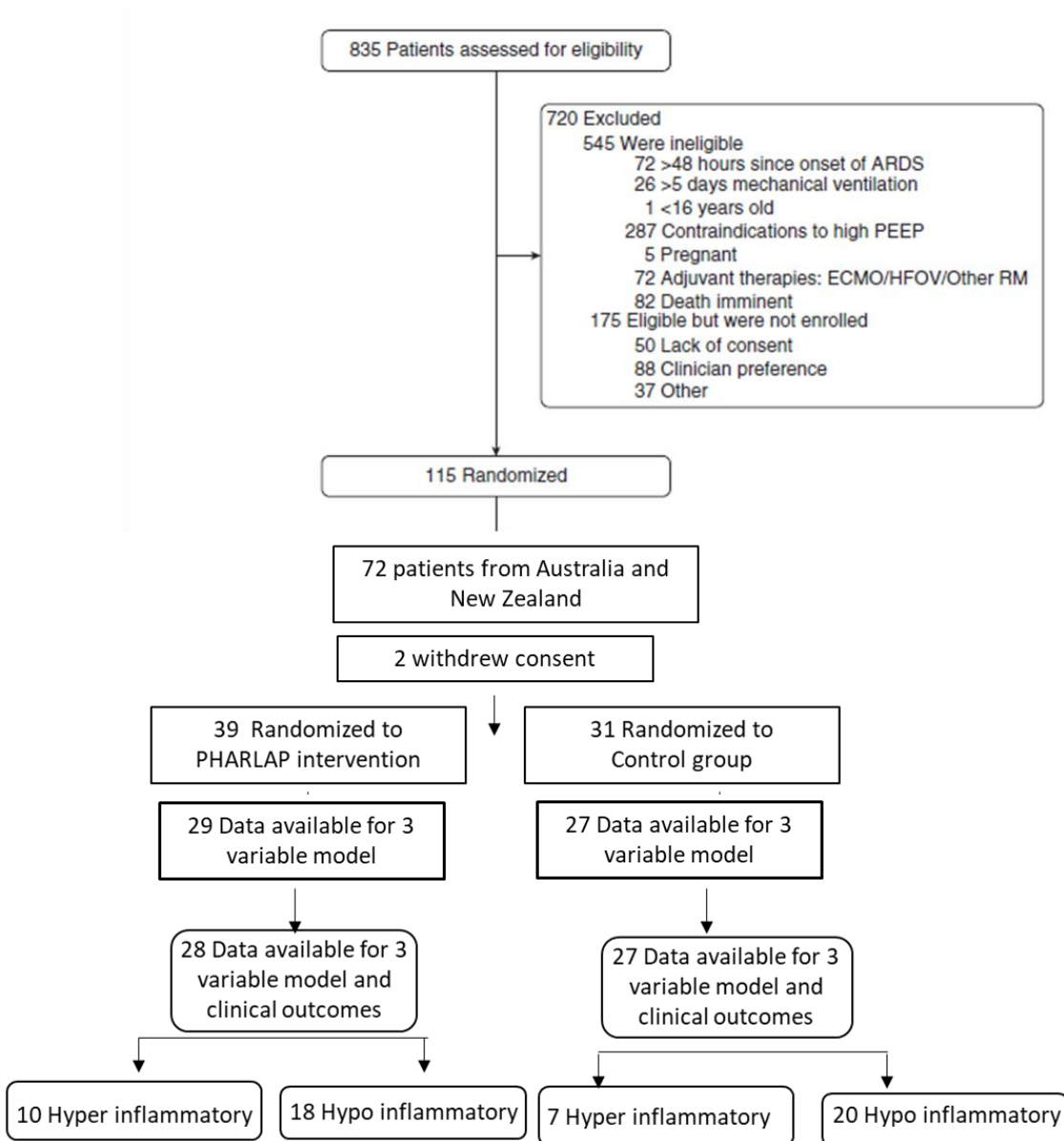


Appendix

This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

Appendix Figure 1: Consolidated flow diagram of the flow of patients through the study.



Three variable model include Day 1 serum IL-8, HCO_3^- and sTNFR1.

ARDS= acute respiratory distress syndrome; ECMO= extracorporeal membrane oxygenation; HFOV= high-flow oscillatory ventilation; PEEP = positive end-expiratory pressure; PHARLAP=Permissive Hypercapnia, Alveolar Recruitment, and Low Airway Pressure; RM= recruitment manoeuvre.

Appendix Table 1: Comparison of patients* in whom day 1 blood samples was available and those it was not available.

	Day 1 samples available (n=58)	Day 1 samples not available (n=56)	P value
Age (years)	52.4 (14.3)	55.1 (13.9)	0.31
Male (n,%)	34 (58.6%)	32 (57.1%)	0.87
Height (cm)	171 (9.15)	168 (10.1)	0.06
Weight (Kg)	87.2 (21.9)	89.1 (29.5)	0.70
APACHE II Score	22.2 (6.79)	23.3 (7.68)	0.39
Blood Gas pH	7.35 (0.1)	7.35 (0.1)	0.84
Blood Gas PaO ₂ mm Hg	73.3 (12.4)	73.7 (17.4)	0.90
Blood Gas PaCO ₂ mm Hg	48.2 (13)	46.5 (8.83)	0.44
Blood Gas HCO ₃ ⁻ mmol/L	25.5 (6.01)	25 (5.55)	0.64
Blood Gas SaO ₂ (%)	93.7 (2.88)	92.9 (4.73)	0.30
Blood Gas FiO ₂	0.59 (0.18)	0.63 (0.18)	0.23
Blood Gas Lactate mmol/L	1.35 [1.1-2]	1.3 [1-1.8]	0.49
Blood Gas base excess	-0.9 [-4.7-4.6]	-0.05 [-4.95-3.2]	0.90
PaO ₂ /FiO ₂	132 (33.3)	124 (38.8)	0.28
SOFA score	8.02 (3.31)	8.63 (3.07)	0.33
ARDS Pulmonary	44 (75.9%)	46 (82.1%)	0.41

* One patient withdrew consent

SOFA; Sequential Organ Failure Assessment, APACHE; Acute Physiologic Assessment and Chronic Health Evaluation

Data presented as number (%), mean (SD) or Median [IQR] based on their distribution.

Fishers Exact, Chi Square, Wilcoxon Rank Sum, Student T-test test were used for comparisons

Appendix Table 2: Comparison of patients with the PHARLAP intervention and control strategy in the hypo-inflammatory phenotype and hyper-inflammatory phenotypes

	Hyper-inflammatory phenotype		P value for interaction with VFD = 0.08
	PHARLAP (n=10)	Control (n=7)	
VFD day 28	0 [0-19]	16 [8-22]	
Mortality at 90 days	4 (40%)	1 (14.3%)	
	Hypo-inflammatory phenotype		
	PHARLAP (n=18)	Control (n=20)	
VFD day 28	19.5 [11-23]	20 [12-24]	
Mortality at 90 days	2 (11.1%)	4 (20%)	

VFD, ventilator free day

Data presented as number (%), mean (SD) or Median [IQR] based on their distribution.

Appendix Table 3: Percent change from day 1 to day 3 in the hypo-inflammatory phenotype and hyper-inflammatory phenotype with serum biomarkers utilising the three variable parsimonious model (IL-8, HCO₃ and sTNFR1).

Serum Change from Day 1 to Day 3	Hyper-inflammatory (n=12)	Hypo-inflammatory (n=33)	P value
Ang- 1	9.32 [-49.1 to 61.8]	-10.5 [-33.5 to 24.1]	0.43
Ang-2	38.8 [13.2 to 49.5]	7.47 [-22.2 to 43.9]	0.07
Ang1:Ang2	-34.2 [-283 to 55.7]	-12.1 [-102 to 26.9]	0.87
IL-6	67.2 [58.8 to 88.8]	35.2 [-288 to 69]	0.012
IL-8	61 [34 to 74]	-8.7 [-76 to 33.8]	0.001
TNF-a	32.9 [10.9 to 53.9]	0 [-31 to 9.89]	0.026
pSP-B	33.5 [-38.9 to 52.7]	-1.89 [-75.7 to 18.4]	0.07
sTNFR1	-11.2 [-53.2 to 39.2]	-9.83 [-36.4 to 16.2]	0.95

Ang, angiopoietin; IL, interleukin; TNF, tumor necrosis factor; sTNFR1, soluble tumor necrosis factor receptor 1; pSP-B, pro surfactant protein

Data presented as Median [IQR] based on their distribution.

Wilcoxon Rank Sum used for comparisons

Appendix Table 4: Percent change from day 1 to day 3 in the hypo-inflammatory phenotype and hyper-inflammatory phenotype lung lavage biomarkers with three variable parsimonious model (IL-8, HCO₃ and sTNFR1).

BAL Change from Day 1 to Day 3	Hyper-inflammatory (n=6)	Hypo-inflammatory (n=14)	P value
Ang-1	-102.5 [-218.6 to 90.3]	8.7 [-10 to 82.1]	0.51
Ang-2	50.2 [0 to 78.8]	34.6 [0 to 82]	0.93
IL-6	86.1 [79.6 to 90.7]	94.1 [0 to 99.3]	0.36
IL-8	-38.8 [-202 to 82.3]	49.4 [-67.9 to 94.4]	0.54
TNF-a	0 [0 to 0]	0 [0 to 87.1]	0.70
pSP-B	52.7 [-323 to 73.6]	0.54 [-271 to 84.9]	0.84
sTNFR1	73.2 [27.5 to 86]	65.6 [16 to 94.2]	0.96
Lavage Protein	54 [-230 to 66.4]	50 [2.28 to 91.7]	0.43

BAL, broncho alveolar lavage

Ang, angiotensin; IL, interleukin; TNF, tumor necrosis factor; sTNFR1, soluble tumor necrosis factor receptor 1; pSP-B, pro surfactant protein

Data presented as Median [IQR] based on their distribution.

Wilcoxon Rank Sum used for comparisons

Appendix Table 5: Serum levels of the examined biomarkers in patients when classified as good and bad outcomes based on the median value of the ventilator free days.

	Day	VFD >15 days (Good)	VFD <15 days (bad)	P value
Day 1				
Ang- 1 (ng/ml)	1	32.2 (16.5)	30.4 (19.6)	0.71
Ang-2 (ng/ml)	1	12 [6.7-18.5]	10.8 [6.2-29.2]	0.64
Ang1:Ang2	1	2.8 [1.48-5.27]	2.45 [0.746-4.05]	0.37
IL-6 (pg/ml)	1	251 [71-708]	256 [74-704]	0.89
IL-8 (pg/ml)	1	57.5 [29.5-170]	105 [68-337]	0.017
TNF-a (pg/ml)	1	17.5 [8-68]	27 [12-45]	0.83
pSP-B (ug/ml)	1	149 [88.6-419]	167 [66.8-507]	0.86
sTNFR1 (pg/ml)	1	4600 [3000-8600]	4800 [3300-12600]	0.33
Day 3				
Ang- 1 (ng/ml)	3	36.2 (25.2)	35.8 (23.2)	0.95
Ang-2 (ng/ml)	3	8.05 [4.2-12.7]	6.6 [5.2-19.2]	0.64
Ang1:Ang2	3	4.16 [1.33-8.2]	2.73 [1.33-6.47]	0.73
IL-6 (pg/ml)	3	78 [22-233]	117 [51-246]	0.29
IL-8 (pg/ml)	3	54 [26-121]	59 [43-117]	0.48
TNF-a (pg/ml)	3	14 [6-82]	17.5 [8-40]	0.76
pSP-B (ug/ml)	3	278 [107-630]	151 [74.7-290]	0.14
sTNFR1 (pg/ml)	3	4100 [2600-7400]	5800 [4500-11500]	0.07

Ang, angiopoietin; IL, interleukin; TNF, tumor necrosis factor; sTNFR1, soluble tumor necrosis factor receptor 1; pSP-B, pro surfactant protein

Data presented as Mean (SD) or Median [IQR] based on their distribution.

Student T-test or Wilcoxon Rank Sum used for comparisons based on their distribution.

Appendix Table 6: Lung lavage levels of the examined biomarkers in patients when classified as good and bad outcomes based on the median value of the ventilator free days.

	Day	VFD >15 days (Good)	VFD <15 days (bad)	P value
Day 1				
Ang- 1 (ng/ml)	1	0.223 [0.031-0.693]	0.251 [0.094-0.563]	0.9
Ang- 2 (ng/ml)	1	0.346 [0.077-0.837]	0.529 [0.025-0.954]	0.95
IL-6 (pg/ml)	1	1869 [940-8286]	607 [288-787]	0.003
IL-8 (pg/ml)	1	973 [641-1877]	824 [652-935]	0.14
TNF-a (pg/ml)	1	27 [4-157]	4 [4-12]	0.05
pSP-B (ug/ml)	1	137 [26.9-215]	93.6 [51.6-148]	0.24
Protein (ug/ml)	1	2089 [1202-5503]	1404 [815-2174]	0.19
sTNFR1 (pg/ml)	1	757 [461-2980]	801 [458-2462]	0.97
Day 3				
Ang- 1 (ng/ml)	3	0.107 [0.02-0.66]	0.154 [0.02-0.487]	0.91
Ang- 2 (ng/ml)	3	0.041 [0.025-0.52]	0.221 [0.025-0.693]	0.40
IL-6 (pg/ml)	3	104 [7-229]	161 [4-993]	0.85
IL-8 (pg/ml)	3	417 [148-807]	575 [189-1167]	0.46
TNF-a (pg/ml)	3	4 [2-10]	4 [4-22]	0.41
pSP-B (ug/ml)	3	44.6 [26.6-50.6]	60.1 [6.3-114]	0.89
Protein (ug/ml)	3	490 [119-2517]	1462 [212-2011]	0.83
sTNFR1 (pg/ml)	3	139 [102-521]	332 [100-1784]	0.55

BAL, broncho alveolar lavage

Ang, angiotensin; IL, interleukin; TNF, tumor necrosis factor; sTNFR1, soluble tumor necrosis factor receptor 1; pSP-B, pro surfactant protein

Data presented as Median [IQR] based on their distribution.

Wilcoxon Rank Sum used for comparisons