PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Exploring biomarkers in routine diagnostics for the risk stratification of older patients in the chest pain unit: A prospective cohort study.
AUTHORS	Kunz, Anna Lisa; Schönstein, Anton; Bahrmann, Philipp; Giannitsis, Evangelos; Wahl, Hans-Werner; Katus, Hugo; Frey, Norbert; Bahrmann, Anke

VERSION 1 – REVIEW

REVIEWER	Sharma, Umesh University at Buffalo
REVIEW RETURNED	21-Dec-2021

GENERAL COMMENTS	In this prospective study, Kunz and associates have studied the over the prognostic value of commonly used biomarkers (troponin, Hgb, and CRP) as the risk predictors of all-cause mortality in older subjects presenting to a tertiary care ED. 1. The enrolment criteria are unclear. Was this study designed for a neurocognitive study? If so, data analytical aspects are not clear. 2. Expected survival of >1day is a very unusual criterion. This raises significant concerns regarding the heterogeneity of the subjects included in this study. 3. For such a generic study (albeit meaningful), larger sample size is needed. Such questions are only addressed by population-based studies or registries. Therefore, the current study can be considered as a pilot or preliminary, or hypothesis-generating. 4. Please provide Kaplan Meier curves for your survival data.

REVIEWER	Herlitz, Johan Faculty of Caring Science, Work Life and Social Welfare, University of Borås
REVIEW RETURNED	18-Jan-2022

GENERAL COMMENTS	This is an interestig paper. I have some comments. Line 7.What do the authors mean with "routinely applied? Line 25 :Expected life expectancy >24 hourts must be defined Line 30.Since all included patients were admitted to a chest pain unit, this is a selected cohort.This needs to b highlighted. Line 43. The final diagnosis is a confoundiong factor. It is not clear how many patients had a myocardial infarction.If some of these patients had a myocardial infarction this might explain the increased mortaliy. Twelve percent had a pneumonia which may explain some of the mortality risk.How do we know that the patients with anemia did not have an underlying cancer disease.

	My point is that elevation of these biomarkers may be explained by underlying diseases rather than aging alone.Thios needs to be highlighted If analysis of these biomarkers became a routine among the elderly what would the authors recommend us to do if elevated values excet lookíng for a cardiac disease, an infection and an undderlying cancer disease?.What more should we do? Line 133 The authors write "older ED patients. Shouldnt they be called "older chest pain unit patients"? My problem with the discussion is that am not convinced that elevation of these biomarkers simply reflect biological aging.I think that elevation of these biomarkers often reflect an underlying disease.This needs to be discussed.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Dr. Umesh Sharma, University at Buffalo
Thank you very much your most helpful remarks and precise corrections which helped to further improve the manuscript.

Comments (reviewer 1)	**Author's response**
1. "The enrolment criteria are unclear. Was this study designed for a neurocognitive study? If so, data analytical aspects are not clear."	Thank you very much for this helpful comment. This is important to consider. The study was designed for geriatric risk-stratification in an emergency department. Thus, it is not a neurocognitive study per se. We add this aspect to the first section of the methodological part. On **page 8**, **first paragraph**, **line 33**, we added: "Therefore, the study is designed for older chest pain unit patients in order to improve geriatric risk-stratification."
2. "Expected survival of >1day is a very unusual criterion. This raises significant concerns regarding the heterogeneity of the subjects included in this study."	Thank you very much for this comment. Inclusion criteria of this study were the admission to the chest pain unit, a minimum age of 70 years and an informed consent. Patients were excluded if they had life-expectancy < 24 hours, or if they did not consent to providing a blood sample for use by the research team. Since our study included a questionnaire, we excluded patients who are in life-threatening conditions. Since the comment is coherent, we replace „expected survival of > 1 day" with „the capability to informed consent" in the **Abstract**, because critical ill patients are likely not able to give their informed

	consent. We now also elaborate on this in the Methods section. On **page 8, line 35**, we added: “Patients were recruited only after the first examination by the ED physician. Also, on the basis of this initial examination, patients were excluded in cases of missing informed consent or an expected life expectancy of less than 24h. Thus, patients who were in a life-threatening condition and therefore unable to be interviewed or to benefit in any way from a more geriatric risk stratification were excluded.”
3. “For such a generic study (albeit meaningful), larger sample size is needed. Such questions are only addressed by population-based studies or registries. Therefore, the current study can be considered as a pilot or preliminary, or hypothesis-generating.“	Thank you for this helpful comment. This study was conducted with an exploratory, hypothesis-generating character. Due to its moderate size and in light of what data may be available in registries etc., it can indeed also be seen as having a pilot character. Some limitations of this study have therefore to be acknowledged: this study draws form only a relatively moderate sample size of older patients admitted to the ED for various symptoms. Thus, a generalization of the study results should be done with caution. A validation of the current findings in larger sample sizes of older patients is certainly needed, which we note in the Discussion section. We highlight this aspect in the methodological section on **page 8, line 30**: “The study’s design was a single-center exploratory, prospective cohort study.” In the Discussion on **page 14, line 124**, we added: “This exploratory, prospective cohort study examined the association of the three biomarkers CRP, hs-TnT and Hb with mortality in order to improve older patient’s risk stratification in the ED.” On **page 17, line 208**, we added in the limitation section: “In this context, one could argue that this study has due to the low number of cases more of a pilot character.” On **page 18, line 226**, we added: “Future research should address the validation of risk stratification approaches based on biological aging biomarkers in large, multicenter, and across the clinical characteristic diverse samples, which would also allow for a deeper examination of the interactions of the different

	aging-related biomarkers than it was possible in this small study with pilot character.”
4. “Please provide Kaplan Meier curves for your survival data.”	Thank you very much for this important comment. We included Kaplan Meier curves for each marker alone as well as for the composite. During submission however we had to upload it separately from the manuscript. Furthermore, we added a Kaplan Meier curve for the entire cohort. We also added the confidence intervals of the curves for the composite variable, which highlight the uncertainty that comes with our estimates.

Reviewer 2: Prof. Johan Herlitz, Faculty of Caring Science, Work Life and Social Welfare
Thank you very much for your encouraging comments and most helpful suggestions which helped to further improve the manuscript.

Comments (reviewer 2)	**Author’s response**
1. „Line 7. What do the authors mean with "routinely applied?“	Thank you very much for this helpful comment. With „routinely applied“ we tried to express that the biomarkers are already used in daily clinical practice. However, we rephrase it in the Abstract on **page 3**: " ***Objectives:*** This study aims to estimate the association of the often in daily clinical practice used biological age-related biomarkers hs-TnT, CRP and Hemoglobin (Hb) with all-cause mortality for the purpose of older patient’s risk stratification in the emergency department (ED).”
2. „Line 25 :Expected life expectancy >24 hours must be defined“	Thank you very much for this comment. Inclusion criteria of this study were the admission to the chest pain unit, a minimum age of 70 years and an informed consent. Patients were excluded if they had life-expectancy < 24 hours. Due to the fact that our study included a questionnaire, we excluded patients who are in life-threatening conditions. Since the comment is coherent,

	we replace „expected survival of > 1 day“ with „the capability to informed consent“ in the **Abstract**, because critical ill patients are likely not able to give their informed consent. On **page 8, line 35**, we added: “Patients were recruited only after the first examination by the ED physician. Also, on the basis of this initial examination, patients were excluded in cases of missing informed consent or an expected life expectancy of less than 24h. Thus, patients who were in a life-threatening condition and therefore unable to be interviewed or to benefit in any way from a more geriatric risk stratification were excluded.”
3. „Line 30. Since all included patients were admitted to a chest pain unit, this is a selected cohort. This needs to be highlighted.“	Thank you for noting this. We add this aspect and highlight it in the first section of the methodological part. On **page 8, line 39**, we added: “Since all included participants were treated in a chest pain unit, the cohort of this study consists of selected cardiological patients.”
4. „Line 43. The final diagnosis is a confounding factor. It is not clear how many patients had a myocardial infarction. If some of these patients had a myocardial infarction this might explain the increased mortality. Twelve percent had a pneumonia which may explain some of the mortality risk. How do we know that the patients with anemia did not have an underlying cancer disease.“	Thank you very much for this helpful comment. Overall comorbidities as well as the diagnoses were important confounders and were controlled with the CACI. Therefore also the final diagnoses from the discharge letters were extracted and included in this score. We added this aspect to the methodological section and highlighted it on **page 9, line 74**: “These diagnoses, which are important confounding factors, were extracted from the discharge letter after treatment in the chest pain unit, therefore including the diagnosed acute issue for which the patient presented. Higher values of the CACI indicate more severe comorbidity.”
5. „My point is that elevation of these biomarkers may be explained by underlying diseases rather than aging alone. This needs to be highlighted. If analysis of these biomarkers became a routine among the	Thank you very much for this significant comment. We have thought about it carefully. The aim of the study was to identify older patients in the chest pain unit, who were at high risk of

elderly what would the authors recommend us to do if elevated values except lookíng for a cardiac disease, an infection and an underlying cancer disease? What more should we do? Line 133 The authors write "older ED patients. Shouldn't they be called "older chest pain unit patients"? My problem with the discussion is that am not convinced that elevation of these biomarkers simply reflect biological aging. I think that elevation of these biomarkers often reflect an underlying disease. This needs to be discussed.“	adverse outcomes as in our case mortality. For this aim we used the three biomarkers hs-TnT, CRP and Hb, looking at the issue from a biomarkers of aging framework. The classification of these biomarkers as biomarkers of aging stems primarily from the cited paper by Wagner and colleagues. We could show that elevation/decrease of these markers were associated with increased risk of all-cause mortality, and that a cmbination of such markers from a biological aging framework appears to make sense. Of course, this is only a longitudinal association and therefore no causal inferences can be drawn. Practically symptoms of the underlying diseases in older patients are often ambiguous and challenging due to atypical presentation in older patients. In addition, diagnostic measures such as echocardiographic imaging is time consuming and challenging in older patients. Thus, it is tempting to speculate that the use of a biomarker approach in a general risk-stratification may provide a way for more targeted allocation of resources. Since we regard the points raised in your comment as really important aspects, we added a new section to the Discussion balancing the pros and cons. On **page 16, line 171**, we added: “In this context, it should be considered that this study has an exploratory, hypothesis-generating character. Wagner et al. noted there are many theories trying to explain the aging process but as of yet it is not fully understood [3], though the three biomarkers focused on this study are assumed to be strong candidates for an involvement. However, their elevation or decrease could also be caused by underlying diseases such as of the cardiovascular, oncological, or infectious type. As has been noted previously, despite many efforts it remains difficult to identify “pure” biomarkers of aging due to their overlap with disease markers [34]. Hence, it is important to rule out such underlying diseases by using further diagnostic tools. For this purpose, it would be meaningful to expand on the results of this research by examining the biomarkers

	in a large cohort where precise health data are available. This health data should be based on a comprehensive health assessment, and include several indicators for latent diseases which may not have yet manifested in a diagnosis [35]. Practically one could argue that in case of conspicuous values of the biomarkers an increased risk can be assumed and further diagnostic tools are needed. Therefore, the biomarkers can serve as a good adjunct in risk-stratification but they cannot replace further diagnostic measures. As Madhavan et al. stated, a biomarker elevation should be considered in clinical context rather than in isolation [36]. Still, as numerous studies showed conspicuous values of the used biomarkers reflect an increased risk of adverse outcomes in line with our results [4, 13, 25], one could argue that regardless of whether the elevations are caused by underlying diseases or by the aging process, they should be taken into account." On **page 17, line 209**, we added in the limitation section: "Also strong conclusions about whether the values of these biomarkers definitely reflect the biological aging process or whether the effects are attributable to underlying diseases is not possible, though we made a strong effort to control for possible confounders available in our data."

VERSION 2 – REVIEW

REVIEWER	Herlitz, Johan Faculty of Caring Science, Work Life and Social Welfare, University of Borås
REVIEW RETURNED	12-Apr-2022

GENERAL COMMENTS	I think that my previous concerns have been adressed.