are in red script in the manuscript.

1. Abstract. The first line of the results section is not very clear. Do the authors mean that diagnostic accuracy with reduced as time to onset of chest pain gets less?

We rephrased the first line in the revised manuscript. The diagnostic accuracy is reduced when time to onset of chest pain gets less.

2. Page 3 gold standard diagnosis. The authors state that they have used the universal definition of myocardial infarction. Assuming that they used the methods stated in the troponin measurements section, both of these methods are contemporary sensitive troponin assays with a 99th percentile above the 10% CV for the assay. The authors then go on to state that they use a rise/fall pattern above the 10% CV. Since the universal definition uses the 99th percentile and even for assays with a 99th percentile in the 10 to 20% CV range, the 99th percentile can be used, the choice of a 10% CV seems rather odd. Especially as both of the assays meet acceptable diagnostic criteria. Please will the authors clarify exactly what they

mean. Are they using the 99th percentile (in accordance with universal definition) or are they using a diagnostic threshold above the 99th percentile. If this is the case, this is highly significant as the diagnostic sensitivity for myocardial infarction will be reduced as the decision threshold is being set at a higher value. Conversely, if they are using a 10% CV which is below the 99th percentile, they will be understating the case.

We added in our revised manuscript that the universal definition that was used was the version in force at the time of inclusion ([ref. 18], version 2012):

"AMI was diagnosed according to the universal definition that was in force at the time of inclusions and adapted to the use of a conventional cTn (18). Thus, patients with a cTnI increase (or a rise/fall pattern) above the 10%CV…"

Thus, the diagnostic sensitivity for MI was reduced in comparison to the use of cTn HS: this limitation was reported and discussed in our Limitation section.

3. Laboratory methods (troponin and copeptin). This section is most unclear and the authors are inconsistent and incorrect in their use of analytical terminology. For all of the assays, the authors need to clearly state the following information, the limit of detection of the assay and the limit of quantitation of the assay (using the conventionally accepted definition of the clinical laboratory standards Institute, the 20% imprecision point is an FDA construct), the 20% imprecision point on the imprecision curve, the 10% imprecision point for the assay, the 99th percentile for the assay, the measuring range of the assay and the expected imprecision values across the measuring range. As they are mixing contemporary sensitive and high sensitive assays, I would suggest that they provide units for comparison and provide units in parentheses as nanograms/L for the conventional sensitive assays. For example 0.04 µg/L (40 ng/L).

As demanded by the reviewer, we clearly stated the listed information for each cTn assay in our revised manuscript.

We also provided units in parentheses as nanograms/L for the conventional sensitive assays, for comparison.

4. Statistical analysis. The authors are mixing two methodologically different troponin I (cTnI) assays. Whilst I have no trouble with the use of appropriate decision thresholds for diagnosis of myocardial infarction (subject to the caveats mentioned above) and to the use of diagnostic tabulation (as shown in table 4), I have some concerns about combining the data for ROC analysis. Certainly, the data from those sites using the same cTnI method can be combined, but the lack of standardisation between methodologies does not really support pooling all cTnI data regardless of method without some form of data transformation. If the data was normalised by factorising to the 99th percentile, then log transformed prior to ROC analysis that would likely remove any bias due to methodological differences. I would wish to see some statistical methodological justification for simple pooling of all cTnI data. Clearly, this does not apply to the troponin T (cTnT) and copeptin data. In addition, will the authors clearly state in this section the diagnostic thresholds being used for classification of the data. This information (which is crucial to the analysis) is scattered across the laboratory methodology and the tables and not clearly gathered together in one place.

We fully understand the concerns raised by the reviewer. In fact, we already mixed the cTnI results from these two similar assays in a previous work, and this was a limitation of the study (Freund et al. Crit Care 2011, 15 :R147). These two cTnI are known to be very similar, and there is a good correlation between both methods: indeed, Christenson et al. found that Access (x axis) and X-pand

(y axis) values were correlated with a slope at 1.00, an y-intercept at 0.00 and a Pearson's coefficient at 0.94 (Christenson et al., JALM 2017; volume 1, Issue 5: 544-561).

However, and as demanded, we normalized cTnI results by factorizing to the 99th percentile before repeating analysis in our revised manuscript. These results are included in our revised version. Of note, we did not find any significant difference between AUCs :

	Biomarker	AUC	95% CI
CPO <2h	cTnI cTnI normalized	0.815 0.841, p=0.288 vs cTnI	0.746 to 0.872 0.775 to 0.894
	cTnI + copeptin cTnI normalized + copeptin	0.866 0.880, p=0.196 vs cTnI	0.804 to 0.915 0.819 to 0.926
CPO 2-4h	cTnI cTnI normalized	0.856 0.886, p=0.207 vs cTnI	0.788 to 0.909 0.823 to 0.933
	cTnI + copeptin cTnI normalized + copeptin	0.906 0.915, p=0.271 vs cTnI	0.846 to 0.948 0.857 to 0.955
CPO >4h	cTnI cTnI normalized	0.989 0.995, p=0.432 vs cTnI	0.955 to 0.999 0.965 to 1.000
	cTnI + copeptin cTnI normalized + copeptin	0.978 0.979, p=0.800 vs cTnI	0.939 to 0.995 0.940 to 0.995

Table 3 and Figure 2 were revised in consequence, replacing previous cTnI data with normalized cTnI data.

We also clearly state in this revised section the diagnostic thresholds being used for classification of the data, as suggested by the reviewer.

5. A diagnostic discriminant of 3 ng/L has also been used in the literature as a rule out threshold for cTnT and I am surprised the authors have not included in this analysis.

The threshold of 3 ng/L has indeed been used in the literature as a rule out threshold. However, its imprecision is worse than 5 ng/L and we do not recommend to use it. As suggested, we included it in our analysis and discussed the results obtained with this low threshold in our revised manuscript.

6. The areas under the ROC curves should be included in the figures or the figure legends.

We included the AUC values in the revised figures, as suggested.

REVIEWER	Jasper Boeddinghaus
	Cardiovascular Research Institute Basel (CRIB), Department of Cardiology, University Hospital Basel, Switzerland
REVIEW RETURNED	24-Oct-2018

GENERAL COMMENTS	I would like to thank the authors for their comments. However, there are some issues that should be further addressed.
	1.) Using a more careful wording. As the available evidence is controversial regarding a safe rule-out of NSTEMI in early presenters and as the proportion of very early presenters in the present study is quite small, this reviewer would recommend a more careful wording like "may" or "seems like" in some parts of the manuscript. This reviewer would also recommend to change the title to "seems not safe enough", as the generalizability of the present findings is limited (due to the limitations discussed).
	2.) Recently, Twerenbold et al. investigated the performance of the ESC 0/1h algorithms in early presenters. This study used a subgroup of early presenters which was the largest subgroup investigated so far. This reviewer would recommend to include this paper in your discussions.
	Twerenbold R, Neumann JT, Sörensen NA, Ojeda F, Karakas M, Boeddinghaus J, Nestelberger T, Badertscher P, Rubini Giménez M, Puelacher C, Wildi K, Kozhuharov N, Breitenbuecher D, Biskup E, du Fay de Lavallaz J, Flores D, Wussler D, Miró Ò, Martín Sánchez FJ, Morawiec B, Parenica J, Geigy N, Keller DI, Zeller T, Reichlin T, Blankenberg S, Westermann D, Mueller C. Prospective Validation of the 0/1-h Algorithm for Early Diagnosis of Myocardial Infarction. J Am Coll Cardiol. 2018;72:620–632.
	3.) Page 15: "In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h but not for earlier presenters (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not evaluated." This statement is not entirely true. The ESC guidelines do not only recommend the 0/3h-algorithms(which uses a CPO threshold of 6h), but also recommends the 0/1h-algorithm taking early presenters into account. Please revise this sentence.
	4.) Discussion/Limitations. "Therefore, the use of a 0/1h-algorithm might be used for a safe rule-out in patients that are not very early presenters." The ESC 0/1h-algorithm CAN be applied to early presenters. The recommended direct rule-out criterion of the ESC 0/1h-algorithm should not be applied to early presenters, but the alternative rule-out criteria (combination of baseline concentrations and 1h-change) can be used in very early presenters. Please revise this sentence.

REVIEWER	Prof Paul Collinson
	St George's Hospital, London
REVIEW RETURNED	08-Jan-2019

| GENERAL COMMENTS | All concerns adddressed |

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer #1

Reviewer Name: Jasper Boeddinghaus

Institution and Country: University Hospital Basel, Cardiovascular Research Institute Basel (CRIB), Basel, Switzerland

I would like to thank the authors for their comments. However, there are some issues that should be further addressed.

Answer to Reviewer #1: We would like to thank reviewer #1 for his comments. We have revised our manuscript so as to take account of all these comments. We have no rebuttal. All changes are in red script in the manuscript.

1.) Using a more careful wording. As the available evidence is controversial regarding a safe rule-out of NSTEMI in early presenters and as the proportion of very early presenters in the present study is quite small, this reviewer would recommend a more careful wording like "may" or "seems like" in some parts of the manuscript. This reviewer would also recommend to change the title to "seems not safe enough", as the generalizability of the present findings is limited (due to the limitations discussed).

As suggested, we revised our manuscript so as to take into account the reviewer's comment (see revised title, revised abstract and revised discussion). The conclusion was already cafully worded with "seems not sensitive enough".

2.) Recently, Twerenbold et al. investigated the performance of the ESC 0/1h algorithms in early presenters. This study used a subgroup of early presenters which was the largest subgroup investigated so far. This reviewer would recommend to include this paper in your discussions.

Twerenbold R, Neumann JT, Sörensen NA, Ojeda F, Karakas M, Boeddinghaus J, Nestelberger T, Badertscher P, Rubini Giménez M, Puelacher C, Wildi K, Kozhuharov N, Breitenbuecher D, Biskup E, du Fay de Lavallaz J, Flores D, Wussler D, Miró Ò, Martín Sánchez FJ, Morawiec B, Parenica J, Geigy N, Keller DI, Zeller T, Reichlin T, Blankenberg S, Westermann D, Mueller C. Prospective Validation of the 0/1-h Algorithm for Early Diagnosis of Myocardial Infarction. J Am Coll Cardiol. 2018;72:620–632.

We thank reviewer"1 for this information, and we agree with the recommendation. Thus, we revised our manuscript so as to include the cited reference (see Discussion section and References section). The reference list was renumbered in consequence.

3.) Page 15: "In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h but not for earlier presenters (1). We believe that this proportion is not negligible and the impact of this very early population might be underestimated in studies were the CPO is not

evaluated." This statement is not entirely true. The ESC guidelines do not only recommend the 0/3h-algorithms (which uses a CPO threshold of 6h), but also recommends the 0/1h-algorithm taking early presenters into account. Please revise this sentence.

As suggested, the sentence was revised:

In the ESC guidelines, a different strategy is recommended for patients with versus without CPO<6h (0/3h-algorithm) (1). Earlier presenters are taken into account in the rapid rule-out 0/1h-algorithm, but in this strategy the rapid exclusion with a unique measurement at admission (H0) is only applicable if CPO>3h (1).

4.) Discussion/Limitations. "Therefore, the use of a 0/1h-algorithm might be used for a safe rule-out in patients that are not very early presenters." The ESC 0/1h-algorithm CAN be applied to early presenters. The recommended direct rule-out criterion of the ESC 0/1h-algorithm should not be applied to early presenters, but the alternative rule-out criteria (combination of baseline concentrations and 1h-change) can be used in very early presenters. Please revise this sentence.

As suggested, the sentence was revised:

The current ESC guidelines incorporate an additional criterion for direct rule-out of patients that are not very early presenters; indeed, the rapid rule-out using a single measurement at admission is possible only if CPO is >3h (1). Furthermore, this rapid algorithm can be used only for 3 HS-cTn assays, including HS-cTnT. Considering our data about patients with CPO >4h, we note that our conclusions are in line with the recommendations. Therefore, the use of a single measurement at admission might be used for a safe rule-out in patients that are not very early presenters. The alternative rule-out criteria, combining baseline concentration and 1h-change, should be used in early presenters.

Reviewer #2

Reviewer Name: Prof Paul Collinson

All concerns addressed.

Answer to Reviewer #2: We would like to thank reviewer #1 for his comment.

VERSION 3 - REVIEW

REVIEWER	Jasper Boeddinghaus Cardiovascular Research Institute Basel and Department of Cardiology, University Hospital Basel, Switzerland
REVIEW RETURNED	24-Feb-2019

GENERAL COMMENTS	I would like to thank the authors for addressing my comments. I have no further comments.