EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original research article

Age-related changes in plasma biomarkers and their association with mortality in COVID-19

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Please cite this article as: Michels EHA, Appelman B, de Brabander J, *et al*. Age-related changes in plasma biomarkers and their association with mortality in COVID-19. *Eur Respir J* 2023; in press (https://doi.org/10.1183/13993003.00011-2023).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

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TITLE PAGE

Title

Age-related changes in plasma biomarkers and their association with mortality in COVID-19

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Take home message

In COVID-19, specific ageing-related alterations in the host response likely contribute to the increased mortality in older patients. We provide evidence for potential age-specific immunomodulatory targets across four pathophysiological COVID-19 domains.

Manuscript details:

Word count: 3468, table count: 2, figure count: 5, reference count: 45

Abstract

Background COVID-19-induced mortality occurs predominantly in older patients. Several immunomodulating therapies seem less beneficial in these patients. The biological substrate behind these observations is unknown. The aim of this study was to obtain insight into the association between ageing, the host response, and mortality in patients with COVID-19.

Methods We determined 43 biomarkers reflective of alterations in four pathophysiological domains: endothelial cell and coagulation activation, inflammation and organ damage, and cytokine and chemokine release. We used mediation analysis to associate ageing-driven alterations in the host response with 30-day mortality. Biomarkers associated with both ageing and mortality were validated in an intensive care unit and external cohort.

Results 464 general ward patients with COVID-19 were stratified according to age decades. Increasing age was an independent risk factor for 30-day mortality. Ageing was associated with alterations in each of the host response domains, characterised by greater activation of the endothelium and coagulation system and stronger elevation of inflammation and organ damage markers, which was independent of an increase in age-related comorbidities. Soluble tumor necrosis factor receptor 1, soluble triggering receptor expressed on myeloid cells 1, and soluble thrombomodulin showed the strongest correlation with ageing and explained part of the ageing-driven increase in 30-day mortality (proportion mediated: 13.0%, 12.9% and 12.6%, respectively).

Conclusion Ageing is associated with a strong and broad modification of the host response to COVID-19, and specific immune changes likely contribute to increased mortality in older patients. These results may provide insight into potential age-specific immunomodulatory targets in COVID-19.

Introduction

From the start of the COVID-19 pandemic, epidemiological data indicate that COVID-19-related mortality sharply increases with old age [1–3]. In the Netherlands, individuals aged 50 and younger account for only ~1% of COVID-19 mortality cases, while individuals aged ≥70 account for ~89% of these deaths [4]. The biological substrate behind this steep increase is currently unknown. Ageing-related comorbidities mediate only a fraction of the increased mortality [2, 5].

COVID-19 is associated with increased concentrations of inflammation markers and cytokines, coagulation disturbances, and endotheliopathy [6, 7]. Multiple randomised controlled trials (RCTs) that sought to ameliorate these host response disturbances have reported improved clinical outcomes [8]. However, in the largest RCTs, the two current cornerstones of treatment for hospitalized COVID-19 patients, namely dexamethasone and tocilizumab, showed little if any beneficial effect in the oldest patients [9–11]. In general, ageing is associated with changes in innate and adaptive immunity; in this context, “inflammageing” refers to a state of sustained low-grade inflammation, whilst “immunosenescence” refers to a gradual decline in the ability to generate an effective immune response to new antigens [12]. Although the impact of ageing on the immune system has been implicated in the pathogenesis of COVID-19 in elderly patients [13, 14], studies analysing the influence of older age on host response disturbances in COVID-19 are scarce. Previous studies focused on plasma cytokine levels in a limited number of patients across various disease severities, showing an association of ageing with an increased inflammatory response [15–18]. The influence of ageing on endothelial and coagulation responses in COVID-19 has yet to be evaluated. Exploring age-driven differences in these key host response domains and their association with ageing-driven mortality in COVID-19 may lay a foundation for identifying new immunomodulating targets.

The primary objective of this study was to gain insight into the association between age and aberrations in key pathophysiological pathways implicated in COVID-19 in patients admitted to a general hospital ward (i.e., non-critically ill). To this end, we determined 43 biomarkers reflective of alterations in the endothelial and coagulation response, inflammation and organ damage, and cytokine and chemokine release. Our secondary objective was to associate the host response aberrations detected in older patients with the increased 30-day mortality rate in this age group.

Methods

Study design and population

Data were derived from the ELDER-BIOME study (ClinicalTrials.gov identifier NCT02928367) and the Amsterdam University Medical Centers (Amsterdam UMCs) COVID-19 biobank study (AUMC 2020_065) (see supplementary methods for details). Both studies were approved by the Amsterdam UMC ethics committee. ELDER-BIOME is a prospective observational study in Amsterdam UMC (both locations: Academic Medical Center [AMC] and Vrije Universiteit Medical Center [VUMC], Amsterdam, the Netherlands) and the Flevo Hospital, Almere, the Netherlands [7]. The Amsterdam UMC COVID-19 biobank study is conducted in both AMC and VUMC, according to which leftover blood from clinical care is processed for plasma storage. Written informed consent was obtained from all patients or their legal representatives. Within the AUMC biobank study, an additional option of deferred consent using an opt-out method was implemented for incapacitated patients. We included patients above 18 years of age with COVID-19-related symptoms and a positive SARS-CoV-2 PCR or suspected COVID-19 with a CORADS-CT score >4 reflecting a high (4) or very high (5) suspicion for pulmonary involvement of COVID-19 [19]. Inclusion was done between February 2020 and September 2021. Patients were sampled within 48 hours of general ward admission (or – in a separate cohort - intensive care unit (ICU) admission). Patients were divided according to four “waves” (see ref. [20], and supplementary methods). Biomarker data were validated in an external

cohort; for details see online supplement and ref [21]. Figure 1 depicts an overview of the COVID-19 cohorts. In addition, mortality data were also obtained from patients admitted to a general ward because of community-acquired pneumonia caused by pathogens other than SARS-CoV-2; for details see online supplement and ref [7].

Assays

Forty-three host response biomarkers were measured in EDTA anticoagulated plasma using Luminex (R&D, USA) and the Bio-Plex 200 System (Bio-Rad Laboratories Inc., Hercules, CA) (see supplementary data for the selection rationale). Biomarkers were stratified into four pathophysiological domains (see Table S1 for details). Reference values were obtained from 10 heathy and 19 age- and sex-matched non-infectious outpatient clinic controls (ClinicalTrials.gov identifier NCT02928367).

Statistics

Patients were stratified according to age decades: <50, ≥50 - <60, ≥60 - <70, and ≥70 years. This method was chosen to facilitate visualization and clinical interpretation. Differences in 30-day survival were visualised by Kaplan-Meier curves. Biomarker data were log-transformed. Overall differences between age decades among biomarkers within one host response domain were visualised by a Principal Component Analysis (PCA), as described previously [7]. Differences in the principal components (PC) scores between age groups were analysed by analysis of variance (ANOVA). The correlation of PC scores with ageing on a continuous scale was determined by Spearman's correlation test.

Differences in individual biomarker levels between age decades were quantified using the Hedges' *g* effect size and visualised using heatmaps [22]. Additionally, in a regression analysis, age was modelled as a continuous variable. The association's strength was analysed using Spearman's correlation. In a separate analysis, the association of individual biomarkers with ageing was also explored upon admission to the ICU.

We investigated if age-driven alterations in biomarker concentrations were associated with the age-driven increase in 30-day mortality rates by mediation analysis [5, 23]. A biomarker or PC score needed to be significantly associated with both ageing and 30-day mortality to enter the mediation analysis (see supplementary methods for assumptions and details) [24]. Biomarkers associated with both ageing and mortality were also externally validated in an independent cohort entailing 196 hospitalised non-intubated COVID-19 patients (see supplementary methods for the cohort description) (Figure 1) [21]. At last, we performed a cluster analysis on patients aged ≥70 to assess the uniformity of their host response, using the Ward's method [25]. Furthermore, we investigated whether biomarker's mediating effects were influenced by the patient's host response phenotype.

The regression and mediation models all consisted of an unadjusted and an adjusted approach. Adjusted models evaluating the association of ageing with biomarker concentrations contained covariates associated with either ageing or COVID-19 care and included: demographics (inclusion hospital, sex, and inclusion wave), age-related comorbidities (hypertension, diabetes, malignancies, immunosuppression, and chronic cardiac, neurologic, respiratory, and kidney disease), age- and biomarker-related chronic medication prior to admission (antiplatelet and anticoagulant drugs), and COVID-19-related immunomodulating treatments before sampling (corticosteroids, anti-IL-6, imatinib). When evaluating the association of ageing with mortality, we used the same covariates plus covariates that may impact mortality in the adjusted model: immunomodulating treatments on

admission but after sampling and the use of antibiotics and remdesivir (see supplementary methods for details and assumption testing).

Details on missingness and handling of missingness are described in the supplementary methods and Supplementary Excel file (sheet 1), Table S2 and Table S3. P-values of all analyses were multiple testing corrected using the Benjamini-Hochberg (BH) procedure. A BH-adjusted p-value <0.05 was considered statistically significant.

Results

Patients, presentation and outcome

464 patients with COVID-19 admitted to a general hospital ward were included (Table 1). Of these, 89 patients (19.2%) were younger than 50 years at hospital admission, 111 (23.9%) ≥50 - <60 years, 135 (29.1%), ≥60 - <70 years, and 129 (27.8%) ≥70 years. The distribution of sex did not differ between age decades. Most patients were enrolled during the second and third COVID-19 waves in the Netherlands, in which the SARS-CoV-2 alpha variant occurred and became dominant. The age distribution was similar between waves (Table S4). The proportion of patients with comorbidities increased with age. While the duration of symptoms prior to admission did not differ between age groups, older patients presented with higher disease severity scores [26]. Routine laboratory values were comparable between age groups except for an age-dependent decrease in lymphocyte counts, and an age-dependent increase in the neutrophil-lymphocyte ratio and creatinine. COVID-19-related treatments, primarily supplemental oxygen and dexamethasone, were comparable between age groups (Table 2). Mortality rates increased with age in a non-linear fashion (Table 2 and Figure 2). For example, an increase in age from 60 to 70 was associated with an increased 30-day mortality (odds ratio (OR) 1.15, 95% confidence interval (CI) 1.12 – 1.18) (Figure 2b), which was independent

of demographics, age-related comorbidities, and COVID-related treatments (adjusted OR 1.14 [1.11 – 1.17] (Figure 2c). In pneumonia patients with pathogens other than SARS-CoV-2 (Table S5), a similar increase in age (from 60 to 70) was not associated with an increase in 30-day mortality odds (OR: 1.01 [0.996 – 1.025]). Moreover, older patients with pneumonia caused by pathogens other than SARS-CoV-2 showed significantly lower mortality rates compared to those with SARS-CoV-2; ≥60-<70 ($p<0.01$) and ≥70 ($p<0.001$) (Figure S1).

Association of ageing with aberrations in distinct host response domains

We determined 43 host response biomarkers in plasma obtained within 48 hours after admission and stratified these in four pathophysiological domains. First, we performed PCA to visualise overall differences between age decades among biomarkers within each pathophysiological domain (Figure 3). All domains showed significant differences between age groups. The strongest association with ageing was observed for PC1 of the systemic inflammation and organ damage domain (Rho=-0.41), which was mainly driven by increased plasma concentrations of soluble tumor necrosis factor receptor 1 (sTNF-RI), soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) and soluble receptor for advanced glycation end-products (sRAGE) in older patients (Figure 3b). The second strongest association was that of ageing with PC2 of the endothelial and coagulation domain (Rho=0.30), which was driven mainly by increased plasma angiopoietin-2/1 ratio's, sThrombomodulin and sE-selectin in older patients (Figure 3a). The cytokine domain was characterised by two groups of cytokines: one in which the levels increased with ageing (Figure 3c, PC2, primarily driven by granulocyte-macrophage colony-stimulating factor [GM-CSF] and the anti-inflammatory cytokines IL-1RA and IL-10), and one in which the levels were relatively similar between age groups (Figure 3c, PC1). A mixed pattern was observed for the chemokine domain in which some chemokine levels increased with ageing (e.g., C-X-C motif chemokine ligand 10 (CXCL10)) while others decreased with ageing (e.g., chemokine C-C motif ligand 5 chemokine (CCL5))

(Figure 3d, PC2). The complete contribution of each biomarker to a PC score is depicted in Table S6. As a sensitivity analysis, we further stratified patients ≥70 into patients ≥70 – <80 (n=86) and ≥80 (n=43), which did not modify the age-dependent trends to a significant extent (Figure S2).

Association of ageing with individual host response biomarkers

We next compared individual host response biomarkers across age decades. Of the 43 determined biomarkers, 36 were significantly different between patients and controls (Supplementary Excel file, sheet 2). Figure 4a shows biomarker concentrations in patients ≥70 years of age compared to the other age groups, expressed as Hedges' g, a commonly used effect size measure [22]. This analysis confirmed that most biomarkers reflective of endothelial and coagulation activation, as well as those indicating systemic inflammation and organ damage, were higher in patients ≥70, while chemokines and cytokines demonstrated a mixed pattern. A sensitivity analysis in which we further stratified patients ≥70 into patients ≥70 – <80 and ≥80 yielded similar results (Figure S2). For almost all biomarkers that had a significant association with ageing in the unadjusted model, significance was maintained after adjusting for demographics, comorbidities, and COVID-19-related immunomodulating treatments before sampling (see methods for details). On a continuous scale, sTNF-RI, sTREM-1, sThrombomodulin and tenascin-C demonstrated the strongest (positive) correlation with ageing (Figure 4b). The strength and direction of the associations of each biomarker with ageing showed strong resembles across inclusion waves (Figure S3).

In a separate analysis, we evaluated the age distribution of the same biomarkers in a cohort of critically ill COVID-19 patients sampled on ICU admission (n=157, Table S8 and Table S9; Supplementary Excel file, sheet 3). Differences between patients ≥70 and younger patients were largely reproduced in this cohort of critically ill patients, especially in comparison with the youngest age group (<50), although, due to the relatively low sample size, statistical significance was not

always reached (Figure S4a). Akin to the patients admitted to a general ward, sTNF-RI, sTREM-1, sThrombomodulin, tenascin-C, and soluble vascular cellular adhesion molecule-1 (sVCAM-1) demonstrated a significant positive correlation with ageing (Figure S4b).

Association of age-driven host response alterations with mortality

We used mediation analysis to evaluate whether age-driven alterations in biomarker concentrations were associated with the age-driven increase in 30-day mortality rates [23]. First, we identified biomarkers associated with both ageing and 30-day mortality before and after adjustment for possible confounding and ageing-related factors (Figures 5a and 5b). Then, we estimated which proportion of the age effect on mortality was associated with age-related alterations in PC scores of pathophysiological domains and individual host response biomarkers (Figure 5c). In the unadjusted model (Figure 5d, left), PC scores of the endothelial and coagulation (PC2), systemic inflammation and organ damage (PC1), chemokine (PC2) and cytokine domains (PC2) explained a significant part of the age effect on mortality. With regard to individual biomarkers, ageing-related differences in sThrombomodulin, sVCAM-1, sTNF-RI, sTREM-1, sRAGE were most associated with the age-driven increase in 30-day mortality. Notably, in an external cohort, sThrombomodulin, sVCAM-1, sTNF-RI, and Tenascin-C showed the strongest association with ageing and 30-day mortality (Figure S5); sTREM-1 was not measured [21]. In the adjusted model, only ageing-driven changes in sVCAM-1, sRAGE, CXCL10, GM-CSF, and IL-10 were associated with the ageing-driven increase in 30-day mortality. Collectively, these results demonstrate that a proportion of the association between age and mortality is explained by specific host response differences.

Given that the host response in older patients may not be uniform, we performed a cluster analysis in patients ≥70. We identified three clusters (Figure S6). Cluster 3 had the highest age and 30-day mortality, with high endothelial cell activation, inflammation, and organ damage markers but low

cytokine concentrations (Table S10, Table S11 and Supplementary excel file sheet 4). Cluster 1 had high coagulation markers and cytokine concentrations (Figure S6). Cluster 2 had a mixed host response. The top 3 mediating biomarkers in the primary analysis (sThrombomodulin, sTNF-RI, and sTREM-1) were important for assigning patients to Cluster 3 (Table S12). Furthermore, the majority of biomarkers and all PC scores that were significant mediators in the primary analysis remained predictors of mortality in patients ≥70, which was, with a few exceptions, independent of the assigned cluster (Table S13).

Discussion

We here report the association of ageing with 43 biomarkers reflective of host response disturbances in four key pathophysiological domains relevant for the pathogenesis of COVID-19 and relate these immune deviations to 30-day mortality. While our results confirm previously reported increased plasma concentrations of IL-6, IL-10, IL-15, TNFα and CXCL8 in older COVID-19 patients [15–18], we additionally show that many cytokines are hardly affected by age. More importantly, we provide novel evidence that ageing is associated with greater activation of the endothelial cell and coagulation system and elevation of inflammation and organ damage markers in COVID-19. Mediation analysis indicated that specific host response alterations explained part of the age effect on mortality. Our study is the most comprehensive analysis of the association between ageing, the host response and mortality in COVID-19.

Older COVID-19 patients showed strongly increased plasma concentrations of several biomarkers in the endothelial cell and coagulation activation and inflammation and organ damage domains. Likely, alterations in these pathophysiological areas are at least in part intertwined. These (and all other) analyses were done in an unadjusted model and a model adjusted for variables associated with ageing. While these two types of analyses yielded largely similar results, we consider the unadjusted

analyses a better reflection of the clinical setting of older patients, who (as an example) inherently have more comorbidities and co-medication than young patients. Ageing results in endothelial senescence, characterised by vascular inflammation and endothelial dysfunction; age-associated increases in endothelial cell NFκB activity have been implicated herein [27, 28]. Endotheliopathy is a key feature of COVID-19, and earlier studies reported elevated plasma levels of a variety of biomarkers indicating endothelial cell activation and dysfunction [29]. The current results suggest that COVID-19 is associated with more profound endotheliopathy in older patients. Indeed, patients ≥70 had higher plasma levels of biomarkers reflective of endothelial cell activation (sThrombomodulin, sVCAM-1), a more disturbed glycocalyx function (syndecan), and a more disrupted barrier function (angiopoietin-2). Loss of glycocalyx is an important feature of endothelial dysfunction in COVID-19 [29, 30]. The glycocalyx consists of proteoglycans and glycosaminoglycans that inhibit immune activation and provide an anticoagulant surface [30]. Hence, a more disturbed glycocalyx function in older COVID-19 patients may contribute to systemic hyperinflammation and coagulation activation. Higher angiopoietin-2 and angiopoietin-2/1 ratio's in older COVID-19 patients point in the same direction: angiopoietin-2 promotes endothelial inflammation and vascular leakage, high plasma angiopoietin-2 has been associated with a worse outcomes in COVID-19 [31], and high angiopoietin-2/1 ratio's strongly correlated with mortality in critically ill patients with acute lung injury [32]. Of note, the association between older age and increased endotheliopathy seems specific for COVID-19, since we recently reported lower endothelial cell activation and dysfunction in old as compared to young patients with sepsis [33].

Ageing was an independent risk factor for mortality in this study group of non-critically ill COVID-19 patients, confirming previous investigations that analysed populations with more diverse baseline disease severities [1, 2, 5]. More importantly, we showed that the age-dependent increase in mortality at day 30 was associated with certain age-associated host response changes. sTNF-RI has been identified as a cornerstone biomarker for the unfavourable "hyperinflammation" subtype of

acute respiratory distress syndrome [34]. sTNF-RI levels may be a surrogate marker for the activation of cell-associated TNF-RI, which can trigger vascular leakage and neutrophilic inflammation in the lung [35–37]. Blockade of TNF-RI in healthy individuals led to a decrease in neutrophil transmigration and endothelial injury upon inhalation of endotoxin [35], and TNF-RI deficient mice were strongly protected against the formation of pulmonary edema in an acute lung injury model [37]. Collectively, these data suggest that targeting the TNF-RI pathway may improve the outcome especially in older COVID-19 patients. TREM-1 is a receptor on innate immune cells that amplifies the immune response triggered by Toll-like and NOD-receptors [38]. Increased concentrations of sTREM-1 are reflective of TREM-1 pathway activity [39] and are associated with increased cytokine levels and disease severity in patients with COVID-19 [40]. In our study, ageing was associated with enhanced sTREM-1 levels, which was a main determinant in the ageing-driven increase in 30-day mortality. Of interest, in a recently completed study in COVID-19 patients requiring ventilator support infusion of an inhibitor of the TREM-1 pathway improved clinical outcomes, including 28-day mortality (data from a press release by the sponsor; see ref [41]).

Plasma IL-6 was associated with both ageing and COVID-19 mortality. However, in the mediation analysis, the age-dependent increase in 30-day mortality was not associated with increased IL-6 concentrations, suggesting that IL-6 blockade may not benefit older COVID-19 patients. In agreement, the beneficial effect of tocilizumab in the overall population of COVID-19 patients enrolled in a large RCT was not observed in the oldest patients [11]. Similarly, dexamethason - the other cornerstone immunomodulatory treatment in COVID-19 – did not improve outcome in old patients [9, 10]. Elevated inflammation in older patients may result in steroid resistance [42], which could contribute to a lower response to a standard dose of dexamethasone. Collectively, these findings suggest that age-associated changes in the host response during COVID-19 influence the effect of immune modulation in this disease and that age should be taken into account for patient selection.

Our study has strengths and limitations. We provide comprehensive insight into aberrations in host response pathways considered important for the pathogenesis of COVID-19 in a large population. Our primary analysis only included non-critically ill patients, yet we provided preliminary evidence that ageing is associated with similar host response changes in COVID-19 patients admitted to the ICU. Our study was purely observational and no definitive conclusion can be drawn on causality. Nonetheless, by using mediation analysis, we provide novel insights into which host response pathways contribute to ageing-associated mortality. While admission plasma biomarkers are used to guide immunomodulating trails [43], they do not cover the full spectrum of the host response, and we may have missed host response alterations that were not (yet) visible on a protein level. Our study was conducted before the broad availability of vaccines and the dominance of the Omicron variant. Yet, in populations with a high vaccination coverage, a significant portion of hospitalised COVID-19 patients remains unvaccinated [44]. While the Omicron variant still disproportionally affects older patients [45], robustness of the ageing-associated alterations remain to be validated.

This study documents that ageing is associated with substantial and broad alterations in the host response to COVID-19 across several pathophysiological domains. Increased concentrations of several biomarkers (in particular, sTNF-RI and sTREM-1) were associated with the ageing-driven increase in 30-day mortality. These results may lay a foundation for new immunomodulatory targets in older patients.

Acknowledgments

We thank Barbara Smids-Dierdorp, Tamara Dekker and Anita Tuip for their technical assistance in the performance of the Luminex assays. Both the graphical abstract and Figure 1 were made with BioRender.com.

Footnote

Ethics approval: The ethical boards of the participating hospitals approved the collection of data for the study purposes.

Data sharing: Data can be shared upon reasonable request after approval of a proposal with a signed data access agreement and always in collaboration with the study group.

Conflicts of interest: All authors declare no conflicts of interest.

Funding information

E.H.A.M., J.d.B, and C.C.A.v.L. received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 847786 (FAIR). H.P.S. was supported by the Dutch Kidney Foundation (Kolff Grant Nr. 19OK009). O.C. was supported by the Landsteiner Foundation (LSBR # 1901). T.D.Y.R. was supported by the research program 'NACTAR', project 'MDR-phage' (grant number 16447), which is financed by the Dutch research council (NWO). WJW was supported by the Talud Foundation (Stichting Talud) for the Amsterdam UMC Corona Research Fund, the Netherlands Organisation for Health Research and Development (ZonMw; TURN-COVID grant number 10430142110001). LDJB reports grants from the Dutch Lung Foundation, grants from the Dutch Lung Foundation and Health Holland (Public-Private Partnership grant), grants from the Dutch Lung Foundation (Dirkje Postma Award), grants from the IMI COVID19 initiative, and grants from Amsterdam UMC fellowship. The funders of this study had no role in study design, data collection, data analysis, data interpretation or writing of the report.

Authorship contributions

EHAM, BA, HPS and TvdP contributed to the concept and design of the study. EHAM, BA, JdB, RBEvA, CCAvL, OC, ARS, TDYR, TALS, AMK, RAD, Amsterdam UMC COVID-19 biobank study group were responsible for the collection of the data and samples and the sample preparation. EHAM performed the data analysis. HPS verified the analytical methods. EHAM drafted the first manuscript in consultation with BA, HPS and TvdP. TvdP supervised the project and provided the funding. All authors provided intellectual input and both revised and approved the final version of the manuscript.

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Tables

Table 1: Baseline characteristics of COVID-19 patients on admission to the general ward

	<50	≥50 - <60	≥60 - <70	≥70	p-value
n	89	111	135	129	
Demographics					
Age (median [IQR])	44 [37, 47]	55[53, 57]	64 [62, 6]	76 [72, 82]	<0.001
Male sex, n (%)	49 (55.1)	68 (61.3)	83 (61.5)	78 (60.5)	0.784
Body Mass Index (median [IQR])	30.0 [27.4, 33.2]	27.8 [25.2, 32.1]	28.7 [25.6, 32.8]	27.5 [24.3, 30.1]	0.002
Duration of symptoms*, days (median [IQR])	8 [7, 10]	9 [7, 11]	8 [6, 11]	8 [5, 10]	0.109
Smoking status, n (%)					0.009
Yes	4 (4.5)	4 (3.6)	8 (5.9)	8 (6.2)	
Former smoker	17 (19.1)	27 (24.3)	48 (35.6)	53 (41.1)	
Never smoked	60 (67.4)	70 (63.1)	73 (54.1)	55 (42.6)	
Unknown	8 (9.0)	10 (9.0)	6 (4.4)	13 (10.1)	
COVID-19 vaccination status, n (%)					0.262
Yes	2 (2.2)	2 (1.8)	8 (5.9)	7 (5.4)	
No	86 (96.6)	106 (95.5)	120 (88.9)	119 (92.2)	
Unknown	1 (1.1)	3 (2.7)	7 (5.2)	3 (2.3)	
Comorbidities and (selected) chronic medication					
Charlson score† (median [IQR])	0 [0, 1.0]	1.0 [1.0, 2.0]	3.0 [2.0, 4.0]	4.0 [4.0, 6.0]	<0.001
Hypertension, n (%)	17 (19.1)	32 (28.8)	64 (47.4)	68 (52.7)	<0.001
Cardiac disease, n (%)	13 (14.6)	16 (14.4)	44 (32.6)	51 (39.5)	<0.001
Respiratory disease, n (%)	14 (15.7)	20 (18.0)	25 (18.5)	33 (25.6)	0.261
Diabetes, n (%)	19 (21.3)	13 (11.7)	35 (25.9)	41 (31.8)	0.003
Kidney disease, n (%)	5 (5.6)	4 (3.6)	8 (5.9)	19 (14.7)	0.005
Neurologic disease, n (%)	5 (5.6)	3 (2.7)	9 (6.7)	21 (16.3)	0.001
Prior malignancy, n (%)	3 (3.4)	1 (0.9)	13 (9.6)	14 (10.9)	0.005
Immunosuppression‡, n (%)	10 (11.2)	4 (3.6)	16 (11.9)	5 (3.9)	0.016
Antiplatelet drugs, n (%)	1 (1.1)	5 (4.5)	28 (20.7)	19 (14.7)	<0.001
Anticoagulant drugs, n (%)	5 (5.6)	4 (3.6)	7 (5.2)	24 (18.6)	<0.001
Disease severity on admission (median [IQR])					
4C Mortality§	4 [3, 5]	3 [2, 5]	4 [3, 6]	6 [4, 7]	<0.001
qSOFA	1 [0, 1]	1 [0, 1]	1 [0, 1]	1 [0, 1]	0.002
MEWS	3 [2, 4]	3 [2, 4]	3 [2, 4]	3 [2, 4]	0.102
CURB ll	0 [0, 1]	0 [0, 1]	0 [0, 1]	1 [0, 1]	<0.001
Treatment at day of admission, n (%)					
Supplementary oxygen therapy	80 (89.9)	103 (92.8)	121 (89.6)	115 (89.1)	0.784
High-flow nasal cannula	0 (0.0)	0 (0.0)	2 (1.5)	2 (1.6)	0.382
Non-invasive ventilation	1 (1.1)	0 (0.0)	2 (1.5)	1 (0.8)	0.647
Routine laboratory markers (median [IQR])					
Leukocyte counts (x10^9/L)	6.1 [4.6, 8.3]	6.0 [4.8, 8.6]	6.4 [4.9, 7.9]	6.9 [5.2, 8.6]	0.306
Neutrophil counts (x10^9/L)	4.8 [3.3, 5.9]	4.4 [3.2, 6.6]	5.0 [3.4, 6.3]	5.2 [3.9, 6.9]	0.074
Lymphocyte counts (x10^9/L)	1.00 [0.70, 1.20]	0.97 [0.70, 1.20]	0.93 [0.70, 1.20]	0.75 [0.50, 1.05]	0.001
Neutrophil-Lymphocyte ratio	4.8 [2.8, 7.2]	4.5 [2.9, 8.6]	4.8 [3.3, 7.5]	6.4 [4.1, 10.5]	0.002
C-reactive protein (mg/L)	98 [49, 126]	76 [43, 136]	83 [51, 141]	100 [57, 147]	0.369
Platelet counts (x10^9/L)	220 [184, 255]	225 [181, 276]	215 [149, 281]	207 [151, 266]	0.434
Creatinine (µmol/L)	74 [63, 92]	79 [65, 90]	82 [67, 101]	87 [70, 113]	0.001

Abbreviations: qSOFA: quick sequential organ failure assessment; MEWS: modified early warning score.

* Prior to admission

† The Charlson score was calculated without the age component

‡ Defined as a history of an organ transplant, immune deficiency, or chronic use of immunosuppressants (see Table S7 for details).

§ The 4C mortality score, a validated COVID-19 score [26], was calculated without the age and obesity component

ll The CURB score was calculated without the age component.

Table 2: Treatments, disease course and outcome of COVID-19 patients after admission to a general ward

	<50	≥50 - <60	≥ 60 - <70	≥70	p-value
n	89	111	135	129	
Treatments, n (%)					
Supplementary oxygen therapy	84 (94.4)	107 (96.4)	131 (97.0)	125 (96.9)	0.737
High-flow nasal cannula	2 (2.2)	8 (7.2)	19 (14.1)	17 (13.2)	0.012
Non-invasive ventilation	1 (1.1)	0 (0.0)	3 (2.2)	8 (6.2)	0.015
Invasive ventilation	8 (9.0)	10 (9.0)	22 (16.3)	9 (7.0)	0.073
Remdesivir	12 (13.5)	10 (9.0)	14 (10.4)	17 (13.2)	0.670
Chloroquine	0 (0.0)	2 (1.8)	0 (0.0)	1 (0.8)	0.284
Monoclonal antibodies against SARS-CoV-2	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0.457
Antibiotics in the first 7 days of admission	31 (34.8)	40 (36.0)	70 (51.9)	65 (50.4)	0.010
Immunomodulating therapies, n (%)					
Dexamethasone 6mg	72 (80.9)	89 (80.2)	102 (75.6)	103 (79.8)	0.730
Of which before sampling	62 (69.7)	79 (71.2)	96 (71.1)	93 (72.1)	0.985
Other corticosteroids*	4 (4.5)	3 (2.7)	6 (4.4)	3 (2.3)	0.713
Of which before sampling	3 (3.4)	2 (1.8)	5 (3.7)	1 (0.8)	0.392
Interleukin-6 inhibitors	10 (11.2)	11 (9.9)	13 (9.6)	12 (9.3)	0.971
Of which before sampling	4 (4.5)	1 (0.9)	3 (2.2)	3 (2.3)	0.426
Anti-C5a antibody	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.8)	0.677
Of which before sampling	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	>1.000
Imatinib	1 (1.1)	5 (4.5)	3 (2.2)	11 (8.5)	0.027
Of which before sampling	0 (0.0)	1 (0.9)	2 (1.5)	4 (3.1)	0.279
Clinical course					
Thrombosis	5 (5.6)	12 (10.8)	9 (6.7)	18 (14.0)	0.110
Of which pulmonary embolism†	5 (5.6)	10 (9.0)	9 (6.7)	17 (13.2)	0.175
Of which deep venous thrombosis†	0 (0.0)	4 (3.6)	0 (0.0)	2 (1.6)	0.054
Length of hospital stay (median [IQR])	4 [2, 7]	4 [3, 8]	6 [3, 11]	7 [4, 11]	<0.001
ICU admission‡, n (%)	9 (10.1)	15 (13.5)	26 (19.3)	13 (10.1)	0.113
ICU stay, days (median [IQR])	15 [8, 29]	11 [9, 13]	8.00 [5, 14]	8.00 [1, 17]	0.450
Readmission§, n (%)	4 (4.5)	5 (4.5)	6 (4.4)	10 (7.8)	0.581
Mortality ll, n (%)					
30 day	1 (1.1)	2 (1.8)	16 (11.9)	39 (30.2)	<0.001
90 day	2 (2.2)	2 (1.8)	16 (11.9)	43 (33.3)	<0.001

Abbreviations: ICU: intensive care unit.

* Prednisolone and hydrocortisone

† Numbers do not add up to a 100% as some patients suffered from both pulmonary and deep venous thrombosis

‡ ICU admission after sampling

§ For any cause within 28 days of the initial admission

ll For 98.2% of patients, worsening of COVID-19 was reported as a causal element for mortality by the treating physician

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Figure legend

Figure 1: Overview of COVID-19 cohorts

Description Figure 1: Overview of COVID-19 cohorts in which plasma biomarkers were measured. The external validation cohort was derived from a publicly available data set of non-intubated COVID-19 patients in whom plasma proteins were measured by Olink Proximity Extension Assay [21].

Figure 2: Mortality analysis of COVID-19 patients admitted to the general ward stratified by age decades

Description Figure 2: a) Kaplan-Meier plot of patients stratified by age group. b) The risk of 30-day mortality with age modelled as a continuous variable. Given the non-linear relationship between age and mortality, a restricted cubic spline function with three inner knots at default quantile locations was used. To calculate the odds ratio, the reference was set to 60 years of age. The red shading represents the 95% confidence interval of the 30-day mortality odds ratio. c) The same method as panel b, but now the 30-day mortality odds ratio is adjusted for demographics (inclusion hospital, sex, and inclusion wave), age-related comorbidities (hypertension, diabetes, malignancies, immunosuppression, and chronic cardiac, neurologic, respiratory, and kidney disease), age-related chronic medication (antiplatelet and anticoagulant drugs), and COVID-19-related treatments both before and after sampling (corticosteroids including dexamethasone, anti-IL-6, imatinib, remdesivir, and antibiotics).

Figure 3: Principal component analysis of host response domain differences between age groups

Description Figure 3: Principal component analysis (PCA) in which principal components (PC) 1 and 2 are plotted per domain. For each domain, the x- and y-axis are labelled with the % of the total

variance within that domain that is explained by PC1 and PC2 respectively. The complete contribution of each biomarker to a PC score is depicted in Table S6. The ellipse indicates the central 10% of each age group, colour coded as indicated in the bottom part of the figure. The arrows indicate the direction (arrow orientation) and strength (arrow length) of the correlation between each biomarker and the PCs. Next to each PCA plot are boxplots with 1.5 interquartile range whiskers of PC1 and PC2. Herein upper p-values were obtained by ANOVA between age groups: rho's with accompanying p-values were generated using a Spearman's correlation with ageing on a continuous scale. Note that a negative association of a principal component with ageing may still reflect a positive association with biomarkers concentrations, as reflected by the direction of the arrows. Post-hoc testing was done with a Tukey Test. *** $p<0.001$, ** $p<0.01$, * $p<0.05$. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon

Figure 4: Association of host response biomarkers with ageing

Description Figure 4: a) Heatmap depicting the magnitude of biomarker differences (Hedges' g) between patients ≥70 years and the other age groups. P-values were obtained from a linear (if linear) or cubic spline regression analysis (if non-linear) in which age was modelled as a continuous variable. The adjusted model included demographics, age-related comorbidities, age and biomarker-related chronic medication and COVID-19-related immunomodulating treatments before sampling,

see Method for details. Red indicates higher levels in patients ≥70: blue indicates lower levels in this age group. b) Volcano plot depicting the strength of the correlation between a biomarker and ageing. Red dots represent a significant positive correlation, blue dots a significant negative correlation, and grey dots a non-significant correlation. Both the adjusted and unadjusted p-values are multiple testing corrected using the Benjamini-Hochberg (BH) procedure for testing 43 biomarkers. *** p<0.001, ** p<0.01, * p<0.05. † Biomarkers with a non-linear relationship with ageing on a continuous scale. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon

Figure 5: Mediation analysis ageing-associated mortality and host response biomarkers upon admission to the general ward

Description Figure 5: a) Quadrant plot. The x-axis depicts the increase in the 30-day mortality odds ratio per 25% increase of the biomarker derived from an unadjusted logistic regression with the log-transformed biomarker as the explanatory variable and 30-day mortality as the response variable. The y-axis shows the percentage change in the biomarker concentration per one-year increase in age which was derived from an unadjusted linear regression analysis with the log-transformed biomarker as the response variable. Biomarkers in both the top right and bottom left corner are most likely associated with an age-dependent increase in 30-day mortality. The significance of the coefficient was multiple testing corrected using the Benjamini-Hochberg (BH) procedure for testing

43 biomarkers. b) The same method as panel A, however, both coefficients are now adjusted for demographics (inclusion hospital, sex, and inclusion wave), age-related comorbidities (hypertension, diabetes, malignancies, immunosuppression, and chronic cardiac, neurologic, respiratory, and kidney disease), age and biomarker-related chronic medication (antiplatelet and anticoagulant drugs), and COVID-19-related treatments both before and after sampling (corticosteroids including dexamethasone, anti-IL-6, imatinib, remdesivir, and antibiotics). c) Diagram of meditation analysis. The adjusted model contained the same covariates as panel B. d) Unadjusted (left) and adjusted (right) mediation analysis results. Only biomarkers and principal components significantly associated with ageing and 30-day mortality were analysed. Confidence intervals were obtained from 1000x bootstrapping. The higher the proportion of mediation, the stronger the association of the age-dependent differences in that biomarker and the age-dependent increase in 30-day mortality. The principal components and their contributing biomarker are depicted in figure 3. The complete contribution of each biomarker to a PC score is depicted in Table S6. Abbreviations: Endocoag score: endothelial and coagulation score; ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon.

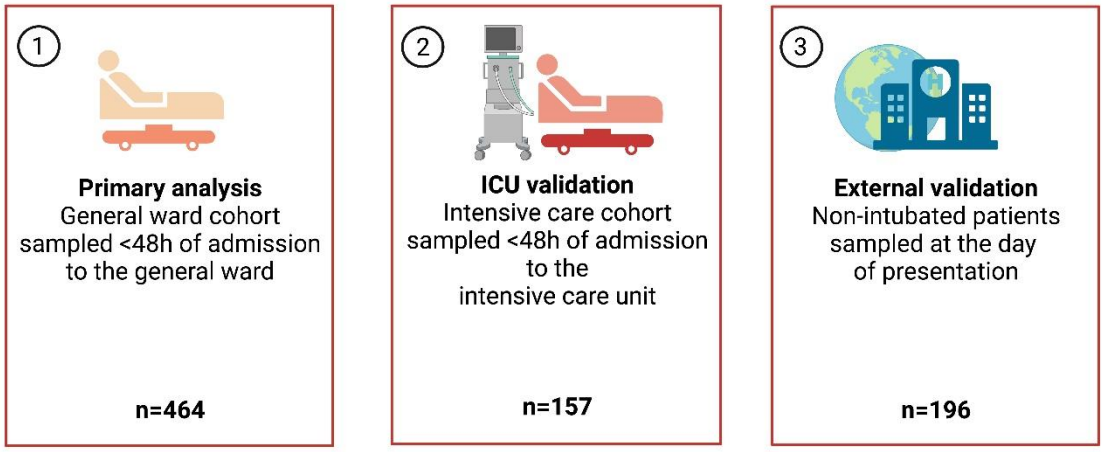
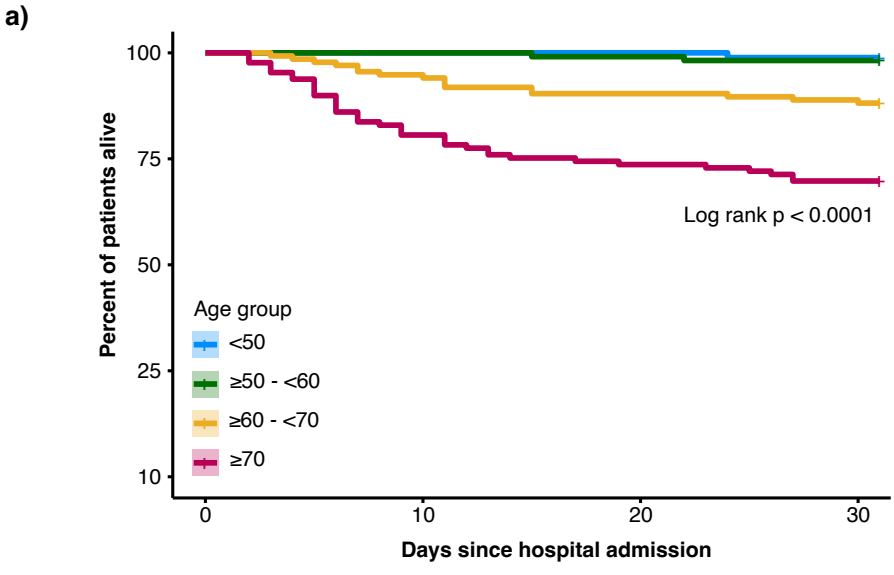
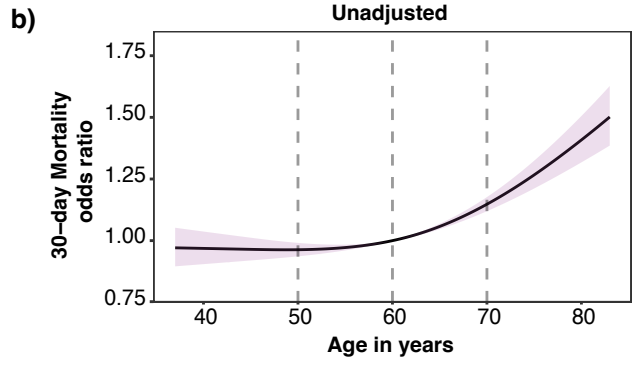


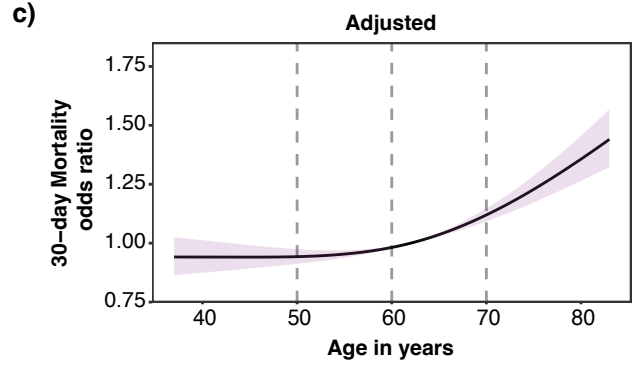
Figure 1: Overview of COVID-19 cohorts Description Figure 1: Overview of COVID-19 cohorts in which plasma biomarkers were measured. The external validation cohort was derived from a publicly available data set of non-intubated COVID-19 patients in whom plasma proteins were measured by Olink Proximity Extension Assay [21].

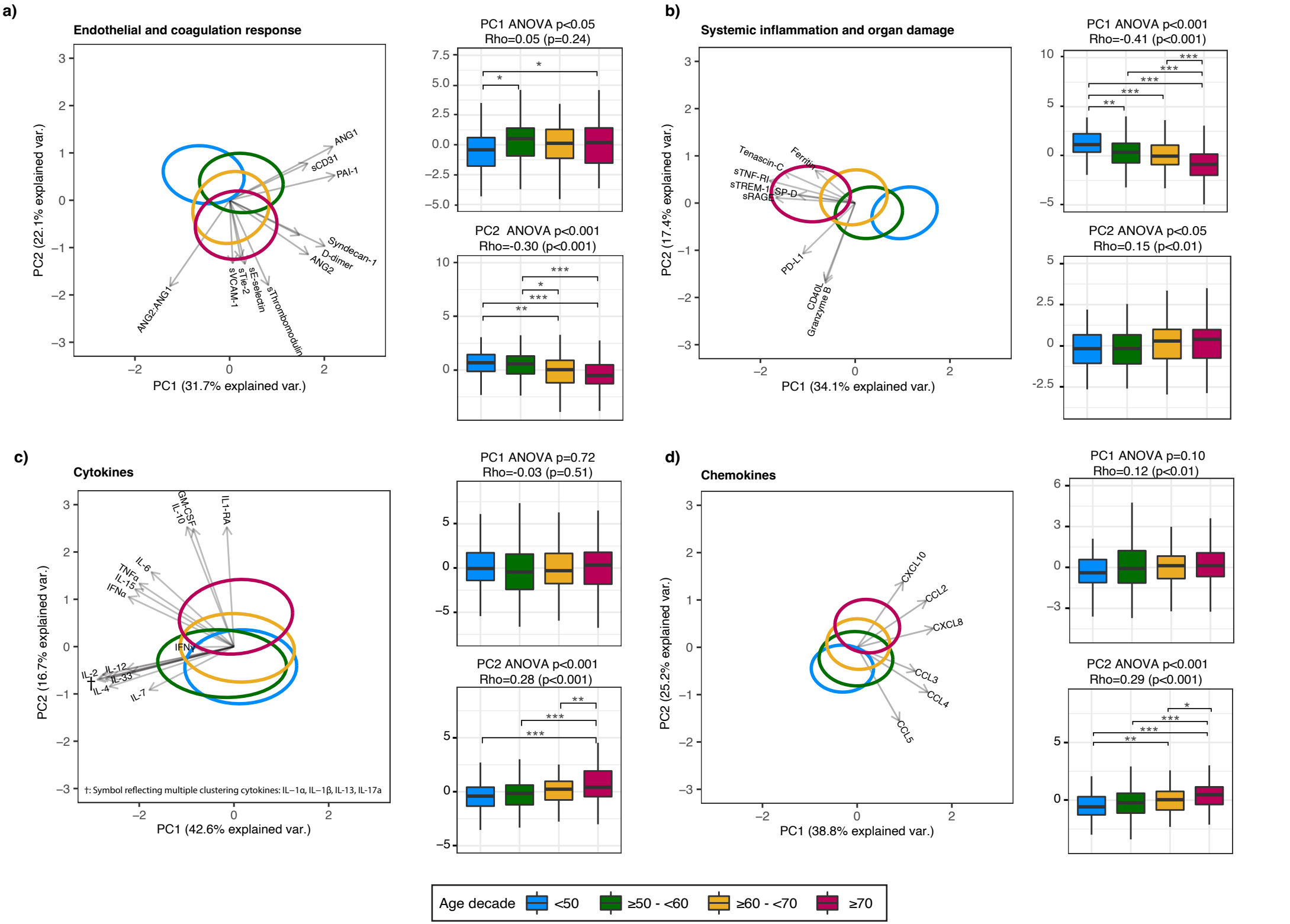


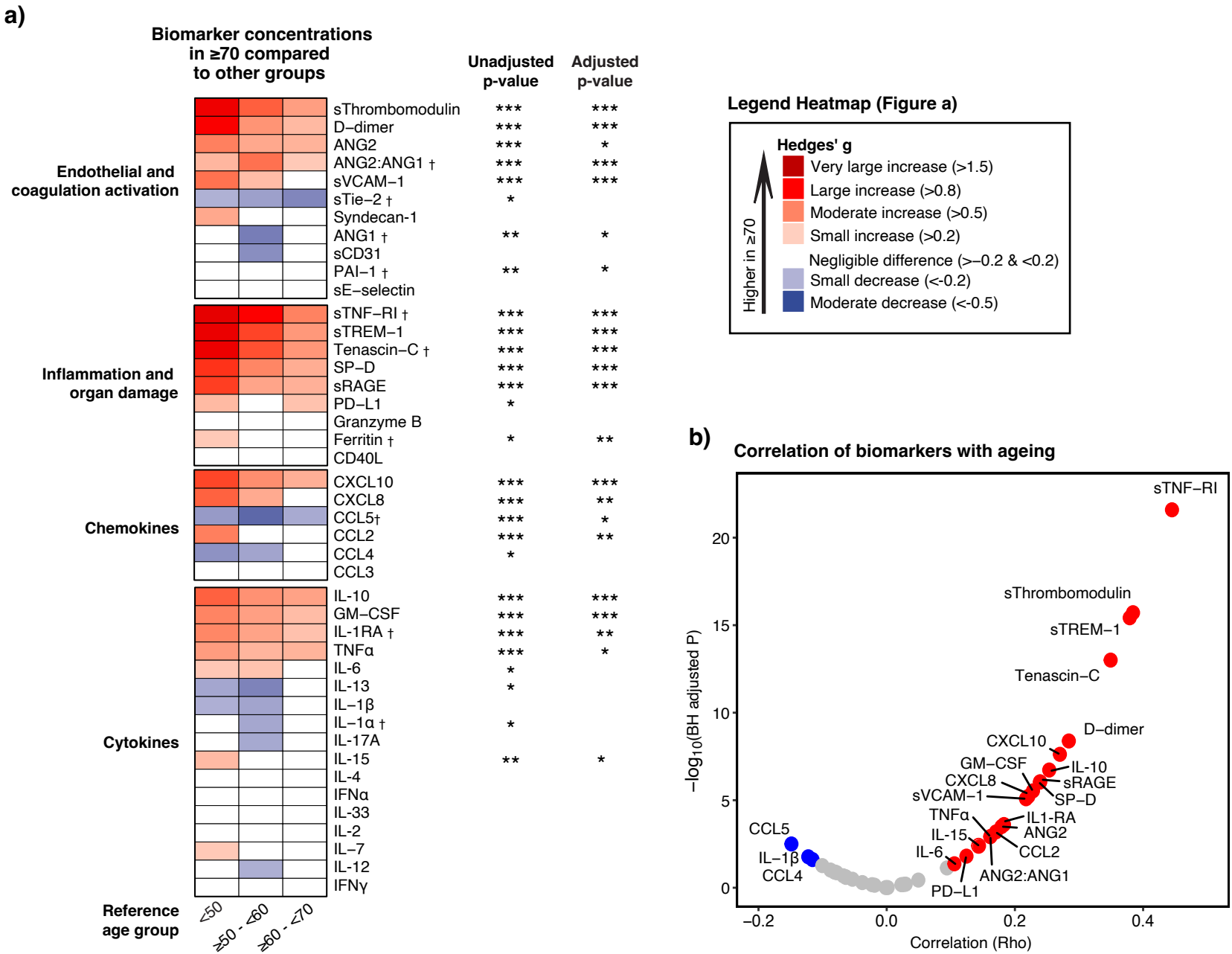
No. at risk

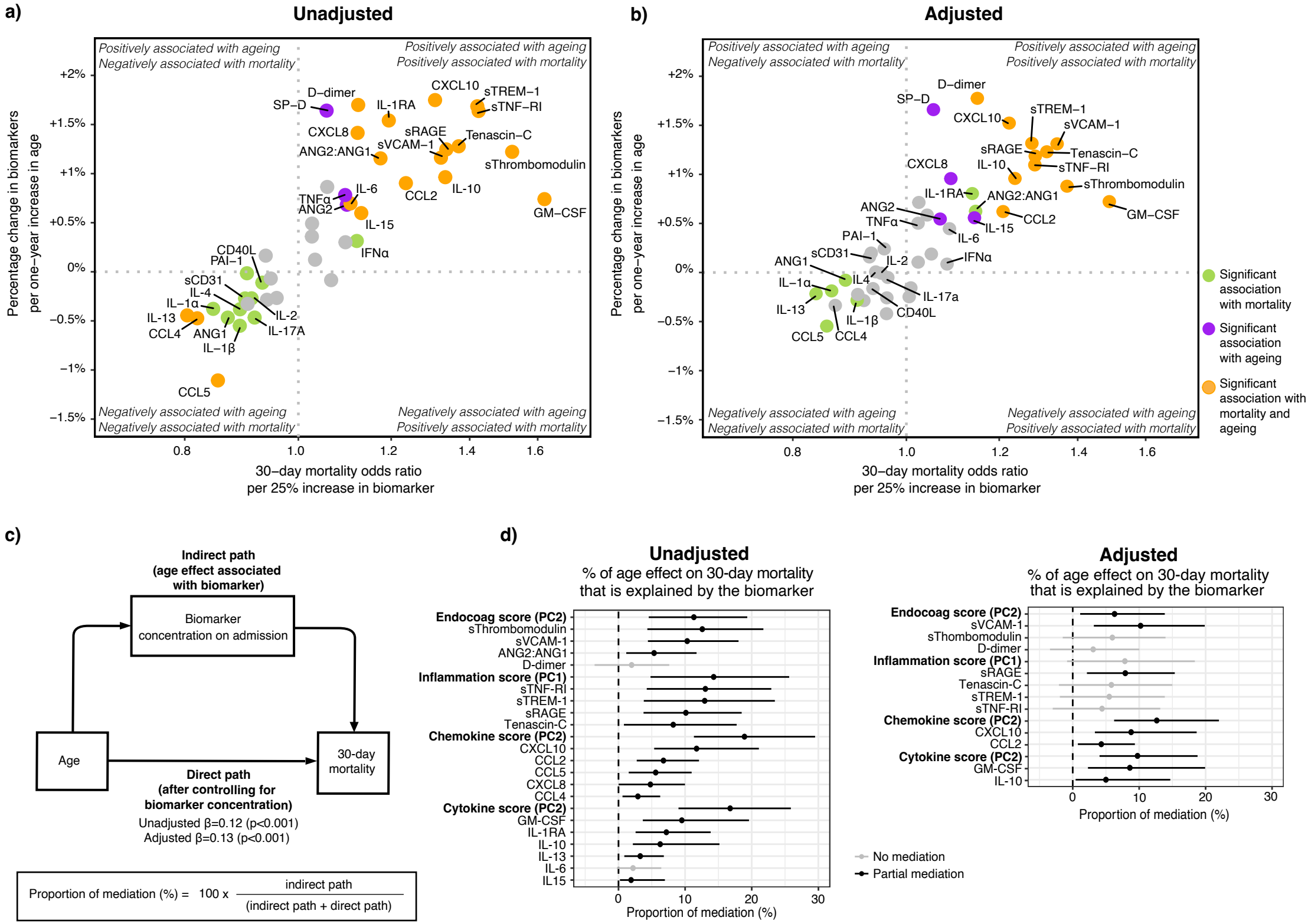
<50	89	89	89	88
≥50 - <60	111	111	110	109
≥60 - <70	135	128	122	120
≥70	129	104	95	90






a)
Endothelial and coagulation response
PC2 (22.1% explained var.)
PC1 (31.7% explained var.)
ANG1
sCD31
PAI-1
Syndecan-1
D-dimer
ANG2
sE-selectin
sThrombomodulin
sVCAM-1
sTie-2
ANG2:ANG1
PC1 ANOVA p<0.05
Rho=0.05 (p=0.24)
PC2 ANOVA p<0.001
Rho=-0.30 (p<0.001)
b)
Systemic inflammation and organ damage
PC2 (17.4% explained var.)
PC1 (34.1% explained var.)
Tenascin-C
Ferritin
sTNF-RI
sTREM-1
SP-D
sRAGE
PD-L1
CD40L
Granzyme B
PC1 ANOVA p<0.001
Rho=-0.41 (p<0.001)
PC2 ANOVA p<0.05
Rho=0.15 (p<0.01)
c)
Cytokines
PC2 (16.7% explained var.)
PC1 (42.6% explained var.)
GM-CSF
IL-10
IL1-RA
IL-6
TNFα
IL-15
IFNα
IFNγ
IL-2
IL-12
IL-33
IL-4
IL-7
†: Symbol reflecting multiple clustering cytokines: IL−1α, IL−1β, IL-13, IL-17a
PC1 ANOVA p=0.72
Rho=-0.03 (p=0.51)
PC2 ANOVA p<0.001
Rho=0.28 (p<0.001)
d)
Chemokines
PC2 (25.2% explained var.)
PC1 (38.8% explained var.)
CXCL10
CCL2
CXCL8
CCL3
CCL4
CCL5
PC1 ANOVA p=0.10
Rho=0.12 (p<0.01)
PC2 ANOVA p<0.001
Rho=0.29 (p<0.001)
Age decade
<50
≥50 - <60
≥60 - <70
≥70


a)
Biomarker concentrations in ≥70 compared to other groups
Unadjusted p-value
Adjusted p-value
Endothelial and coagulation activation
sThrombomodulin *** ***
D–dimer *** ***
ANG2 *** *
ANG2:ANG1 † *** ***
sVCAM–1 *** ***
sTie–2 † *
Syndecan-1
ANG1 † ** *
sCD31
PAI–1 † ** *
sE–selectin
Inflammation and organ damage
sTNF–RI † *** ***
sTREM–1 *** ***
Tenascin–C † *** ***
SP–D *** ***
sRAGE *** ***
PD–L1 *
Granzyme B
Ferritin † * **
CD40L
Chemokines
CXCL10 *** ***
CXCL8 *** **
CCL5† *** *
CCL2 *** **
CCL4 *
CCL3
Cytokines
IL-10 *** ***
GM–CSF *** ***
IL-1RA † *** **
TNFα *** *
IL-6 *
IL-13 *
IL–1β
IL–1α † *
IL-17A
IL-15 ** *
IL-4
IFNα
IL-33
IL-2
IL–7
IL-12
IFNγ
Reference age group
<50
≥50 - <60
≥60 - <70
Legend Heatmap (Figure a)
Hedges' g
Very large increase (>1.5)
Large increase (>0.8)
Moderate increase (>0.5)
Small increase (>0.2)
Negligible difference (>–0.2 & <0.2)
Small decrease (<-0.2)
Moderate decrease (<-0.5)
Higher in ≥70
b)
Correlation of biomarkers with ageing
sTNF–RI
sThrombomodulin
sTREM–1
Tenascin–C
D–dimer
CXCL10
GM–CSF
IL-10
CXCL8
sRAGE
sVCAM–1
SP–D
TNFα
IL1-RA
IL-15
ANG2
IL-6
CCL2
ANG2:ANG1
PD–L1
CCL5
IL–1β
CCL4
−log10(BH adjusted P)
Correlation (Rho)
20
15
10
5
0
–0.2
0.0
0.2
0.4


a)
Unadjusted
Positively associated with ageing
Negatively associated with mortality
Positively associated with ageing
Positively associated with mortality
Negatively associated with ageing
Negatively associated with mortality
Negatively associated with ageing
Positively associated with mortality
Percentage change in biomarkers per one−year increase in age
30−day mortality odds ratio per 25% increase in biomarker
D−dimer
SP−D
IL-1RA
CXCL10
sTREM−1
sTNF−RI
CXCL8
sRAGE
Tenascin−C
ANG2:ANG1
sVCAM−1
sThrombomodulin
IL-6
CCL2
IL-10
TNFα
ANG2
IL-15
GM−CSF
IFNα
CD40L
PAI−1
sCD31
IL-4
IL−1α
IL-13
IL-2
CCL4
ANG1
IL-17A
IL−1β
CCL5
b)
Adjusted
Significant association with mortality
Significant association with ageing
Significant association with mortality and ageing
c)
Indirect path
(age effect associated with biomarker)
Biomarker concentration on admission
Age
30-day mortality
Direct path
(after controlling for biomarker concentration)
Unadjusted β=0.12 (p<0.001)
Adjusted β=0.13 (p<0.001)
Proportion of mediation (%) = 100 x indirect path / (indirect path + direct path)
d)
Unadjusted
% of age effect on 30-day mortality that is explained by the biomarker
Endocoag score (PC2)
sThrombomodulin
sVCAM-1
ANG2:ANG1
D-dimer
Inflammation score (PC1)
sTNF-RI
sTREM-1
sRAGE
Tenascin-C
Chemokine score (PC2)
CXCL10
CCL2
CCL5
CXCL8
CCL4
Cytokine score (PC2)
GM-CSF
IL-1RA
IL-10
IL-13
IL-6
IL15
Proportion of mediation (%)
Adjusted
% of age effect on 30-day mortality that is explained by the biomarker
Endocoag score (PC2)
sVCAM-1
sThombomodulin
D-dimer
Inflammation score (PC1)
sRAGE
Tenascin-C
sTREM-1
sTNF-RI
Chemokine score (PC2)
CXCL10
CCL2
Cytokine score (PC2)
GM-CSF
IL-10
Proportion of mediation (%)
No mediation
Partial mediation

Online Data Supplement

Age-related changes in plasma biomarkers and their association with mortality in COVID-19

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Methods

ELDER-BIOME and Amsterdam UMC Biobank COVID-19 cohorts

Plasma samples were obtained as part of two studies: ELDER-BIOME and the Amsterdam UMC biobank study. The Amsterdam UMC COVID-19 biobank study collected leftover blood drawn as part of clinical care, which was processed for plasma storage. From all patients that retrospectively complied with the inclusion criteria of the ELDER-BIOME study (as described in the Methods section of the main manuscript), plasma samples were retrieved for the current investigation. Researchers of the ELDER-BIOME study were involved in setting up the Amsterdam UMC COVID-19 biobank study. Moreover, the primary investigators of the ELDER-BIOME (Michels) and COVID-19 biobank study (Appelman) worked together to ensure that the clinical variables' definitions were concurrent. Inclusion criteria, blood sampling and clinical data collection were done in exactly the same manner for both cohorts.

Patients with pneumonia caused by pathogens other than SARS-CoV-2

For comparison of mortality rates we used patients admitted to a general hospital ward with community-acquired pneumonia caused by pathogens other than SARS-CoV-2. These patients were recruited in the ELDER-BIOME study, a prospective observational investigation in Amsterdam UMC [1]. Patients were eligible if admitted to a general hospital ward with a clinical suspicion of community-acquired pneumonia and meeting all following criteria: one or more systemic symptoms (fever or hypothermia, leukocytosis or leukopenia), one or more respiratory symptoms (new cough or sputum production, chest pain, dyspnea, tachypnea, abnormal lung examination, or respiratory failure), and an evident new or progressive infiltrate, consolidation or pleural effusion on chest X-ray or computed tomography scan [1].

The rationale for the biomarker selection

Pathophysiological mechanisms implicated in COVID-19 include endothelial cell and coagulation activation, excessive inflammation and organ damage, and activation of cytokine and chemokine networks; within each of these domains we selected representative biomarkers in accordance with literature [2–9] and previous publications from our group [10–15] in order to obtain insight into possible associations between ageing and the host response and ageing-associated mortality (Table S1).

Description of COVID-19 waves in the Netherlands

To tackle the variability of the viral strain and immunomodulating therapies over time patients were divided according to four "waves" in keeping with information collected by the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) [16, 17]: wave 1 consisted of patients included before the implementation of dexamethasone (<1st of July 2020); wave 2 were patients included during the implementation of dexamethasone and the appearance of the alpha stain (June 1, 2020 – February 1, 2021); wave 3 contained patients included during the implementation of vaccinations, anti-interleukin-6 treatment, and dominance of the alpha stain (February 1, 2021 – July 1, 2021); wave 4 entailed patients included during the implementation of monoclonal antibodies against SARS-CoV-2 and dominance of the delta stain (July 1, 2021 - January 1, 2022) [16].

Assays

Ethylenediamine tetraacetic acid (EDTA) anticoagulated plasma was stored within 4 hours after the blood draw at -80 °C. Biomarkers were measured by Luminex multiplex assays (R&D Systems Inc., Minneapolis, United States), using the Bio-Plex 200 System (Bio-Rad Laboratories Inc., California, United States); the following multiplex assays were used: the Human Immunotherapy 24-plex

Luminex Performance Assay (LKTM010) and two custom build discovery assays: a Discovery 16-plex (R&D Luminex code: dDHNfKDr) and a Discovery 4-plex (R&D Luminex code: NlqcGJUe). The quality of the measurements is depicted in Supplementary Excel file, sheet 2. Due to a low number of measurable beats for soluble P-selectin and an inadequate standard curve with only 4 points within range for interleukin (IL)-5, these markers were removed from the analysis. Concentrations below the lower limit of quantification were set to the lower limit of quantification. Concentrations above the upper limit of quantification were set to the upper limit of quantification. Samples with an insufficient bead count (<25 beats) were classified as missing (Supplementary Excel file 1).

Statistics, including assumption testing

Data were analysed in R version 4.0.3 (R Core Team 2013, Vienna, Austria), including the R mediation package [18]. Data distributions were assessed by histograms and quantile-quantile plots. Categorical variables were analysed using a Chi-square test of independence. Non-normal continuous data were analysed using a Kruskal-Wallis test, normally distributed continuous data using an analysis of variance (ANOVA). For post hoc testing of non-normal data, a Dunn's test of multiple comparisons using rank sums was used; for post hoc testing of normally distributed continuous data, a Tukey post hoc test was conducted. The differences in 30-day survival were visualised by Kaplan-Meier curves.

All biomarkers were log-transformed. Linearity of a biomarker with ageing was visually inspected by using scatterplots and formally tested by a Wald test of the nonlinear terms of a restricted cubic spline function ($p<0.05$ indicating nonlinearity). Biomarkers that showed a linear relationship with age were analysed using linear regression analysis; nonlinear biomarkers were analysed using a restricted cubic spline function with three inner knots at default quantile locations. Age group differences in biomarker concentrations were visualised by a Hedges g heatmap [19]. The strength of

the correlation of biomarkers with age on a continuous scale was analysed using Spearman's correlation.

We used mediation analysis to examine the extent to which ageing-related alterations in biomarkers contribute to the age-related increase in 30-day mortality [18, 20]. A biomarker or PC score needed to be significantly associated with ageing and 30-day mortality in both cohorts to enter the mediation analysis.

Next, we evaluated if an association between a biomarker and mortality varies with age. To address this, we evaluated the interaction term (Age * log-transformed biomarker) in a logistic regression model with 30-day mortality as the outcome. A significant interaction term would suggest that the association between the biomarker and mortality differs for different age groups, making it an unsuitable candidate for mediation analysis [21]. In mediation analysis, it is assumed that the association between the biomarker and mortality should be similar for each age group for the biomarker to explain a certain proportion of the mortality associated with ageing. Therefore, only biomarkers showing a consistent association between mortality and ageing were considered valid for the mediation analysis [21].

Next, we performed a cluster analysis on patients aged ≥70 to assess the uniformity of their host response using Ward's method [22]. The optimal number of clusters was determined by the majority ruling of the NbClust R package [23]. This approach has been previously used to identify biomarker phenotypes in COVID-19 patients that may benefit from treatment with imatinib [12]. The importance of each biomarker to the cluster assignment was determined by performing a least absolute shrinkage and selection operator regression. To optimise the performance of the lasso regression model, we used k-fold cross-validation to find the optimal lambda value. At last, we investigated whether the biomarkers' mediating effects were still present in this subgroup of

patients aged ≥70 and whether the mediation depended on the patients' host response phenotype. For this, we conducted a logistic regression with 30-day mortality as an outcome, the log-transformed biomarker or PC score as a predictor and the cluster assignment as a covariate.

Each analysis (association of ageing with biomarkers concentrations, mediation analysis) consisted of an unadjusted and an adjusted model. First, we sought to address the crude association of ageing with biomarker concentrations. This method was chosen as we wished to explore the association of ageing with biomarkers before correcting for covariates that are known to be strongly associated with ageing (e.g., older age and diabetes). We consider this a better reflection of the actual clinical population. Next, we performed an adjusted model in which we additionally corrected for demographics (inclusion hospital, sex, and inclusion wave), age-related comorbidities (hypertension, diabetes, malignancies, immunosuppression, and chronic cardiac, neurologic, pulmonary, and kidney disease), age and biomarker-related chronic medication (antiplatelet and anticoagulant drugs), and COVID-19-related immunomodulating treatments before sampling (corticosteroids including dexamethasone, anti-IL6, imatinib [24]). We consider the adjusted model a reflection of the remaining explained variance of ageing with biomarkers when correcting for variables that are strongly associated with ageing. When evaluating the association of ageing with mortality, we used the previous described adjusted model with the addition of covariates that may impact mortality; immunomodulating treatments after sampling, the use of antibiotics and remdesivir. Conserning immunomodulating treatments after samples, we believed immortal-time and lead-time bias to be minimal as corticosteroids and imatinib were almost exclusively given within 48h of admission. Moreover, anti-Il6 treatment was mostly given early in the disease course with a median of 1 day after admission [IQR: 0,1].

Missing value analysis

Clinical variables and routine lab markers

Most variables showed <5% missingness. For the ward cohort, missingness was only higher than 5% for BMI (9%) and smoking status (8%). For the ICU cohort, missingness was higher than 5% for BMI (8%), the 4c mortality score (9%), smoking status (19%) and vaccination status (12%). We performed a Pearson's chi-squared test to explore missingness patterns for these variables. All clinical variables showed no significant differences in the proportion of missingness among age groups and were therefore classified as missing at random (Table S2). The proportion of missing values of each routine lab marker per age group is displayed in Table S3. For the routine lab markers, missing values were comparable between groups with the exception of activated partial thromboplastin time upon ICU admission; >5% missingness and more prominent missingness in the <50 age group).

Biomarkers

All analysed biomarkers showed <1% missingness, except for CD31. CD31 was missing in 9% of non-critically and 6% of critically ill patients (Supplementary Excel file, sheet 2). In both cohorts, the proportion of missing CD31 was similar between age groups (based on a Pearson's chi-squared test) and was therefore classified as missing at random. For each analysis, missing biomarkers were tackled using pairwise deletion. For the principal component analysis (PCA), the very few missing biomarkers were imputed using multiple imputations by Chained Equations using the classification and regressions tree method with 10 iterations and 10 imputations. For this, the MICE R package was used. Based on a random number generator, one dataset was selected to construct the PCA plots.

Description of the independent cohort

We utilised a publicly available cohort with proteomic data to validate a biomarker's association with ageing and 30-day mortality (direction and magnitude) [25]. Plasma proteins were measured at multiple days using the Olink Proximity Extension Assay (PEA), which combines DNA reporter sequences with real-time PCR [25]. The cohort entailed 306 patients with a positive SARS-CoV-2 PCR included between the 24th of March 2020 and the 30th of April 2020 in a large urban academic hospital in Boston, USA. Given that enrollment occurred early in the pandemic, immunomodulating therapies were not part of standard care. To analyse a population most reflective of our general ward cohort, we only selected hospitalised patients who were not mechanically ventilated or deceased on the day of admission and had available protein data at admission (n=196).

Observing a similar association of a biomarker with ageing and 30-day mortality in both cohorts (our cohort and this independent cohort) would greatly underpin the robustness of the association. To clarify, there are many noticeable differences between both cohorts. Our cohort (vs. this independent cohort [25]) entailed COVID-19 patients enrolled in the Netherlands (vs. the United States), primarily during the occurrence and dominance of the alpha strain (vs. dominance of the wild-type variant), with the majority of patients receiving dexamethasone before sampling (vs. no immunomodulating therapies), in which proteins were measured using Luminex multiplex assays (vs. Olink PEA). Collectively, robustness of the association of the biomarker with ageing and mortality in both cohorts would suggest that such association is independent of the geographical location, COVID-19 strain, immunomodulating therapies before sampling, and the method of protein measurement.

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Supplementary tables

Table S1: Stratification of biomarkers in host response domains

Main analysis			
Endothelium and coagulation activation	Inflammation and organ damage	Cytokines	Chemokines
ANG1	sRAGE	IL-1RA	CCL2 (MCP1)
ANG2	Ferritin	IL-1α	CCL3 (MIP-1α)
ANG2:ANG1	sTNF-RI	IL-1β	CCL4 (MIP-1β)
sTie-2	sTREM-1	IL-2	CCL5 (Rantes)
sE-Selectin	Tenascin-C	IL-4	CXCL8 (IL-8)
sThrombomodulin	SP-D	IL-6	CXCL10 (IP-10)
sVCAM-1	Granzyme B	IL-7	
Syndecan-1	CD40L	IL-10	
D-dimer	PD-L1	IL-12 p70	
PAI-1		IL-13	
sCD31		IL-15	
		IL-17a	
		IL-33	
		TNFα	
		GM-CSF	
		IFN-γ	
		IFN-α	

Names within brackets reflect a frequently used synonym of a biomarker. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFα: tumor necrosis factor alpha; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon

Table S2: Missing pattern analysis of clinical variables with more than 5% missingness displaying the proportion of missingness per age group

	<50	≥50 - <60	≥60 - <70	≥70	P-value
Ward cohort, n (%)					
Sample size	89	111	135	129	
BMI	10 (11.2)	8 (7.2)	9 (6.7)	16 (12.4)	0.312
Smoking status	8 (9.0)	10 (9.0)	6 (4.4)	13 (10.1)	0.340
ICU cohort, n (%)					
Sample size	30	37	59	31	
BMI	3 (10.0)	2 (5.4)	3 (5.1)	4 (12.9)	0.520
Smoking status	5 (16.7)	10 (27.0)	10 (16.9)	5 (16.1)	0.578
Vaccination status	4 (13.3)	6 (16.2)	5 (8.5)	4 (12.9)	0.710
4c mortality*	4 (13.3)	4 (10.8)	5 (8.5)	1 (3.2)	0.545

Abbreviations: BMI: Body Mass Index. P-values are obtained from Pearson's chi-squared test.

* The 4c mortality score is a validated COVID-19 score [26].

Table S3: Missing pattern analysis of routine lab markers displaying the proportion of missingness per age group

	<50	≥50 - <60	≥60 - <70	≥70	P-value
Ward cohort, n (%)					
Sample size	89	111	135	129	
Lymphocyte count	1 (1.1)	2 (1.8)	0 (0.0)	6 (4.7)	0.046
NLR	2 (2.2)	3 (2.7)	2 (1.5)	10 (7.8)	0.033
Leukocyte count	2 (2.2)	1 (0.9)	0 (0.0)	3 (2.3)	0.306
Neutrophil count	2 (2.2)	3 (2.7)	2 (1.5)	10 (7.8)	0.033
CRP	2 (2.2)	2 (1.8)	1 (0.7)	2 (1.6)	0.819
Platelet count	4 (4.5)	1 (0.9)	1 (0.7)	6 (4.7)	0.088
Creatinine	4 (4.5)	1 (0.9)	1 (0.7)	3 (2.3)	0.187
ICU cohort, n (%)					
Sample size	30	37	59	31	
CRP	1 (3.3)	4 (10.8)	2 (3.4)	1 (3.2)	0.352
WBC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Platelets	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
APTT	14 (46.7)	12 (32.4)	8 (13.6)	4 (12.9)	0.001
Creatinine	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Urea	2 (6.7)	5 (13.5)	9 (15.3)	0 (0.0)	0.110
Bilirubin	13 (43.3)	10 (27.0)	12 (20.3)	7 (22.6)	0.126
Lactate	0 (0.0)	0 (0.0)	1 (1.7)	0 (0.0)	0.643
pH	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	0.353

Abbreviations: NLR: neutrophil to lymphocyte ratio; WBC: white blood cell count; CRP: C-reactive protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; APTT: activated partial thromboplastin time. P-values are obtained from Pearson's chi-squared test.

Table S4: Age distribution of patients per COVID-19 wave in the Netherlands of the general ward cohort

	<50	≥50 - <60	≥ 60 - <70	≥70	p-value
n	89	111	135	129	
Inclusion wave, n (%)					0.280
Wave 1	10 (11.2)	16 (14.4)	23 (17.0)	22 (17.1)	
Wave 2	43 (48.3)	45 (40.5)	56 (41.5)	71 (55.0)	
Wave 3	34 (38.2)	47 (42.3)	53 (39.3)	33 (25.6)	
Wave 4	2 (2.2)	3 (2.7)	3 (2.2)	3 (2.3)	

Wave 1: <1st of July 2020, Wave 2: June 1, 2020 – February 1, 2021, Wave 3: February 1, 2021 – July 1, 2021, Wave 4: July 1, 2021 - January 1, 2022.

Table S5: Baseline characteristics, treatments and outcome of patients with community-acquired pneumonia due to pathogens other than SARS-CoV-2

	<50	≥50 - <60	≥60 - <70	≥70	p-value
n	32	43	62	121	
Demographics					
Age (median [IQR])	42 [34, 46]	55 [53, 58]	65 [62, 67]	78 [75, 83]	<0.001
Male sex, n (%)	21 (65.6)	28 (65.1)	34 (54.8)	66 (54.5)	0.476
Body Mass Index (median [IQR])	30.0 [27.4, 33.2]	27.8 [25.2, 32.1]	28.7 [25.6, 32.8]	27.5 [24.3, 30.1]	0.002
Duration of symptoms*, days (median [IQR])	3 [2, 6]	4 [2, 7]	3 [1, 7]	4 [2, 7]	0.864
Smoking status, n (%)					<0.001
Yes	9 (28.1)	8 (18.6)	14 (22.6)	12 (9.9)	
Former smoker	8 (25.0)	11 (25.6)	27 (43.5)	75 (62.0)	
Never smoked	14 (43.8)	17 (39.5)	20 (32.3)	26 (21.5)	
Unknown	1 (3.1)	7 (16.3)	1 (1.6)	8 (6.6)	
Comorbidities and (selected) chronic medication					
Charlson score† (median [IQR])	0 [0.00, 1.00]	1 [1.00, 3.00]	4 [3.00, 5.00]	5 [4.00, 7.00]	<0.001
Hypertension, n (%)	5 (15.6)	12 (27.9)	22 (35.5)	66 (54.5)	<0.001
Cardiac disease, n (%)	8 (25.0)	13 (30.2)	27 (43.5)	61 (50.4)	0.020
Respiratory disease, n (%)	8 (25.0)	22 (51.2)	35 (56.5)	64 (52.9)	0.024
Diabetes, n (%)	6 (18.8)	4 (9.3)	16 (25.8)	28 (23.1)	0.183
Kidney disease, n (%)	1 (3.1)	1 (2.3)	10 (16.1)	16 (13.2)	0.053
Neurologic disease, n (%)	2 (6.2)	2 (4.7)	4 (6.5)	7 (5.8)	0.983
Prior malignancy, n (%)	3 (9.4)	7 (16.3)	16 (25.8)	39 (32.2)	0.026
Immunosuppression‡, n (%)	11 (34.4)	11 (25.6)	24 (38.7)	13 (10.7)	<0.001
Antiplatelet drugs, n (%)	2 (6.2)	5 (11.6)	23 (37.1)	41 (33.9)	<0.001
Anticoagulant drugs, n (%)	2 (6.2)	4 (9.3)	11 (17.7)	37 (30.6)	0.002
Disease severity on admission (median [IQR])					
4C Mortality§	2 [1, 3]	3 [2 , 4]	4 [3 , 5]	4 [3 , 6]	<0.001
qSOFA	1 [0, 1]	1 [0 , 1]	1 [0 , 1]	1 [0 , 1]	0.773
MEWS	3 [3, 4]	4 [2 , 5]	3 [2 , 5]	3 [2 , 4]	0.393
CURB ll	0 [0, 1]	0 [0 , 1]	0 [0 , 1]	1 [0 , 1]	0.015
Treatment at day of admission, n (%)					
Supplementary oxygen treatment	15 (46.9)	29 (67.4)	50 (80.6)	94 (77.7)	0.002
Non-invasive ventilation	0 (0.0)	1 (2.3)	0 (0.0)	5 (4.1)	0.264
Routine laboratory markers (median [IQR])					
Leukocyte counts (x10^9/L)	12.1 [7.8, 16.3]	13.4 [9.1, 18.1]	11.6 [8.6, 17.2]	13.7 [10.1, 17.7]	0.286
Neutrophil counts (x10^9/L)	9.3 [6.1, 14.4]	11.3 [6.9, 14.0]	9.8 [6.6, 15.3]	11.1 [7.7, 14.5]	0.555
Lymphocyte counts (x10^9/L)	0.94 [0.60, 1.56]	0.97 [0.61, 1.66]	0.90 [0.60, 1.20]	0.81 [0.60, 1.30]	0.862
Neutrophil-Lymphocyte ratio	7.6 [4.4, 14.3]	10.0 [5.9, 18.4]	9.7 [6.3, 14.7]	11.6 [7.4, 18.7]	0.217
C-reactive protein (mg/L)	153 [40, 235]	148 [87, 300]	118 [64, 259]	110 [50, 250]	0.519
Platelet counts (x10^9/L)	227 [187, 284]	258 [186, 343]	241 [170, 347]	239 [174, 321]	0.615
Creatinine (µmol/L)	86 [69, 111]	78 [68, 108]	88 [66, 127]	97 [70, 12]	0.144
Treatments, n (%)					
Supplementary oxygen therapy	15 (46.9)	33 (76.7)	52 (83.9)	101 (83.5)	<0.001
Non-invasive ventilation	0 (0.0)	3 (7.0)	1 (1.6)	9 (7.5)	0.168
Invasive ventilation	0 (0.0)	1 (2.3)	1 (1.6)	2 (1.7)	0.877
Antibiotics in the first 7 days of admission	32 (100.0)	41 (95.3)	60 (96.8)	118 (97.5)	0.661
Clinical course					
Length of hospital stay (median [IQR])	4 [2, 7]	4 [3, 8]	6 [3, 11]	7 [4, 11]	<0.001
ICU admission**, n (%)	0 (0.0)	4 (9.3)	2 (3.2)	9 (7.4)	0.234
ICU stay, days (median [IQR])	N.A.	3.50 [2.75, 5.75]	8.50 [4.75, 12.25]	1.50 [1.00, 4.00]	0.381
Readmission, n (%)	0 (0.0)	1 (2.3)	0 (0.0)	1 (0.8)	0.556
Mortality, n (%)					
30 day	1 (3.1)	0 (0.0)	0 (0.0)	7 (5.8)	0.100
90 day	1 (3.1)	0 (0.0)	0 (0.0)	9 (7.4)	0.038

Abbreviations: qSOFA: quick sequential organ failure assessment; MEWS: modified early warning score; N.A.; not applicable.

* Prior to admission

† The Charlson score was calculated without the age component

‡ Defined as a history of an organ transplant, immune deficiency, or chronic use of immunosuppressants

§ The 4C mortality score, a validated COVID-19 score [26], was calculated without the age and obesity component

ll The CURB score was calculated without the age component.

** ICU admission after sampling

Table S6: Contribution of each biomarker to principal components 1 and 2 of each host response domain upon admission to the general ward

Endothelial and coagulation response		Inflammation and organ damage		Cytokines		Chemokines	
PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
20.8% PAI-1	1.7% PAI-1	20.9% sTNF-RI	2.8% sTNF-RI	10.8% IL-1α	1.6% IL-1α	25.3% CXCL8	2.3% CXCL8
20.0% ANG1	7.7% ANG1	20.3% sTREM-1	0.7% sTREM-1	10.5% IL-13	1.5% IL-13	21.9% CCL4	13.6% CCL4
17.0% Syndecan-1	5.7% Syndecan-1	17.8% sRAGE	0.2% sRAGE	10.0% IL-2	1.2% IL-2	20.9% CCL2	14.7% CCL2
11.6% ANG2	7.9% ANG2	14.5% Tenascin-C	5.6% Tenascin-C	9.5% IL-17a	1.6% IL-17a	14.8% CCL3	3.9% CCL3
11.2% sCD31	3.8% sCD31	9.1% SP-D	0.4% SP-D	9.0% IL-4	2.4% IL-4	9.3% CXCL10	28.9% CXCL10
9.1% D-dimer	3.3% D-dimer	7.7% PD-L1	14.4% PD-L1	8.7% IL-1β	1.4% IL-1β	7.8% CCL5	36.5% CCL5
6.7% ANG2:ANG1	19.8% ANG2:ANG1	4.5% Ferritin	6.0% Ferritin	6.6% IL-12	0.7% IL-12		
2.9% sThrombomodulin	19.3% sThrombomodulin	2.6% CD40L	33.8% CD40L	6.5% IFN-α	3.7% IFN-α		
0.4% sE-Selectin	11.0% sE-Selectin	2.5% Granzyme B	36.0% Granzyme B	6.1% IL-33	1.0% IL-33		
0.2% sTie-2	9.1% sTie-2			5.7% IL-15	4.9% IL-15		
0.0% sVCAM-1	10.7% sVCAM-1			5.2% TNF-α	5.9% TNF-α		
				4.2% IL-7	2.8% IL-7		
				4.0% IL-6	8.2% IL-6		
				1.3% IL-10	21.2% IL-10		
				1.0% GM-CSF	20.6% GM-CSF		
				0.9% IFN-γ	0.0% IFN-γ		
				0.0% IL-1RA	21.2% IL-1RA		

The top 3 contributing biomarkers per principal component score are marked with blue shading. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFα: tumor necrosis factor alpha; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon

Table S7: Subdivision of immunosuppression as comorbidity

	<50	≥50 - <60	≥ 60 - <70	≥70	p-value
n	89	111	135	129	
Immunosuppression, n (%)	10 (11.2)	4 (3.6)	16 (11.9)	5 (3.9)	0.016
Immunosuppression due to disease, n (%)					
Hypogammaglobinaemia	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Asplenia	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Neutropenic after CAR T-cell transfusion	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.238
Kidney transplant receiver	4 (4.5)	2 (1.8)	3 (2.2)	2 (1.6)	0.517
Chronic use of immunosuppressives*, n (%)					
High dose prednisone (≥7.5mg) or equivalent	1 (1.1)	3 (2.7)	4 (3.0)	1 (0.8)	0.510
Mycophenolate mofetil	3 (3.4)	2 (1.8)	3 (2.2)	1 (0.8)	0.584
Tacrolimus	4 (4.5)	2 (1.8)	3 (2.2)	1 (0.8)	0.315
Azathioprine	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Mercaptopurine	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.238
Methotrexate	0 (0.0)	0 (0.0)	3 (2.2)	1 (0.8)	0.197
Vedolizumab	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.238
Adalimumab	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0.457
Ustekinumab	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Rituximab	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Nilotinib	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0.486
Hydroxyurea	1 (1.1)	0 (0.0)	1 (0.7)	0 (0.0)	0.506
Active chemotherapy	1 (1.1)	0 (0.0)	2 (1.5)	1 (0.8)	0.647
Included in an antineoplastic trail	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.8)	0.677

Immunosuppression was defined as a history of an organ transplant, immune deficiency, or chronic use of immunosuppressives. Immunosuppressives as part of COVID-19 treatment were excluded from this table and are shown in Table 2 section "immunomodulating therapies"

* Numbers do not add up to a 100% as some patients used multiple immunosuppressive medications.

Table S8: Baseline characteristics of the intensive care unit patients upon admission to the Intensive care unit including wave distribution

	<50	≥50 - <60	≥60 - <70	≥70	p-value
n	30	37	59	31	
Demographics					
Wave, n (%)	3 (10.0)	9 (24.3)	9 (15.3)	2 (6.5)	0.295
Wave 1	3 (10.0)	9 (24.3)	9 (15.3)	2 (6.5)	
Wave 2	3 (10.0)	6 (16.2)	15 (25.4)	10 (32.3)	
Wave 3	20 (66.7)	19 (51.4)	31 (52.5)	17 (54.8)	
Wave 4	4 (13.3)	3 (8.1)	4 (6.8)	2 (6.5)	
Age (median [IQR])	42 [36, 46]	55 [52, 58]	64 [62, 67]	72 [72, 74]	<0.001
Male sex, n (%)	15 (50.0)	21 (56.8)	37 (62.7)	26 (83.9)	0.035
Body Mass Index (median [IQR])	28.3 [25.7, 35.5]	31.7 [27.8, 35.1]	28.0 [25.0, 31.5]	26.6 [24.4, 31.0]	0.021
Duration of symptoms*, days (median [IQR])	10 [7, 11]	11 [7, 14]	9 [7, 13]	9 [6, 12]	0.294
Smoking status, n (%)					0.021
Yes	3 (10.0)	2 (5.4)	3 (5.1)	2 (6.5)	
Former smoker	4 (13.3)	3 (8.1)	22 (37.3)	14 (45.2)	
Never smoked	18 (60.0)	22 (59.5)	24 (40.7)	10 (32.3)	
Unknown	5 (16.7)	10 (27.0)	10 (16.9)	5 (16.1)	
Vaccination status, n (%)					0.561
Yes	2 (6.7)	1 (2.7)	7 (11.9)	1 (3.2)	
No	24 (80.0)	30 (81.1)	47 (79.7)	26 (83.9)	
Unknown	4 (13.3)	6 (16.2)	5 (8.5)	4 (12.9)	
Comorbidities and (selected) chronic medication					
Charlson score† (median [IQR])	0 [0, 0]	1.0 [1.0, 2.0]	3.0 [2.0, 4.0]	5.0 [4.0, 6.0]	<0.001
Hypertension, n (%)	3 (10.0)	15 (40.5)	33 (55.9)	13 (41.9)	0.001
Cardiac disease, n (%)	0 (0.0)	5 (13.5)	12 (20.7)	12 (38.7)	0.001
Respiratory disease, n (%)	2 (6.7)	3 (8.1)	5 (8.5)	6 (19.4)	0.306
Diabetes, n (%)	4 (13.3)	9 (24.3)	22 (37.3)	14 (45.2)	0.028
Neurologic disease, n (%)	0 (0.0)	1 (2.7)	6 (10.2)	4 (12.9)	0.118
Kidney disease, n (%)	2 (6.7)	3 (8.1)	10 (16.9)	6 (19.4)	0.301
Prior malignancy, n (%)	1 (3.3)	2 (5.4)	2 (3.4)	4 (12.9)	0.275
Immunosupression, n (%)	1 (3.3)	1 (2.7)	5 (8.5)	3 (9.7)	0.512
Antiplatelet drugs, n (%)	0 (0.0)	1 (2.7)	6 (10.2)	7 (22.6)	0.008
Anticoagulant drugs, n (%)	0 (0.0)	0 (0.0)	4 (6.8)	4 (12.9)	0.049
Severity score on ICU admission (median [IQR])					
4c mortality ‡	5[4, 6]	5 [4, 7]	6 [5, 7]	6 [5, 8]	0.009
SOFA §	5 [3, 5]	5 [4, 7]	6 [5, 8]	6 [5, 7]	0.007
APACHE IV APS score	46 [39, 54]	45 [37, 59]	63 [55, 72]	65 [59, 71]	<0.001
Treatment at day of admission, n (%)					
Supplementary oxygen therapy	30 (100.0)	37 (100.0)	59 (100.0)	31 (100.0)	>0.999
High-flow nasal cannula	13 (43.3)	8 (21.6)	22 (37.3)	13 (41.9)	0.208
Non-invasive ventilation	2 (6.7)	2 (5.4)	4 (6.8)	2 (6.5)	0.994
Invasive ventilation	13 (43.3)	27 (73.0)	33 (55.9)	16 (51.6)	0.089
Routine laboratory markers (median [IQR])*					
Leukocyte counts (x10^9/L)	9.3 [6.5, 11.7]	11.0 [8.3, 13.4]	10.7 [7.8, 15.8]	11.4 [8.1, 16.0]	0.195
C-Reactive Protein (mg/L)	109 [61, 193]	124 [58.2, 189]	132 [73, 257]	103 [52, 245]	0.675
Platelet counts (x10^9/L)	250 [204, 293]	243 [172, 345]	239 [173, 326]	205 [161, 260]	0.240
aPTT (sec)	26 [23, 31]	26 [23, 31]	26 [22, 33]	30 [25, 34]	0.284
Creatinine (µmol/L)	74 [57, 97]	88 [68, 125]	88 [68, 134]	117 [87, 151]	0.002
Urea (mmol/L)	5.0 [3.4, 7.3]	8.0 [6.0, 11.3]	9.0 [7.0, 11]	9.0 [8.0, 13.0]	<0.001
Bilirubin (mg/dL)	11.0 [6.0, 16.0]	7.0 [5.5, 10]	11.0 [7.0, 14.5]	9.0 [7.0, 12.3]	0.111
Lactate (mmol/L)	1.60 [1.2, 2.22]	1.70 [1.30, 2.30]	2.00 [1.60, 2.85]	1.80 [1.50, 2.20]	0.020
pH	7.48 [7.44, 7.50]	7.44 [7.38, 7.47]	7.43 [7.34, 7.49]	7.45 [7.39, 7.50]	0.028

Abbreviations: AUMC: Amsterdam University Medical Centers; SOFA: quick sequential organ failure assessment; aPTT: activated partial thromboplastin time.

* At ICU admission

† The Charlson score was calculated without the age component

‡ The 4c mortality score a validated COVID-19 score [26], was calculated without the age, obesity and Glasgow coma scale component

§ The SOFA score was calculated without the central nervous system component

Table S9: Treatments and outcomes of the intensive care unit patients

	<50	≥50 - <60	≥ 60 - <70	≥70	P-values
n	30	37	59	31	
Treatments, n (%)					
Supplementary oxygen therapy	30 (100.0)	37 (100.0)	59 (100.0)	31 (100.0)	>0.999
High-flow nasal cannula	25 (83.3)	23 (65.7)	34 (59.6)	23 (74.2)	0.125
Non-invasive ventilation	4 (13.8)	5 (13.5)	9 (15.3)	10 (32.3)	0.143
Invasive ventilation	15 (50.0)	28 (75.7)	47 (79.7)	23 (74.2)	0.026
Remdesivir	0 (0.0)	1 (2.7)	3 (5.1)	1 (3.2)	0.636
Chloroquine	0 (0.0)	1 (2.7)	3 (5.1)	1 (3.2)	0.636
Monoclonal antibodies against SARS-CoV-2	1 (3.3)	1 (2.7)	0 (0.0)	1 (3.2)	0.597
Antibiotics in the first 7 days of admission	25 (83.3)	33 (89.2)	54 (91.5)	28 (90.3)	0.696
Immunomodulating therapies, n (%)					
Dexamethasone 6mg	24 (80.0)	28 (75.7)	48 (81.4)	28 (90.3)	0.479
Of which before sampling *	24 (80.0)	27 (75.0)	43 (76.8)	25 (86.2)	0.699
Other corticosteroids	8 (26.7)	6 (16.2)	13 (22.0)	10 (32.3)	0.449
Of which before sampling	2 (6.7)	0 (0.0)	6 (10.2)	4 (12.9)	0.183
Interleukin-6 inhibitors	16 (53.3)	15 (40.5)	18 (30.5)	9 (29.0)	0.136
Of which before sampling †	11 (36.7)	14 (37.8)	12 (21.4)	7 (22.6)	0.215
Anti-C5a antibody	3 (10.0)	5 (13.5)	12 (20.3)	5 (16.1)	0.613
Of which before sampling	1 (3.3)	2 (5.4)	2 (3.4)	1 (3.2)	0.954
Included in imatinib trial ‡	2 (6.7)	1 (2.7)	8 (13.6)	2 (6.5)	0.270
Clinical course					
Thrombosis	4 (13.3)	13 (35.1)	21 (35.6)	8 (25.8)	0.132
Of which Pulmonary embolism §	2 (6.7)	8 (21.6)	16 (27.1)	5 (16.1)	0.133
Of which deep venous thrombosis §	3 (10.0)	9 (24.3)	13 (22.0)	4 (12.9)	0.333
Length of hospital stay, days (median [IQR])	13 [10, 15]	14 [10, 28]	21 [15, 41]	18 [12, 25]	<0.001
ICU admission, n (%)	30 (100.0)	37 (100.0)	59 (100.0)	31 (100.0)	1.000
ICU stay, days (median [IQR])	5 [3, 9]	9 [5, 15]	13 [6, 26]	11 [7, 18]	<0.001
Readmission, n (%)	1 (3.3)	2 (5.4)	3 (5.1)	0 (0.0)	0.624
Mortality, n (%) ll					
30-day	1 (3.3)	7 (18.9)	23 (39.0)	20 (64.5)	<0.001
90-day	1 (3.3)	5 (13.5)	16 (27.1)	19 (61.3)	<0.001

Abbreviations ICU: intensive care unit.

* For 3.8% of patients it was unknown if dexamethasone was started before sampling

† For 1.9% of patients it was unknown if Interleukin-6 inhibitors were started before sampling

‡ Clinical trial (ClinicalTrials.gov Identifier: NCT04794088)

§ Numbers do not add up to a 100% as some patients suffered from both pulmonary and deep venous thrombosis

ll Starting from ICU admission

Table S10: Baseline characteristics of patients ≥70 years of age stratified by biomarker subphenotype

	Cluster 1	Cluster 2	Cluster 3	p-value
n	48	66	15	
Demographics				
Wave, n (%)				0.226
Wave 1	8 (16.7)	14 (21.2)	0 (0.0)	
Wave 2	25 (52.1)	38 (57.6)	8 (53.3)	
Wave 3	13 (27.1)	13 (19.7)	7 (46.7)	
Wave 4	2 (4.2)	1 (1.5)	0 (0.0)	
Age (median [IQR])	75.50 [72.00, 81.00]	75.00 [72.00, 83.00]	80.00 [74.00, 81.00]	0.503
Male sex, n (%)	28 (58.3)	42 (63.6)	8 (53.3)	0.709
Body Mass Index (median [IQR])	27.68 [24.97, 29.52]	26.86 [24.33, 30.93]	26.97 [21.73, 31.09]	0.908
Duration of symptoms*, days (median [IQR])	8.00 [6.75, 11.00]	7.00 [5.00, 10.00]	5.00 [1.00, 10.00]	0.016
Smoking status, n (%)				0.856
Yes	3 (6.2)	3 (4.5)	2 (13.3)	
Former smoker	18 (37.5)	30 (45.5)	5 (33.3)	
Never smoked	22 (45.8)	27 (40.9)	6 (40.0)	
Unknown	5 (10.4)	6 (9.1)	2 (13.3)	
Vaccination status, n (%)				0.514
Yes	2 (4.2)	3 (4.5)	2 (13.3)	
No	44 (91.7)	62 (93.9)	13 (86.7)	
Unknown	2 (4.2)	1 (1.5)	0 (0.0)	
Comorbidities and (selected) medication				
Charlson score† (median [IQR])	4.00 [3.00, 6.00]	4.00 [3.00, 6.00]	6.00 [4.00, 7.00]	0.103
Hypertension, n (%)	19 (39.6)	38 (57.6)	11 (73.3)	0.039
Cardiac disease, n (%)	20 (41.7)	23 (34.8)	8 (53.3)	0.388
Respiratory disease, n (%)	12 (25.0)	19 (28.8)	2 (13.3)	0.461
Diabetes, n (%)	20 (41.7)	16 (24.2)	5 (33.3)	0.142
Kidney disease, n (%)	3 (6.2)	7 (10.6)	9 (60.0)	<0.001
Neurologic disease, n (%)	6 (12.5)	11 (16.7)	4 (26.7)	0.428
Prior malignancy, n (%)	5 (10.4)	7 (10.6)	2 (13.3)	0.947
Immunosuppression‡, n (%)	1 (2.1)	4 (6.1)	0 (0.0)	0.394
Antiplatelet drugs, n (%)	8 (16.7)	10 (15.2)	1 (6.7)	0.628
Anticoagulant drugs, n (%)	5 (10.4)	14 (21.2)	5 (33.3)	0.102
Disease severity on admission, median [IQR]				
4C Mortality§	5.00 [4.00, 7.00]	6.00 [3.75, 7.00]	8.50 [7.00, 10.75]	0.003
qSOFA	1.00 [0.00, 1.00]	1.00 [1.00, 1.00]	1.00 [0.25, 1.00]	0.960
MEWS	3.00 [2.00, 3.25]	3.00 [2.00, 4.00]	3.00 [2.00, 5.00]	0.357
CURB ll	1.00 [0.00, 1.00]	1.00 [0.00, 1.00]	1.00 [1.00, 2.00]	0.162
Treatment at day of admission, n (%)				
Supplementary oxygen therapy	45 (93.8)	58 (87.9)	12 (80.0)	0.293
High-flow nasal cannula	0 (0.0)	1 (1.5)	1 (6.7)	0.189
Non-invasive ventilation	0 (0.0)	1 (1.5)	0 (0.0)	0.618
Routine laboratory markers (median [IQR])				
Leukocyte counts ($x10^9$/L)	7.10 [5.90, 8.10]	6.25 [4.60, 8.33]	8.10 [5.50, 9.50]	0.296
Neutrophil counts ($x10^9$/L)	5.34 [4.60, 6.82]	5.08 [3.81, 6.73]	6.54 [3.21, 8.05]	0.631
Lymphocyte counts ($x10^9$/L)	0.78 [0.56, 1.09]	0.75 [0.53, 1.00]	0.55 [0.41, 0.96]	0.242
Neutrophil-Lymphocyte ratio	6.43 [4.18, 11.40]	6.42 [4.25, 8.72]	10.06 [4.45, 19.57]	0.321
C-reactive protein (mg/L)	104.00 [73.25, 149.62]	87.35 [41.75, 138.43]	146.60 [72.15, 205.40]	0.028
Platelet counts ($x10^9$/L)	241.50 [181.00, 306.25]	184.00 [148.50, 229.50]	157.50 [123.25, 249.50]	0.007
Creatinine (µmol/L)	85.00 [68.00, 100.50]	85.00 [70.00, 109.25]	174.00 [121.50, 254.00]	<0.001

Abbreviations: qSOFA: quick sequential organ failure assessment; MEWS: modified early warning score.

* Prior to admission

† The Charlson score was calculated without the age component

‡ Defined as a history of an organ transplant, immune deficiency, or chronic use of immunosuppressants

§ The 4C mortality score, a validated COVID-19 score [26], was calculated without the age and obesity component

ll The CURB score was calculated without the age component.

Table S11: Treatments, disease course and outcome of patients ≥70 years of age stratified by biomarker subphenotype

	Cluster 1	Cluster 2	Cluster 3	p-value
n	48	66	15	
Treatments, n (%)				
Supplementary oxygen therapy	47 (97.9)	63 (95.5)	15 (100.0)	0.576
High-flow nasal cannula	2 (4.2)	9 (13.6)	6 (40.0)	0.002
Non-invasive ventilation	2 (4.2)	6 (9.1)	0 (0.0)	0.320
Invasive ventilation	4 (8.3)	4 (6.1)	1 (6.7)	0.894
Remdesivir	4 (8.3)	12 (18.2)	1 (6.7)	0.225
Chloroquine	0 (0.0)	1 (1.5)	0 (0.0)	0.618
Monoclonal antibodies against SARS-CoV-2	1 (2.1)	0 (0.0)	0 (0.0)	0.427
Antibiotics in the first 7 days of admission	22 (45.8)	33 (50.0)	10 (66.7)	0.369
Immunomodulating therapies, n (%)				
Dexamethasone 6mg	39 (81.2)	50 (75.8)	14 (93.3)	0.295
Of which before sampling	34 (70.8)	47 (71.2)	12 (80.0)	0.767
Other corticosteroids	1 (2.1)	1 (1.5)	1 (6.7)	0.485
Of which before sampling	0 (0.0)	1 (1.5)	0 (0.0)	0.618
Interleukin-6 inhibitors	6 (12.5)	2 (3.0)	4 (26.7)	0.011
Of which before sampling	1 (2.1)	1 (1.5)	1 (6.7)	0.485
Anti-C5a antibody	1 (2.1)	0 (0.0)	0 (0.0)	0.427
Of which before sampling	0 (0.0)	0 (0.0)	0 (0.0)	>1.000
Imatinib	4 (8.3)	7 (10.6)	0 (0.0)	0.413
Of which before sampling	2 (4.2)	2 (3.0)	0 (0.0)	0.718
Clinical course				
Thrombosis	10 (20.8)	8 (12.1)	0 (0.0)	0.105
Of which Pulmonary embolism*	10 (20.8)	7 (10.6)	0 (0.0)	0.077
Of which deep venous thrombosis*	0 (0.0)	2 (3.0)	0 (0.0)	0.379
Length of hospital stay (median [IQR])	6.00 [3.00, 8.00]	7.00 [4.25, 11.00]	9.00 [4.50, 13.00]	0.132
ICU admission†, n (%)	4 (8.3)	8 (12.1)	1 (6.7)	0.720
ICU stay, days (median [IQR])	29.00 [17.50, 70.00]	2.00 [1.00, 15.50]	23.00 [23.00, 23.00]	0.146
Readmission‡, n (%)	5 (10.4)	5 (7.6)	0 (0.0)	0.419
Mortality, n (%)				
30 day	12 (25.0)	18 (27.3)	9 (60.0)	0.027
90 day	15 (31.2)	19 (28.8)	9 (60.0)	0.064

Abbreviations: ICU: intensive care unit.

* Numbers do not add up to a 100% as some patients suffered from both pulmonary and deep venous thrombosis

† ICU admission after sampling

‡ For any cause within 28 days of the initial admission

Table S12: Results of the multinomial least absolute shrinkage and selection operator (lasso) regression model reflecting the importance of biomarker for assignment to a cluster

Biomarker	Cluster 1 Coefficient	Cluster 2 Coefficient	Cluster 3 Coefficient
Intercept	0,42	1,49	-1,90
Endothelium and coagulation activation			
ANG2:ANG1	.	.	.
sTie-2	.	.	.
sE-Selectin	.	.	0,09
Soluble Thrombomodulin	.	-0,58	0,06
sVCAM-1	-0,36	.	.
Syndecan-1	.	.	1,83
D-dimer	0,71	-0,45	.
PAI-1	.	.	.
sCD31	0,80	.	.
Inflammation and organ damage			
sRAGE	.	.	.
Ferritin	.	.	.
sTNF-RI	.	.	1,28
sTREM-1	.	.	0,20
Tenascin-C	.	.	.
SP-D	.	-0,49	.
Granzyme B	.	-0,06	0,19
CD40L	1,20	.	.
PD-L1	.	.	.
Cytokines			
IL-1RA	.	-0,07	0,63
IL-1α	0,47	.	.
IL-1β	0,50	.	.
IL-2	.	.	.
IL-4	0,31	.	.
IL-6	.	.	.
IL-7	1,36	.	.
IL-10	.	.	.
IL-12 p70	0,26	.	.
IL-13	.	.	.
IL-15	.	-0,28	.
IL-17a	.	.	.
IL-33	0,40	.	.
TNFα	-0,12	.	0,47
GM-CSF	.	.	.
IFNα	.	.	.
IFNɣ	0,63	.	.
Chemokines			
CCL2	.	-0,04	.
CCL3	0,19	-0,82	.
CCL4	.	.	.
CCL5	.	.	.
CXCL8	.	.	.
CXCL10	.	.	.

When no coefficient is displayed, the variable coefficient is shrunk to zero by the lasso regression algorithm. These predictors (when combined with all the other predictors) were deemed irrelevant by the algorithm to the prediction of cluster membership. A positive value indicates that an increase of that biomarker makes assignment to the cluster more likely, a negative value the opposite. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFα: tumor necrosis factor alpha; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon

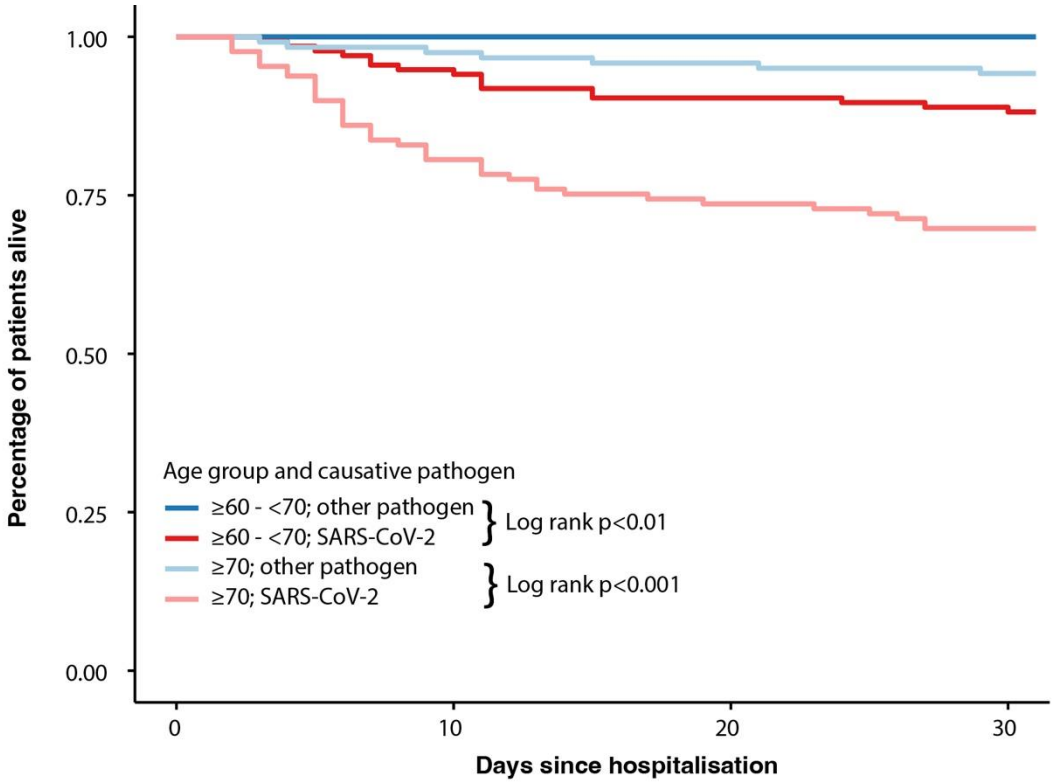
Table S13: Results of a logistic regression with 30-mortaliy as an outcome in patients >70 years

Mediating PC score or biomarker	Unadjusted p-value	Adjusted for assigned cluster p-value
Endothelium and coagulation activation		
Endocoag score (PC2)	<0.001	<0.05
sThrombomodulin	<0.01	<0.05
sVCAM-1	<0.01	<0.05
ANG2:ANG1	<0.01	<0.05
Systemic inflammation and organ damage		
Inflammation score (PC1)	<0.001	<0.01
sTNF-RI	<0.001	<0.01
sTREM-1	<0.001	<0.01
sRAGE	<0.001	<0.01
Tenascin-C	<0.05	0.22
Cyokines		
Cytokine score (PC2)	<0.001	<0.01
GM-CSF	<0.05	0.06
IL-1RA	<0.001	<0.05
IL-10	0.12	0.26
IL-13	<0.05	0.14
Chemokines		
Chemokine score (PC2)	<0.001	<0.001
CXCL10	<0.01	<0.05
CCL2	<0.01	<0.01
CCL5	<0.01	<0.05
CXCL8	<0.05	0.12
CCL4	0.16	0.16

The principal components and their contributing biomarker are depicted in figure 3. Abbreviations Endocoag: endothelial and coagulation score; PC: principal component; sThrombomodulin: soluble thrombomodulin; sVCAM: soluble vascular cellular adhesion molecule-1; ANG: angiopoietin; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; sRAGE: soluble receptor for advanced glycation end-products; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand.

Supplementary figures

Figure S1 – Comparison of 30-day mortality in patients admitted to the general ward with pneumonia caused by SARS-CoV-2 (COVID-19) vs community-acquired pneumonia caused by other pathogens

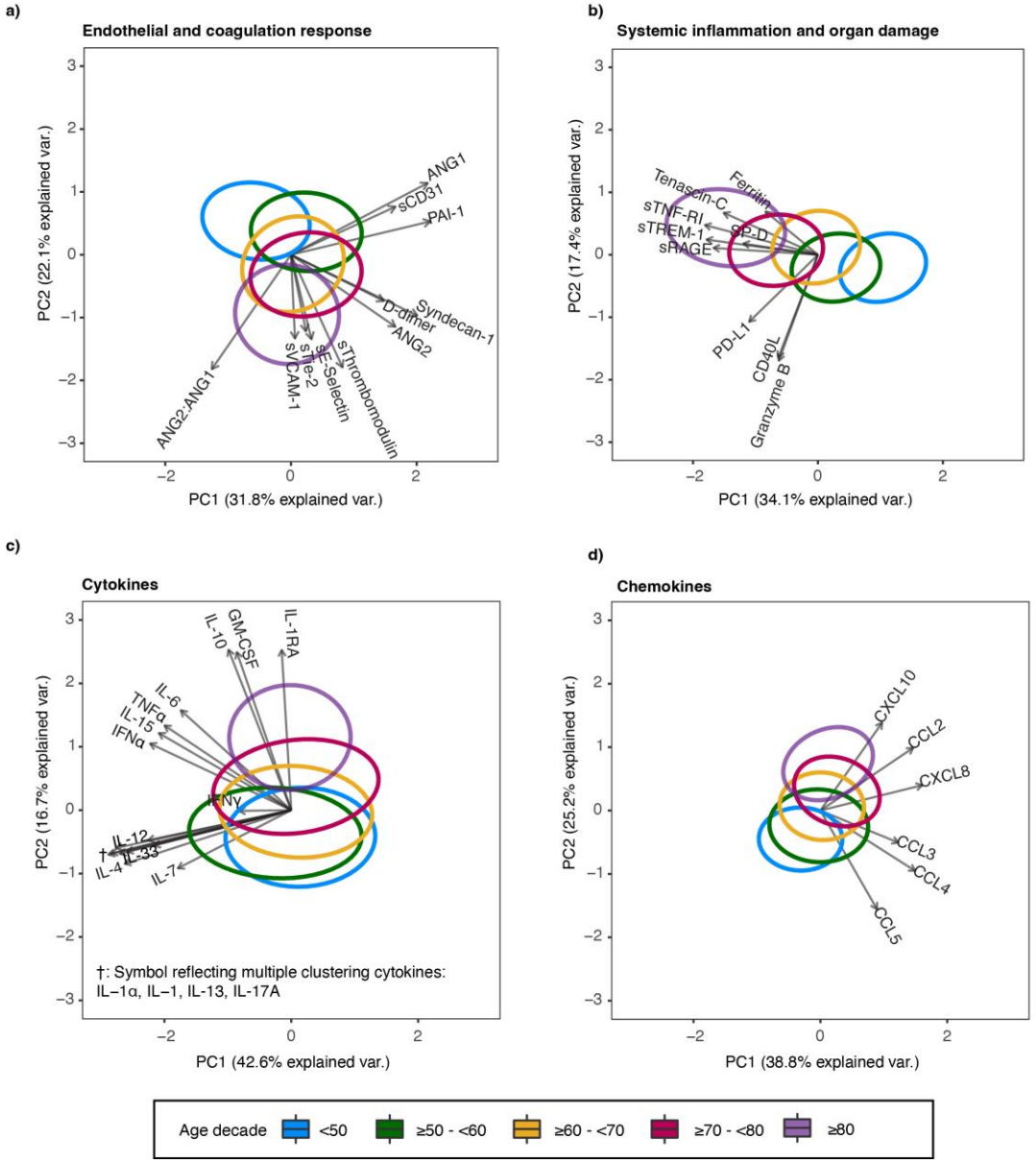


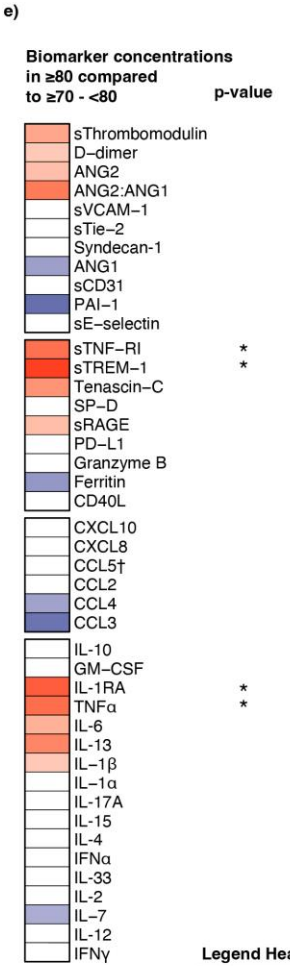
Number at risk				
≥60 - <70; other pathogen	62	62	62	62
≥60 - <70; SAR S-CoV-2	135	128	122	114
≥70; other pathogen	121	118	116	120
≥70; SARS-CoV-2	129	104	95	90

Number at risk (curves not shown because of low mortality)				
<50; other pathogen	32	32	32	31
<50; SARS-CoV-2	89	89	89	88
≥50 - <60; other pathogen	43	43	43	43
≥50 - <60;SARS-CoV-2	111	111	110	109

Description figure S1: Kaplan-Meijer plot of patients stratified by age group and cause of pneumonia (SARS-CoV-2 versus other pathogens). Patients with pneumonia not caused by SARS-CoV-2 that were included during the COVID-19 pandemic had a negative SARS-CoV-2 PCR and a CORADS-CT score <4 [19].

Figure S2 -- Principal component analysis of host response domain differences including a ≥80 age group





Description Figure S2 a-d) Principal components analysis (PCA) in which principal components (PC) 1 and 2 are plotted per domain. For each domain, the x- and y-axis are labelled with the % of the total variance within that domain that is explained by PC1 and PC2 respectively. The ellipse indicates the central 10% of each age group, colour coded as indicated in the bottom part of the figure. e) Heatmap depicting the magnitude of biomarker differences (Hedges' g) between patients ≥80 and patients ≥70 - <80 years of age. P-values were obtained from a t-test and are multiple testing corrected using the Benjamini-Hochberg (BH) procedure for testing 43 biomarkers. The arrows indicate the direction (arrow orientation) and strength (arrow length) of the correlation between each biomarker and the PCs. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon.

Legend Heatmap (Figure e)

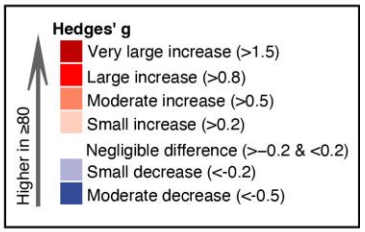
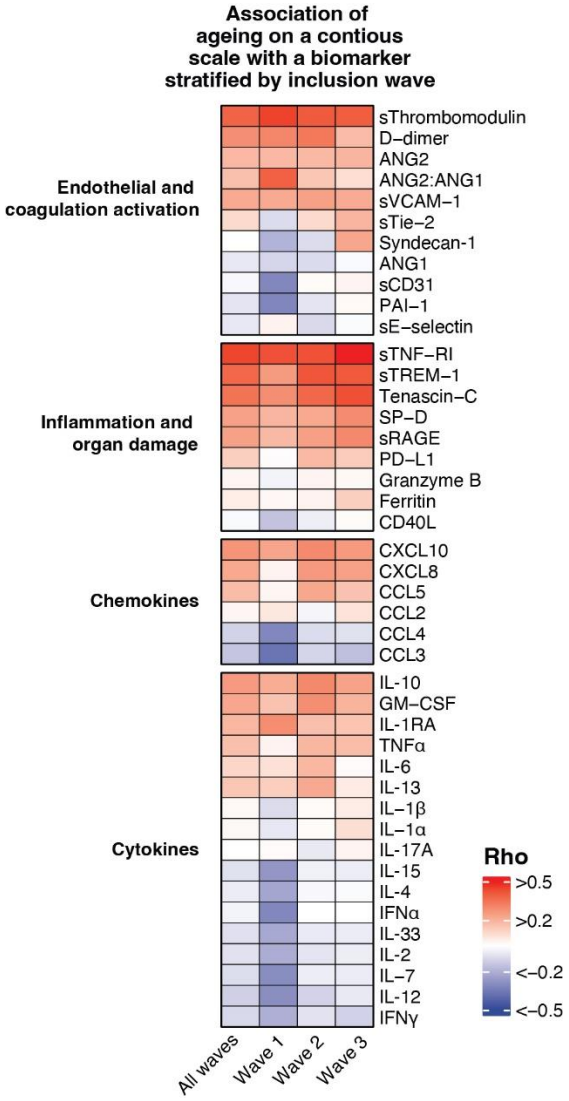
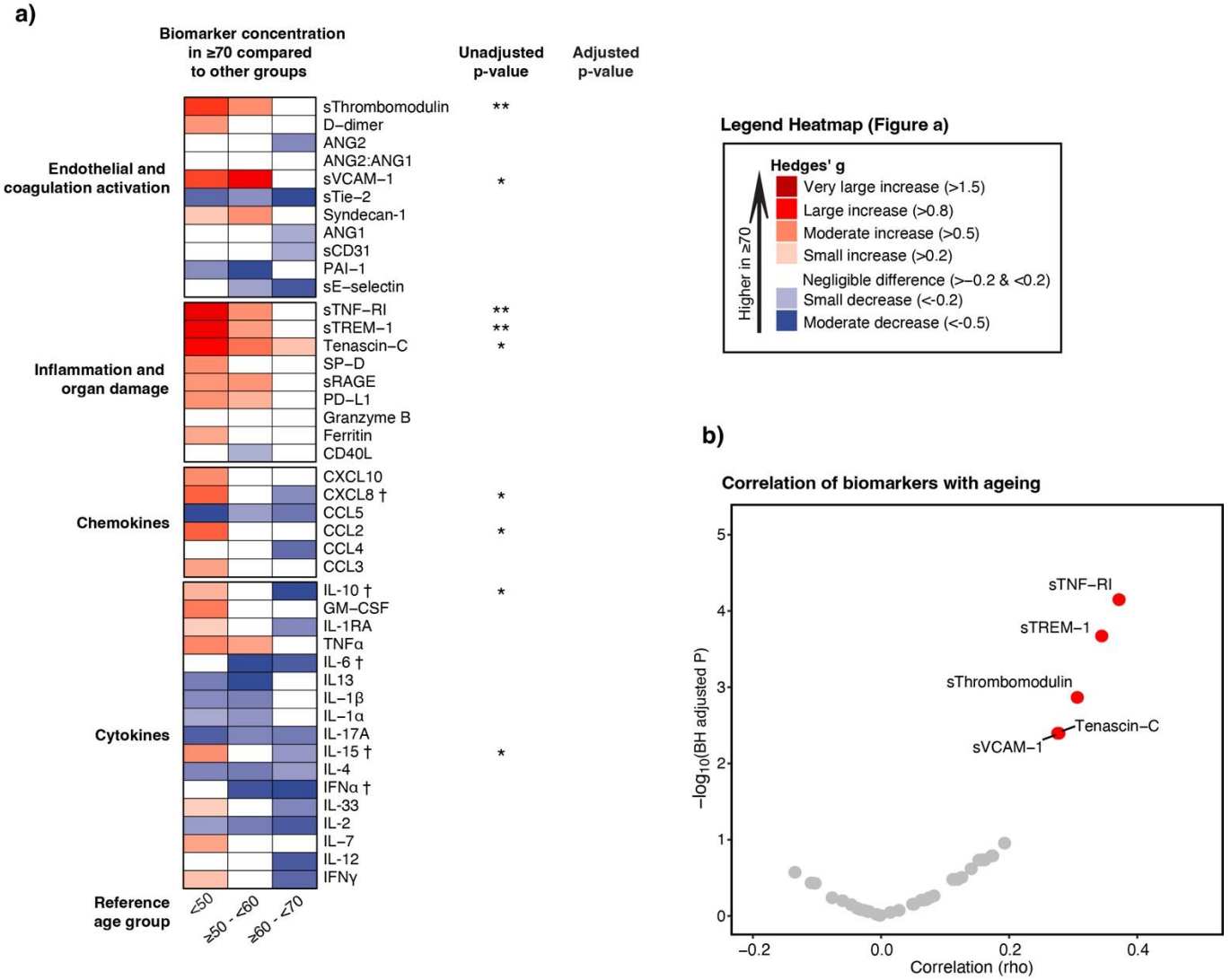


Figure S3 – Strenght of the association of ageing with biomarkers across inclusion waves



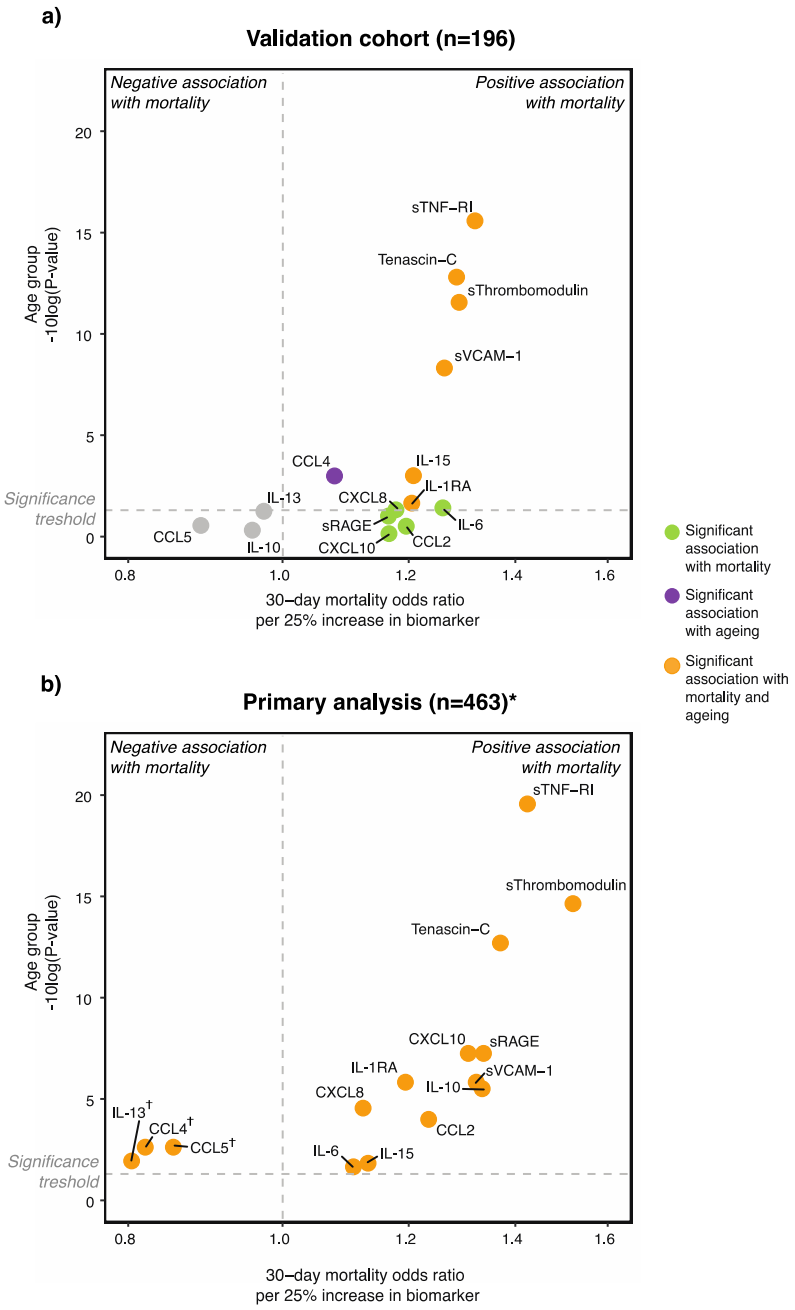
Description Figure S3: Heatmap depicting the strength of the association of ageing on a continuous scale with individual biomarker concentrations across inclusion waves. Rho's were generated using a Spearman's correlation. The first collum represents the association of ageing with biomarker concentrations across all inclusion waves. See supplementary methods for a description of the inclusion waves. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon.

Figure S4: Association of host response biomarkers with ageing upon admission to the intensive care unit



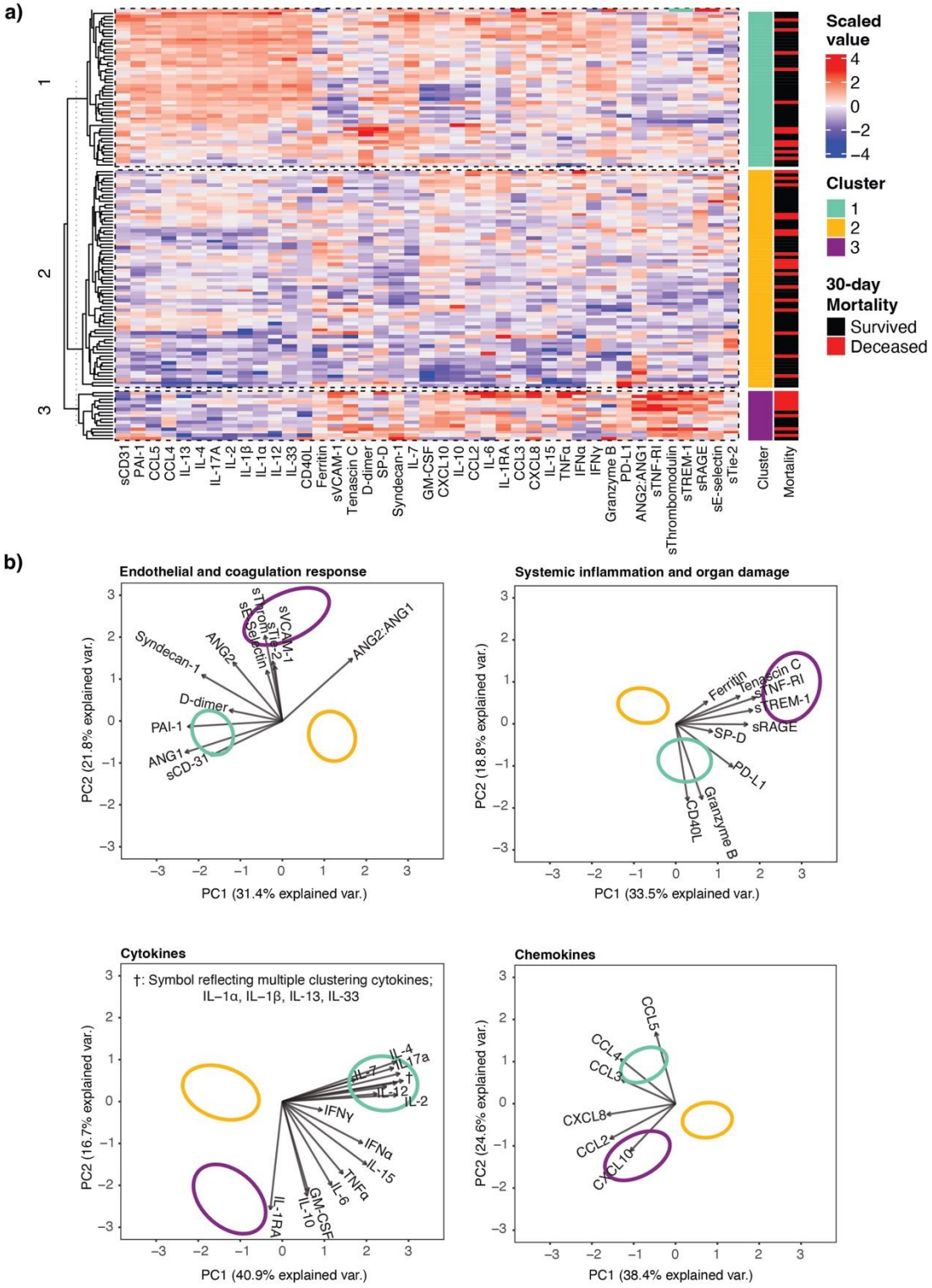
Description Figure S4: a) Heatmap depicting the magnitude of biomarker differences (Hedges' g) between patients ≥70 and the other age groups. P-values were obtained from a linear (if linear) or cubic spline regression analysis (if non-linear) in which age was modelled as a continuous variable. The adjusted model included demographics, age-related comorbidities, age and biomarker-related chronic medication and COVID-19-related immunomodulating treatments before sampling, see Methods for details. Red indicates higher levels in patients ≥70: blue indicates lower levels in this age group. b) Volcano plot depicting the strength of the correlation between a biomarker and ageing. Red dots represent a significant positive correlation, blue dots a significant negative correlation, and grey dots a non-significant correlation. Both the adjusted and unadjusted p-values are multiple testing corrected using the Benjamini-Hochberg (BH) procedure for testing 43 biomarkers. *** p<0.001, ** p<0.01, * p<0.05. † Biomarkers with a non-linear relationship with ageing on a continuous scale. Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-selectin: soluble E-selectin; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; sCD31: soluble cluster of differentiation 31; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; sTREM-1: soluble triggering receptor expressed on myeloid cells 1; SP-D: surfactant protein D; CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IFN: interferon.

Figure S5: External validation (in an independent cohort both direction and magnitude) of biomarkers with a significant association with ageing and 30-day mortality in the primary analysis



Desciption Figure S5: Validation (both direction and magnitude) of biomarkers with a significant association with ageing and 30-day mortality in the primary analysis (Fig. 4a). The validation cohort did not contain TREM-1, GM-CSF, D-dimer, and the ANG2:ANG1 ratio. Only age group data was available in the validation cohort (≥20-<35 , ≥36-<50, ≥50-65, ≥65-<80, ≥80 years of age). Therefore, the age groups were matched in the primary cohort to facilitate a direct comparison. The x-axis depicts the increase in the 30-day mortality odds ratio per 25% increase of the biomarker derived from an unadjusted logistic regression with the log-transformed biomarker as the explanatory variable and 30-day mortality as the response variable. The y-axis depicts the -10log(p-value) obtained from an ANOVA between age groups. All p-values and coefficients were multiple testing corrected using the Benjamini-Hochberg (BH) procedure for testing 15 biomarkers. * One patient was excluded from the primary cohort as the patient was aged <20. † Biomarkers with a negative association with ageing. All other biomarkers show a positive or non-significant association with ageing. All biomarkers with a significant association with mortality in both cohorts show a similar direction and magnitude of the association. Abbreviations: ANOVA; analysis of variance; sThrombomodulin: soluble thrombomodulin; sVCAM-1: soluble vascular cellular adhesion molecule-1; sRAGE: soluble receptor for advanced glycation end-products; sTNF-R1: soluble tumor necrosis factor receptor 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin.

Figure S6 – Biomarker cluster analysis in patients ≥70 years of age



Description figure S6: a) Biomarker heatmap. Rows represent patients; columns represent biomarkers. Red values indicate a higher concentration of a biomarker in that patient compared to the other included patients, while blue values indicate lower concentrations. The first column right of the heatmap depicts the cluster assignment: cluster 1 (aquamarine), cluster 2 (yellow), and cluster 3 (purple). The second column right of the heatmap shows 30-day mortality. B) Principal component analysis of host response domain differences between the three host response phenotypes. Each plot's x- and y-axis portrays the % of the total variance within that domain explained by Principal component (PC) 1 and PC2, respectively. The ellipses indicate the central 10% of each group and are colour coded as indicated at the bottom part of the figure. The arrows indicate the direction (arrow orientation) and strength (arrow length) of the association between each biomarker and the PCs.

Sheet 1: Summary of the quality of the luminex assay

Biomarkers in alphabetic order	Ward cohort (n=464)						Intensive care unit cohort (n=157)						Healthy controls (n=29) †					
	Within all limits	>ULQ extrapolated based on standard	>ULQ set to max of standar curve	<LLQ extraplolated based on standard curve	<LLQ set to minimum of standard curve	<25 beads measured*	Within all limits	>ULQ extrapolated based on standard	>ULQ set to max of standar curve	<LLQ extraplolated based on standard curve	<LLQ set to minimum of standard curve	<25 beads measured*	Within all limits	>ULQ extrapolated based on standard	>ULQ set to max of standar curve	<LLQ extraplolated based on standard curve	<LLQ set to minimum of standard curve	<25 beads measured*
ANG1	463	0	0	1	0	0	157	0	0	0	0	0	29	0	0	0	0	0
ANG2	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
CCL2 (MCP1)	463	0	0	0	0	1	157	0	0	0	0	0	29	0	0	0	0	0
CCL3 (MIP-1α)	234	0	0	52	178	0	85	0	0	15	57	0	1	0	0	22	6	0
CCL4 (MIP-1β)	448	0	0	2	14	0	142	0	0	3	12	0	25	0	0	4	0	0
CCL5 (Rantes)	463	0	0	0	1	0	157	0	0	0	0	0	29	0	0	0	0	0
CD-31	422	2	0	0	0	40	147	0	0	0	0	10	28	0	0	0	0	1
CD40L	229	0	0	16	219	0	71	0	0	14	72	0	4	0	0	25	0	0
CXCL10 (IP-10)	458	6	0	0	0	0	144	0	13	0	0	0	29	0	0	0	0	0
CXCL8 (IL-8)	457	0	0	3	4	0	157	0	0	0	0	0	27	0	0	1	1	0
D-dimer	460	3	0	0	0	1	150	3	4	0	0	0	28	0	0	0	0	1
Ferritin	389	68	7	0	0	0	111	10	36	0	0	0	29	0	0	0	0	0
GM-CSF	462	0	0	1	1	0	157	0	0	0	0	0	26	0	0	3	0	0
Granzyme B	230	0	0	54	180	0	82	0	0	18	57	0	3	0	0	19	7	0
IFNa	456	0	0	4	3	1	155	0	0	2	0	0	26	0	0	1	2	0
IFNγ	136	0	0	17	311	0	51	0	0	2	104	0	3	0	0	25	1	0
IL-1α	411	0	0	7	46	0	125	0	0	8	24	0	20	0	0	8	1	0
IL-10	463	0	0	1	0	0	157	0	0	0	0	0	28	0	0	1	0	0
IL-12 p70	367	0	0	47	50	0	107	0	0	20	30	0	17	0	0	8	4	0
IL-13	455	0	0	1	8	0	142	0	0	3	12	0	28	0	0	1	0	0
IL-15	458	0	0	3	3	0	156	0	0	1	0	0	26	0	0	3	0	0
IL-17a	407	0	0	38	19	0	96	0	0	34	27	0	21	0	0	4	4	0
IL-1RA	461	2	0	0	1	0	153	0	3	0	0	0	26	0	0	2	1	0
IL-1β	427	0	0	12	25	0	136	0	0	5	16	0	24	0	0	5	0	0
IL-2	453	0	0	3	8	0	143	0	0	5	9	0	28	0	0	1	0	0
IL-33	332	0	0	49	83	0	107	0	0	14	36	0	11	0	0	14	4	0
IL-4	364	0	0	50	50	0	70	0	0	33	54	0	18	0	0	6	5	0
IL-5 (inadequate standard curve)	199	0	0	30	235	0	82	0	0	5	70	0	11	0	0	18	0	0
IL-6	459	0	1	2	2	0	155	0	2	0	0	0	27	0	0	1	1	0
IL-7	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
PAI-1	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
PD-L1	357	0	0	15	92	0	131	0	0	6	20	0	13	0	0	16	0	0
sE-Selectin	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
Soluble P-Selectin	200	3	0	1	0	251	97	0	0	0	1	57	23	0	0	0	0	6
Soluble Thrombomodulin	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
SP-D	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
sRAGE	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
sTie-2	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
sTNF-RI	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
sTREM-1	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
sVCAM-1	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
Syndecan-1	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
Tenascin-C	464	0	0	0	0	0	157	0	0	0	0	0	29	0	0	0	0	0
TNFα	441	0	0	18	5	0	148	0	0	9	0	0	25	0	0	2	2	0

*Measurments with less <25 measured beads were set to missing due to lack of quality

†Biomarker data of the non-infectious and young healthy controls were merged due to similar biomarker concentrations

Abbreviations: ULQ: upper limit of quantification; LLQ: lower limit of quantification; ANG: angiopoietin; sTie-2: soluble Tie-2; sE-Selectin: soluble E-Selectin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1;

CD-31: cluster of differentiation 31; sRAGE: soluble Receptor for Advanced Glycation End-products; sTNF-R1: soluble Tumor Necrosis Factor Receptor 1; sTREM-1: soluble Triggering Receptor Expressed on Myeloid cells 1; SP-D: surfactant protein D

CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFa: Tumor necrosis factor alpha; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IFN: interferon

Sheet 2: Biomarker concentrations (median [IQR] in picogram/ml) in healthy individuals and patients of the ward cohort

	Non-infectious controls + young healthy individuals*	Ward cohort stratified by age decade				Mann-Whitney U test, p-value
		<50	≥50 - <60	≥60 - <70	≥70	Healthies vs all ward patients
Sample size	29	89	111	135	129	29 vs 464
Endothelium and coagulation activation						
ANG1	3415.19 [2299.48, 8029.90]	7307.12 [3856.48, 11283.35]	10358.07 [5105.99, 18068.24]	7903.76 [3956.03, 13390.08]	6911.37 [3029.59, 14619.19]	0.002
ANG2	3592.19 [1928.37, 4729.31]	3139.11 [2235.31, 5438.39]	3702.79 [2600.22, 5922.77]	3551.70 [2787.64, 5749.92]	4706.42 [3259.79, 6605.68]	0.074
ANG2:ANG1	0.86 [0.37, 1.44]	0.44 [0.30, 0.87]	0.43 [0.23, 0.63]	0.47 [0.29, 0.97]	0.60 [0.35, 1.33]	0.048
sTie-2	5659.43 [4757.28, 8053.91]	6712.21 [4856.53, 9136.75]	6608.90 [4871.04, 9151.03]	6676.39 [4861.62, 10802.82]	6454.46 [3875.81, 8558.47]	0.381
sE-Selectin	23346.47 [14898.52, 28250.25]	22281.48 [16726.60, 27710.24]	21635.71 [17037.11, 29067.58]	23128.02 [16559.43, 31169.90]	21681.63 [16666.61, 27751.09]	0.457
Soluble Thrombomodulin	4812.58 [3931.62, 6059.42]	3944.78 [3215.55, 5481.83]	4919.53 [3956.36, 6089.56]	5562.12 [4538.12, 6815.59]	6393.73 [5060.81, 8192.74]	0.282
sVCAM-1	440839.41 [358773.27, 580402.27]	1774700.00 [1177900.00, 2901000.00]	2121600.00 [1491300.00, 3279750.00]	2372200.00 [1648300.00, 3876950.00]	2826500.00 [1703900.00, 4023500.00]	<0.001
Syndecan-1	4960.69 [4195.96, 6811.30]	8575.64 [6014.02, 11664.32]	9375.56 [6977.63, 13322.96]	9848.06 [7399.48, 12565.76]	9472.79 [6900.06, 13686.08]	<0.001
D-dimer	1190700.00 [868204.79, 1621750.00]	2488000.00 [1578000.00, 3386400.00]	3302700.00 [2064900.00, 4412750.00]	3402600.00 [2244600.00, 5569050.00]	4080000.00 [2772050.00, 7058750.00]	<0.001
PAI-1	20291.87 [10974.59, 36699.22]	35160.18 [20247.71, 47216.39]	51452.01 [26043.06, 67807.97]	38989.93 [25237.22, 60456.46]	34635.30 [21854.75, 59028.18]	<0.001
sCD-31	209621.00 [157677.26, 367531.25]	344792.10 [160694.25, 518980.55]	347718.95 [152128.10, 527586.97]	365225.88 [146596.14, 654696.96]	285306.26 [124924.68, 507895.99]	0.175
Inflammation and organ damage						
sRAGE	2590.21 [2008.49, 3724.74]	3951.61 [2759.15, 5623.56]	4875.12 [3525.56, 7623.10]	5101.84 [3320.65, 7660.82]	6317.21 [3861.58, 10472.65]	<0.001
Ferritin	78497.11 [34073.63, 153334.44]	551412.71 [238290.55, 932996.35]	752916.73 [358480.99, 1365856.82]	665530.29 [356970.05, 1325237.20]	710961.02 [344411.73, 1256677.21]	<0.001
sTNF-RI	159.16 [125.60, 206.68]	172.69 [121.86, 218.09]	205.15 [138.32, 286.09]	235.40 [184.55, 302.78]	294.96 [200.41, 382.90]	0.001
sTREM-1	22028.32 [17177.38, 27732.06]	31963.73 [22926.61, 41740.89]	37965.71 [26800.76, 46197.11]	39963.88 [30242.25, 56602.26]	49528.45 [37737.89, 68220.16]	<0.001
Tenascin-C	4606.16 [2481.93, 6710.74]	3193.49 [2342.88, 5720.89]	3914.35 [2749.78, 7644.53]	4965.37 [2567.27, 9964.41]	6533.85 [3247.15, 12922.33]	0.629
SP-D	890.95 [727.58, 1021.65]	1292.31 [1012.68, 1594.75]	1534.51 [1235.95, 1935.42]	1699.95 [1393.21, 2100.95]	2132.76 [1579.63, 3005.68]	<0.001
Granzyme B	6.82 [4.96, 11.44]	12.22 [6.20, 22.92]	16.16 [7.11, 29.31]	12.22 [6.20, 27.54]	15.12 [7.17, 28.93]	<0.001
CD40L	418.39 [341.96, 530.51]	530.51 [394.77, 2123.06]	1219.93 [424.01, 2799.87]	530.51 [391.54, 2071.61]	582.97 [418.39, 3037.58]	<0.001
PD-L1	37.11 [17.42, 65.39]	115.08 [48.52, 173.69]	128.55 [53.18, 192.58]	119.72 [39.22, 199.54]	155.49 [82.29, 236.34]	<0.001
Cytokines						
IL-1RA	8.56 [6.36, 13.98]	18.16 [11.28, 26.05]	18.50 [11.49, 31.91]	19.74 [10.31, 27.34]	15.68 [9.24, 24.15]	<0.001
IL-1α	4.90 [2.96, 7.58]	9.59 [6.75, 15.06]	11.41 [7.37, 16.70]	9.81 [6.68, 14.23]	8.40 [5.45, 14.52]	<0.001
IL-1β	236.69 [117.67, 330.27]	694.25 [486.23, 1230.97]	907.40 [546.87, 1368.00]	949.84 [576.12, 1912.94]	1030.84 [599.72, 2730.78]	<0.001
IL-2	3.69 [2.15, 5.92]	6.60 [4.52, 10.76]	8.28 [5.23, 12.61]	7.52 [4.80, 10.76]	6.96 [4.31, 10.34]	<0.001
IL-4	0.47 [0.31, 0.81]	0.70 [0.42, 1.30]	0.80 [0.41, 1.55]	0.76 [0.33, 1.16]	0.67 [0.33, 1.26]	0.026
IL-6	14.94 [8.30, 23.80]	38.78 [27.40, 63.03]	46.38 [28.68, 67.12]	47.06 [30.98, 68.30]	47.07 [31.07, 78.26]	<0.001
IL-7	57.22 [42.82, 93.61]	71.14 [49.98, 109.08]	81.33 [53.69, 120.71]	86.48 [57.22, 121.40]	77.82 [51.31, 130.10]	0.041
IL-10	84.61 [62.10, 106.65]	368.89 [276.06, 453.95]	413.00 [293.84, 495.41]	428.12 [318.31, 546.20]	487.40 [355.09, 665.22]	<0.001
IL-12 p70	6.46 [4.51, 10.52]	11.14 [6.56, 16.56]	11.69 [7.44, 18.12]	10.52 [5.35, 16.87]	10.52 [5.64, 16.96]	<0.001
IL-13	36.69 [26.20, 52.60]	56.22 [38.95, 68.89]	56.84 [41.35, 83.47]	52.77 [41.22, 69.62]	50.90 [36.23, 67.71]	<0.001
IL-15	1.89 [1.17, 2.64]	4.60 [3.50, 6.09]	5.20 [3.78, 6.55]	5.23 [4.02, 6.61]	5.37 [4.13, 6.74]	<0.001
IL-17a	4.01 [2.06, 5.55]	6.90 [3.66, 9.90]	7.22 [3.99, 11.83]	6.39 [4.13, 10.04]	5.73 [2.98, 9.70]	0.001
IL-33	3.27 [2.37, 5.86]	9.05 [3.27, 15.22]	9.75 [3.27, 17.51]	8.53 [3.27, 14.60]	6.09 [3.04, 16.86]	<0.001
TNFα	10.62 [5.94, 14.09]	17.55 [12.60, 23.13]	17.73 [12.43, 27.10]	18.44 [13.94, 25.11]	22.47 [15.57, 29.11]	<0.001
GM-CSF	20.28 [12.51, 29.08]	125.89 [77.11, 148.99]	128.05 [89.76, 158.38]	140.45 [103.33, 174.61]	155.87 [110.52, 180.97]	<0.001
IFNγ	4.66 [1.93, 6.28]	5.71 [4.64, 12.48]	6.28 [4.66, 14.25]	6.28 [3.80, 13.23]	6.28 [2.02, 14.25]	0.040
IFNα	3.96 [2.16, 6.41]	10.18 [7.04, 13.59]	11.19 [6.18, 16.67]	10.06 [6.79, 13.68]	11.24 [6.78, 15.02]	<0.001
Chemokines						
CCL2 (MCP1)	146.87 [107.19, 182.66]	177.84 [119.43, 263.72]	209.60 [137.43, 350.77]	228.77 [149.44, 361.93]	244.18 [170.15, 368.20]	<0.001
CCL3 (MIP-1α)	8.65 [7.64, 10.72]	11.65 [9.87, 17.42]	11.35 [8.06, 18.69]	11.65 [8.86, 16.82]	11.65 [8.81, 20.28]	<0.001
CCL4 (MIP-1β)	186.96 [136.71, 292.67]	321.46 [263.99, 395.58]	327.00 [232.92, 434.23]	296.97 [231.84, 369.60]	296.97 [217.81, 384.50]	<0.001
CCL5 (Rantes)	27337.79 [16370.48, 54755.54]	40882.91 [23094.24, 79684.63]	51170.23 [24639.67, 83878.31]	38279.87 [22557.18, 72426.40]	34392.97 [14175.54, 58294.74]	0.201
CXCL8 (IL-8)	1.64 [0.92, 3.10]	4.67 [2.38, 7.81]	5.62 [3.37, 9.54]	6.08 [3.49, 12.20]	7.37 [5.02, 12.44]	<0.001
CXCL10 (IP-10)	69.58 [53.10, 105.36]	730.62 [354.98, 1105.67]	948.73 [457.14, 1717.08]	1152.15 [545.05, 1681.73]	1398.16 [699.72, 2262.52]	<0.001

* Biomarker data of the non-infectious and young healthy controls were merged due to similar biomarker concentrations

Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-Selectin: soluble E-Selectin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1;

CD-31: cluster of differentiation 31; sRAGE: soluble Receptor for Advanced Glycation End-products; sTNF-R1: soluble Tumor Necrosis Factor Receptor 1; sTREM-1: soluble Triggering Receptor Expressed on Myeloid cells 1; SP-D: surfactant protein D

CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFa: Tumor necrosis factor alpha; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IFN: interferon

Sheet 3: Biomarker concentrations (median [IQR] in picogram/ml) of patients in the intensive care cohort

	Intensive care unit cohort statified by age decade			
	<50	≥50 - <60	≥60 - <70	≥70
Sample size	30	37	59	31
Endothelium and coagulation activation				
ANG1	6458.96 [2446.78, 11132.69]	5774.67 [2335.97, 12230.33]	6344.47 [3099.86, 15422.94]	5056.33 [2462.19, 11923.15]
ANG2	2710.70 [2090.10, 7197.98]	4036.63 [2712.92, 5343.96]	4558.03 [3296.24, 6441.80]	4129.18 [2313.69, 5721.99]
ANG2:ANG1	0.58 [0.30, 1.29]	0.50 [0.31, 1.44]	0.63 [0.33, 1.64]	0.70 [0.39, 1.66]
sTie-2	6566.86 [4596.19, 11480.54]	6252.53 [4939.39, 9709.82]	7821.22 [5484.04, 10406.22]	5593.83 [4679.14, 7575.81]
sE-Selectin	19338.79 [12519.65, 39939.83]	25148.13 [20374.47, 33710.41]	28687.90 [20664.48, 46118.36]	23544.38 [18139.72, 29264.17]
Soluble Thrombomodulin	5356.56 [4567.59, 7193.68]	6616.63 [4900.74, 8078.97]	7746.92 [5717.34, 11252.36]	8368.97 [5695.35, 11107.37]
sVCAM-1	2138850.00 [1601400.00, 3224025.00]	1466700.00 [1269500.00, 2805400.00]	2868100.00 [1770650.00, 5366750.00]	3097100.00 [2291100.00, 4690450.00]
Syndecan-1	12437.56 [9201.09, 21966.17]	12908.27 [9035.08, 17094.17]	14092.81 [10764.44, 20530.55]	14717.17 [11086.03, 22403.92]
D-dimer	3881000.00 [3087025.00, 5050925.00]	8559800.00 [3325100.00, 14386000.00]	5918100.00 [3728850.00, 11961000.00]	8349600.00 [2332550.00, 17461500.00]
PAI-1	41051.01 [25081.40, 58650.13]	49186.94 [23701.11, 68123.34]	47419.05 [28540.12, 94343.18]	42883.17 [31710.62, 63157.97]
sCD31	202304.18 [118108.95, 414784.61]	263389.87 [159655.21, 408292.52]	199617.45 [97007.45, 333331.54]	198044.32 [107821.87, 272482.23]
Inflammation and organ damage				
sRAGE	8670.10 [5564.13, 14249.73]	8820.37 [5266.43, 13668.51]	13256.25 [6021.66, 25604.70]	11062.92 [6851.23, 22556.43]
Ferritin	672988.30 [214763.52, 1888844.06]	1048608.65 [529045.04, 1762905.23]	1067960.46 [515751.43, 1581972.94]	1426000.00 [672092.63, 1791633.38]
sTNF-RI	210.64 [180.20, 287.22]	300.26 [237.67, 406.59]	430.24 [266.19, 724.03]	440.16 [275.47, 571.72]
sTREM-1	52388.68 [34260.88, 62737.55]	53379.88 [45984.14, 67523.08]	65660.34 [44764.53, 91252.37]	72492.68 [53870.30, 89451.98]
Tenascin-C	5941.32 [3466.27, 14079.71]	8537.92 [5314.99, 21451.87]	12201.24 [6530.38, 25985.90]	9567.60 [5924.54, 18648.00]
SP-D	1712.06 [1273.20, 2210.05]	2045.67 [1680.70, 2687.12]	2546.92 [1941.71, 4008.79]	2637.19 [2098.28, 3558.01]
Granzyme B	15.61 [7.51, 31.90]	14.10 [7.63, 27.25]	13.58 [6.82, 24.49]	12.34 [10.58, 31.47]
CD40L	442.30 [359.42, 1461.93]	838.29 [424.01, 1662.92]	460.60 [366.63, 1589.85]	460.60 [338.77, 1661.35]
PD-L1	126.58 [61.34, 199.40]	159.67 [80.96, 227.40]	171.20 [99.43, 277.80]	172.29 [104.95, 289.99]
Cytokines				
IL-1RA	10.27 [7.78, 19.74]	11.61 [7.57, 19.47]	13.41 [7.52, 20.73]	9.24 [7.02, 13.85]
IL-1α	6.52 [3.37, 10.11]	6.33 [4.25, 7.83]	7.33 [3.15, 10.80]	4.29 [3.03, 8.94]
IL-1β	1391.92 [833.00, 2561.49]	2343.41 [972.28, 3539.35]	2101.36 [1211.68, 5371.40]	1753.07 [973.05, 2835.02]
IL-2	3.64 [2.49, 6.76]	3.69 [2.24, 6.98]	5.47 [2.72, 7.08]	2.92 [1.24, 5.40]
IL-4	0.28 [0.24, 0.76]	0.31 [0.26, 0.61]	0.39 [0.27, 0.70]	0.26 [0.22, 0.51]
IL-6	90.58 [39.07, 297.84]	273.71 [67.87, 659.10]	163.41 [52.44, 569.51]	66.30 [28.04, 382.12]
IL-7	54.16 [45.79, 91.83]	86.47 [51.16, 134.07]	78.81 [55.83, 126.59]	92.06 [55.98, 140.70]
IL-10	468.13 [342.35, 556.70]	496.57 [405.70, 612.58]	539.71 [455.89, 668.65]	463.12 [418.24, 537.35]
IL-12 p70	6.67 [4.77, 8.99]	5.35 [4.31, 9.09]	9.09 [6.19, 11.31]	5.35 [4.27, 10.73]
IL-13	37.39 [23.00, 51.35]	41.58 [24.31, 52.50]	37.60 [16.60, 51.48]	29.36 [20.41, 37.99]
IL-15	4.32 [2.06, 6.13]	4.90 [3.97, 6.22]	5.50 [4.27, 7.55]	4.84 [4.28, 5.97]
IL-17a	2.50 [2.05, 5.25]	2.62 [1.76, 4.13]	3.66 [1.68, 6.19]	2.15 [1.42, 3.74]
IL-33	5.84 [2.86, 13.29]	5.69 [2.79, 14.21]	10.50 [3.64, 19.91]	6.33 [3.21, 13.04]
TNFα	16.21 [9.41, 27.88]	23.19 [10.20, 27.62]	23.62 [15.37, 31.17]	21.95 [16.36, 31.44]
GM-CSF	156.03 [91.01, 185.54]	176.51 [135.19, 199.17]	160.85 [142.39, 187.88]	169.88 [133.51, 202.53]
IFNɣ	5.01 [1.79, 7.75]	5.36 [1.93, 8.50]	6.38 [5.36, 14.62]	6.02 [4.54, 10.78]
IFNα	7.41 [4.02, 14.21]	9.06 [6.08, 15.23]	10.22 [6.64, 16.99]	6.73 [4.58, 12.13]
Chemokines				
CCL2 (MCP1)	301.44 [191.29, 432.51]	423.64 [185.55, 672.56]	467.15 [296.71, 867.89]	295.90 [223.23, 670.88]
CCL3 (MIP-1α)	10.72 [9.07, 14.23]	11.65 [9.52, 23.95]	12.91 [8.81, 20.50]	11.65 [9.09, 20.39]
CCL4 (MIP-1β)	255.92 [134.79, 353.40]	238.41 [174.79, 324.07]	267.26 [174.79, 356.14]	241.45 [147.33, 319.98]
CCL5 (Rantes)	31966.53 [14032.87, 62250.42]	22309.28 [11606.86, 44106.33]	26476.19 [13967.57, 48156.24]	16065.13 [5716.45, 40574.14]
CXCL8 (IL-8)	6.56 [2.36, 10.81]	8.83 [6.04, 18.91]	11.97 [6.01, 23.70]	9.15 [4.77, 13.78]
CXCL10 (IP-10)	1315.88 [804.42, 2173.02]	2101.11 [1022.93, 2803.65]	2192.24 [1250.52, 2775.43]	1951.72 [1131.75, 2588.43]

Abbreviations: ANG: angiopoietin; sTie-2: soluble Tie-2; sE-Selectin: soluble E-Selectin; sVCAM: soluble vascular cellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1;

CD-31: cluster of differentiation 31; sRAGE: soluble Receptor for Advanced Glycation End-products; sTNF-R1: soluble Tumor Necrosis Factor Receptor 1; sTREM-1: soluble Triggering Receptor Expressed on Myeloid cells 1; SP-D: surfactant protein D

CD40L: CD40-ligand; PD-L1: programmed death-ligand 1; CCL: Chemokine C-C motif ligand; CXCL: C-X-C motif chemokine ligand; IL: interleukin; TNFa: Tumor necrosis factor alpha; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IFN: interferon

Sheet 4: Biomarker concentrations (median [IQR] in picogram/ml) in clusters				
	Cluster 1	Cluster 2	Cluster 3	p-value
Sample size	48	66	15	
Endothelium and coagulation activation				
ANG1	16064.94 [10103.11, 23588.65]	4261.88 [2373.96, 6873.13]	3971.60 [1244.22, 8831.71]	<0.001
ANG2	5698.42 [4396.97, 7932.05]	3747.32 [2551.18, 5079.50]	5768.51 [3495.69, 7621.26]	<0.001
ANG2:ANG1	0.36 [0.27, 0.59]	0.88 [0.44, 1.75]	0.96 [0.63, 5.81]	<0.001
sTie-2	6582.92 [3770.40, 8665.77]	6067.29 [3454.85, 8647.46]	6454.46 [5511.46, 7960.85]	0.701
sE-Selectin	22089.46 [17453.29, 27548.68]	19862.29 [15362.08, 24301.28]	34629.39 [22879.24, 39309.65]	0.002
Soluble Thrombomodulin	6720.91 [5523.75, 8185.20]	5551.54 [4317.85, 7090.50]	14522.41 [10245.01, 20491.43]	<0.001
sVCAM-1	2497550.00 [1500850.00, 3572750.00]	2699700.00 [1669250.00, 3678900.00]	4317900.00 [3011900.00, 8573100.00]	0.007
Syndecan-1	12818.84 [9163.17, 16431.09]	7210.00 [5990.80, 9134.50]	16198.33 [12627.92, 20218.51]	<0.001
D-dimer	6266950.00 [3973950.00, 14139750.00]	3144200.00 [2138950.00, 4784650.00]	4160600.00 [3526000.00, 7521050.00]	<0.001
PAI-1	62806.25 [43148.03, 92324.52]	25573.62 [18411.34, 36572.07]	32258.88 [16794.17, 67865.87]	<0.001
sCD-31	505034.00 [329145.79, 887524.54]	169060.38 [113081.86, 341817.54]	100113.51 [59716.87, 132891.17]	<0.001
Inflammation and organ damage				
sRAGE	6758.66 [4568.72, 10449.69]	5474.14 [3313.69, 8557.97]	14229.25 [9814.09, 23188.80]	<0.001
Ferritin	569273.72 [339582.18, 1186663.58]	724802.58 [250889.73, 1375477.26]	744094.39 [584717.55, 1363228.06]	0.446
sTNF-RI	308.36 [237.53, 384.85]	250.57 [169.33, 319.15]	687.37 [436.30, 1034.17]	<0.001
sTREM-1	52763.58 [37351.69, 66300.30]	44004.98 [37328.92, 55007.03]	85860.52 [62038.63, 137466.86]	<0.001
Tenascin-C	9437.54 [5540.55, 18143.89]	4041.46 [2515.61, 9380.60]	6533.85 [3453.82, 21561.49]	0.001
SP-D	2119.78 [1696.83, 2775.64]	1957.13 [1433.62, 2743.79]	6156.71 [4760.46, 12629.17]	<0.001
Granzyme B	26.62 [10.60, 36.13]	11.56 [6.82, 16.16]	18.03 [8.51, 51.29]	<0.001
CD40L	3402.48 [1639.19, 4227.88]	424.01 [358.79, 591.99]	418.39 [302.19, 1208.82]	<0.001
PD-L1	193.50 [131.06, 255.15]	106.70 [35.38, 158.97]	302.41 [241.93, 339.79]	<0.001
Cytokines				
IL-1RA	30.24 [21.26, 35.63]	11.71 [7.70, 15.61]	8.91 [8.15, 14.77]	<0.001
IL-1α	15.93 [11.73, 18.28]	6.33 [4.53, 8.68]	5.51 [3.68, 6.97]	<0.001
IL-1β	756.91 [536.16, 1691.11]	1024.03 [594.10, 2309.48]	4855.08 [2841.88, 10364.91]	<0.001
IL-2	12.21 [9.86, 15.48]	5.72 [3.03, 6.96]	4.68 [2.59, 7.26]	<0.001
IL-4	1.40 [0.99, 1.70]	0.49 [0.28, 0.73]	0.31 [0.20, 0.40]	<0.001
IL-6	61.38 [43.67, 90.98]	33.71 [26.24, 52.49]	77.17 [49.04, 82.13]	<0.001
IL-7	134.24 [110.24, 169.36]	55.40 [43.53, 71.48]	65.54 [54.01, 89.91]	<0.001
IL-10	487.35 [367.42, 639.27]	446.53 [349.71, 551.64]	769.84 [594.88, 1022.32]	0.001
IL-12 p70	17.66 [10.93, 24.61]	6.91 [4.66, 11.09]	8.22 [4.59, 10.52]	<0.001
IL-13	73.18 [61.02, 88.75]	41.50 [31.08, 52.89]	32.93 [21.74, 37.14]	<0.001
IL-15	6.33 [5.10, 7.62]	4.45 [3.52, 5.92]	7.34 [4.83, 9.30]	<0.001
IL-17a	10.95 [8.18, 13.87]	4.61 [2.62, 6.46]	2.05 [1.67, 3.62]	<0.001
IL-33	18.06 [13.29, 24.06]	3.27 [2.63, 6.09]	5.86 [3.28, 8.90]	<0.001
TNFα	23.40 [17.44, 33.04]	18.13 [13.64, 25.42]	33.56 [26.91, 46.69]	<0.001
GM-CSF	157.32 [116.50, 181.83]	141.44 [106.54, 171.38]	178.55 [152.70, 214.38]	0.022
IFNγ	8.80 [6.28, 16.91]	4.90 [1.93, 7.23]	5.71 [1.77, 15.79]	0.002
IFNα	13.46 [10.51, 16.47]	8.26 [6.04, 12.62]	8.78 [4.66, 29.25]	0.001
Chemokines				
CCL2 (MCP1)	265.60 [178.27, 415.33]	209.38 [154.29, 297.58]	335.59 [206.64, 522.65]	0.014
CCL3 (MIP-1α)	19.69 [13.20, 24.55]	10.28 [7.67, 11.65]	13.41 [10.28, 27.59]	<0.001
CCL4 (MIP-1β)	392.47 [340.52, 454.80]	242.82 [166.11, 296.65]	269.36 [200.19, 373.37]	<0.001
CCL5 (Rantes)	58058.76 [46806.19, 97907.74]	21751.96 [10343.85, 37970.59]	9041.33 [4355.52, 22030.41]	<0.001
CXCL8 (IL-8)	8.40 [6.48, 12.59]	5.90 [3.37, 9.48]	11.78 [5.71, 25.03]	0.001
CXCL10 (IP-10)	1223.78 [509.83, 1991.02]	1256.77 [707.21, 2153.15]	3289.11 [1736.67, 4557.36]	0.001