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Exploring biomarkers in routine diagnostics for the risk stratification of older patients in the chest pain unit: A prospective cohort study.

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Abstract:

Objectives: This study aims to estimate the association of the routinely applied 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).

Design: Exploratory, prospective cohort study with a follow-up at 2.5 years after recruitment start. For the predictors, data from the hospital files including the routinely applied biological age-related biomarkers hs-TnT, CRP and Hemoglobin (Hb) were supplemented by a questionnaire.

Setting: A cardiological ED, chest pain unit, of the university hospital in Heidelberg, Germany.

Participants: N=256 cardiological ED patients with a minimum age of 70 years and with an expected life-expectancy above 24h.

Primary outcome measures: The primary outcome of this study was all-cause mortality which was assessed by requesting registry office information.

Results: Among *N*=256 patients 63 died over the follow-up period. Positive results in each of the three biomarkers alone as well as the combination were associated with increased all-cause mortality at follow-up. The number of positive age-related biomarkers appeared to be strongly indicative of the risk of mortality, even when controlled for major confounders (age, sex, BMI, creatinine clearance, and comorbidity).

Conclusions: In older ED patients, biomarkers explicitly related to biological aging processes such as hs-TnT, CRP and Hb were independently of each other as well as combined associated with an increased risk of all-cause mortality. Thus, they may have the potential to be used to supplement the general risk stratification of older patients in the ED. Validation of the results in a large dataset is needed.

Key words: troponin, CRP, hemoglobin, mortality, routine diagnostics.

Strengths and limitations of this study:

- The prospective design and the 2.5 years long follow-up period with few censored observations at early times are strengths of this study.

- As information about medical history and diagnoses received from the visit to the ED were available, effect estimates could be controlled for major confounders.
- Limitations of this study are mainly the exploratory approach, the single-center design, and the relatively small number of events.

Declarations:

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Code availability: The code for the analyses conducted in this study can be made available on request.

Author contributions:

Study concept and design:

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Acquisition of data:

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Analysis and interpretation of data:

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Drafting of the manuscript:

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Critical revision of the manuscript for important intellectual content:

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Ethics approval:

The research reported in this paper was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and the protocol was approved by the ethics committee of the medical faculty at the University of Heidelberg (S-455/2016).

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Introduction

Older adults are an increasing population and frequent users of the emergency department (ED) setting [1]. As a challenging clientele they frequently present with atypical symptoms, polymedication, and unclear medical history in the ED [2] and physicians then face the challenge of quickly identifying those patients with a high-risk of adverse outcomes. Strategies to combine biomarkers indicative of biological age (see Wagner et al. [3]) may enhance risk stratification of older and geriatric patients in the ED, due to their objectivity and clear clinical implications. From a biomarkers of aging framework three distinct biomarkers (C-reactive protein, high-sensitive troponin-T, and hemoglobin) are frequently encountered in routine diagnostic procedures.

Established theories of immune-senescence and *„inflammaging"* describe biological aging as a chronic, low-grade inflammatory status [4]. In accordance with this, markers such as C-reactive protein (CRP) have shown their potential for the prognosis of older people in terms of frailty and mortality [5] as well as in terms of events such as myocardial infarction or stroke [6].

Similarly, high-sensitivity troponins can serve as biomarkers of age. Troponins are regulator proteins of the contractile apparatus and enter the bloodstream in case of cardiomyocyte damage. The high-sensitivity troponins (hs-TnT) showed a notable predictive validity regarding mortality as well as other outcomes such as myocardial infarction or stroke in numerous studies [7–11]. It was also found that these effects of elevated troponins are independent of their etiology [7]. They thus mark a general risk factor that must be urgently noticed before any further treatment.

Further, geriatric patients frequently have decreased levels of hemoglobin in form of anemia [12]. Recently, a study by Han et al. found that in apparently healthy older people the occurrence of anemia is associated with an increased rate of hospitalization and mortality [13], irrespective of the underlying comorbidities [14].

In this study we therefore aim to estimate the association of the biomarkers C-reactive protein, high-sensitive troponin-T, and hemoglobin, with older ED patient's all-cause mortality. Accordingly, we expect an increased all-cause mortality with elevated cardiac or inflammation markers or low hemoglobin levels, even when age, overall comorbidity and other variables (sex, BMI, renal functioning) are controlled for. Given the differential mechanisms in the pathways of these biomarkers, we also hypothesize that the risk of mortality will increase even further when these different markers interact, and a patient therefore shows more than one abnormal result.

Methods

Study design, setting, and participants

The study's design was a single-center prospective cohort study. It was conducted in the chest pain unit (CPU) with 12 beds as part of the cardiological ED of a university hospital (114 beds in the cardiological department overall). In total, 260 cardiological ED patients with a minimum age of 70 years were recruited (participation rate ≈ 76%). The first patient was recruited in July 2017 and the last one in May 2018. Patients were excluded in cases of missing informed consent or an expected life expectancy of less than 24h. Further, patients that were isolated due to transmission-based precautions had to be excluded from the study.

Baseline information was assessed by using a questionnaire, in which the Short Portable Mental Questionnaire (SPMSQ) [15] was integrated, and additionally by extracting relevant data from the hospital files. Follow-up data for this study was collected by requesting registry office information on mortality roughly 2.5 years after recruitment of the first patient.

Based on the cognitive performance on the Short Portable Mental Status Questionnaire [15], about 23% of the participants had at least a minor form of cognitive impairment. Participants presented to the cardiological ED with numerous symptoms, including dyspnea (59%), thorax pain (20%), and angina pectoris (18%), vertigo (9%) and palpitations (8%). Frequent primary diagnoses were suspicion/exclusion of myocardial infarction (26%), cardiac decompensation (23%), and heart rhythm disturbances (19%). Further information on primary and secondary diagnoses of the sample are presented in detail in Supplementary Tables 1 and 2.

This research was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. The protocol was approved by the ethics committee of the medical faculty at the University of Heidelberg (S-455/2016).

Patient and public involvement

No patient involved.

Study variables

The biomarkers relevant to this study were part of the routinely applied diagnostic procedures in the ED. To classify the lab results as positive or negative, the following cut offs were used.

For CRP a cut-off > 5 mg/l was used. This cut off is an established reference value [16, 17] and was used for both sexes, as studies showed a similar distribution of this marker among men and women [18].

A value of hs-TnT > 14 ng/L (i.e. exceeding the 99th percentile of a multicenter reference study) [19] describes the cut-off for both sexes. Increased levels showed a higher risk for cardiovascular events [19] and the prognostic value of including gender difference for hs-TnT was detected only modest in other studies [20].

Due to the fact that mild anemia was defined as a hemoglobin (Hb) concentration between 10,0 g/dl and 11,9 g/dl in women and between 10,0 g/dl and 12,9 g/dl in men [21], we used a cut-off of < 12 g/dl for women and < 13 g/dl for men. This identifies anemia as defined by the World Health Organization [22].

To assess the effect of multiple biomarkers of interest being positive in a given patient, a combined predictor variable ranging from 0-3 was created, with zero indicating a positive result in none of the biomarkers, 1 indicating a positive result in either one of them, et cetera. This strategy of combining the predictors was chosen as the sample size is relatively small and the pathways of the biomarker effects are assumed to be different. Also, the resulting groups were fairly balanced.

Overall comorbidity and age of the patients as a central control variable was accounted for using the Charlson-Age Comorbidity Index (CACI) [23] based on the patient's medical history which included the diagnoses assigned by the ED physician. Higher values indicate more severe comorbidity.

In order to more precisely account for patient's renal functioning as possible confounder, an estimate of the creatinine clearance based on the formula by Cockroft & Gault [24] was calculated based on serum creatinine.

For the purpose of sample description, participants' result (error score) on the cognitive screening SPMSQ is also reported. The theoretical range of the SPMSQ is 0-10 and higher values indicate worse cognitive performance.

The primary outcome examined in this study was the patient's all-cause mortality (time-to-event, days) after their initial ED visit. This information was collected using registry office information.

Statistical methods

To describe the characteristics of the sample, means, standard deviations, medians and interquartile ranges were calculated for continuous or discrete variables. Absolute and relative frequencies were calculated for categorical variables.

Kaplan-Meier curves were used to illustrate the associated effect of positive hs-TnT, CRP, anemia (Hb), as well as the composite predictor of all three on overall survival after the initial ED visit. Cox proportional hazards models were then used to first, get uncontrolled hazard ratio estimates for those effects and second, to control those effects for patient's body-mass index, age, creatinine clearance, sex and overall comorbidity. Schoenfeld residuals did not show evidence against the proportional hazards assumption.

Missing values were rare (max.1.5% or 4/260 per variable) and were therefore handled by listwise deletion, which reduced the total sample to *N*=256 patients.

Uncertainty was quantified using 95% confidence intervals and as this study is of exploratory nature, provided p-values are to be interpreted as descriptive.

All analyses were conducted using R version 3.6.1 (R Core Team, Vienna, Austria).

Results

About 343 patients were eligible, 260 were recruited (participation rate ≈ 76%); 4 patients had missing data for major baseline variables due to them leaving the ED against medical advice.

In total, the final cohort of *N*=256 participants can be described as a high age but, as measures of dispersion for example for variables such as the number of medications indicate, heterogenous sample (detailed descriptive characteristics in Table 1). Bivariate correlations show that there was only a weak association between the results in each of the three biomarkers (hs-TnT with CRP: r_{φ}= .30, *95% CI*= .19-.41; hs-TnT with Hb: r_{φ}= .35, *95% CI*= .24-.45; and CRP with Hb: r_{φ} =.24, *95% CI*=.13-.36, respectively).

Table 1

Descriptive statistics of the cohort (N=256).

Variable	n (%)	Mean (SD)	Median (IQR)
Age		79.27 (6.0)	79.00 (75, 83)
Sex			
female	97 (38%)		
male	159 (62%)		
School years			
< 9 years	116 (45%)		
≥ 9 years	140 (55%)		
Falls in the past 12 months			
At least one	80 (31%)		
None	176 (69%)		
Smoker			
Current/former	104 (41%)		
Never	152 (59%)		
Diabetes			
Yes	73 (29%)		
No	183 (71%)		
Malignancy in Anamnesis			
Active	21 (8%)		
Inactive	19 (7%)		
None	216 (84%)		
BMI		26.85 (4.8)	26.37 (23.74, 29.76)
SPMSQ		1.50 (1.6)	1 (0, 2)
No. medications		7.64 (3.8)	7 (5, 9.75)
CACI		5.72 (2.0)	5 (4, 7)
Creatinine clearance (ml/min)		60.42 (24.8)	60.26 (42.25, 76.69)
Increased Troponine-T	166 (65%)		
Increased CRP	115 (45%)		
Decreased Hemoglobin	102 (40%)		

Note: BMI= body mass index; SPMSQ= Short Portable Mental Status Questionnaire (number of errors); CACI= Charlson Age Comorbidity Index. Creatinine clearance as calculated by Cockroft & Gault formula.

The median follow-up time calculated by reverse Kaplan-Meier method was 835 days. 63 patients (25% of the sample) died during the follow-up period. Separate controlled and uncontrolled estimates of the association between each biomarker and all-cause mortality are presented in Table 2, associated Kaplan-Meier curves can be found in Supplementary Figure 1. As can be taken from Table 2 and Supplementary Figure 1, positive results in each of the three biomarkers were associated with increased all-cause mortality at follow-up. Hemoglobin showed the weakest association to mortality and did not remain statistically significant when adjusted for confounders.

Table 2

Uncontrolled and controlled associations (hazard-ratio) of biological aging related biomarkers and mortality from separate Cox-regression models (N=256).

Variable	Crude HR	95% CI	Adjusted HR	95% CI
Increased Troponine-T	7.28	[2.92, 18.16]	4.41	[1.71, 11.43]
Increased CRP	3.67	[2.12, 6.34]	2.97	[1.69, 5.23]
Decreased Hemoglobin	2.64	[1.60, 4.38]	1.55	[0.89, 2.68]

Note: HR= Hazard Ratio; adjusted estimates are controlled for sex, BMI, creatinine clearance, age and comorbidity (CACI-score).

When all three biomarkers were combined in a joint model and therefore controlled for each other (details in Supplementary Table 3), they were still all associated with mortality at follow-up.

In the main analyses, a summary score was used to predict mortality indicating how many of the three biomarkers showed positive results in a patient (theoretical range: no positive result – three positive results). Kaplan-Meier curves for this grouping are presented in Figure 1. The number of positive biomarkers appeared to be strongly associated to the patient's all-cause mortality. Even when controlled for sex, BMI, creatinine clearance, comorbidity and age as scored by the CACI, the summary score of positive biomarkers was still associated with mortality (see Table 3). While the point-estimates for the effect sizes were notably high, this estimation comes with considerable uncertainty as indicated by the wide 95% confidence intervals (e.g., for the patients with three positive biomarkers vs. the no positive biomarkers reference *HR*=7.46 and *95% CI*= 2.12-26.28).

Table 3
Summary of cox-regression model using the composite predictor (number of positive biomarkers, theoretical range 0-3, N=256).

Variable	Hazard-Ratio	95% CI
Biomarker Composite		
None (*n*=60)	*Ref.*	*Ref.*
One (*n*=63)	1.54	[0.39, 6.04]
Two (*n*=79)	4.29	[1.25, 14.75]
Three (*n*=54)	7.46	[2.12, 26.28]
Sex		
Female	*Ref.*	*Ref.*
Male	1.11	[0.64, 1.92]
BMI	0.94	[0.90, 1.00]
CACI	1.32	[1.16, 1.51]
Creatinine Clearance	1.00	[0.98, 1.01]

Note: Ref. = Reference category; BMI= Body mass index; CACI= Charlson Age Comorbidity Index. Creatinine clearance estimation as calculated by Cockroft-Gault formula. Biomarker composite describes the number of biomarkers with a positive result.

Discussion

This 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. The main results of this study can be summarized as follows: First, each marker alone was associated with an increased risk of all-cause mortality during a more than 2-year follow-up. Second, these biomarkers were to a certain degree independently associated to patient's all-cause mortality. Third, the number of positive results on age-related biomarkers was strongly indicative of the risk of patient's all-cause mortality.

As geriatric patients in the ED represent a complex clientele [2], the quick identification of those with high-risk of adverse outcomes constitutes a considerable challenge. There are few studies which have examined risk stratification of ED patients using a biological aging markers framework, especially from a dedicated geriatric perspective. As Bahrmann et al. [25] similar to Wagner et al. [3] argue on the issue of this approach, combining such biomarkers reflecting different pathophysiological pathways might be better than those which have the same general mechanism [26]. Therefore, and based on theoretical considerations such as *inflammaging* and existent

previous research tying CRP, hs-TnT, and Hb to biological aging processes across different pathways, we aimed to provide a joint examination of those biomarkers in terms of their predictive validity regarding the mortality of a heterogeneous sample of older ED patients, including both, frail and well performing older adults.

As we found in our study CRP, hs-TnT and Hb were individually all associated with older ED patient's all-cause mortality, CRP and hs-TnT even when controlled for major confounders such as comorbidity, age, creatinine clearance, sex, and BMI. Our study therefore largely conforms with previous research, which illustrated an association for each marker to mortality.

For instance, Bahrmann et al. showed that older patients presenting to the ED with hs-TnT levels of 108 ng/L or higher had a continuous increased risk of cardiovascular death [27]. It should be considered that elevated serum troponin concentrations can be caused by any type of heart muscle cell injury, affected by a multitude of factors. Aside from myocardial infarction reasons for an increase can be due to other cardiac as well as non-cardiac diseases, such as for example myocarditis, arrhythmias, sepsis, renal failure, severe acute neurological diseases and critical illness [28]. A recent study by Zelis et al. also estimated the predictive value of several biomarkers, including hs-TnT, for adverse outcomes in older ED patients. In line with our results, hs-TnT showed one of the strongest associations to mortality [37].

CRP as an inflammatory marker was shown to be an independent predictor of overall, cardiovascular and inhospital mortality, as studies by Zimmermann et al. [5] as well as by Yoshinaga et al. have indicated [29]. These findings support the theory of *inflammaging*. The aging process leads to a paradoxically hyper-activated but at the same time defective immune system. Depending on which of these dominates, inflammatory markers could help to distinguish between healthy aging and age-associated pathologies or the occurrence of comorbidities [30].

Moreover, Han et al. recently examined apparently healthy older men and could show the association of decreased levels of Hb with overall and cancer-related mortality [13]. Other studies have also linked lowered Hb to mortality [12, 31].

In our study, we were able to show that positive results on the presumably independent biomarkers CRP, hs-TnT, and Hb were indicative of the older ED patient's risk of all-cause mortality during the follow-up period, even when their effects were controlled for each other. This is in line with the assumption that they exert effects at least in part across their own respective pathways [5, 13, 32]. We could also show that a strong association of the number of positive biomarkers to mortality existed, even when major confounders were controlled for. These

results support the idea that risk stratification may especially benefit from the incorporation of a multitude of biological age-related markers that exert their effects over different physiological pathways [26]. Our exploratory approach to examine the association of the number of positive results to mortality implies equal weighting of each positive result. However, effect sizes for the separate biomarkers indicate that the relationship might be stronger for selected markers (in our case, hs-TnT). This should be considered in future work, which may build on large multicenter datasets that would resolve the issue of the high uncertainty associated with the estimates and provide the opportunity for construction of clinical risk-scores involving these biomarkers.

Previous approaches to risk-stratification of older patients in the ED frequently employed questionnaire-based methods (e.g. Identification of Seniors at Risk [33]). Although there is quite a body of research on these approaches, they were found to be limited in their predictive validity [34, 35]. In comparison, the biological age-related biomarkers showed relatively solid associations to the patient's all-cause mortality in this and numerous other studies [7, 13, 29]. Concerning this matter, the cohort presented in this study was previously examined with regards to the usefulness of a cognitive screening method for the patient's risk stratification. Even when the time-frame of the current study was restrained to 1-year after initial ED visit, we found the association for biomarkers of aging to mortality to be considerably stronger than the association of the cognitive measure to mortality [36].

Thus, incorporation of biomarkers into the risk stratification of older patients in the ED appears to be a convenient, feasible and low-cost approach, as blood collection is already implemented into the diagnostic routines. In general, the assessment of biomarkers of age can also be considered a much more objective approach than (self-) report-based measures.

Limitations

Several limitations of this study must be taken into account. Due to the study being a prospective cohort study with exploratory character, we refrain from causal inferences. While a cohort of older ED patients is difficult to recruit, and the follow-up time in this study was considerably long, this cohort only constitutes a relatively small sample. This rather small sample size, and consequently the low number of overall events, prevented us from examining in more detail the specific interactions of the separate biomarkers. The small number of events also causes the effects found in this study to come with considerable uncertainty, as indicated by the wide confidence intervals. For this reason, weighting approaches required to construct clinical risk scores for application in clinical practice were not feasible. The recruitment of the study sample was also limited to one

specific CPU and is therefore as a convenience sample not representative of the more heterogeneous patient populations present in the hospital. The CPU ward relates, amongst other things, to several specific health problems, associated treatment approaches and reasons of hospital confinement. The preconditioned informed consent procedure also constitutes a selection effect. Finally, while we did not use age-specific cut-offs for hs-TnT as their use is discouraged for convenience in the guideline of the European Society of Cardiology (ESC)[38], age-related variation of 99th percentile values might provide useful information in risk-stratification processes and should be examined in further research.

Conclusions

As the results in this study show, biomarkers with an explicit relation to biological aging processes such as CRP, hs-TnT and Hb were strongly associated to older ED patient's all-cause mortality. Therefore, they should be considered as potential candidates to supplement or replace risk stratification approaches based on e.g., questionnaires, especially since, unlike questionnaire related methods, some of these biomarkers are already implemented into the routine diagnostic process within the ED. Given their other properties such as a clear clinical interpretation and objectivity, they could become part of low-cost, feasible risk-stratification methods. 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.

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REFERENCES

1 Aminzadeh F DWB. Older adults in the emergency department: A systematic review of patterns of use, adverse outcomes, and effectiveness of interventions 2002. Available at: https://www.sciencedirect.com/science/article/pii/S0196064402530614 Accessed November 02, 2019.

2 Salvi F, Morichi V, Grilli A, et al. The elderly in the emergency department: a critical review of problems and solutions. *Intern Emerg Med* 2007;2(4):292–301. doi:10.1007/s11739-007-0081-3 [published Online First: 28 November 2007].

3 Wagner K-H, Cameron-Smith D, Wessner B, et al. Biomarkers of Aging: From Function to Molecular Biology. *Nutrients* 2016;8(6). doi:10.3390/nu8060338 [published Online First: 2 June 2016].

4 De Martinis, M. et al. Inflammation markers predicting frailty and mortality in the elderly. *Experimental and Molecular Pathology* 2006;80(3):219–27. doi:10.1016/j.yexmp.2005.11.004 [published Online First: Juni 2006].

5 Zimmermann, J., Herrlinger, S., Pruy, A., Metzger, T., & Wanner, C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients 1999. Available at: https://www.sciencedirect.com/science/article/pii/S0085253815460090 Accessed October 13, 2019.

6 Ridker, M P, Cushman, et al. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men | NEJM 2009. Available at: https://www.nejm.org/doi/full/10.1056/nejm199704033361401 Accessed October 13, 2019.

7 Meigher S, Thode HC, Peacock WF, et al. Causes of Elevated Cardiac Troponins in the Emergency Department and Their Associated Mortality. *Acad Emerg Med* 2016;23(11):1267–73. doi:10.1111/acem.13033 [published Online First: 20 June 2016].

8 Bardaji, A., Cediel, G., Carrasquer, A., de Castro, R., Sánchez, R., & Boqué, C. Troponin Elevation in Patients Without Acute Coronary Syndrome 2015. Available at: https://www.sciencedirect.com/science/article/pii/S188558571500033X Accessed October 13, 2019.

9 Groot B de, Verdoorn RCW, Lameijer J, et al. High-sensitivity cardiac troponin T is an independent predictor of inhospital mortality in emergency department patients with suspected infection: a prospective observational derivation study. *Emerg Med J* 2014;31(11):882–88. doi:10.1136/emermed-2013-202865 [published Online First: 21 August 2013].

10 deFilippi CR, Lemos JA de, Christenson RH, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA* 2010;304(22):2494–502. doi:10.1001/jama.2010.1708 [published Online First: 8 December 2010].

11 Kaura A, Panoulas V, Glampson B, et al. Association of troponin level and age with mortality in 250 000 patients: cohort study across five UK acute care centres. *BMJ* 2019;367:l6055 (accessed 28 Aug 2020).

18

12 Culleton BF, Manns BJ, Zhang J, et al. Impact of anemia on hospitalization and mortality in older adults. *Blood* 2006;107(10):3841–46. doi:10.1182/blood-2005-10-4308 [published Online First: 15 May 2006].

13 Han SV, Park M, Kwon Y-M, et al. Mild Anemia and Risk for All-Cause, Cardiovascular and Cancer Deaths in Apparently Healthy Elderly Koreans. *Korean J Fam Med* 2019;40(3):151–58. doi:10.4082/kjfm.17.0089 [published Online First: 17 January 2019].

14 den Elzen WPJ, Willems JM, Westendorp RGJ, et al. Effect of anemia and comorbidity on functional status and mortality in old age: results from the Leiden 85-plus Study. *CMAJ* 2009;181(3-4):151–57. doi:10.1503/cmaj.090040 [published Online First: 4 August 2009].

15 Pfeiffer E. A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. *J Am Geriatr Soc* 1975;23(10):433–41.

16 Luppa P.B. SW. Referenzwerte der wichtigsten Laborparameter 2020. Available at: https://link.springer.com/chapter/10.1007/978-3-662-54507-2_159#citeas Accessed June 11, 2020.

17 Morrow David A, Rifai Nader, Antman Elliott M, et al. C-Reactive Protein Is a Potent Predictor of Mortality Independently of and in Combination With Troponin T in Acute Coronary Syndromes: A TIMI 11A Substudy. *J Am Coll Cardiol* 1998;31(7):1460–65 (accessed 27 May 2021).

18 Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. *Clin Chem* 2003;49(4):666–69 (accessed 13 Jun 2020).

19 Giannitsis E, Kurz K, Hallermayer K, et al. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem* 2010;56(2):254–61 (accessed 11 Jun 2020).

20 Mueller-Hennessen M, Lindahl B, Giannitsis E, et al. Diagnostic and prognostic implications using age- and gender-specific cut-offs for high-sensitivity cardiac troponin T - Sub-analysis from the TRAPID-AMI study. *Int J Cardiol* 2016;209:26–33 (accessed 11 Jun 2020).

21 Riva E, Tettamanti M, Mosconi P, et al. Association of mild anemia with hospitalization and mortality in the elderly: the Health and Anemia population-based study. *Haematologica* 2009;94(1):22–28 (accessed 11 Jun 2020).

22 Weltgesundheitsorganisation. Research into environmental pollution: Report of five WHO scientific groups. Geneva: World Health Organization 1968.

23 Charlson M, Szatrowski TP, Peterson J, et al. Validation of a combined comorbidity index. *Journal of Clinical Epidemiology* 1994;47(11):1245–51 (accessed 11 Jun 2020).

24 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16(1):31–41 (accessed 30 Aug 2020).

25 Bahrmann P, Bahrmann A, Hofner B, et al. Multiple biomarker strategy for improved diagnosis of acute heart failure in older patients presenting to the emergency department. *European Heart Journal: Acute Cardiovascular Care* 2015;4(2):137–47 (accessed 8 Aug 2020).

26 Manzano L, Escobar C, Cleland JGF, et al. Diagnosis of elderly patients with heart failure. *Eur J Heart Fail* 2012;14(10):1097–103 (accessed 10 Aug 2020).

27 Bahrmann P, Christ M, Bahrmann A, et al. A 3-hour diagnostic algorithm for non-ST-elevation myocardial infarction using high-sensitivity cardiac troponin T in unselected older patients presenting to the emergency department. *J Am Med Dir Assoc* 2013;14(6):409–16 (accessed 7 Aug 2020).

28 Suzuki K, Komukai K, Nakata K, et al. The Usefulness and Limitations of Point-of-care Cardiac Troponin Measurement in the Emergency Department. *Intern Med* 2018;57(12):1673–80 (accessed 28 Aug 2020).

29 Yoshinaga R, Doi Y, Ayukawa K, et al. High-sensitivity C reactive protein as a predictor of inhospital mortality in patients with cardiovascular disease at an emergency department: a retrospective cohort study. *BMJ Open* 2017;7(10):e015112. https://bmjopen.bmj.com/content/7/10/e015112.citation-tools.

30 Larbi A, Franceschi C, Mazzatti D, et al. Aging of the immune system as a prognostic factor for human longevity. *Physiology (Bethesda)* 2008;23:64–74 (accessed 27 May 2021).

31 Zakai NA, Katz R, Hirsch C, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch Intern Med* 2005;165(19):2214–20.

32 Bahrmann P, Bahrmann A, Breithardt O-A, et al. Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. *Clin Chem Lab Med* 2013;51(6):1307–19 (accessed 7 Aug 2020).

33 McCusker J, Bellavance F, Cardin S, et al. Detection of older people at increased risk of adverse health outcomes after an emergency visit: the ISAR screening tool. *J Am Geriatr Soc* 1999;47(10):1229–37.

34 Galvin R, Gilleit Y, Wallace E, et al. Adverse outcomes in older adults attending emergency departments: a systematic review and meta-analysis of the Identification of Seniors At Risk (ISAR) screening tool. *Age Ageing* 2017;46(2):179–86. doi:10.1093/ageing/afw233 [published Online First: 15 December 2016].

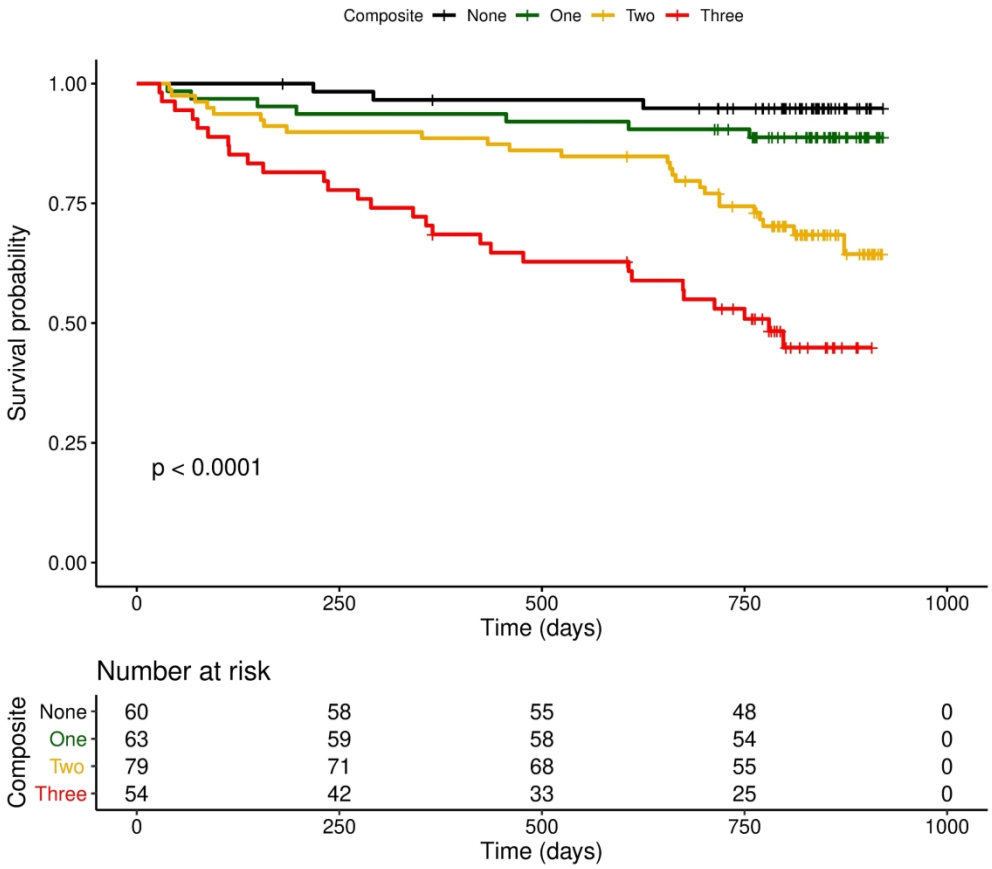
35 Carpenter CR, Shelton E, Fowler S, et al. Risk factors and screening instruments to predict adverse outcomes for undifferentiated older emergency department patients: a systematic review and meta-analysis. *Acad Emerg Med* 2015;22(1):1–21. doi:10.1111/acem.12569 [published Online First: 6 January 2015].

36 Schönstein A, Wahl H-W, Katus HA, et al. SPMSQ zur Risikostratifizierung älterer Patienten in der Notaufnahme Eine explorative prospektive Kohortenstudie. *Z Gerontol Geriatr* 2019;52(Suppl 4):222–28 (accessed 10 Aug 2020).

37 Zelis N, Hundscheid R, Buijs J, et al. Value of biomarkers in predicting mortality in older medical emergency department patients: a Dutch prospective study. *BMJ Open* 2021;11(1):e042989. doi:10.1136/bmjopen-2020-042989 [published Online First: 31 January 2021].

38 Collet J-P, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J* 2020. doi:10.1093/eurheartj/ehaa575 [published Online First: 29 August 2020].

20



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Supplement

Supplementary Table 1

Primary diagnoses in the sample (N=256).

Primary diagnoses	Number (%) of patients (total *N*=256)
Group 1: Suspicion/Exclusion of myocardial infarction or Myocardial infarction	66 (26%)
Group 2: Cardiac decompensation	60 (23%)
Group 3: Heart rhythm disturbances*	48 (19%)
Group 4: Hypertensive derailment	17 (7%)
Group 5: Others	65 (25%)

Note: *(Atrial fibrillation/atrial flutter, tachyarrhythmia absoluta).

Supplementary Table 2

Frequent secondary diagnoses (N=256).

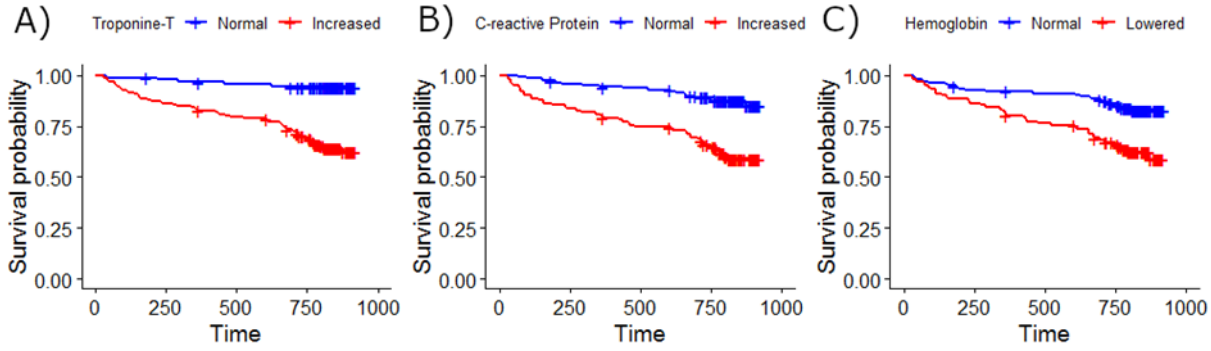
Most common secondary diagnoses	Number (%) of patients (total *N*=256)
Renal insufficiency / kidney disease	51 (20%)
Heart failure	33 (13%)
Anemia	21 (8%)
Pneumonia	30 (12%)

Supplementary Table 3

Uncontrolled and controlled associations (hazard-ratio) of biological aging related biomarkers and mortality from Cox-Regression models including all biomarkers as predictors simultaneously (N=256).

Variable	HR (Model 1)	95% CI	HR (Model 2)	95% CI
Increased Troponine-T	4.84	[1.90, 12.32]	3.61	[1.39, 9.40]
Increased CRP	2.57	[1.47, 4.48]	2.64	[1.50, 4.66]
Decreased Hemoglobin	1.68	[1.00, 2.81]	1.20	[0.69, 2.07]

Note: HR= Hazard Ratio; Model 1: Survival predicted by the three dummy coded biomarker variables. Model 2: Survival predicted by the three dummy coded biomarker variables as well as covariates sex, BMI, creatinine clearance, age and comorbidity (CACI-score). Estimates within a column are therefore from the same model.



Supplementary Figure 1. Kaplan-Meier curves for patients grouped by A) increased Troponine-T B) increased C - reactive Protein and C) lowered Hemoglobin. Time units are days.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			**7**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			**8-10**
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8 and 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8 and 9
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	8 and 10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	10
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	9, 10

Results			**9-12**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9 and 10
		(b) Indicate number of participants with missing data for each variable of interest	10, Fig. 1
		(c) Summarise follow-up time (eg, average and total amount)	12
Outcome data	15*	Report numbers of outcome events or summary measures over time	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, Tables & Figures
		(*b*) Report category boundaries when continuous variables were categorized	8, 9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	see Fig. 1
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			**13-16**
Key results	18	Summarise key results with reference to study objectives	13
Limitations			**15-16**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15, 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15, 16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

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1

Exploring biomarkers in routine diagnostics for the risk stratification of older patients in the chest pain unit: A prospective cohort study.

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3

Abstract:

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).

Design: Exploratory, prospective cohort study with a follow-up at 2.5 years after recruitment start. For the predictors, data from the hospital files including the routinely applied biological age-related biomarkers hs-TnT, CRP and Hemoglobin (Hb) were supplemented by a questionnaire.

Setting: A cardiological ED, chest pain unit, of the university hospital in Heidelberg, Germany.

Participants: N=256 cardiological ED patients with a minimum age of 70 years and the capability to informed consent.

Primary outcome measures: The primary outcome of this study was all-cause mortality which was assessed by requesting registry office information.

Results: Among *N*=256 patients 63 died over the follow-up period. Positive results in each of the three biomarkers alone as well as the combination were associated with increased all-cause mortality at follow-up. The number of positive age-related biomarkers appeared to be strongly indicative of the risk of mortality, even when controlled for major confounders (age, sex, BMI, creatinine clearance, and comorbidity).

Conclusions: In older ED patients, biomarkers explicitly related to biological aging processes such as hs-TnT, CRP and Hb were independently of each other as well as combined associated with an increased risk of all-cause mortality. Thus, they may have the potential to be used to supplement the general risk stratification of older patients in the ED. Validation of the results in a large dataset is needed.

Key words: troponin, CRP, hemoglobin, mortality, routine diagnostics.

Strengths and limitations of this study:

- The prospective design and the 2.5 years long follow-up period with few censored observations at early times are strengths of this study.

- As information about medical history and diagnoses received from the visit to the ED were available, effect estimates could be controlled for major confounders.
- Limitations of this study are mainly the exploratory approach, the single-center design, and the relatively small number of events.

Declarations:

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Code availability: The code for the analyses conducted in this study can be made available on request.

Author contributions:

Study concept and design:

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Acquisition of data:

Schönstein, A., Kunz, A. L.

Analysis and interpretation of data:

Schönstein A., Kunz, A. L., Bahrmann, P., Bahrmann, A.

Drafting of the manuscript:

Kunz, A. L., Schönstein, A., Bahrmann, P., Giannitsis, E., Wahl, H.-W., Katus, H. A., Frey, N., Bahrmann, A.

Critical revision of the manuscript for important intellectual content:

Kunz, A. L., Schönstein, A., Bahrmann, P., Giannitsis, E., Wahl, H.-W., Katus, H. A., Frey, N., Bahrmann, A.

Ethics approval:

The research reported in this paper was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and the protocol was approved by the ethics committee of the medical faculty at the University of Heidelberg (S-455/2016).

7

Consent to participate: Informed consent was obtained for all individual participants included in the study.

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Introduction

Older adults are an increasing population and frequent users of the emergency department (ED) setting [1]. As a challenging clientele they frequently present with atypical symptoms, polymedication, and unclear medical history in the ED [2] and physicians then face the challenge of quickly identifying those patients with a high-risk of adverse outcomes. Strategies to combine biomarkers indicative of biological age (see Wagner et al. [3]) may enhance risk stratification of older and geriatric patients in the ED, due to their objectivity and clear clinical implications. From a biomarkers of aging framework three distinct biomarkers (C-reactive protein, high-sensitive troponin-T, and hemoglobin) are frequently encountered in routine diagnostic procedures.

Established theories of immune-senescence and *„inflammaging"* describe biological aging as a chronic, low-grade inflammatory status [4]. In accordance with this, markers such as C-reactive protein (CRP) have shown their potential for the prognosis of older people in terms of frailty and mortality [5] as well as in terms of events such as myocardial infarction or stroke [6].

Similarly, high-sensitivity troponins can serve as biomarkers of age. Troponins are regulator proteins of the contractile apparatus and enter the bloodstream in case of cardiomyocyte damage. The high-sensitivity troponins (hs-TnT) showed a notable predictive validity regarding mortality as well as other outcomes such as myocardial infarction or stroke in numerous studies [7–11]. It was also found that these effects of elevated troponins are independent of their etiology [7]. They thus mark a general risk factor that must be urgently noticed before any further treatment.

Further, geriatric patients frequently have decreased levels of hemoglobin in form of anemia [12]. Recently, a study by Han et al. found that in apparently healthy older people the occurrence of anemia is associated with an increased rate of hospitalization and mortality [13], irrespective of the underlying comorbidities [14].

In this study we therefore aim to estimate the association of the biomarkers C-reactive protein, high-sensitive troponin-T, and hemoglobin, with older ED patient's all-cause mortality. Accordingly, we expect an increased all-cause mortality with elevated cardiac or inflammation markers or low hemoglobin levels, even when age, overall comorbidity and other variables (sex, BMI, renal functioning) are controlled for. Given the differential mechanisms in the pathways of these biomarkers, we also hypothesize that the risk of mortality will increase even further when these different markers interact, and a patient therefore shows more than one abnormal result.

Methods

Study design, setting, and participants

The study's design was a single-center exploratory, prospective cohort study. It was conducted in the chest pain unit (CPU) with 12 beds as part of the cardiological ED of a university hospital (114 beds in the cardiological department overall). In total, 260 cardiological ED patients with a minimum age of 70 years were recruited (participation rate ≈ 76%). Therefore, the study is designed for older chest pain unit patients in order to improve geriatric risk-stratification. The first patient was recruited in July 2017 and the last one in May 2018. 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. Further, patients that were isolated due to transmission-based precautions had to be excluded from the study. Since all included participants were treated in a chest pain unit, the cohort of this study consists of selected cardiological patients.

Baseline information was assessed by using a questionnaire, in which the Short Portable Mental Questionnaire (SPMSQ) [15] was integrated, and additionally by extracting relevant data from the hospital files. Follow-up data for this study was collected by requesting registry office information on mortality roughly 2.5 years after recruitment of the first patient.

Based on the cognitive performance on the Short Portable Mental Status Questionnaire [15], about 23% of the participants had at least a minor form of cognitive impairment. Participants presented to the cardiological ED with numerous symptoms, including dyspnea (59%), thorax pain (20%), and angina pectoris (18%), vertigo (9%) and palpitations (8%). Frequent primary diagnoses were suspicion/exclusion of myocardial infarction (26%), cardiac decompensation (23%), and heart rhythm disturbances (19%). Further information on primary and secondary diagnoses of the sample are presented in detail in Supplementary Tables 1 and 2.

This research was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. The protocol was approved by the ethics committee of the medical faculty at the University of Heidelberg (S-455/2016).

Patient and public involvement

No patient involved.

Study variables

The biomarkers relevant to this study were part of the routinely applied diagnostic procedures in the ED. To classify the lab results as positive or negative, the following cut offs were used.

For CRP a cut-off > 5 mg/l was used. This cut off is an established reference value [16, 17] and was used for both sexes, as studies showed a similar distribution of this marker among men and women [18].

A value of hs-TnT > 14 ng/L (i.e. exceeding the 99th percentile of a multicenter reference study) [19] describes the cut-off for both sexes. Increased levels showed a higher risk for cardiovascular events [19] and the prognostic value of including gender difference for hs-TnT was detected only modest in other studies [20].

Due to the fact that mild anemia was defined as a hemoglobin (Hb) concentration between 10,0 g/dl and 11,9 g/dl in women and between 10,0 g/dl and 12,9 g/dl in men [21], we used a cut-off of < 12 g/dl for women and < 13 g/dl for men. This identifies anemia as defined by the World Health Organization [22].

To assess the effect of multiple biomarkers of interest being positive in a given patient, a combined predictor variable ranging from 0-3 was created, with zero indicating a positive result in none of the biomarkers, 1 indicating a positive result in either one of them, et cetera. This strategy of combining the predictors was chosen as the sample size is relatively small and the pathways of the biomarker effects are assumed to be different. Also, the resulting groups were fairly balanced.

Overall comorbidity and age of the patients as a central control variable was accounted for using the Charlson-Age Comorbidity Index (CACI) [23] based on the patient's medical history which included the diagnoses assigned by the ED physician. 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.

In order to more precisely account for patient's renal functioning as possible confounder, an estimate of the creatinine clearance based on the formula by Cockroft & Gault [24] was calculated based on serum creatinine.

For the purpose of sample description, participants' result (error score) on the cognitive screening SPMSQ is also reported. The theoretical range of the SPMSQ is 0-10 and higher values indicate worse cognitive performance.

The primary outcome examined in this study was the patient's all-cause mortality (time-to-event, days) after their initial ED visit. This information was collected using registry office information.

Statistical methods

To describe the characteristics of the sample, means, standard deviations, medians and interquartile ranges were calculated for continuous or discrete variables. Absolute and relative frequencies were calculated for categorical variables.

Kaplan-Meier curves were used to illustrate the associated effect of positive hs-TnT, CRP, anemia (Hb), as well as the composite predictor of all three on overall survival after the initial ED visit. Cox proportional hazards models were then used to first, get uncontrolled hazard ratio estimates for those effects and second, to control those effects for patient's body-mass index, age, creatinine clearance, sex and overall comorbidity. Schoenfeld residuals did not show evidence against the proportional hazards assumption.

Missing values were rare (max.1.5% or 4/260 per variable) and were therefore handled by listwise deletion, which reduced the total sample to *N*=256 patients.

Uncertainty was quantified using 95% confidence intervals and as this study is of exploratory nature, provided p-values are to be interpreted as descriptive.

All analyses were conducted using R version 3.6.1 (R Core Team, Vienna, Austria).

Results

About 343 patients were eligible, 260 were recruited (participation rate ≈ 76%); 4 patients had missing data for major baseline variables due to them leaving the ED against medical advice.

In total, the final cohort of *N*=256 participants can be described as a high age but, as measures of dispersion for example for variables such as the number of medications indicate, heterogenous sample (detailed descriptive characteristics in Table 1). Bivariate correlations show that there was only a weak association between

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the results in each of the three biomarkers (hs-TnT with CRP: r_{φ}= .30, *95% CI*= .19-.41; hs-TnT with Hb: r_{φ}= .35, *95% CI*= .24-.45; and CRP with Hb: r_{φ} =.24, *95% CI*=.13-.36, respectively).

Table 1

Descriptive statistics of the cohort (N=256).

Variable	n (%)	Mean (SD)	Median (IQR)
Age		79.27 (6.0)	79.00 (75, 83)
Sex			
female	97 (38%)		
male	159 (62%)		
School years			
< 9 years	116 (45%)		
≥ 9 years	140 (55%)		
Falls in the past 12 months			
At least one	80 (31%)		
None	176 (69%)		
Smoker			
Current/former	104 (41%)		
Never	152 (59%)		
Diabetes			
Yes	73 (29%)		
No	183 (71%)		
Malignancy in Anamnesis			
Active	21 (8%)		
Inactive	19 (7%)		
None	216 (84%)		
BMI		26.85 (4.8)	26.37 (23.74, 29.76)
SPMSQ		1.50 (1.6)	1 (0, 2)
No. medications		7.64 (3.8)	7 (5, 9.75)
CACI		5.72 (2.0)	5 (4, 7)
Creatinine clearance (ml/min)		60.42 (24.8)	60.26 (42.25, 76.69)
Increased Troponine-T	166 (65%)		
Increased CRP	115 (45%)		
Decreased Hemoglobin	102 (40%)		

Note: BMI= body mass index; SPMSQ= Short Portable Mental Status Questionnaire (number of errors); CACI= Charlson Age Comorbidity Index. Creatinine clearance as calculated by Cockroft & Gault formula.

The median follow-up time calculated by reverse Kaplan-Meier method was 835 days. 63 patients (25% of the sample) died during the follow-up period. Separate controlled and uncontrolled estimates of the association between each biomarker and all-cause mortality are presented in Table 2, associated Kaplan-Meier curves can be found in Supplementary Figure 1. As can be taken from Table 2 and Supplementary Figure 1, positive results in each of the three biomarkers were associated with increased all-cause mortality at follow-up. Hemoglobin showed the weakest association to mortality and did not remain statistically significant when adjusted for confounders.

Table 2

Uncontrolled and controlled associations (hazard-ratio) of biological aging related biomarkers and mortality from separate Cox-regression models (N=256).

Variable	Crude HR	95% CI	Adjusted HR	95% CI
Increased Troponine-T	7.28	[2.92, 18.16]	4.41	[1.71, 11.43]
Increased CRP	3.67	[2.12, 6.34]	2.97	[1.69, 5.23]
Decreased Hemoglobin	2.64	[1.60, 4.38]	1.55	[0.89, 2.68]

Note: HR= Hazard Ratio; adjusted estimates are controlled for sex, BMI, creatinine clearance, age and comorbidity (CACI-score).

When all three biomarkers were combined in a joint model and therefore controlled for each other (details in Supplementary Table 3), they were still all associated with mortality at follow-up.

In the main analyses, a summary score was used to predict mortality indicating how many of the three biomarkers showed positive results in a patient (theoretical range: no positive result – three positive results). Kaplan-Meier curves for this grouping are presented in Figure 1. Kaplan-Meier curve with 95% confidence interval for the entire cohort is illustrated in Figure 2. The number of positive biomarkers appeared to be strongly associated to the patient's all-cause mortality. Even when controlled for sex, BMI, creatinine clearance, comorbidity and age as scored by the CACI, the summary score of positive biomarkers was still associated with mortality (see Table 3). While the point-estimates for the effect sizes were notably high, this estimation comes with considerable uncertainty as indicated by the wide 95% confidence intervals (e.g., for the patients with three positive biomarkers vs. the no positive biomarkers reference *HR*=7.46 and *95% CI*= 2.12-26.28).

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Table 3
Summary of cox-regression model using the composite predictor (number of positive biomarkers, theoretical range 0-3, N=256).

Variable	Hazard-Ratio	95% CI
Biomarker Composite		
None (*n*=60)	*Ref.*	*Ref.*
One (*n*=63)	1.54	[0.39, 6.04]
Two (*n*=79)	4.29	[1.25, 14.75]
Three (*n*=54)	7.46	[2.12, 26.28]
Sex		
Female	*Ref.*	*Ref.*
Male	1.11	[0.64, 1.92]
BMI	0.94	[0.90, 1.00]
CACI	1.32	[1.16, 1.51]
Creatinine Clearance	1.00	[0.98, 1.01]

Note: Ref. = Reference category; BMI= Body mass index; CACI= Charlson Age Comorbidity Index. Creatinine clearance estimation as calculated by Cockroft-Gault formula. Biomarker composite describes the number of biomarkers with a positive result.

Discussion

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. The main results of this study can be summarized as follows: First, each marker alone was associated with an increased risk of all-cause mortality during a more than 2-year follow-up. Second, these biomarkers were to a certain degree independently associated to patient's all-cause mortality. Third, the number of positive results on age-related biomarkers was strongly indicative of the risk of patient's all-cause mortality.

As geriatric patients in the ED represent a complex clientele [2], the quick identification of those with high-risk of adverse outcomes constitutes a considerable challenge. There are few studies which have examined risk stratification of ED patients using a biological aging markers framework, especially from a dedicated geriatric perspective. As Bahrmann et al. [25] similar to Wagner et al. [3] argue on the issue of this approach, combining such biomarkers reflecting different pathophysiological pathways might be better than those which have the same general mechanism [26]. Therefore, and based on theoretical considerations such as *inflammaging* and existent

previous research tying CRP, hs-TnT, and Hb to biological aging processes across different pathways, we aimed to provide a joint examination of those biomarkers in terms of their predictive validity regarding the mortality of a heterogeneous sample of older ED patients, including both, frail and well performing older adults.

As we found in our study CRP, hs-TnT and Hb were individually all associated with older ED patient's all-cause mortality, CRP and hs-TnT even when controlled for major confounders such as comorbidity, age, creatinine clearance, sex, and BMI. Our study therefore largely conforms with previous research, which illustrated an association for each marker to mortality.

For instance, Bahrmann et al. showed that older patients presenting to the ED with hs-TnT levels of 108 ng/L or higher had a continuous increased risk of cardiovascular death [27]. It should be considered that elevated serum troponin concentrations can be caused by any type of heart muscle cell injury, affected by a multitude of factors. Aside from myocardial infarction reasons for an increase can be due to other cardiac as well as non-cardiac diseases, such as for example myocarditis, arrhythmias, sepsis, renal failure, severe acute neurological diseases and critical illness [28]. A recent study by Zelis et al. also estimated the predictive value of several biomarkers, including hs-TnT, for adverse outcomes in older ED patients. In line with our results, hs-TnT showed one of the strongest associations to mortality [29].

CRP as an inflammatory marker was shown to be an independent predictor of overall, cardiovascular and inhospital mortality, as studies by Zimmermann et al. [5] as well as by Yoshinaga et al. have indicated [30]. These findings support the theory of *inflammaging*. The aging process leads to a paradoxically hyper-activated but at the same time defective immune system. Depending on which of these dominates, inflammatory markers could help to distinguish between healthy aging and age-associated pathologies or the occurrence of comorbidities [31].

Moreover, Han et al. recently examined apparently healthy older men and could show the association of decreased levels of Hb with overall and cancer-related mortality [13]. Other studies have also linked lowered Hb to mortality [12, 32].

In our study, we were able to show that positive results on the presumably independent biomarkers CRP, hs-TnT, and Hb were indicative of the older ED patient's risk of all-cause mortality during the follow-up period, even when their effects were controlled for each other. This is in line with the assumption that they exert effects at least in part across their own respective pathways [5, 13, 33]. We could also show that a strong association of the number of positive biomarkers to mortality existed, even when major confounders were controlled for. These

results support the idea that risk stratification may especially benefit from the incorporation of a multitude of biological age-related markers that exert their effects over different physiological pathways [26]. Our exploratory approach to examine the association of the number of positive results to mortality implies equal weighting of each positive result. However, effect sizes for the separate biomarkers indicate that the relationship might be stronger for selected markers (in our case, hs-TnT). This should be considered in future work, which may build on large multicenter datasets that would resolve the issue of the high uncertainty associated with the estimates and provide the opportunity for construction of clinical risk-scores involving these biomarkers.

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.

Previous approaches to risk-stratification of older patients in the ED frequently employed questionnaire-based methods (e.g. Identification of Seniors at Risk [37]). Although there is quite a body of research on these approaches, they were found to be limited in their predictive validity [38, 39]. In comparison, the biological age-related biomarkers showed relatively solid associations to the patient's all-cause mortality in this and numerous

other studies [7, 13, 30]. Concerning this matter, the cohort presented in this study was previously examined with regards to the usefulness of a cognitive screening method for the patient's risk stratification. Even when the time-frame of the current study was restrained to 1-year after initial ED visit, we found the association for biomarkers of aging to mortality to be considerably stronger than the association of the cognitive measure to mortality [40].

Thus, incorporation of biomarkers into the risk stratification of older patients in the ED appears to be a convenient, feasible and low-cost approach, as blood collection is already implemented into the diagnostic routines. In general, the assessment of biomarkers of age can also be considered a much more objective approach than (self-) report-based measures.

Limitations

Several limitations of this study must be taken into account. Due to the study being a prospective cohort study with exploratory character, we refrain from causal inferences. While a cohort of older ED patients is difficult to recruit, and the follow-up time in this study was considerably long, this cohort only constitutes a relatively small sample. This rather small sample size, and consequently the low number of overall events, prevented us from examining in more detail the specific interactions of the separate biomarkers. The small number of events also causes the effects found in this study to come with considerable uncertainty, as indicated by the wide confidence intervals. For this reason, weighting approaches required to construct clinical risk scores for application in clinical practice were not feasible. In this context, one could argue that this study has due to the low number of cases more of a pilot character. 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. The recruitment of the study sample was also limited to one specific CPU and is therefore as a convenience sample not representative of the more heterogeneous patient populations present in the hospital. The CPU ward relates, amongst other things, to several specific health problems, associated treatment approaches and reasons of hospital confinement. The preconditioned informed consent procedure also constitutes a selection effect. Finally, while we did not use age-specific cut-offs for hs-TnT as their use is discouraged for convenience in the guideline of the European Society of Cardiology (ESC)[41], age-related variation of 99th percentile values might provide useful information in risk-stratification processes and should be examined in further research.

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Conclusions

As the results in this study show, biomarkers with an explicit relation to biological aging processes such as CRP, hs-TnT and Hb were strongly associated to older ED patient's all-cause mortality. Therefore, they should be considered as potential candidates to supplement or replace risk stratification approaches based on e.g., questionnaires, especially since, unlike questionnaire related methods, some of these biomarkers are already implemented into the routine diagnostic process within the ED. Given their other properties such as a clear clinical interpretation and objectivity, they could become part of low-cost, feasible risk-stratification methods. 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.

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REFERENCES

1 Aminzadeh F DWB. Older adults in the emergency department: A systematic review of patterns of use, adverse outcomes, and effectiveness of interventions 2002 Accessed November 02, 2019.

2 Salvi F, Morichi V, Grilli A, et al. The elderly in the emergency department: a critical review of problems and solutions. *Intern Emerg Med* 2007;2(4):292–301. doi:10.1007/s11739-007-0081-3 [published Online First: 28 November 2007].

3 Wagner K-H, Cameron-Smith D, Wessner B, et al. Biomarkers of Aging: From Function to Molecular Biology. *Nutrients* 2016;8(6). doi:10.3390/nu8060338 [published Online First: 2 June 2016].

4 De Martinis, M. et al. Inflammation markers predicting frailty and mortality in the elderly. *Experimental and Molecular Pathology* 2006;80(3):219–27. doi:10.1016/j.yexmp.2005.11.004 [published Online First: Juni 2006].

5 Zimmermann, J., Herrlinger, S., Pruy, A., Metzger, T., & Wanner, C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients 1999. Available at: https://www.sciencedirect.com/science/article/pii/S0085253815460090 Accessed October 13, 2019.

6 Ridker, M P, Cushman, et al. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men | NEJM 2009. Available at: https://www.nejm.org/doi/full/10.1056/nejm199704033361401 Accessed October 13, 2019.

7 Meigher S, Thode HC, Peacock WF, et al. Causes of Elevated Cardiac Troponins in the Emergency Department and Their Associated Mortality. *Acad Emerg Med* 2016;23(11):1267–73. doi:10.1111/acem.13033 [published Online First: 20 June 2016].

8 Bardaji, A., Cediel, G., Carrasquer, A., de Castro, R., Sánchez, R., & Boqué, C. Troponin Elevation in Patients Without Acute Coronary Syndrome 2015. Available at: https://www.sciencedirect.com/science/article/pii/S188558571500033X Accessed October 13, 2019.

9 Groot B de, Verdoorn RCW, Lameijer J, et al. High-sensitivity cardiac troponin T is an independent predictor of inhospital mortality in emergency department patients with suspected infection: a prospective observational derivation study. *Emerg Med J* 2014;31(11):882–88. doi:10.1136/emermed-2013-202865 [published Online First: 21 August 2013].

10 deFilippi CR, Lemos JA de, Christenson RH, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA* 2010;304(22):2494–502. doi:10.1001/jama.2010.1708 [published Online First: 8 December 2010].

11 Kaura A, Panoulas V, Glampson B, et al. Association of troponin level and age with mortality in 250 000 patients: cohort study across five UK acute care centres. *BMJ* 2019;367:l6055 (accessed 28 Aug 2020).

12 Culleton BF, Manns BJ, Zhang J, et al. Impact of anemia on hospitalization and mortality in older adults. *Blood* 2006;107(10):3841–46. doi:10.1182/blood-2005-10-4308 [published Online First: 15 May 2006].

13 Han SV, Park M, Kwon Y-M, et al. Mild Anemia and Risk for All-Cause, Cardiovascular and Cancer Deaths in Apparently Healthy Elderly Koreans. *Korean J Fam Med* 2019;40(3):151–58. doi:10.4082/kjfm.17.0089 [published Online First: 17 January 2019].

14 den Elzen WPJ, Willems JM, Westendorp RGJ, et al. Effect of anemia and comorbidity on functional status and mortality in old age: results from the Leiden 85-plus Study. *CMAJ* 2009;181(3-4):151–57. doi:10.1503/cmaj.090040 [published Online First: 4 August 2009].

15 Pfeiffer E. A short portable mental status questionnaire for the assessment of organic brain deficit in elderly patients. *J Am Geriatr Soc* 1975;23(10):433–41.

16 Luppa P.B. SW. Referenzwerte der wichtigsten Laborparameter 2020. Available at: https://link.springer.com/chapter/10.1007/978-3-662-54507-2_159#citeas Accessed June 11, 2020.

17 Morrow David A, Rifai Nader, Antman Elliott M, et al. C-Reactive Protein Is a Potent Predictor of Mortality Independently of and in Combination With Troponin T in Acute Coronary Syndromes: A TIMI 11A Substudy. *J Am Coll Cardiol* 1998;31(7):1460–65 (accessed 27 May 2021).

18 Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. *Clin Chem* 2003;49(4):666–69 (accessed 13 Jun 2020).

19 Giannitsis E, Kurz K, Hallermayer K, et al. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem* 2010;56(2):254–61 (accessed 11 Jun 2020).

20 Mueller-Hennessen M, Lindahl B, Giannitsis E, et al. Diagnostic and prognostic implications using age- and gender-specific cut-offs for high-sensitivity cardiac troponin T - Sub-analysis from the TRAPID-AMI study. *Int J Cardiol* 2016;209:26–33 (accessed 11 Jun 2020).

21 Riva E, Tettamanti M, Mosconi P, et al. Association of mild anemia with hospitalization and mortality in the elderly: the Health and Anemia population-based study. *Haematologica* 2009;94(1):22–28 (accessed 11 Jun 2020).

22 Weltgesundheitsorganisation. Research into environmental pollution: Report of five WHO scientific groups. Geneva: World Health Organization 1968.

23 Charlson M, Szatrowski TP, Peterson J, et al. Validation of a combined comorbidity index. *Journal of Clinical Epidemiology* 1994;47(11):1245–51 (accessed 11 Jun 2020).

24 Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;16(1):31–41 (accessed 30 Aug 2020).

25 Bahrmann P, Bahrmann A, Hofner B, et al. Multiple biomarker strategy for improved diagnosis of acute heart failure in older patients presenting to the emergency department. *European Heart Journal: Acute Cardiovascular Care* 2015;4(2):137–47 (accessed 8 Aug 2020).

26 Manzano L, Escobar C, Cleland JGF, et al. Diagnosis of elderly patients with heart failure. *Eur J Heart Fail* 2012;14(10):1097–103 (accessed 10 Aug 2020).

27 Bahrmann P, Christ M, Bahrmann A, et al. A 3-hour diagnostic algorithm for non-ST-elevation myocardial infarction using high-sensitivity cardiac troponin T in unselected older patients presenting to the emergency department. *J Am Med Dir Assoc* 2013;14(6):409–16 (accessed 7 Aug 2020).

28 Suzuki K, Komukai K, Nakata K, et al. The Usefulness and Limitations of Point-of-care Cardiac Troponin Measurement in the Emergency Department. *Intern Med* 2018;57(12):1673–80 (accessed 28 Aug 2020).

22

29 Zelis N, Hundscheid R, Buijs J, et al. Value of biomarkers in predicting mortality in older medical emergency department patients: a Dutch prospective study. *BMJ Open* 2021;11(1):e042989. doi:10.1136/bmjopen-2020-042989 [published Online First: 31 January 2021].

30 Yoshinaga R, Doi Y, Ayukawa K, et al. High-sensitivity C reactive protein as a predictor of inhospital mortality in patients with cardiovascular disease at an emergency department: a retrospective cohort study. *BMJ Open* 2017;7(10):e015112. https://bmjopen.bmj.com/content/7/10/e015112.citation-tools.

31 Larbi A, Franceschi C, Mazzatti D, et al. Aging of the immune system as a prognostic factor for human longevity. *Physiology (Bethesda)* 2008;23:64–74 (accessed 27 May 2021).

32 Zakai NA, Katz R, Hirsch C, et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch Intern Med* 2005;165(19):2214–20.

33 Bahrmann P, Bahrmann A, Breithardt O-A, et al. Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. *Clin Chem Lab Med* 2013;51(6):1307–19 (accessed 7 Aug 2020).

34 American Federation for Aging Research. Biomarkers of Aging 2016. Available at: https://www.afar.org/imported/AFAR_BIOMARKERS_OF_AGING_2016.pdf.

35 Häseler-Ouart K, Arefian H, Hartmann M, et al. Geriatric assessment for older adults admitted to the emergency department: A systematic review and meta-analysis. *Experimental Gerontology* 2021;144:111184. doi:10.1016/j.exger.2020.111184 [published Online First: 3 December 2020].

36 Madhavan MV, Gersh BJ, Alexander KP, et al. Coronary Artery Disease in Patients ≥80 Years of Age. *J Am Coll Cardiol* 2018;71(18):2015–40.

37 McCusker J, Bellavance F, Cardin S, et al. Detection of older people at increased risk of adverse health outcomes after an emergency visit: the ISAR screening tool. *J Am Geriatr Soc* 1999;47(10):1229–37.

38 Galvin R, Gilleit Y, Wallace E, et al. Adverse outcomes in older adults attending emergency departments: a systematic review and meta-analysis of the Identification of Seniors At Risk (ISAR) screening tool. *Age Ageing* 2017;46(2):179–86. doi:10.1093/ageing/afw233 [published Online First: 15 December 2016].

39 Carpenter CR, Shelton E, Fowler S, et al. Risk factors and screening instruments to predict adverse outcomes for undifferentiated older emergency department patients: a systematic review and meta-analysis. *Acad Emerg Med* 2015;22(1):1–21. doi:10.1111/acem.12569 [published Online First: 6 January 2015].

40 Schönstein A, Wahl H-W, Katus HA, et al. SPMSQ zur Risikostratifizierung älterer Patienten in der Notaufnahme Eine explorative prospektive Kohortenstudie. *Z Gerontol Geriatr* 2019;52(Suppl 4):222–28 (accessed 10 Aug 2020).

41 Collet J-P, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J* 2020. doi:10.1093/eurheartj/ehaa575 [published Online First: 29 August 2020].

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Attached Figures:

Figure 1: **Kaplan-Meier curves for composite**

Kaplan-Meier curves with 95% confidence intervals for patients grouped by the number of positive aging related biomarkers (theoretical range 0-3, *N*= 256).

Figure 2: **Kaplan-Meier curve for the entire cohort**

Kaplan-Meier curve with 95% confidence interval for the entire cohort.

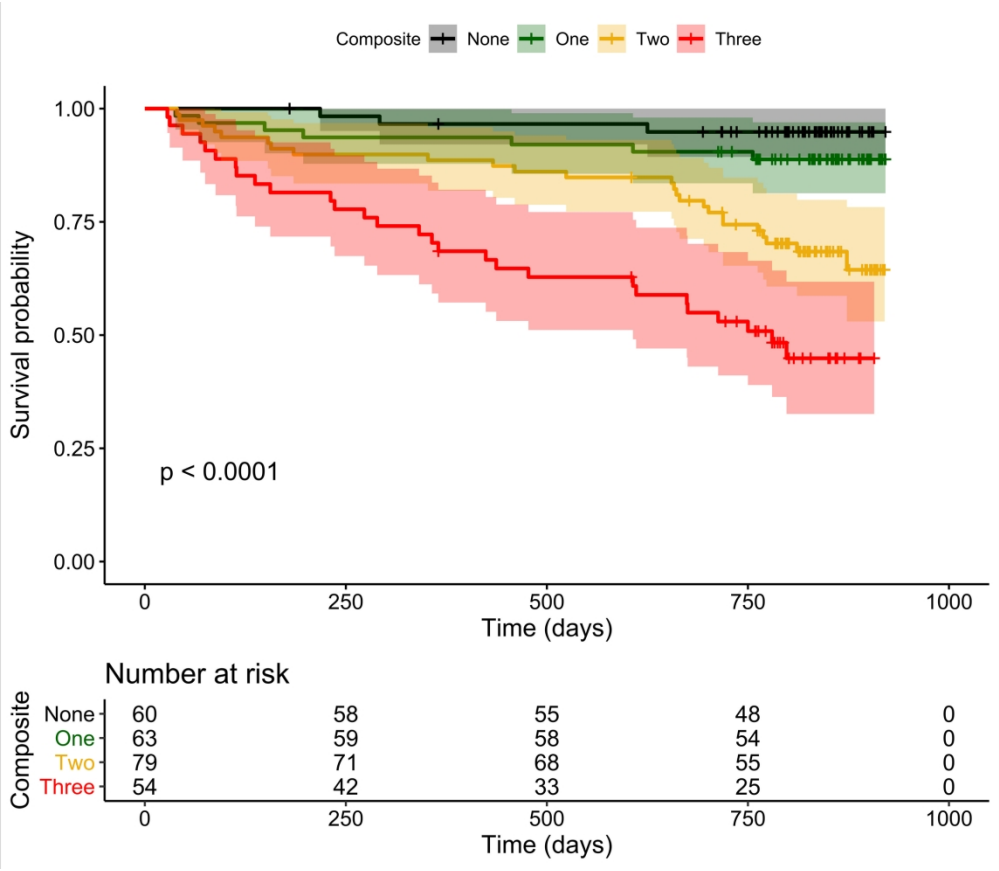


Figure 1: Kaplan-Meier curves for composite
Kaplan-Meier curves with 95% confidence intervals for patients grouped by the number of positive aging related biomarkers (theoretical range 0-3, N= 256).

198x171mm (300 x 300 DPI)

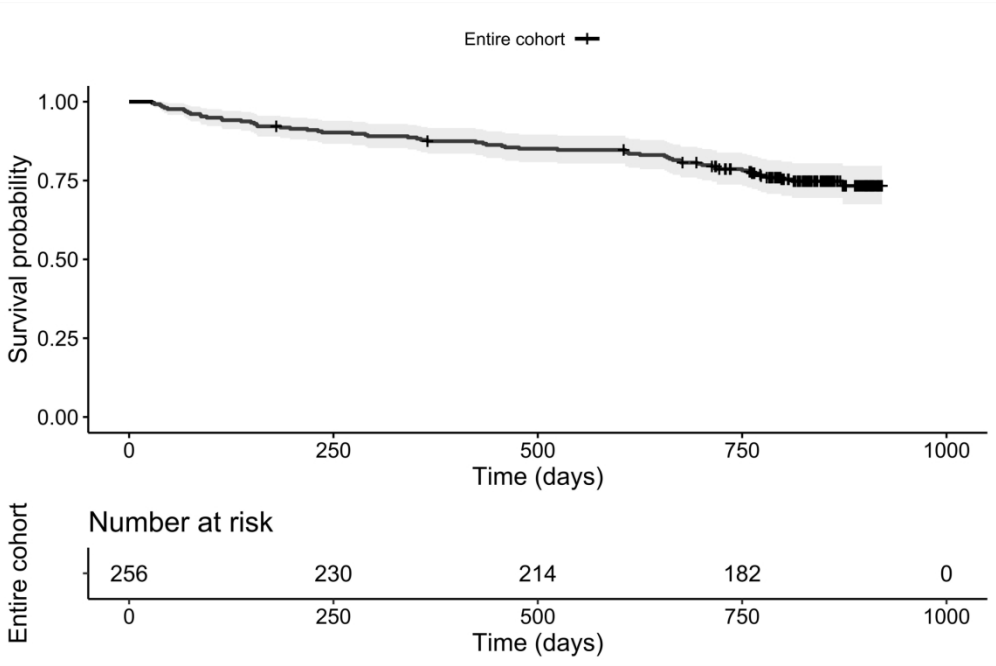


Figure 2: Kaplan-Meier curve for the entire cohort
Kaplan-Meier curve with 95% confidence interval for the entire cohort.

198x132mm (300 x 300 DPI)

Supplement

Supplementary Table 1

Primary diagnoses in the sample (N=256).

Primary diagnoses	Number (%) of patients (total *N*=256)
Group 1: Suspicion/Exclusion of myocardial infarction or Myocardial infarction	66 (26%)
Group 2: Cardiac decompensation	60 (23%)
Group 3: Heart rhythm disturbances*	48 (19%)
Group 4: Hypertensive derailment	17 (7%)
Group 5: Others	65 (25%)

Note: *(Atrial fibrillation/atrial flutter, tachyarrhythmia absoluta).

Supplementary Table 2

Frequent secondary diagnoses (N=256).

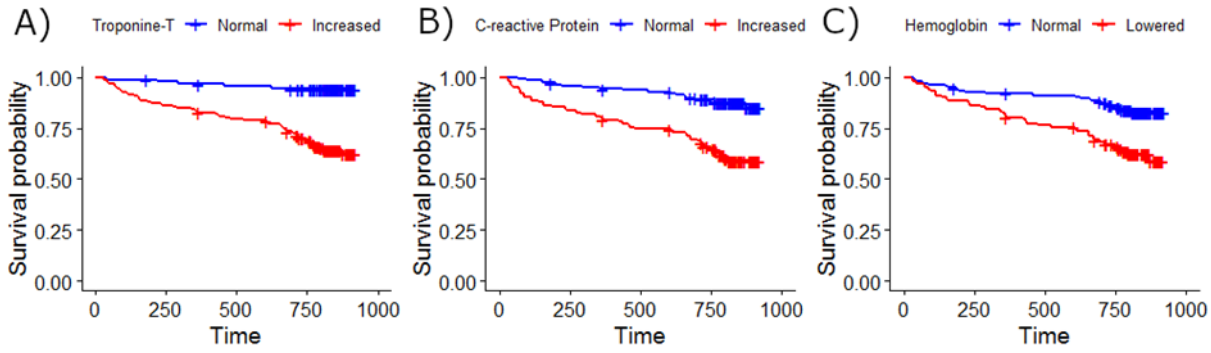
Most common secondary diagnoses	Number (%) of patients (total *N*=256)
Renal insufficiency / kidney disease	51 (20%)
Heart failure	33 (13%)
Anemia	21 (8%)
Pneumonia	30 (12%)

Supplementary Table 3

Uncontrolled and controlled associations (hazard-ratio) of biological aging related biomarkers and mortality from Cox-Regression models including all biomarkers as predictors simultaneously (N=256).

Variable	HR (Model 1)	95% CI	HR (Model 2)	95% CI
Increased Troponine-T	4.84	[1.90, 12.32]	3.61	[1.39, 9.40]
Increased CRP	2.57	[1.47, 4.48]	2.64	[1.50, 4.66]
Decreased Hemoglobin	1.68	[1.00, 2.81]	1.20	[0.69, 2.07]

Note: HR= Hazard Ratio; Model 1: Survival predicted by the three dummy coded biomarker variables. Model 2: Survival predicted by the three dummy coded biomarker variables as well as covariates sex, BMI, creatinine clearance, age and comorbidity (CACI-score). Estimates within a column are therefore from the same model.



Supplementary Figure 1. Kaplan-Meier curves for patients grouped by A) increased Troponine-T B) increased C - reactive Protein and C) lowered Hemoglobin. Time units are days.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			**7**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			**8-10**
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8 and 9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8 and 9
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	8 and 10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 9, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9, 10
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	10
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	9, 10

Results			**9-12**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9 and 10
		(b) Indicate number of participants with missing data for each variable of interest	10, Fig. 1
		(c) Summarise follow-up time (eg, average and total amount)	12
Outcome data	15*	Report numbers of outcome events or summary measures over time	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12, Tables & Figures
		(*b*) Report category boundaries when continuous variables were categorized	8, 9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	see Fig. 1
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	-
Discussion			**13-16**
Key results	18	Summarise key results with reference to study objectives	13
Limitations			**15-16**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15, 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	15, 16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3