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 ‖	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

‖ 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 ‖	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

‖ 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

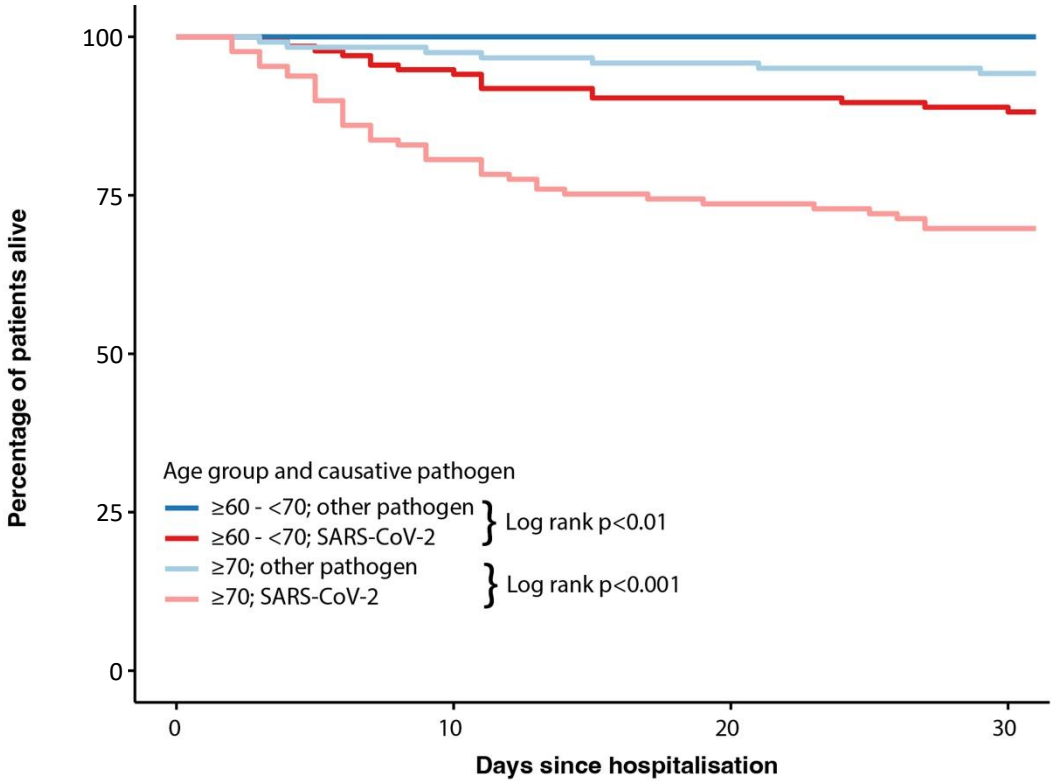
Table S13: Results of a logistic regression with 30-mortaliy as an outcome in patients >70 years

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

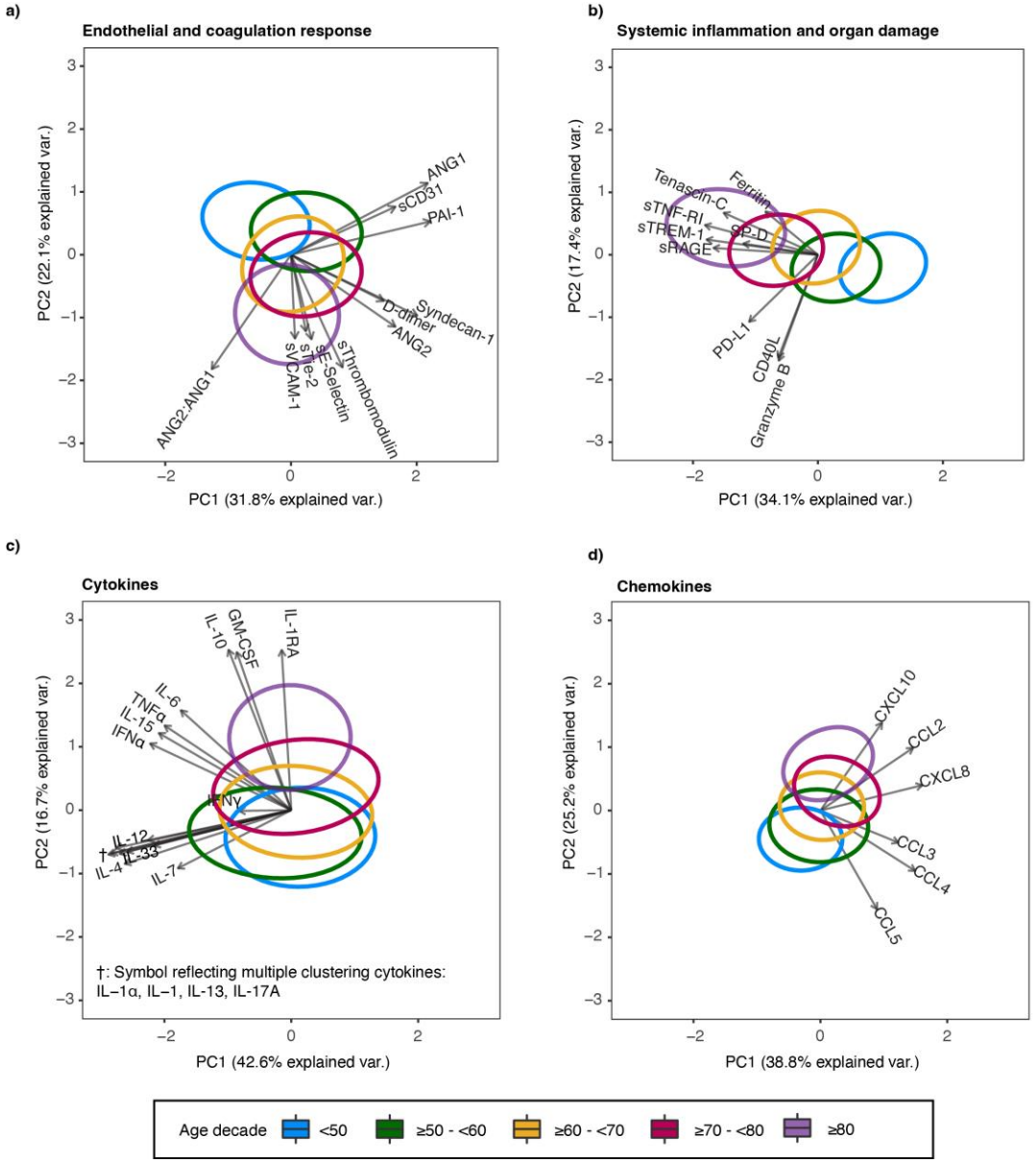


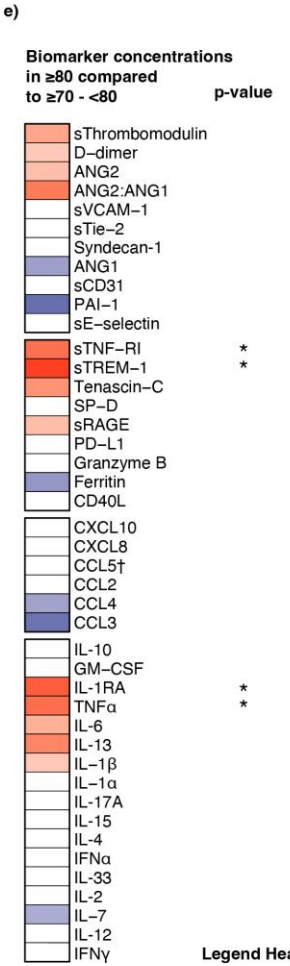
Number at risk				
≥60 - <70; other pathogen	62	62	62	62
≥60 - <70; SAR S-CoV-2	135	128	122	114
≥70; other pathogen	121	118	116	120
≥70; SARS-CoV-2	129	104	95	90

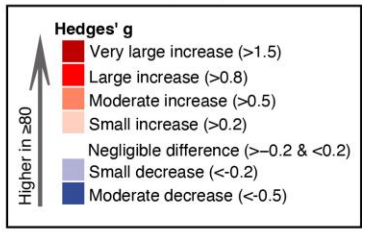
Number at risk (curves not shown because of low mortality)				
<50; other pathogen	32	32	32	31
<50; SARS-CoV-2	89	89	89	88
≥50 - <60; other pathogen	43	43	43	43
≥50 - <60;SARS-CoV-2	111	111	110	109

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

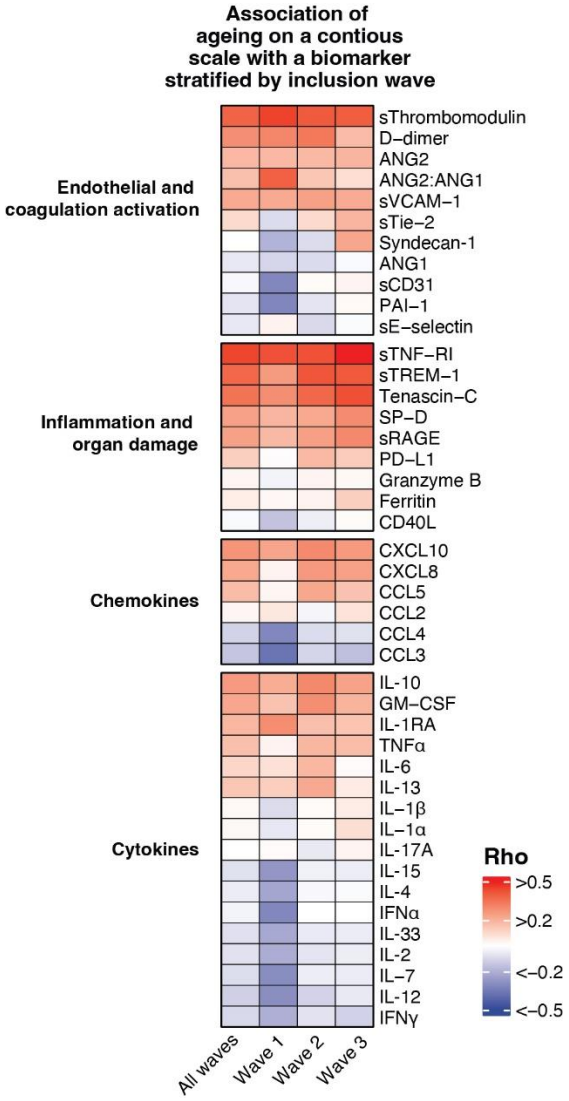






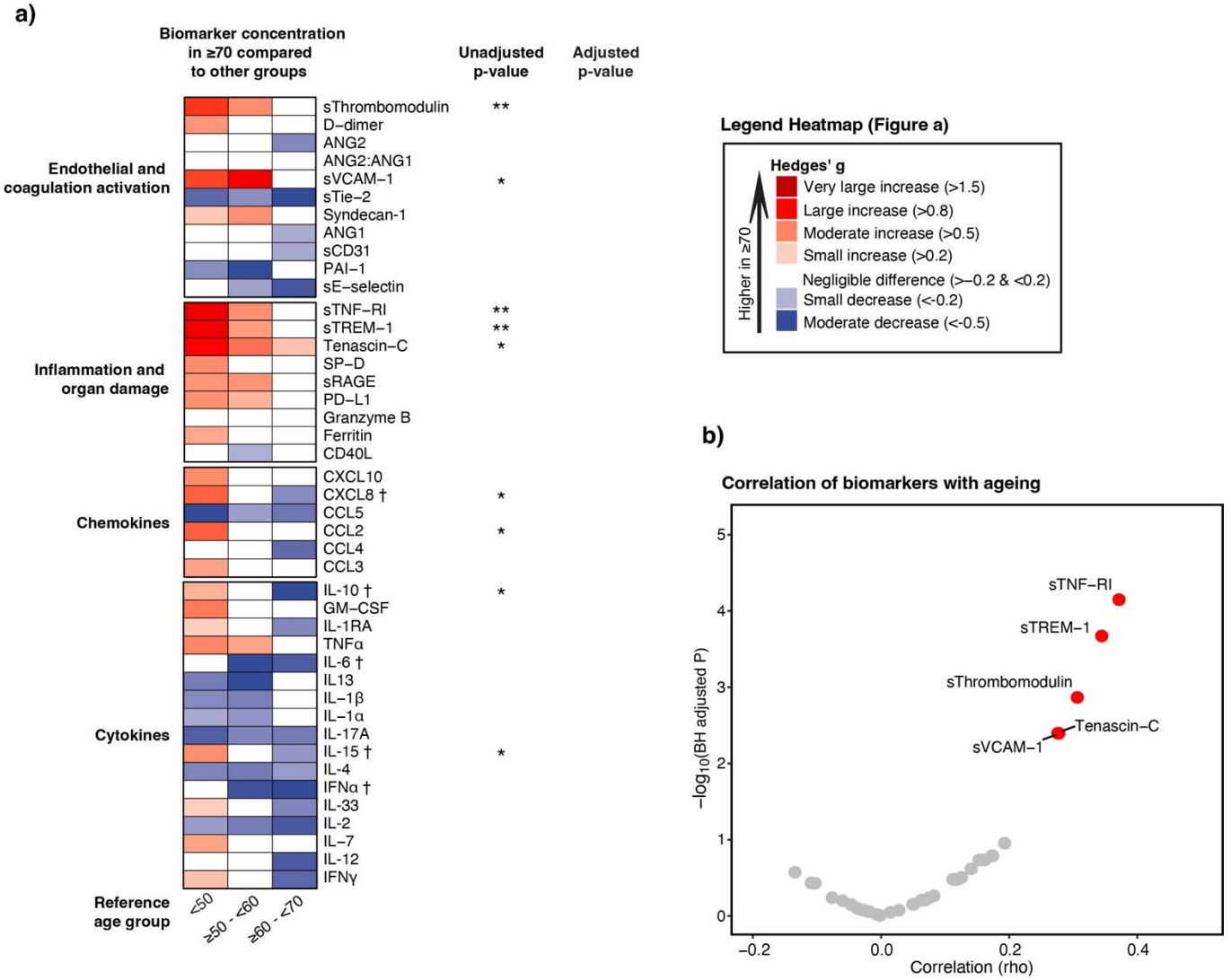
Description Figure S2 a-d) Principal components analysis (PCA) in which principal components (PC) 1 and 2 are plotted per domain. For each domain, the x- and y-axis are labelled with the % of the total variance within that domain that is explained by PC1 and PC2 respectively. The ellipse indicates the 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.

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



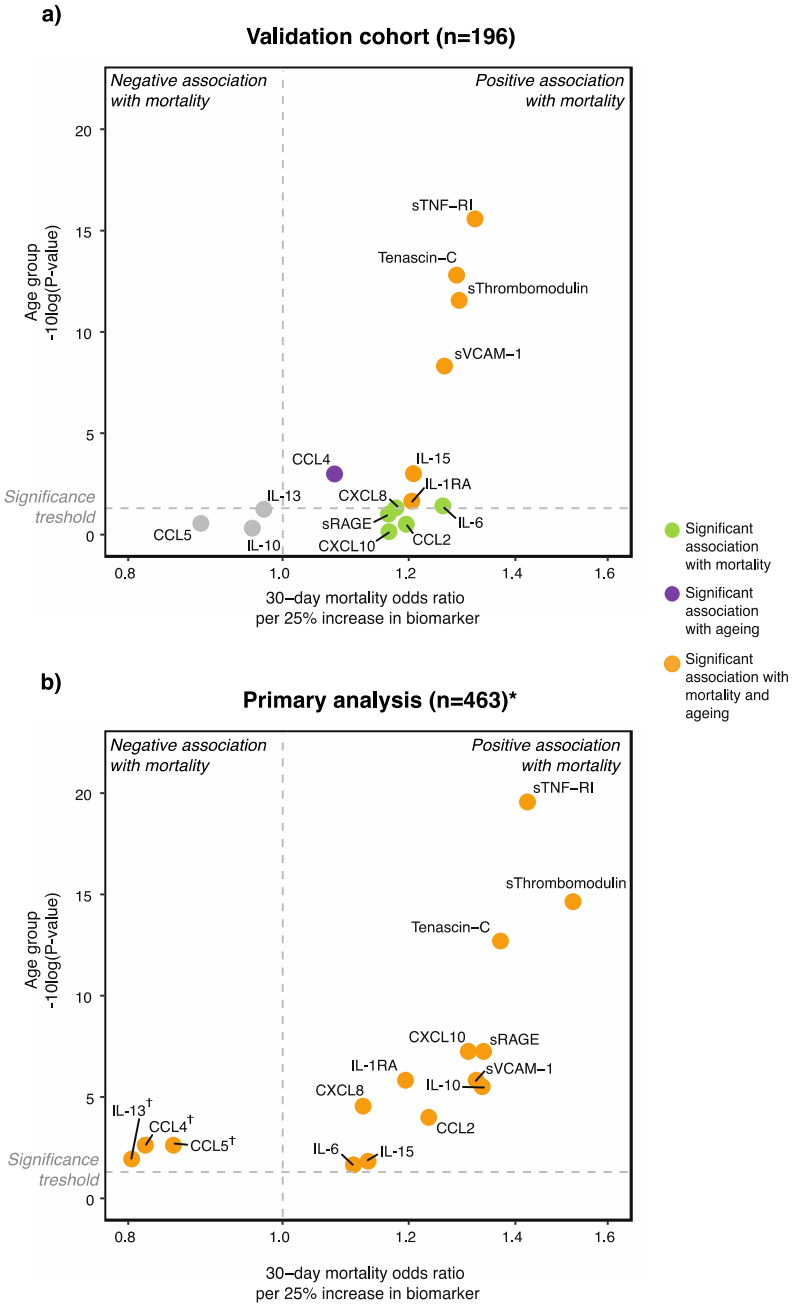
Description Figure S3: Heatmap depicting the strength of the association of ageing on a continuous scale with individual biomarker concentrations across inclusion waves. Rho's were generated using a Spearman's correlation. The first collum represents the association of ageing with biomarker concentrations 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



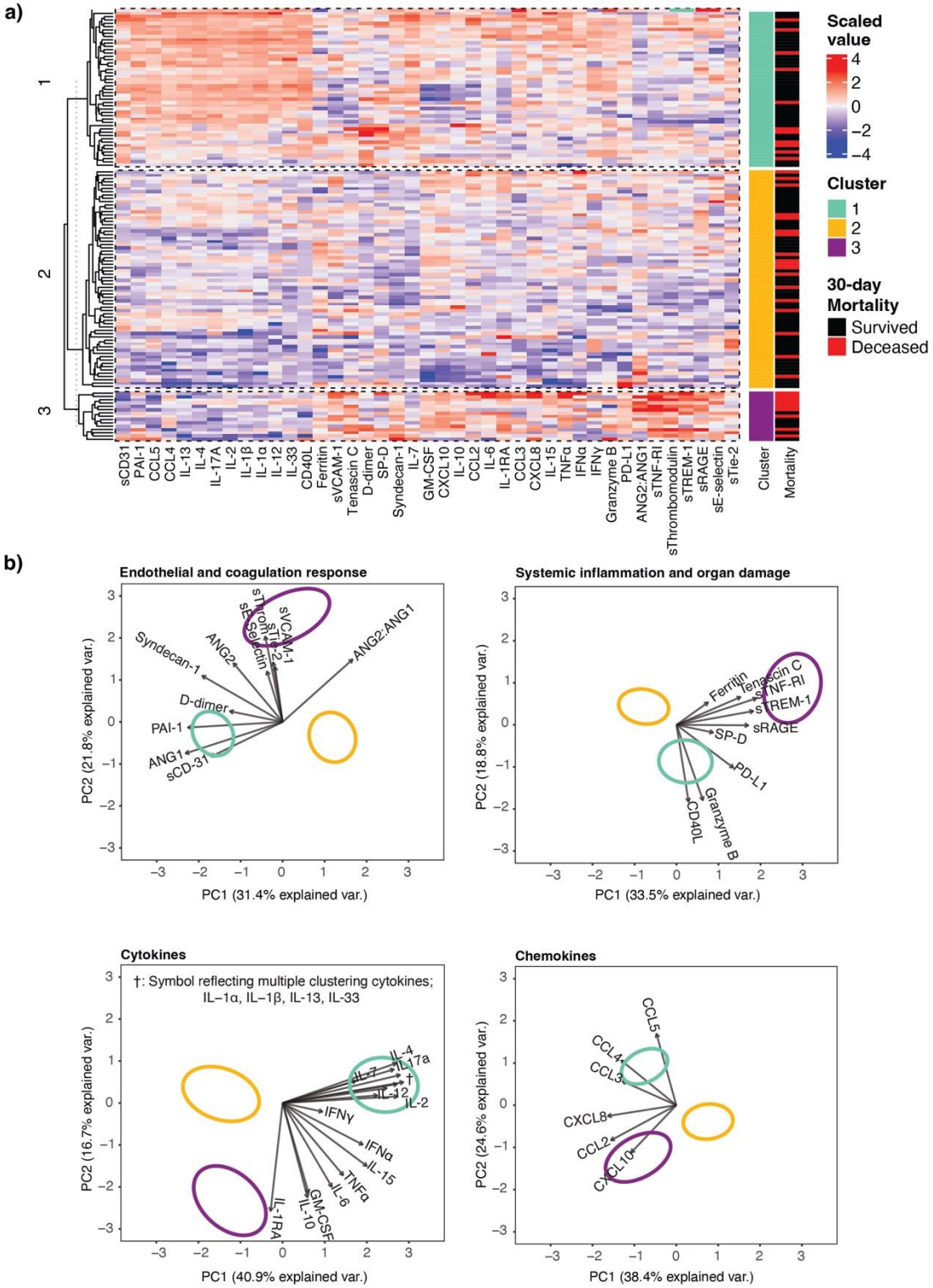
Description Figure S4: a) Heatmap depicting the magnitude of biomarker differences (Hedges' g) between patients ≥70 and the other age groups. P-values were obtained from a linear (if linear) or cubic spline regression analysis (if non-linear) in which age was modelled as a continuous variable. The adjusted 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.