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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Appendix 1

This web appendix formed part of the original submission and has been peer reviewed.
Supplement to: *Predictive performance and clinical applications of COV50, a urinary proteomic biomarker in early COVID-19 infection: a prospective multicentre cohort study*

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Urinary proteomics

Sample preparation and CE-MS analysis

Sample preparation and capillary electrophoresis coupled with mass spectrometry (CE-MS) analysis were performed essentially as described.[1] Urine aliquots were thawed and 700 µl mixed with 700 µl of 2 M urea, 10 mM NH_4OH containing 0·02 % SDS. Subsequently, samples were ultrafiltered using a Centristat 20 kDa cut-off centrifugal filter device (Satorius, Göttingen, Germany) to eliminate high molecular weight proteins. The obtained filtrate was desalted using a PD 10 gel filtration column (GE Healthcare Bio Sciences, Uppsala, Sweden) to remove urea, electrolytes and salts as well as to enrich polypeptides. The samples were lyophilized and stored at 4°C until usage. Shortly before CE-MS analysis, the samples were re-suspended in 10 µl HPLC-grade H_2O. Samples were injected into CE-MS with 2 psi for 99 sec, resulting in injection volumes of ~280 nl.

A P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA) was coupled with a MicrOTOF II MS (Bruker Daltronic, Bremen, Germany). A solution of 20% acetonitrile (Sigma-Aldrich, Taufkirchen, Germany) in HPLC-grade water (Roth, Karlsruhe, Germany) supplemented with 0·94% formic acid (Sigma-Aldrich) was used as running buffer. For CE-MS analysis, the electrospray ionization interface from Agilent Technologies (Palo Alto, CA) was set to a potential of -4·0 to -4·5 kV. Spectra were recorded over an *m*/*z* range of 350-3000 and accumulated every 3 sec.

CE-MS data processing

After the CE-MS analysis, mass spectral ion peaks representing identical molecules at different charge states were deconvoluted into single masses using MosaFinder software.[2] Only signals with z>1 observed in a minimum of 3 consecutive spectra with a signal-to-noise ratio of at least 4 were considered. The resulting peak list characterises each polypeptide by

its mass and migration time. Data were calibrated utilising 3151 internal standards as reference data points for mass and migration time by applying global and local linear regression, respectively. Reference signals of 29 abundant peptides were used as internal standards for calibration of signal intensity using linear regression. This procedure is highly reproducible and addresses both analytical and dilution variances in a single calibration step.[3] Among 60 independent analytic runs of a single urine sample, the coefficient of variation was 1%.[4] The obtained peak list characterises each polypeptide by its calibrated molecular mass [Da], calibrated CE migration time [min] and normalised signal intensity. All detected peptides were deposited, matched, and annotated in a Microsoft SQL database allowing further statistical analysis.

Sequencing of peptides

Candidate biomarkers were sequenced using CE-MS/MS or LC-MS/MS analysis, as described in detail.[5] MS/MS experiments were using an Ultimate 3000 nano-flow system (Dionex/LC Packings, USA) or a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA), both connected a Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (ThermoFisher Scientific, Waltham, Massachusetts, USA). The mass spectrometer is operated in data-dependent mode to automatically switch between MS and MS/MS acquisition. Survey full-scan MS spectra (from *m*/*z* 300–2000) were acquired in the Orbitrap. Ions were sequentially isolated for fragmentation. Data files were searched against the UniProt human nonredundant database using Proteome Discoverer 2.4 and the SEQUEST search engine. Relevant settings were: no fixed modifications, oxidation of methionine and proline as variable modifications. The minimum precursor mass was set to 790 Da, maximum precursor mass to 6000 Da with a minimum peak count of 10. The high-confidence peptides were defined by cross-correlation (Xcorr) >1·9 and rank = 1. Precursor

mass tolerance was 5 ppm and fragment mass tolerance 0·05 Da. For further validation of obtained peptide derivations, the correlation between peptide charge at the working pH of 2 and CE-migration time was utilised to minimise false-positive derivation rates:[6] calculated CE-migration time of the sequence candidate based on its peptide sequence (number of basic amino acids) was compared to the experimental migration time.

Sample classification

A disease-specific peptide-based classifier was developed using support vector machine (SVM)-based MosaCluster software, as described before.[7] The COV50 marker was expressed as a numerical value quantifying the Euclidean distance of the data point to the maximal margin of the separation hyperplane among cases and controls in a multidimensional space.

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Table 1: **Sequenced peptides and parental proteins included in COV50**

Mean amplitude WHO stages 1-3	Mean amplitude WHO stages 6-8	Fold difference	Amino-acid sequence	Parental protein	AUC	p value
1432·28	19·89	72·0101	GGSKRISIGGGS	Keratin, type II cytoskeletal 6B	0·6039	7·7990E-07
10138·45	539·76	18·7833	LmIEQNTKSPLFMGKVVNPTQK	Alpha-1-antitrypsin	0·7638	3·0497E-29
28478·81	2036·69	13·9829	EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAE	Alpha-1-antitrypsin	0·7624	1·8618E-24
123·57	10·81	11·4311	AGPpGKAGEDGHPGKPGRpGERG	Collagen alpha-2(I) chain	0·6714	2·3505E-21
1345·11	142·33	9·4506	AGPpGKAGEDGHpGKpGRpGERG	Collagen alpha-2(I) chain	0·7420	5·9622E-21
1436·74	179·06	8·0238	TGAKGAAGLpGVAGApGLpGPRGIpGPVGAAGATGARG	Collagen alpha-2(I) chain	0·7782	3·4876E-31
234·71	31·09	7·5494	GPpGPKGNSGEpGApGSKGDTGAKGEpGPVG	Collagen alpha-1(I) chain	0·6839	1·6287E-13
2394·78	406·86	5·8860	KGEKGDSGASGREGFPGVpGGTGP	Collagen alpha-1(VII) chain	0·7240	9·3676E-28
2191·92	399·81	5·4824	LQGLPGTGGppGENGKpGEpGpKGDAGApGApGGKGDAG ApGERGpPG	Collagen alpha-1(III) chain	0·7807	5·4736E-27
4321·59	795·43	5·4330	SETAPAAPAAPAPAEKTPVKKKA	Histone H1,4	0·7764	4·3675E-23
5120·09	1034·77	4·9480	GPEGPSGKpGINGKDGIPGAQGImGKpGDRGpKGERGDQ GIP	Collagen alpha-1(XIX) chain	0·7848	4·7943E-29
4391·47	1077·18	4·0768	PpGESGREGApGAEGSpGRDGSPGAKGDRGETGP	Collagen alpha-1(I) chain	0·8086	<1E-25
2086·96	615·11	3·3928	LkGQpGApGVkGEpGApGENGTpGQTGARG	Collagen alpha-2(I) chain	0·7775	9·4967E-24
10861·9	8257·89	1·3153	FDVNDEKNWGLS	Alpha-1-acid glycoprotein 1	0·5220	4·6569E-01
254·23	256·58	0·9908	GLSMDGGGSPKGDVDP	Sodium/potassium-transporting ATPase subunit gamma	0·6484	8·2980E-08
268·76	325·15	0·8266	EEKAVADTRDQADGSRASVDSGSSEEQGGSSRALVST	Polymeric immunoglobulin receptor	0·6941	1·2074E-12
1289·63	1826·87	0·7059	NSGEpGApGSKGDTGAkGEpGPVG	Collagen alpha-1(I) chain	0·6613	1·0102E-08
1573·18	2270·16	0·6930	WVGTGASEAEKTGAQEL	Gelsolin	0·6590	1·4941E-08
819·38	1217·08	0·6732	EAGGGSNSLQNSP	FERM domain-containing protein 4A	0·6709	1·1552E-09
6502·2	9818·1	0·6623	EGSpGRDGSpGAKGDRGETGPA	Collagen alpha-1(I) chain	0·6492	1·1940E-07
4040·6	6368·69	0·6344	pGKDGDTGPTGPQGPQ	Collagen alpha-1(XXII) chain	0·6562	2·9324E-08
349·17	559·11	0·6245	GpKGDpGIpGLDRSGFpGETGSPGIPGHQ	Collagen alpha-3(IV) chain	0·6546	3·1735E-08
2612·3	4260·77	0·6131	ESGREGApGAEGSpGRDGSpGAKGDRGETGP	Collagen alpha-1(I) chain	0·7183	7·3786E-15

Mean amplitude WHO stages 1-3	Mean amplitude WHO stages 6-8	Fold difference	Amino-acid sequence	Parental protein	AUC	p value
3551·59	6057·37	0·5863	PGTpGSPGPAGASGNPG	Collagen alpha-1(II) chain	0·7032	4·4980E-13
1029·17	1956·35	0·5261	GRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEVRGA	Neurosecretory protein VGF	0·7143	1·9560E-14
337·17	645·44	0·5224	DQGPVGRTGEVGAVGPpGFAGEKGpSGEAGTAGPPGTpGPQG	Collagen alpha-2(I) chain	0·7188	3·0958E-15
8454·59	16930·27	0·4994	VGPpGPpGPpGPpGPPS	Collagen alpha-1(I) chain	0·6906	1·1385E-11
1222·72	2550·98	0·4793	PpGPAGFAGPPGADGQPGAKGEpGDAGAKGDAGPPGPAGP	Collagen alpha-1(I) chain	0·7498	<1E-25
381·09	824·39	0·4623	GpAGPRGERGPpGESGA	Collagen alpha-2(I) chain	0·7229	1·6009E-15
2046·46	4449·59	0·4599	VGPpGPPGPpGPpGPPS	Collagen alpha-1(I) chain	0·7167	1·1599E-14
1957·84	4464·36	0·4385	PQGPpGPTGpGGDKGDTGPpGPQGLQGLpGT	Collagen alpha-1(III) chain	0·7188	5·9850E-15
1850·08	4432·16	0·4174	GPpGVPGpPGpGGSPGLP	Collagen alpha-1(XXII) chain	0·7518	<1E-25
570·53	1393·57	0·4094	GpAGPPGPPGPPGTSGHPGSPGSPGYQGPPGEPGQAGPSGPPG	Collagen alpha-1(III) chain	0·7443	<1E-25
244·19	601·77	0·4058	FPGQTGPRGEMGQp	Collagen alpha-1(VII) chain	0·7309	<1E-25
372·75	1136·9	0·3279	GSEGPQGVRGEPGpPGPAGAAGPAGNPGADGQPGAKGANG	Collagen alpha-1(I) chain	0·7684	<1E-25
249·66	771·02	0·3238	ERGEAGIpGVpGAKGEDGKDGSPGEpGANG	Collagen alpha-1(III) chain	0·7779	<1E-25
1797·67	6089·45	0·2952	PpGESGREGApGAEGSpGRDGSpGAKGDRGETGP	Collagen alpha-1(I) chain	0·8310	<1E-25
51·62	179·98	0·2868	NDGApGKNGERGGpGGp	Collagen alpha-1(III) chain	0·7561	<1E-25
306·22	1176·88	0·2602	SGQSSGYTqhGSGSGh	Hornerin	0·7144	3·0958E-15
144·09	559·6	0·2575	ppGSNGNpGPPGPPGPSGKDGPKGARGDSGPPGRAGEPG	Collagen alpha-1(II) chain	0·7509	<1E-25
172·43	709·58	0·2430	EDGHpGKPGRpGERG	Collagen alpha-2(I) chain	0·8101	<1E-25
105·18	478·47	0·2198	DDGEAGKpGRpG	Collagen alpha-1(I) chain	0·7914	<1E-25
14·6	74·52	0·1959	PGPVGpPGSNGPVGEPGPEGPAGNDGTPGRDGAVGERGDRGDPGPAGLPG	Collagen alpha-2(V) chain	0·6604	4·1952E-12
85·74	441·2	0·1943	GTDGpMGpHGpAGPKGERGE	Collagen alpha-1(XXV) chain	0·7865	<1E-25
197·45	1129·00	0·1749	EEDDGEVTEDSDEDFIQP	E3 ubiquitin-protein ligase TRIM33	0·8410	<1E-25
280·74	1745·34	0·1609	DADLADGVSGGEGKGGSDGGGSHRKEGEEADAPGVIPGIVGAVV	CD99 antigen	0·8359	<1E-25

Mean amplitude WHO stages 1-3	Mean amplitude WHO stages 6-8	Fold difference	Amino-acid sequence	Parental protein	AUC	p value
87·85	559·87	0·1569	IDGSpGEKGDPGDVGGPGPPGASGEPGAPGPPGKRGPS	Collagen alpha-3(V) chain	0·7534	<1E-25
11·18	119·19	0·0938	DDPRPPNPPKPMPNPNPNHPSSSGS	CD99 antigen	0·7133	<1E-25
348·81	4044·53	0·0862	EEKAVADTRDQADGSRASVDSGSSEEQGGSSRALVSTLV PLG	Polymeric immunoglobulin receptor	0·8427	<1E-25
5·03	127·72	0·0394	HVSGSGQSSGFGQHESRSGHSSYGQHGFGSSQSSGYG	Filaggrin-2	0·7537	<1E-25

The peptide amino acid sequence and the parental protein of origin are listed. For each peptide, the mean relative abundance in the urine from patients with moderate disease (maximal who grade 1-3) and the urine from patients with critical disease (maximal who grade 6-8) is given, the fold change between these two groups, the AUC, and the p-value (after correction for multiple testing).

***Table 2*: Discriminative performance of COV50 in the interim report based on the initial recruitment**

Outcome	Derivation cohort	Validation cohort
Mortality		
Number events/at risk	23/228	10/99
Continuously distributed COV50		
AUC (95% confidence interval)	0·82 (0·74–0·89)	0·83 (0·71–0·94)
Cross-validated AUC (95% confidence interval)	0·80 (0·72–0·88)	NA
Categorised COV50		
Youden cut-off threshold	0·47	0·47
Sensitivity	87·0 (73·1–100)	80·0 (55·0–1·00)
Specificity	74·6 (68·7–80·6)	70·8 (61·3–80·2)
Progressing WHO score		
Number events/at risk	48/228	23/99
Continuously distributed COV50		
AUC (95% confidence interval)	0·75 (0·67–0·82)	0·70 (0·58–0·81)
Cross-validated AUC (95% confidence interval)	0·74 (0·66–0·81)	NA
Categorised COV50		
Youden cut-off threshold	0·04	0·04
Sensitivity (95% confidence interval)	77·1 (65·2–89·0)	73·9 (56·0–91·9)
Specificity (95% confidence interval)	63·9 (56·9–70·9)	63·2 (52·3–74·0)

AUC indicates area under the curve. The AUC in the validation cohort was derived from the probabilities as predicted by the logistic model in the derivation cohort. Sensitivity and specificity in the validation cohort were based on the thresholds obtained in the derivation cohort. NA indicates not applicable. The validation cohort (n=99) are included in the continued recruitment. Reproduced from Wendt et al., EClinicalMedicine 2021; 36: 100883 (doi: 10.1016/j.eclinm.2021.100883).

Table 3: **Baseline characteristics by quartiles of the baseline COV50 distribution in the full study**

Characteristic	Low	Medium-low	Medium-high	High	p value for trend
COV50 limits	-1·28	[-1·27, -0·30]	[-0·28, 0·79]	≥0·80	
Number in group	253	253	253	253	…
Main study variables					
Maximal WHO score during follow-up					
1-3	216 (85·4)	118 (46·6)	78 (30·8)	33 (13·0)	
4-5	37 (14·6)	132 (52·2)	173 (68·4)	187 (73·9)	<0.0001
6-8	0 (0·0)	3 (1·2)	2 (0·8)	33 (13·0)	
Mean COV50 biomarker level at entry	-2·01 (0·45)	-0·76 (0·28)	0·22 (0·31)	1·63 (0·56)	…
Number with characteristic (%)					
White ethnicity	210 (83·0)	221 (87·4)	226 (89·3)	223 (92·1)	<0.0001
Women	136 (53·7)	122 (48·2)	109 (43·1)	80 (31·6)	<0·0001
Hypertension	78 (30·8)	131 (51·8)	157 (62·1)	191 (75·5)	<0.0001
Heart failure	12 (4·7)	28 (11·1)	64 (25·3)	50 (19·8)	<0.0001
Body mass index ≥30 kg/m^2	56 (22·1)	72 (28·5)	68 (26·9)	55 (21·7)	0·2021
Diabetes mellitus	21 (8·3)	43 (17·0)	81 (32·0)	112 (44·3)	<0.0001
Cancer	10 (4·0)	26 (10·3)	24 (9·5)	46 (18·2)	<0·0001
Use of RAS blockers,	59 (23·3)	102 (40·3)	129 (51·0)	137 (54·2)	<0·0001
Mean (SD) of characteristic					
Age	49·0 (16·9)	59·9 (16·6)	67·9 (15·6)	72·3 (12·2)	<0·0001
Systolic blood pressure, mm Hg	126·1 (18·8)	128·4 (19·1)	129·3 (20·1)	128·8 (22·0)	0·1248
Diastolic blood pressure, mm Hg	78·2 (11·3)	77·8 (12·0)	75·4 (11·9)	73·3 (12·7)	<0·0001
Heart rate, beats per minute	79·2 (13·6)	82·3 (15·5)	82·5 (15·8)	84·8 (16·6)	0·0002
Body mass index, kg/m^2	26·8 (5·2)	28·2 (5·4)	28·2 (5·2)	27·3 (5·1)	0·2510

RAS blockers indicate blocker of the renin-angiotensin system, including angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers. The number of participants with missing blood pressure and heart rate amounted to 11, 7, 8 and 5 in the low, medium-low, medium-high and high groups. An ellipsis indicates not applicable. The p-value for trend was derived by regressing the row entries on a dummy variable ranging from 1 to 4, coding for the increasing categories of COV50. An ellipsis indicates that the p-value was not calculated.

Table 4: **Discriminative performance of COV50 by recruitment phase**

Outcome	Initial	Continued	Full
Mortality			
N° deaths/at risk (%)	25/228 (11·0)	94/784 (12·0)	119/1012 (11·8)
Continuously distributed COV50			
AUC (95% confidence interval)	0·83 (0·77-0·90)	0·83 (0·77-0·90)	0·81 (0·77-0·85)
Categorised COV50			
Youden cut-off threshold	0·47	0·47	0·47
Sensitivity (95% confidence interval)	88·0 (68·8-97·4)	71·3 (61·0-80·1)	74·8 (66·0-82·3)
Specificity (95% confidence interval)	75·4 (68·8-81·1)	74·1 (70·6-77·3)	74·4 (71·4-77·2)
PLR (95% confidence interval)	3·57 (2·70-4·73)	2·75 (2·30-3·29)	2·92 (2·50-3·40)
NLR (95% confidence interval)	0·16 (0·05-0·46)	0·39 (0·28-0·53)	0·34 (0·25-0·46)
Accuracy	76·8 (70·7-82·1)	73·2 (70·5-76·8)	74·4 (71·6-77·1)
Progressing WHO score			
N° events/at risk (%)	50/228 (21·9)	221/784 (28·3)	271/1012 (26·8)
Continuously distributed COV50			
AUC (95% confidence interval)	0·76 (0·69-0·83)	0·76 (0·69-0·83)	0·72 (0·68-0·75)
Categorised COV50			
Youden cut-off threshold	0·04	0·04	0·04
Sensitivity (95% confidence interval)	80·0 (66·3-90·0)	64·7 (58·0-71·0)	67·5 (61·6-73·1)
Specificity (95% confidence interval)	64·6 (57·1-71·6)	68·2 (64·2-72·0)	67·3 (63·8-70·7)
PLR (95% confidence interval)	2·26 (1·77-2·88)	2·04 (1·74-2·38)	2·07 (1·81-2·36)
NLR (95% confidence interval)	0·31 (0·18-0·54)	0·52 (0·43-0·62)	0·48 (0·40-0·58)
Accuracy	68 ·0 (61·5-74·0)	67·2 (63·8-70·5)	67·4 (64·4-70·3)

Initial recruitment lasted from 30 June 2020 until 19 November 2020 and continued recruitment from 30 April 2020 until 14 April 2021. NA indicates not applicable. AUC=area under the curve. PLR is the positive likelihood ratio (true positive rate/false positive rate. NLR is the negative likelihood ratio (false negative rate/true negative rate). Accuracy is the overall probability that a patient is correctly classified. All estimates in this table were unadjusted for other risk factors.

Table 5: **Risk and discriminatory performance associated with single risk factors by recruitment phase**

Outcome		Initial		Continued		Full	
		Estimate	p	Estimate	p	Estimate	p
Mortality							
N° deaths/at risk (%)		25/228 (11·0)		94/784 (12·0)		119/1012 (11·8)	
Female sex	OR	0·32 (0·12-0·89)	0·0284	0·32 (0·12-0·89)	0·0284	0·53 (0·35-0·80)	0·0025
Age, +10 years	OR	1·32 (1·01-1·74)	0·0442	2·39 (1·94-2·95)	<0·0001	2·01 (1·71-2·37)	<0·0001
	AUC	0·62 (0·52-0·72)	0·0442	0·80 (0·77-0·84)	<0·0001	0·77 (0·73-0·80)	<0·0001
BMI, +5 kg/m^2	OR	1·04 (0·72-1·52)	0·8346	1·01 (0·83-1·24)	0·9063	1·02 (0·85-1·22)	0·8514
Comorbidities present	OR	1·81 (0·79-4·17)	0·1633	3·92 (2·42-6·35)	<0·0001	3·27 (2·17-4·92)	<0·0001
GFR, +30 ml/min/1·73 m^2	OR	0·62 (0·41-0·95)	0·0286	0·57 (0·45-0·73)	<0·0001	0·58 (0·47-0·72)	<0·0001
	AUC	0·61 (0·48-0·75)	0·0286	0·65 (0·59-0·72)	<0·0001	0·64 (0·58-0·73)	<0·0001
WHO score, +1 point	OR	2·07 (1·42-3·01)	0·0002	3·23 (2·35-4·44)	<0·0001	2·40 (1·94-2·95)	<0·0001
	AUC	0·75 (0·67-0·83)	0·0002	0·75 (0·71-0·79)	<0·0001	0·74 (0·71-0·78)	<0·0001
COV50 (+1 SD)	OR	2·44 (1·69-3·54)	<0·0001	2·47 (2·02-3·03)	<0·0001	2·44 (2·05-2·92)	<0·0001
	AUC	0·83 (0·77-0·90)	<0·0001	0·80 (0·76-0·85)	<0·0001	0·81 (0·77-0·85)	<0·0001
Worsening WHO score							
N° events/at risk (%)		50/228 (21·9)		221/784 (28·3)		271/1012 (26·8)	
Female sex	OR	0·42 (0·21-0·85)	0·0150	0·69 (0·50-0·94)	0·0210	0·64 (0·48-0·85)	0·0020
Age, +10 years	OR	1·36 (1·10-1·67)	0·0036	1·52 (1·37-1·69)	<0·0001	1·48 (1·35-1·63)	<0·0001
	AUC	0·63 (0·55-0·1)	0·0036	0·69 (0·65-0·73)	<0·0001	0·68 (0·64-0·72)	<0·0001
BMI, +5 kg/m^2	OR	0·87 (0·64-1·19)	0·3832	1·00 (0·87-1·16)	0·9447	0·97 (0·85-1·11)	0·6934
Comorbidities present	OR	1·99 (1·05-3·73)	0·0351	2·74 (1·99-3·78)	<0·0001	2·59 (1·95-3·45)	<0·0001
GFR, +30 ml/min/1·73 m^2	OR	0·99 (0·81-1·20)	0·8842	0·81 (0·68-0·95)	0·0101	0·86 (0·75-0·99)	0·0221
	AUC			0·58 (0·53-0·63)		0·57 (0·53-0·61)	0·0221
WHO score, +1 point	OR	1·46 (1·15-1·86)	0·0018	1·53 (1·32-1·77)	<0·0001	1·45 (1·29-1·63)	<0·0001
	AUC	0·62 (0·54-0·70)	0·0025	0·60 (0·56-0·63)	<0·0001	0·60 (0·56-0·63)	<0·0001
COV50, +1 SD	OR	2·44 (1·69-3·53)	<0·0001	2·47 (2·02-3·03)	<0·0001	2·44 (2·05-2·92)	<0·0001
	AUC	0·83 (0·77-0·90)	<0·0001	0·80 (0·76-0·85)	<0·0001	0·81 (0·77-0·85)	<0·0001

The odds ratio and the area under the curve, both given with 95% confidence interval, estimate the association size and discriminatory performance, respectively. Risk factors were determined at enrolment with the exception of GFR, which was measured after hospitalisation in 816 patients at risk. For GFR the death rates were 13·1% (25/191), 15·0% (94/625), and 14·6% (119/816) in the initial, continued and full recruitment cohorts; the corresponding rates of worsening WHO score were 26·2% (50/191), 34·2% (214/625), and 32·4% (264/816), respectively. Comorbidities include hypertension, heart failure, diabetes and cancer. The AUC was not computed for non-significant odds ratios and for categorical variables with only two levels. OR=odds ratio. AUC=area under the curve. BMI=body mass index. GFR=glomerular filtration rate estimated from serum creatinine using the CKD-EPI formula (*Ann Intern Med.* 2009; 150: 604-12).

Table 6: **Odds ratios relating outcome to COV50 by recruitment phase in hospitalised patients**

Outcome	Initial		Continued		Full	
	OR (95% CI)	p value	OR (95%CI)	p value	OR (95% CI)	p value
Mortality						
N° deaths/at risk (%)	25/191 (13·1)		94/625 (15·0)		119/816 (14·6)	
Unadjusted	2·32 (1·58-3·40)	<0·0001	2·23 (1·79-2·77)	<0.0001	2·23 (1·85-2·69)	<0.0001
Adjusted						
Sex and age	2·16 (1·50-3·28)	<0·0001	1·82 (1·45-2·29)	<0·0001	1·97 (1·62-2·40)	<0·0001
+ baseline WHO score	2·18 (1·31-3·63)	0·0028	1·54 (1·21-1·96)	0·0005	1·65 (1·34-2·04)	<0·0001
+ BMI, comorbidities and GFR	2·36 (1·34-4·15)	0·0030	1·50 (1·16-1·93)	0·0022	1·58 (1·27-1·98)	<0·0001
Progressing WHO score						
N° events/at risk (%)	50/191 (26·2)		214/625 (34·2)		264/819 (32·4)	
Unadjusted	1·74 (1·33-2·28)	<0·0001	1·53 (1·32-1·78)	<0·0001	1·56 (1·38-1·78)	<0·0001
Adjusted						
Sex and age	1·68 (1·27-2·21)	0·0002	1·38 (1·18-1·61)	<0·0001	1·45 (1·27-1·66)	<0·0001
+ baseline WHO score	2·42 (1·62-3·61)	<0·0001	1·50 (1·27-1·77)	<0·0001	1·66 (1·43-1·93)	<0·0001
+ BMI, comorbidities and GFR	2·43 (1·62-3·66)	<0·0001	1·54 (1·29-1·84)	<0·0001	1·68 (1·44-1·97)	<0·0001

Odds ratios given with 95% confidence interval express the risk for 1-SD increment in COV50. Initial recruitment lasted from 30 June 2020 until 19 November 2020 and continued recruitment from 30 April 2020 until 14 April 2021. Comorbidities include hypertension, heart failure, diabetes and cancer. BMI=body mass index; GFR=glomerular filtration rate.

Table 7: **Probability of progressing to a maximal WHO score during follow-up by entry COV50 level, entry WHO score, and age class**

Baseline		Maximal WHO score during follow-up				
COV50 WHO score Age class	**Number**	**1-2**	**3**	**4**	**5**	**6-8**
[-3·26, 3·39]	1012					
1-2	199	0·9849	0	0·0151	0	0
3	246	0	0·6179	0·3089	0·0203	0·0529
4	432	0	0	0·7361	0·0880	0·1759
5	97	0	0	0	0·5773	0·4227
6	38	0	0	0	0	1·0000
<0·04, [19, 96 y]	587					
1-2	194	1·0000	0	0	0	0
3	118	0	0·7024	0·2619	0·0179	0·0178
4	212	0	0	0·8571	0·0714	0·0715
5	41	0	0	0	0·9231	0·0769
6	22	0	0	0	0	1·0000
≥0·04, [19, 96 y]	587					
1-2	194	0·4000	0	0·6000	0	0
3	118	0	0·4359	0·4103	0·0256	0·1282
4	212	0	0	0·6356	0·1017	0·2627
5	41	0	0	0	0·4507	0·5493
6	22	0	0	0	0	1·0000
<0·04, <65 y	120		0			
1-2	2	0·5000	0	0·5000	0	0
3	15	0	0·7143	0·2381	0·0476	0
4	38	0	0	0·6200	0·1800	0·2000
5	26	0	0	0	0·5517	0·4483
6	39	0	0	0	0	1·0000
≥0·04, ≥65 y						
1-2	0	0	0	1·0000	0	0
3	19	0	0·3334	0·4737	0·0175	0·1754
4	147	0	0	0·6398	0·0806	0·2796
5	32	0	0	0	0·3810	0·6190
6	107	0	0	0	0	1·0000

The transition matrix was derived from the participants enrolled in CRIT-Cov-U according to the transition diagram shown on page 17. The number of patients progressing to a higher WHO score during follow-up was simulated by multiplying the baseline distribution vector by the transition matrix as derived from the current dataset, using the IML procedure as implemented in the SAS software and 1000 iterations to determine the distribution around the initial point estimate. The age stratification was only introduced for the patients with the highest risk of progression (COV50 level at entry ≥0·04).

Table 8: **Summary of Markov chain simulation for WHO score progression by entry COV50 level, baseline WHO score, and age class**

COV50 level Percentiles Age class		Number of patients expected with maximal WHO score during follow-up					
		1-2	**3**	**4**	**5**	**6-8**	**Total**
[-3·26, 3·39]	O	196	152	397	99	168	1012
5	S	193	140	383	88	149	953
25	S	195	146	390	94	160	985
50	S	196	152	397	99	168	1012
75	S	197	157	403	103	177	1037
95	S	199	164	411	110	188	1072
<0·04, [19, 96 y]	O	194	118	212	41	22	587
5	S	194	108	203	34	14	553
25	S	194	114	208	38	18	572
50	S	194	118	212	41	22	587
75	S	194	123	216	43	25	601
95	S	194	129	221	48	30	622
≥0·04, [19, 96 y]	O	2	34	185	58	146	425
5	S	0	27	172	49	128	376
25	S	1	31	179	54	139	404
50	S	2	34	184	58	146	424
75	S	3	36	190	62	153	444
95	S	4	41	198	68	164	475
≥0·04, <65 y	O	2	15	38	26	39	120
5	S	0	12	33	21	32	98
25	S	1	14	36	24	36	111
50	S	2	15	38	26	39	120
75	S	3	17	40	28	42	130
95	S	4	18	43	30	47	142
≥0·04, ≥65 y	O	0	19	147	32	107	305
5	S	0	13	134	25	92	264
25	S	0	17	142	29	100	288
50	S	0	19	147	32	107	305
75	S	0	21	153	35	113	322
95	S	0	25	159	39	122	345

Values are the number of patients progressing to a higher WHO score. For each cell in the transition matrix, the distribution of the predicted number of patients progressing to a higher WHO score is characterised by providing the 5th, 25th, 50th, 75th and 95th percentiles. The age stratification was only introduced for the patients with the highest risk of progression (COV50 level at entry ≥0·04). O=observed number of patients. S=simulated number of patients.

Table 9: **Simulated hospitalisation costs by hospital facility at presentation and age class**

Baseline COV50 Entry hospital facility	Item	Hospitalisation costs expressed per 1000 patients hospitalised for 1 week	Cost reduction associated with 1-day less hospitalisation per 1000 patients
[-3·26, 3·39]			
Regular and intermediate care	days	6 (4-14)	
	M€	3·393 (3·633-4·225)	
Intensive care	days	7 (4-13)	
	M€	6·456 (5·710-7·205)	
All care facilities	days	9 (4-15)	
	M€	10·366 (9·343-11·430)	1·481 (1·335-1·633)
<0·04			
Regular and intermediate care	days	7 (3-13)	
	M€	4·617 (4·137-5·103)	
Intensive care	days	6 (4-11)	
	M€	1·591 (0·973-2·170)	
All care facilities	days	8 (4-13)	
	M€	6·208 (5·110-7·273)	0·887 (0·730-1·039)
≥0·04, [19, 96 y]			
Regular and intermediate care	days	11 (5-16)	
	M€	3·740 (3·289-4·257)	
Intensive care	days	6 (4-18)	
	M€	10·946 (9·596-12·296)	
All care facilities	days	11 (5-17)	
	M€	14·686 (12·872-16·553)	2·089 (1·839-2·365)
≥0·04, <65 y			
Regular and intermediate care	days	10 (4-17)	
	M€	5·182 (4·246-5·975)	
Intensive care	days	5 (4-14)	
	m€	9·850 (8·082-11·870)	
All care facilities	days	11 (5-17)	
	M€	15·032 (12·328-17·845)	2·147 (1·761-2·549)
≥0·04, ≥65 y			
Regular and intermediate care	days	10 (4-17)	
	m€	2·805 (2·378-3·220)	
Intensive care	days	5 (4-14)	
	M€	9·296 (7·993-10·600)	
All care facilities	days	11 (5-17)	
	M€	12·101 (10·371-13·820)	1·729 (1·482-1·974)

Hospitalisation costs per care facility (median and 95% percentile interval) were extrapolated from the distributions of patients to be expected by Markov chain simulation reaching follow-up WHO scores of 3-4, 5, and 6-8 and the care facility corresponding with disease severity, i.e., regular, intermediate and intensive care for scores 3-4, 5, and 6-8, respectively. Cost estimates in intensive care units also include the costs of lower care facilities to which patients were admitted before or after they reached their maximal WHO score during follow-up. Days refers to the median number of days (interquartile interval) as observed in the CRIT-Cov-U cohort. M€ indicates million Euro.

Table 10: **Statistics extracted from outcome trials in COVID-19 patients**

Trial	Design	Setting	Timing	Control treatment	Experimental treatment	FU (days)	Endpoint extracted	Results (control *vs* intervention)	p
PRINCIPLE, 2021	SB (B)	A mild	…	usual care	inhaled budesonide	28	hospitalisation	94/1069 (8·8) *vs* 54/787 (6·8)	>99·9
ACTT-1, 2020	DB	H moderate severe	9 (6-12)	placebo	remdesivir	28	days to recovery (WHO scale 0-3)	10 *vs* 15	<0·0001
Hung, 2020	O	H mild moderate	5 (3-7)	lopinavir + ritonavir	lopinavir + ritonavir + interferon b1b	14	hospital days	9 (7-13) *vs* 14 (9-16)	0·016
RECOVERY, 2021	O (B)	H moderate severe	9 (7-14)	usual care	tocilizumab	28	days to discharge	>28 *vs* 19	
							discharged within 28 d	1044/2094 (49·9) *vs* 1150/2022 (56·9)	<0·0001
BLAZE-1, 2021	O	A mild moderate	4 (…)	placebo	bamlanivimab + etesivimab	29	hospitalisation	37/517 (7·2) *vs* 12/518 (2·3)	…
Wang, 2020	DB	H moderate severe	11 (9-12)	placebo	remdesivir	28	days of IMV in survivors	42·0 (17·0-46·0) *vs* 19 (5-42)	…
REMAP-CAP, 2021	O (B)	ICU severe	1·2 (0·8-2·8)	usual care	tocilizumab or sarilumab	21	support-free days	UC: 0 (-1 to 15) T: 10 (-1 to 16) S: 11 (0 to 16)	T: 99.9 S: 99.5
RECOVERY, 2021	O	H moderate severe	9 (5-13)	usual care	dexamethasone	28	cessation of IMV	268/683 (39·2) *vs* 160/324 (49·4	…

Trials are identified by acronym or the surname of the first author, year of publication and the refence number in the article text. Design refers to type of masking (O, open; SB, single blind; DB, double blind; B, a Bayesian statistical approach. Setting refers to the recruitment of ambulatory (A) or hospitalised (H) patients and the disease stage at enrolment according to the WHO scale: mild, <3; moderate, 3-4; severe, 6-8. Timing refers to the median number of day (interquartile range) between symptom-onset and randomisation; for REMAP-CAP, the number of hours between ICU admission and randomisation is given. Control and experimental indicate the treatments administered. Patients randomised to experimental also received usual care. FU is the duration of follow-up in days. Results in the control *vs* experimental group are given in days, -1 indicating death, or as the proportion of patients. UC/T/S indicate usual care/tocilizumab/sarilumab and IMV invasive mechanical ventilation. p is the posterior probability of superiority of the experimental treatment for trials that applied a Bayesian approach or the conventional significance level. An ellipsis indicates data not available in the publication. Full details of the selected (n=8) and non-selected (n=44) trials and corresponding references are available via https://www.appremed.org/Publications8.

Table 11: **Baseline characteristics of the full CRIT-Cov-U cohort and the 2022 substudy**

Characteristic	CRIT-Cov-U	Substudy
Number in group	1012	62
Main study variables		
WHO score		
1-3	445 (44·0)	35 (56·5)
4-5	529 (52·3)	27 (43·5)
6	38 (3·8)	0 (0·0)
COV50 level	-0·23 (1·40)	-0·45 (1·10)
Vaccination status		
None or unknown	1012 (100·0)	18 (29·0)
1		2 (3·2)
2		14 (22·6)
≥3		28 (45·2)
Number with characteristic (%)		
Women	447 (44·2)	26 (41·9)
Hypertension	557 (55·0)	38 (61·3)
Heart failure	154 (15·2)	0 (0·0)
Body mass index ≥30 kg/m^2	251 (24·8)	12 (19·4)
Diabetes mellitus	257 (25·4)	25 (40·3)
Cancer	106 (10·5)	12 (19·4)
Chronic obstructive lung disease	…	7 (11·3)
Immunosuppressed	…	13 (21·0)
Mean (SD) of characteristic		
Age	62·3 (17·8)	64·9 (19·8)
Body mass index, kg/m^2	27·6 (5·2)	27·1 (7·1)
Glomerular filtration, ml/min/1·73 m^2	85·6 (37·6)	69·8 (39·6)

The CRIT-Cov-U cohort was enrolled from 30 June 2020 until 14 April 2021 and the patients enrolled in the substudy from 7 February 2022 until 16 March 2022. One patient was infected by the delta variant and 61 by the omicron strain. The glomerular filtration rate estimated from serum creatinine using the CKD-EPI formula (*Ann Intern Med.* 2009; 150: 604-12). In the CRIT-Cov-U cohort glomerular filtration was unavailable in 196 ambulatory patients. An ellipsis indicates that data were not on file.

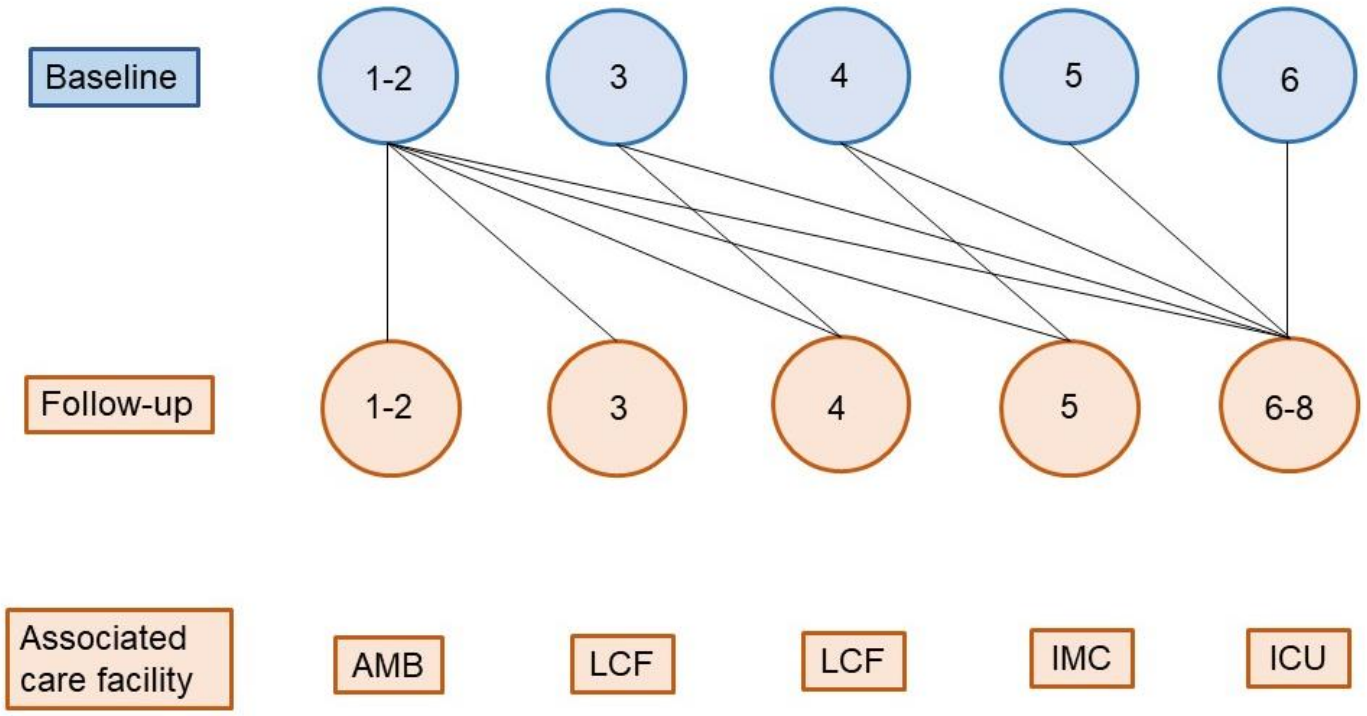

Figure 1:

Transition diagram applied for Markov modelling of the probability of progression of the baseline WHO score during follow-up.

The WHO-score categories are: (1) ambulatory without limitation of activity; (2) ambulatory with limited activity; (3) hospitalised without oxygen therapy; (4) hospitalised on oxygen therapy by mask or nasal prongs; (5) hospitalised receiving non-invasive ventilation or high-flow oxygen therapy; (6) hospitalised with intubation and mechanical ventilation; (7) hospitalised with mechanical ventilation and additional organ support, such as vasopressors, renal replacement therapy, or extracorporeal membrane oxygenation; and (8) death. The associated care facilities are ambulatory care (AMB) and hospitalised care in a regular ward (LCF), intermediate care (IMC) or intensive care (ICU).

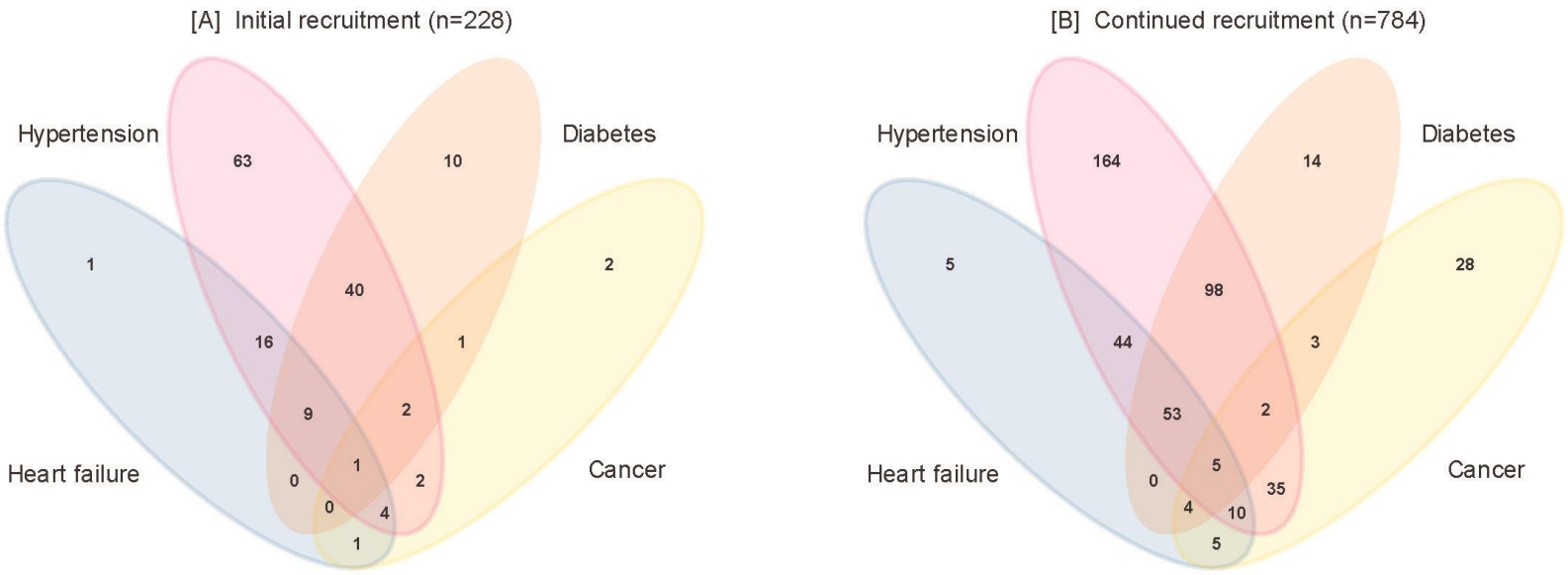

Figure 2:

Overlap in comorbidities in the initial cohort (A) and the continued recruitment cohort (B).

Numbers are not additive, because most patients had several comorbidities.

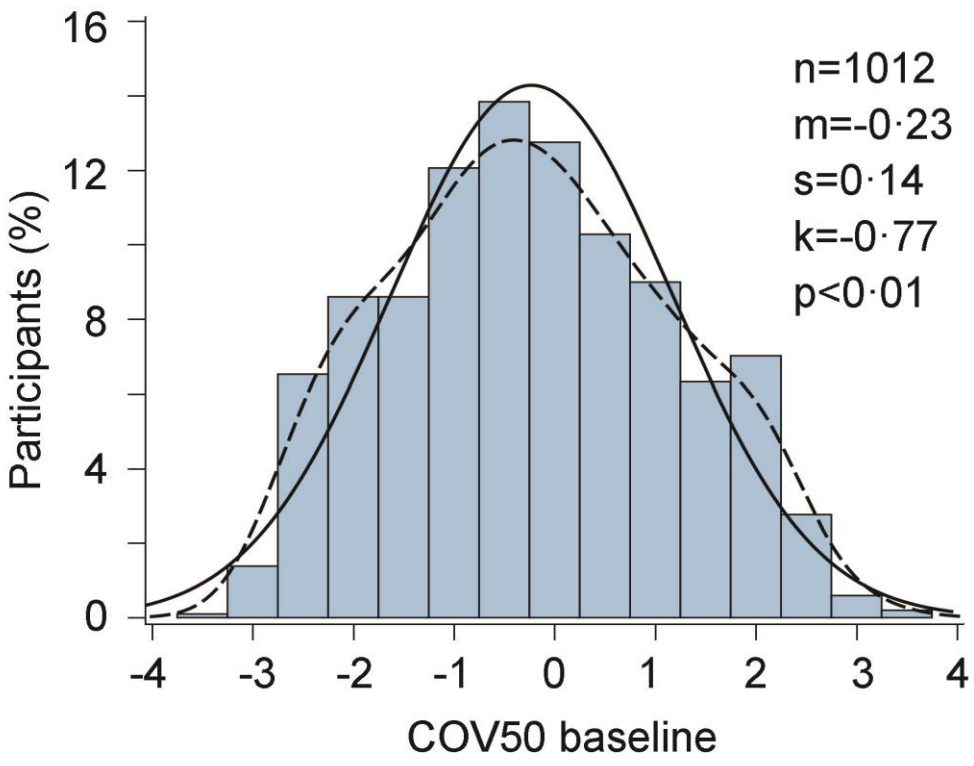

Figure 3:

Distribution of the urinary COV50 marker in the whole study population.

n, m, s and k indicate the number of patients, the arithmetic mean and the coefficients of skewness and kurtosis. The solid and dotted lines represent the normal and kernel density distributions. The p-value is for departure of the actually observed distribution from normality according to the Kolmogorov-Smirnov test.

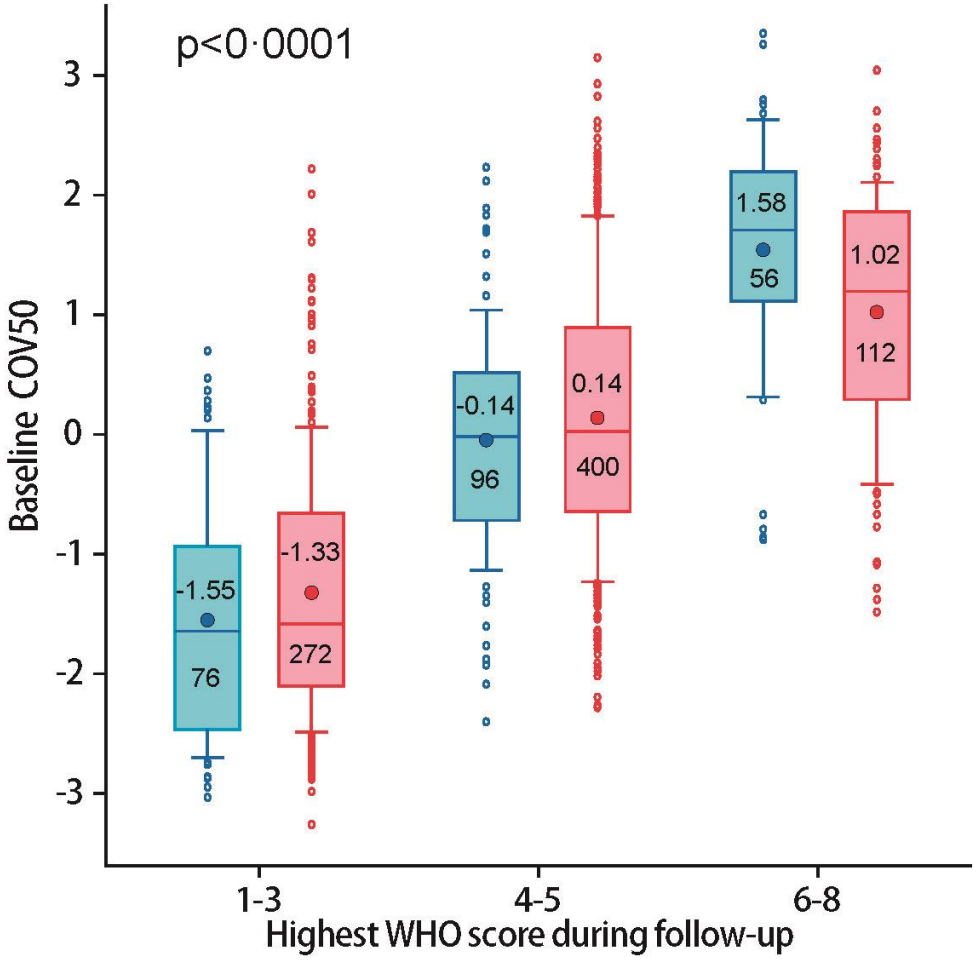

Figure 4:

Boxplots showing the distributions of the urinary biomarker COV50 at baseline by the worst WHO score attained during follow-up in the initial (blue) and continued recruitment (pink) cohorts. The central line, the upper and lower lines, and the upper and lower caps represent the median, interquartile range, and the 10th to 90th percentile interval. The arithmetic means and extreme measurements are represented by circles inside the box and outside the whiskers, respectively. The arithmetic means and the number of data points contributing to each whisker plot is given within the boxes. The p value denotes the overall between-WHO category significance derived by ANOVA.

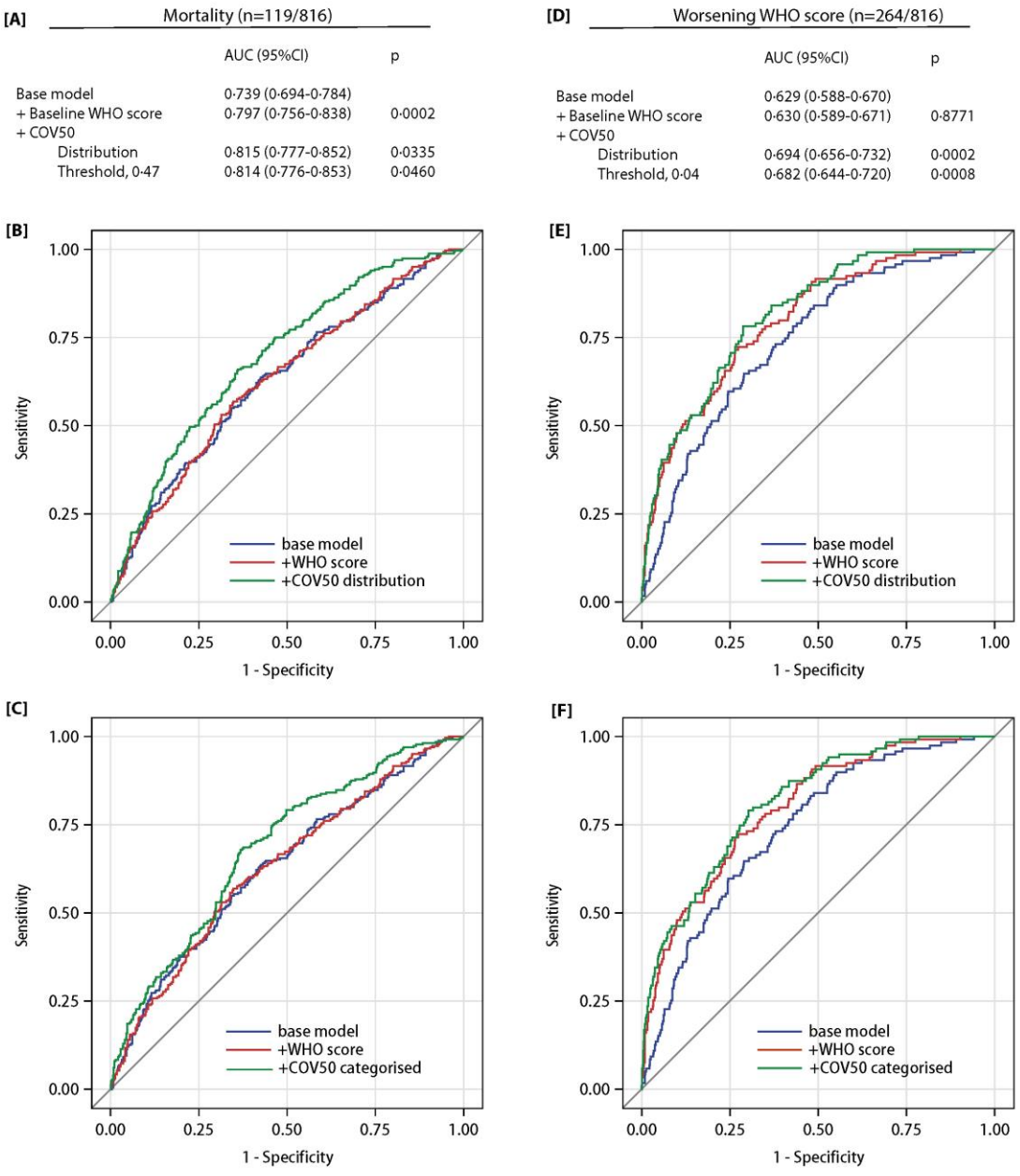

Figure 5:

Performance of the COV50 urinary marker on top of other baseline risk factors in 816 hospitalised patients for contrasting mortality *vs* survival (panels A-C) and for progression *vs* non-progression in the baseline WHO score during follow-up (panels D-F).

The base model included sex, age, body mass index, the presence of comorbidities (hypertension, heart failure, diabetes and cancer), and the glomerular filtration rate. In subsequent steps, the baseline WHO score was added and next COV50 as a continuously distributed variable (panels B and E) or as a categorised variable based on an optimised threshold of 0·47 for mortality (panel C) or 0·04 for a worsening WHO score (panel F). At each step, the p-values are for the comparison with the preceding