## SUPPLEMENTARY MATERIAL

## DOI: 10.4081/monaldi.2024.2829

## Diaphragmatic morphological *post-mortem* findings in critically ill COVID-19 patients: an observational study

Luigi Vetrugno,<sup>1,2</sup> Cristian Deana,<sup>3</sup> Savino Spadaro,<sup>4,5</sup> Gianmaria Cammarota,<sup>6,7</sup> Domenico Luca Grieco,<sup>8,9</sup> Annarita Tullio,<sup>10</sup> Tiziana Bove,<sup>3,11</sup> Carla Di Loreto,<sup>11,12</sup> Salvatore Maurizio Maggiore,<sup>2,13</sup> Maria Orsaria,<sup>12</sup> and the DIASUS study group

<sup>1</sup>Department of Medical, Oral and Biotechnological Sciences, Gabriele d'Annunzio University of Chieti Pescara, Chieti; <sup>2</sup>Department of Anesthesiology, Critical Care Medicine and Emergency, Annunziata Hospital, Chieti; <sup>3</sup>Department of Anesthesia and Intensive Care, Health Integrated Agency Friuli Centrale, Academic Hospital of Udine; <sup>4</sup>Department of Translational Medicine, University of Ferrara; <sup>5</sup>Intensive Care Unit, Azienda Ospedaliera Universitaria Sant'Anna, Ferrara; <sup>6</sup>Department of Translational Medicine, Università degli Studi del Piemonte Orientale, Novara; <sup>7</sup>Department of Anesthesiology and Intensive Care, Azienda Ospedaliero-Universitaria "Maggiore della Carità", Novara; <sup>8</sup>Department of Emergency, Intensive Care Medicine and Anesthesia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome; <sup>9</sup>Department of Anesthesiology and Intensive Care Medicine, Catholic University of The Sacred Heart, Rome; <sup>10</sup>Health Integrated Agency Friuli Centrale, Academic Hospital of Udine; <sup>11</sup>Department of Medicine, University of Udine; <sup>12</sup>Institute of Anatomic Pathology, Health Integrated Agency Friuli Centrale, Academic Hospital of Udine; <sup>13</sup>Department of Innovative Technologies in Medicine and Dentistry, Gabriele d'Annunzio University of Chieti Pescara, Chieti, Italy

**Correspondence**: Luigi Vetrugno, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Via dei Vestini n 33, Chieti, 66100, Italy.

E-mail: <u>luigi.vetrugno@unich.it</u>

**Key words:** COVID-19, invasive mechanical ventilation, diaphragm dysfunction, myosin, ventilation-induce diaphragm injury.





Supplementary Figure 1. Fast fiber content and their number relationship with mechanical ventilation. Fast myosin content showed a positive linear correlation with the percentage of controlled mechanical ventilation on total  $LOS_{ICU}$  (R<sup>2</sup>=0.446, p=0.006 as shown in panel A). On the opposite, their number decreased regarding the same ventilation parameters (R<sup>2</sup>=-0.34, p=0.022 as shown in panel B).

Supplementary table 1. Pre-existing conditions and relevant medical history						
<i>,</i>	OVERALL (n = 29)	CLUSTER 1 (n = 14)	CLUSTER 2 (n=15)	P value		
Cardiovascular	19 (65%)	9 (64%)	10 (66%)	>0.99		
COPD	4 (14%)	2 (14%)	2 (13%)	>0.99		
DM type 2	6 (21%)	4 (28%)	2 (13%)	0.38		
Dyslipidemia	4 (14%)	2 (14%)	2 (13%)	>0.99		
CKD	4 (14%)	2 (14%)	2 (13%)	>0.99		
Solid neoplasia	1 (3.5%)	-	1 (6.5%)	>0.99		
Other	11 (38%)	6 (43%)	5 (33%)	0.71		

COPD, chronic obstructive pulmonary disease; DM, diabetes; CKD, chronic kidney disease.



Correlations		CLUSTER 1	CLUSTER 2	
	slow myosin		2	
% time under assisted ventilation and	fibers%	R <sup>2</sup> =-0.025, p=0.584	R <sup>2</sup> =0.001, p=0.983	
	tast myosin tibers	$R^2$ =-0.355. p=0.014	$R^2=0.246$ , p=0.059	
	n° of fibers	$R^2=0.001$ n=0.839	$R^2 = -0.115 n = 0.216$	
	CSA	$R^2 = 0.006 \text{ p} = 0.804$	$R^2 = -0.028$ p=0.544	
	Porimeter	$R^2 = 0.01$ n = 0.723	$R^2 = 0.020, p = 0.344$ $R^2 = -0.011, p = 0.709$	
	Diameter	$R^2 = 0.01; p = 0.723$ $R^2 = 0.025, p = 0.584$	$R^2 = -0.022$ n = 0.596	
	Eraction	R = 0.023, p = 0.304 $P^2 = 0.211, p = 0.005$	R = -0.022, p = 0.030 $P^2 = 0.282, p = 0.041$	
% time under controlled ventilation and	slow myosin	K = -0.211, p = 0.093	K = 0.202, p = 0.041	
	fibers%	R <sup>2</sup> =-0.025, p=0.586	R <sup>2</sup> =-0.001, p=0.921	
	fast myosin fibers			
	%	R <sup>2</sup> =0.003, p=0.834	R <sup>2</sup> =0.446, p=0.006	
	n° of fibers	R <sup>2</sup> =0.013, p=0.689	R <sup>2</sup> =-0.34, p=0.022	
	CSA	R <sup>2</sup> =-0.085, p=0.310	R <sup>2</sup> =0.000, p=0.995	
	Perimeter	R <sup>2</sup> =-0.097, p=0.277	R <sup>2</sup> =0.006, p=0.773	
	Diameter	R <sup>2</sup> =-0.106, p=0.255	R <sup>2</sup> =0.008, p=0.738	
	Fraction	R <sup>2</sup> =0.015, p=0.671	R <sup>2</sup> =0.100, p=0.249	
% time under pronation	slow myosin fibers%	$R^2 = 0.005 \text{ p} = 0.799$	$R^2$ 0.08 n=0.288	
	fast myosin fibers	K = 0.005, p = 0.755	K =-0.00, p=0.200	
	%	R <sup>2</sup> =-0.015, p=0.672	R <sup>2</sup> =0.046, p=0.441	
	n° of fibers	R <sup>2</sup> =-0.218, p=0.092	R <sup>2</sup> =-0.263, p=0.060	
	CSA	R <sup>2</sup> =-0.24, p=0.075	R <sup>2</sup> =-0.031, p=0.529	
anu	Perimeter	R <sup>2</sup> =-0.247, p=0.07	R <sup>2</sup> =-0.010, p=0.710	
	Diameter	R <sup>2</sup> =-0.257, p=0.064	R <sup>2</sup> =-0.014, p=0.664	
	Fraction	R <sup>2</sup> =0.096, p=0.280	R <sup>2</sup> =0.439, p=0.007	
	slow myosin fibers%	$R^2 = -0.014$ , p=0.682	$R^2 = -0.034$ , p=0.508	
	fast myosin fibers			
% time under sedation and	%	R <sup>2</sup> =-0.429, p=0.011	R <sup>2</sup> =0.248, p=0.058	
	n° of fibers	R <sup>2</sup> =0.023, p=0.599	R <sup>2</sup> =-01.58, p=0.142	
	CSA	R <sup>2</sup> =-0.127, p=0.21	R <sup>2</sup> =0.001, p=0.946	
	Perimeter	R <sup>2</sup> =-0.144, p=0.180	R <sup>2</sup> =0.003, p=0.825	
	Diameter	R <sup>2</sup> =0.125, p=0.213	R <sup>2</sup> =0.001, p=0.890	
	Fraction	R <sup>2</sup> =0.037, p=0.504	R <sup>2</sup> =0.06, p=0.378	
% time under curarization and	slow myosin fibers%	$R^2$ =-0.01, p=0.732	R <sup>2</sup> =-0.184, p=0.11	
	fast myosin fibers		, , , , , , , , , , , , , , , , , , ,	
	%	R <sup>2</sup> =-0.004, p=0.824	R <sup>2</sup> =0.001, p=0.932	
	n° of fibers	R <sup>2</sup> =-0.077, p=0.333	R <sup>2</sup> =0.039, p=0.479	
	CSA	R <sup>2</sup> =-0.078, p=0.330	R <sup>2</sup> =0.091, p=0.272	
	Perimeter	$R^2$ =-0.071, p=0.354	R <sup>2</sup> =0.118, p=0.208	

Supplementary table 2. Correlations between ventilatory parameters and anatomopathological findings.



	Diameter	R <sup>2</sup> =-0.06, p=0.397	R <sup>2</sup> =0.129, p=0.187
	Fraction	R <sup>2</sup> =0.028, p=0.563	R <sup>2</sup> =-0.031, p=0.526
	slow myosin fibers%	R <sup>2</sup> =-0.005, p=0.799	R <sup>2</sup> =0.026, p=0.565
Tidal	fast myosin fibers %	R <sup>2</sup> =-0.37, p=0.022	R <sup>2</sup> =0.312, p=0.046
volume	n° of fibers	R <sup>2</sup> =0.016, p=0.659	R <sup>2</sup> =0.008, p=0.74
(based on IBW) and	CSA	R <sup>2</sup> =0.013, p=0.691	R <sup>2</sup> =-0.001, p=0.956
	Perimeter	R <sup>2</sup> =0.022, p=0.611	R <sup>2</sup> =0.004, p=0.822
	Diameter	R <sup>2</sup> =0.028, p=0.566	R <sup>2</sup> =0.002, p=0.851
	Fraction	R <sup>2</sup> =0.057, p=0.41	R <sup>2</sup> =0.065, p=0.358
	slow myosin fibers%	R <sup>2</sup> =0.009, p=0.974	R <sup>2</sup> =-0.088, p=0.282
	fast myosin fibers %	R <sup>2</sup> =0.010, p=0.743	R <sup>2</sup> =0.054, p=0.40
PEEP and	n° of fibers	R <sup>2</sup> =-0.038, p=0.50	R <sup>2</sup> =0.054, p=0.40
	CSA	R <sup>2</sup> =0.020, p=0.623	R <sup>2</sup> =0.027, p=0.558
	Perimeter	R <sup>2</sup> =0.028, p=0.499	R <sup>2</sup> =0.028, p=0.544
	Diameter	R <sup>2</sup> =0.062, p=0.387	R <sup>2</sup> =0.025, p=0.57
	Fraction	R <sup>2</sup> =-0.034, p=0.522	R <sup>2</sup> =0.001, p=0.952
	slow myosin fibers%	R <sup>2</sup> =-0.089, p=0.344	R <sup>2</sup> =0.012, p=0.688
PaO2/FiO2 and	fast myosin fibers %	R <sup>2</sup> =0.016, p=0.695	R <sup>2</sup> =0.001, p=0.936
	n° of fibers	R <sup>2</sup> =0.514, p=0.004	R <sup>2</sup> =-0.007, p=0.766
	CSA	R <sup>2</sup> =-0.005, p=0.825	R <sup>2</sup> =0.016, p=0.644
	Perimeter	R <sup>2</sup> =-0.004, p=0.834	R <sup>2</sup> =0.006, p=0.779
	Diameter	R <sup>2</sup> =0.001, p=0.973	R <sup>2</sup> =0.002, p=0.859
	Fraction	R <sup>2</sup> =-0.153, p=0.208	R <sup>2</sup> =0.051, p=0.414
	slow myosin fibers%	R <sup>2</sup> =-0.031, p=0.623	R <sup>2</sup> =-0.086, p=0.288
MP and	fast myosin fibers %	R <sup>2</sup> =-0.459, p=0.031	R <sup>2</sup> =0.219, p=0.078
	n° of fibers	R <sup>2</sup> =-0.739, p=0.414	R <sup>2</sup> =-0.013, p=0.676
	CSA	R <sup>2</sup> =0.003, p=0.866	R <sup>2</sup> =0.110, p=0.225
	Perimeter	R <sup>2</sup> =0.008, p=0.795	R <sup>2</sup> =0.146, p=0.158
	Diameter	R <sup>2</sup> =0.089, p=0.771	R <sup>2</sup> =0.155, p=0.145
	Fraction	$R^2$ =-0.021, p=0.685	R <sup>2</sup> =-0.013, p=0.677

CSA, cross sectional area; IBW, ideal body weight; PEEP, positive end expiratory pressure; MP, mechanical power.

