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Supplementary appendix

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Supplemental Material

Prevalence of ARDS Phenotypes in Critically-Ill COVID-19 Patients: A Prospective Observational Cohort Study.

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Content

Methods

Study sites

The Royal Gwent Hospital (RGH) is a medium-size district general hospital with 800 inpatient beds in Newport, Wales, serving approximately 480,000 population with the highest number of deprived areas compared to any other Welsh hospital. The critical care unit is normally a 16-bedded combined ICU/HDU located in two areas on the same floor, admitting both invasively ventilated patients and patients needing other organ support. During the COVID-19 surge this has been transformed to a 40 bedded ICU spanning three separate areas with relaxed nurse-to-patient ratios, where only invasively ventilated patients were admitted. At the height of the first wave the unit was occupied by 28 COVID-19 patients and 6 patients with other pathologies. 38 patients were screened for the study at RGH. Five patients were not enrolled due to lack of research staff and early death prior to recruitment. One patient was withdrawn from the study due to being SARS-COV-2 negative on multiple testing.

University College Hospital (UCH) is a 600-bed inner city University Hospital. It is one of the largest cancer centres in the UK delivering advanced therapies such as bone marrow transplantation, CAR T-cells and shortly proton beam. It houses a large Hyper Acute Stroke Unit, an active Emergency Department and a range of other surgical and medical specialties. The hospital became a Critical Care COVID-19 Hub for the North Central London Sector transforming it's 35 bed critical care unit up to 65 beds with a separate 20 bed CPAP ward, at the peak. Over half the admissions were transferred in from other Critical Care Units within the sector. 50 patients were screen at UCH. Failure of study recruitment in these patients were due Multistat analyser malfunction/calibration (23 patients), patients had ARDS for longer than 48 hours (13 patients), and due to lack of research staff (7 patients).

Point of care biomarker analyser

The Evidence MultiSTAT analyser is a fully automated Biochip Array System (Randox Laboratories, Country Antrim, UK). For the purposes of this study a bespoke assay was developed to enable rapid quantification of interleukin-6 (IL-6) and soluble tumour necrosis factor receptor-1 (sTNFR-1). The semi-automated assay uses a cartridge containing both the assay reagents and a biochip, a solid state device with discrete test regions onto which antibodies for IL-6 and sTNFR-1 are bound and stabilised. Plasma is added to the cartridge and secondary antibody binding rapidly quantified by chemiluminescence. Blood samples are collected in a lithium heparinised tube and centrifuged at 2000rcf for 10 minutes to separate plasma and cells. Plasma is then added to the MultiSTAT cartridge, which is inserted in the Evidence MultiSTAT analyser for biomarker quantification. The bespoke assay values, measured using the Evidence MultiSTAT analyser have been validated against ELISA (R&D systems; Minneapolis, MN) quantification for both biomarkers. There was high correlation between the biomarker levels and the probabilities for phenotype classification using both measurement systems.[1]

Parsimonious Classifier Model

The classifier model is a logistic regression-based models comprised of the IL-6, sodium bicarbonate and sTNFR-1.[2] For the model, IL-6 and sTNFR-1 were both log-transformed from their original values after the addition of one (+1). The coefficients used for the model were as follows:

Intercept	IL-6	Sodium Bicarbonate	sTNFR-1
-11.9593	1.0138	-0.2436	1.1903

Propensity Matching

Given the importance of age and gender as prognostic markers in COVID-19, we wanted to match the populations using these factors. In terms of disease severity, we matched using a marker of ARDS severity- PaO_2/FiO_2. The APACHE II score was deliberately excluded from the matching given the higher APACHE II scores in the HARP-2 cohort overall and we wanted to test how the APACHE II scores differed in the matched cohort compared to the COVID-19 ARDS cohort. A logistic regression-based score was developed using COVID-19 as the outcome and age, gender and PaO_2/FiO_2 as the predictor variables. Initial analysis led to an imbalance of gender. Given the known importance of gender in outcome of ARDS, matching was performed within gender based on a logistic-regression model with age and PaO_2/FiO_2 as the predictor variables. The HARP-2 cohort was matched to the COVID-19 cohort using nearest neighbour matching where the distance was estimated based on the logistic regression model. R package "MatchIt" was used for the analysis.

References

1. Parker JC, Higgins M, Torrisi M, et al. Development of a Point-of-Care (POC) Theranostic Assay to Stratify Patients with Acute Respiratory Distress Syndrome (ARDS). European Respiratory Scoiety Research Seminar: Personalised Medicine in Acute Respiratory Distress Syndrome; 2020; Barcelona, Spain.
2. Sinha P, Delucchi KL, McAuley DF, O'Kane CM, Matthay MA, Calfee CS. Development and validation of parsimonious algorithms to classify acute respiratory distress syndrome phenotypes: a secondary analysis of randomised controlled trials. *Lancet Respir Med.* 2020;8(3):247-257.

Tables

Table S1. Differences in selected baseline characteristics between the two sites, Royal Gwent Hospital (RGH) and University College Hospital (UCH) from which the cohort were derived.

		RGH N=32	UCH N = 7
Age (Years)		55 (50 – 59)	63 (61 – 69)
Gender: Male		19 (59%)	6 (86%)
Race			
	White	16 (50%)	3 (42%)
	Asian	7 (22%)	2 (29%)
	Black	2 (6%)	2 (29%)
	Other	7 (12%)	0
Diabetes Mellitus		9 (28%)	0
Hypertension		2 (6%)	4 (57%)
PaO_2/FiO_2 (kPa)		19 (16 – 22)	20 (17 – 24)
Interleukin-6 (pg/mL)		218 (140 – 642)	149 (84 – 270)
sTNFR-1 (pg/mL)		3490 (2475 – 4482)	2735 (2323 – 3705)
Vasopressor-use (baseline)		23 (72%)	1 (14%)
Invasive Ventilation (baseline)		32 (100%)	3 (43%)
SOFA Score		6 (6 – 8)	5 (4 – 6)
APACHE II score		12 (10 – 15)	16 (10 – 18)
Mortality at Day 28		12 (37.5%)	5 (71%)

sTNFR-1 = Soluble Tumour Necrosis Factor-1.

Table S2 Difference in baseline characteristics between the hypoinflammatory and hyperinflammatory phenotypes using a probability cut-off of ≥ 0.5 to assign class. Note p-value are not presented due to the small sample size and imbalance between the two groups.

		Hypoinflammatory (n= 35)	**Hyperinflammatory (n= 4)**
Age (Years)		56 (52 – 61)	59 (56 – 60)
Gender: Male		22 (63%)	3 (75%)
Race			
	White	18 (51%)	1 (25%)
	Asian	7 (20%)	2 (50%)
	Black	3 (9%)	1 (25%)
	Other	7 (20%)	0 (0%)
Diabetes Mellitus		7 (20%)	2 (50%)
Hypertension		6 (17%)	0 (0%)
Heart Rate (beats/min)		102 (81 – 142)	104 (97 – 121)
Mean Arterial Pressure (mmHg)		64 (61 – 71)	73 (69 – 76)
PaO_2/FiO_2 (kPa)		18 (15 – 21)	16 (10 – 21)
Minute Ventilation (L/min)		10.3 (9.4 – 10.9)	13.0 (11.6 – 14.8)
Plateau Pressure ($cm.H_2O$)		30 (26 – 34)	34 (32 – 34)
PEEP ($cm.H_2O$)		12 (12 – 15)	13.5 (11 – 15)
Compliance ($mL/cm.H_2O$)		24 (20 – 28)	24 (21 – 28)
White Blood Cells ($x\ 10^9/L$)		9.9 (7.8 – 12)	10.6 (9.8 – 11.8)
Lymphocytes ($x\ 10^9/L$)		0.8 (0.6 – 1.1)	1.3 (1.1 – 1.45)
Platelets ($x\ 10^9/L$)		280 (219 – 339)	219 (129 – 219)
Albumin (g/L)		23 (20 – 26)	24 (22 – 25)
Bilirubin (μmol/L)		10 (6 – 21)	18 (8 – 30)
Creatinine (μmol/L)		78 (63 – 151)	301 (201 – 386)
Troponin (ng/L)		13 (5 – 29)	221 (122 – 3959)
Lactate Dehydrogenase (units/L)		449 (315 – 545)	867 (732 – 899)
Procalcitonin (ng/mL)		0.9 (0.4 – 2.9)	6.7 (2.2 – 205)
Fibrinogen (g/L)		6.6 (6.0 – 6.8)	5.4 (5.1 – 5.8)
D-Dimer (ng/mL)		1583 (867 – 3636)	3747 (2686 – 3762)
Ferritin (mcg/L)		807 (418 – 2649)	4877 (3527 – 4881)
C-Reactive Protein (mg/L)		214 (158 – 304)	244 (144 – 360)
Vasopressor-use (baseline)		22 (63%)	2 (50%)
Invasive Ventilation (baseline)		31 (90%)	4 (100%)
SOFA Score		6 (5 – 8)	11 (7 – 16)
APACHE II score		12 (10 –16)	17 (16 – 19)
Mortality at Day 28		14 (40%)	3 (75%)

Table S3. Comparison of the variables that were used to match the COVID-19 cohort with a subset of HARP-2 patient with pneumonia as their primary risk factor for ARDS. P-values compared the data from COVID-19 with the HARP-2 Matched cohort and represent the Wilcoxon Rank test unless annotated. Values for the subset of HARP-2 used for matching and the unmatched portion of the subset are also presented.

	COVID-19 (n =39)	HARP-2 Matched (n =39)	P-value	SMD	HARP-2 Subset[a] (n =97)	HARP-2 Unmatched (n = 58)
PaO_2/FiO_2 (kPa)	18 (15 – 21)	18 (12 – 23)	0.97	-0.002	15 (11 – 21)	13 (10 – 19)
Gender: Male	25 (64%)	24 (62%)	0.99*	—	50 (52%)	26 (45%)
Age (years)	57 (52 – 61)	58 (42 – 68)	0.50	0.043	56 (43 – 68)	55 (43 – 69)

SMD = Standardized mean difference. *Chi-squared test. a = The subset of HARP-2 presented here are those patients that had IL-6 and sTNFR-1 measured using the Multistat assay.

Table S4. Baseline characteristics of the HARP-2 Cohort and HARP-2 matched cohort. Variables are further stratified mortality at day 28. Note that in the HARP-2 trial only a limited set of data were collected and only these are presented.

	HARP-2 (whole cohort)			HARP-2 Matched		
	Total Population (n = 539)	**Survivors (n = 407)**	**Non-survivors (n = 132)**	**Total Population (n = 39)**	**Survivors (n = 28)**	**Non-survivors (n = 11)**
Age (Years)	54 (42 – 66)	52 (40 – 63)	63 (49 – 73)	58 (42 – 68)	57 (39 – 68)	63 (52 – 68)
Gender: Male	307 (57%)	233 (57%)	74 (56%)	24 (62%)	15 (54%)	9 (82%)
Plateau Pressure ($cm.H_2O$)	24 (20 – 28)	23 (19 – 27)	25 (20 – 30)	22 (19 – 25)	22 (19 – 23)	26 (25 – 28)
Platelets ($x\ 10^9/L$)	174 (106 – 254)	181 (118 – 257)	136 (79 – 246)	184 (106 – 273)	216 (162 – 298)	96 (89 – 109)
Bilirubin (μmol/L)	12 (6 – 24)	11 (6 – 21)	15 (8 – 29)	10 (5 – 27)	9 (5 – 22)	19 (7 – 62)
Creatinine (μmol/L)	78 (58 – 126)	76 (57 – 118)	95 (66 – 148)	75 (57 – 120)	73 (52 – 112)	91 (69 – 141)
Vasopressor-use	356 (66%)	254 (62%)	102 (77%)	23 (59%)	15 (54%)	8 (73%)
PaO_2/FiO_2 (kPa)	15 (11 – 21)	16 (12 – 22)	15 (10 – 20)	18 (12 – 23)	19 (13 – 25)	16 (11 – 19)
APACHE II score	18 (14 – 24)	17 (13 – 23)	22 (16 – 27)	19 (14 – 23)	18 (14 – 23)	20 (16 – 26)