Combined diaphragm and limb muscle atrophy is associated with increased mortality in mechanically ventilated patients: a pilot study.

Supplementary material

Methods

Study Design and Subjects. This observational, prospective, single-center study was approved by the Maciel Hospital's Research Ethics Committee. All included subjects or their relatives provided written informed consent. The study was conducted between August 2016 and November 2018 in an 18-bed medical and surgical ICU (Hospital Maciel, Montevideo, Uruguay). Subjects were eligible for enrollment if they had received invasive mechanical ventilation for less than 24 hours. Subjects were excluded if one of the following criteria were present: age of less than 18 years, expected time under mechanical ventilation of less than 72 hours or history of neuromuscular disease.

Demographic, clinical and therapeutic data were collected, including sex, age, diagnosis, Simplified Acute Physiology Score III (SAPS III), sepsis, use of vasopressors, neuromuscular blocking agents, corticosteroids or aminoglycosides antibiotics, duration of mechanical ventilation, duration of ICU and hospital stay.[1]

Diaphragm thickness. Diaphragm thickness was measured by ultrasound, using a previously validated technique.[2, 3] With subjects in a semi-recumbent position (30º) a 13-MHz linear probe (LOGIQ e, GE Healthcare, Milwakee, WI)

was placed between the right anterior and mid-axillary lines, over the 8th – 10th intercostal space, perpendicular to the rib cage. At this site, the right hemi-diaphragm was identified as the relatively non-echogenic muscular layer bound by two echogenic membranes (peritoneum and parietal pleura, Figure 1A). After identifying the diaphragm, measurements were performed 2 cm below the costo-phrenic sinus at end-expiration. Diaphragm thickness was calculated as the distance between the inner edges of the echogenic membranes.

Peripheral muscle thickness. Peripheral muscle thickness was determined on right limbs, with the patient in the same position (30º). Muscle thickness was measured at the mid-upper arm anteriorly (midway between the acromion and the olecranon, i.e. biceps muscle), mid-forearm (midway between the antecubital skin crease and ulnar styolid) and mid-thigh (midway between the greater trochanter and the joint line of the knee, i.e. quadriceps muscle) as previously described.[4] Using the same 13-MHz linear probe, muscle thickness was measured as the distance between the superficial fat-muscle interface and the bone or interosseous membrane (Figure 1B-D).

The first diaphragm and peripheral muscle ultrasound measurement was performed within 24 hours after the initiation of mechanical ventilation, and repeated on days three and seven, unless the patient was discharged from ICU or died. At each time-point, measurements were repeated three times and the mean value was recorded. The same investigator performed all the ultrasound

examinations; intraobserver reproducibility assessed by the intraclass correlation coefficient (95% confidence intervals) was 0.996 (0.989-0.999).

Definitions. Diaphragmatic atrophy was defined as a decrease in diaphragm thickness of at least 5% from baseline. Limb muscle atrophy was considered to be present when any of the peripheral muscles had a decrease in thickness of at least 5% from baseline. Subjects associating diaphragmatic and limb muscle atrophy were considered to present combined muscle atrophy.

Statistical analysis. Categorical variables were reported as absolute numbers (percentage) and compared using Chi-square test or Fisher exact test, as appropriate. Continuous variables were expressed as mean ± standard deviation if normally distributed, or median (25^{th}, 75^{th} percentile) if not. Student *t* test was used to compare initial muscle thickness between groups. Mann-Whitney *U* test was performed to compare muscle thickness change from baseline between atrophy and no-atrophy groups at each time point. The Spearman correlation was used to analyze bivariate correlations. A *P* value of less than 0.05 was considered statistically significant.

Supplementary Figure Legends

Supplementary Figure 1. Representative ultrasound images used to measure muscle thickness of diaphragm (A), mid-upper arm (B), mid-forearm (C) and mid-thigh (D). Red lines represent muscle thickness measurements. Diaphragm was measured between the pleura and peritoneum. Limb muscle thickness was measured between the superficial fat-muscle interface and the corresponding bone or interosseous membrane.

Supplementary Figure 2. Baseline muscle thickness in patients that developed muscle atrophy or not. Baseline diaphragm thickness was compared between patients developing diaphragm atrophy or not (A). Baseline arm (B), forearm (C) and thigh (D) muscle thickness was compared between patients that developed limb muscle atrophy and those who did not. Data is presented as mean and standard deviation.

Supplementary Figure 3. Baseline diaphragm (A), arm (B), forearm (C) and thigh (D) muscle thickness in survivors and non-survivors. Data is presented as mean and standard deviation.

References

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Supplementary Table 1. Main patients' characteristics.

Variable	N = 32
Gender (men/women)	23/9
Age (years)	59 (32, 71)
SAPS III, mean ± SD	62 ± 16
Reason for ICU admission	
Sepsis	13 (40.6)
Neurologic dysfunction	10 (31.2)
Trauma	3 (9.4)
Cardiovascular dysfunction	3 (9.4)
Other	3 (9.4)
Medication exposure	
Aminoglycoside antibiotics	5 (15.6)
Norepinephrine	17 (53.1)
Neuromuscular blocking agents	2 (6.3)
Corticosteroids	7 (21.9)
Maximum glycemia (mg/dL)	137 (114, 180)
Days on mechanical ventilation	11 (5, 13)
ICU length of stay (days)	14 (8, 21)
Hospital length of stay (days)	21 (9, 37)
ICU mortality	8 (25)

Data are presented as n (%) for categorical variables and median (25^{th}, 75^{th} percentile) for continuous variables, unless otherwise indicated.

Supplementary Table 2. Main characteristics of patients with and without diaphragm atrophy.

Variable	No diaphragmatic atrophy N = 9	Diaphragmatic atrophy N = 20	*P* value
Gender (men/women)	4/5	4/16	0.209
Age (years)	41 (27, 73)	59 (33, 68)	0.429
SAPS III, mean ± SD	61 ± 17	61 ± 15	0.976
Reason for ICU admission			0.773
Sepsis	4 (44.4)	6 (30)	
Neurologic dysfunction	3 (33.3)	7 (35)	
Trauma	1 (11.1)	2 (10)	
Cardiovascular dysfunction	0 (0)	3 (15)	
Other	1 (11.1)	2 (10)	
Medication exposure			
Aminoglycoside antibiotics	0 (0)	5 (25)	0.131
Norepinephrine	4 (44.4)	10 (50)	0.550
Neuromuscular blocking agents	2 (22.2)	0 (0)	0.089
Corticosteroids	1 (11.1)	3 (15.0)	0.636
Maximum glycemia (mg/dL)	122 (110, 205)	154 (116, 172)	0.979
Days on mechanical ventilation	6 (2, 13)	12 (6, 15)	0.167
ICU length of stay (days)	11 (7, 27)	17 (7, 21)	0.871
Hospital length of stay (days)	12 (5, 36)	22 (9, 38)	0.562
ICU mortality	1 (11.1)	6 (30.0)	0.273

Data are presented as n (%) for categorical variables and median (25^{th}, 75^{th} percentile) for continuous variables, unless otherwise indicated.

In three patients the evolution of diaphragm thickness could not be determined because of technical difficulties. Therefore the comparison between patients with or without diaphragm atrophy was performed including 29 patients.

Supplementary Table 3. Main characteristics of patients with and without limb muscle atrophy.

Variable	No limb muscle atrophy N = 12	Limb muscle atrophy N = 19	*P* value
Gender (men/women)	9/3	13/6	0.999
Age (years)	64 (32, 75)	58 (31, 69)	0.524
SAPS III, mean ± SD	68 ± 13	59 ± 17	0.138
Reason for ICU admission			0.267
Sepsis	6 (50.0)	7 (36.8)	
Neurologic dysfunction	5 (41.7)	4 (21.1)	
Trauma	0 (0)	3 (15.8)	
Cardiovascular dysfunction	1 (8.3)	2 (10.5)	
Other	0 (0)	3 (15.8)	
Medication exposure			
Aminoglycoside antibiotics	1 (8.3)	4 (21.1)	0.342
Norepinephrine	6 (50.0)	10 (52.6)	0.589
Neuromuscular blocking agents	1 (8.3)	1 (5.3)	0.632
Corticosteroids	2 (16.7)	5 (26.3)	0.435
Maximum glycemia (mg/dL)	120 (95, 175)	163 (121, 181)	0.211
Days on mechanical ventilation	10 (4, 15)	11 (6, 13)	0.795
ICU length of stay (days)	15 (8, 21)	14 (8, 23)	0.617
Hospital length of stay (days)	19 (2, 35)	25 (11, 38)	0.287
ICU mortality	1 (8.3)	6 (31.6)	0.143

Data are presented as n (%) for categorical variables and median (25^{th}, 75^{th} percentile) for continuous variables, unless otherwise indicated.

In one patient the evolution of peripheral muscle thickness could not be determined because of technical difficulties. Therefore the comparison between patients with or without limb muscle atrophy was performed including 31 patients.

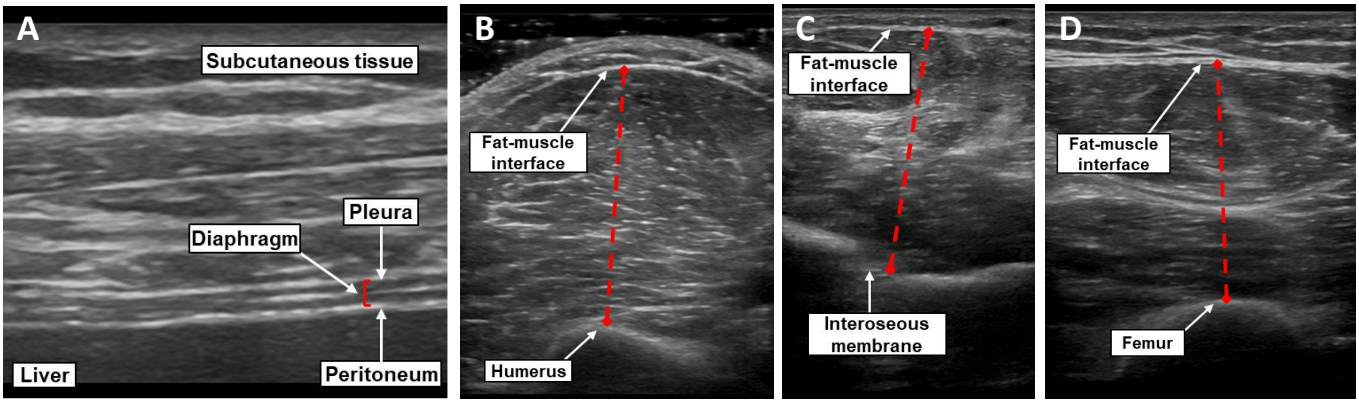
Supplementary Table 4. Main characteristics of patients with and without combined muscle atrophy.

Variable	No combined muscle atrophy N = 16	Combined muscle atrophy N = 13	*P* value
Gender (men/women)	12/4	9/4	0.526
Age (years)	55 (30, 73)	59 (33, 68)	0.705
SAPS III, mean ± SD	63 ± 15	60 ± 18	0.657
Reason for ICU admission			0.638
Sepsis	7 (43.8)	4 (30.8)	
Neurologic dysfunction	6 (37.5)	3 (23.1)	
Trauma	1 (6.3)	2 (15.4)	
Cardiovascular dysfunction	1 (6.3)	2 (15.4)	
Other	1 (6.3)	2 (15.4)	
Medication exposure			
Aminoglycoside antibiotics	1 (6.3)	4 (30.8)	0.107
Norepinephrine	7 (43.8)	7 (53.8)	0.434
Neuromuscular blocking agents	2 (12.5)	0 (0)	0.296
Corticosteroids	2 (12.5)	3 (23.1)	0.396
Maximum glycemia (mg/dL)	118 (98, 144)	168 (133, 181)	0.083
Days on mechanical ventilation	9 (4, 14)	11 (8, 15)	0.329
ICU length of stay (days)	15 (7, 23)	17 (9, 21)	0.650
Hospital length of stay (days)	19 (9, 36)	25 (11, 41)	0.398
ICU mortality	1 (6.3)	5 (38.5)	0.047

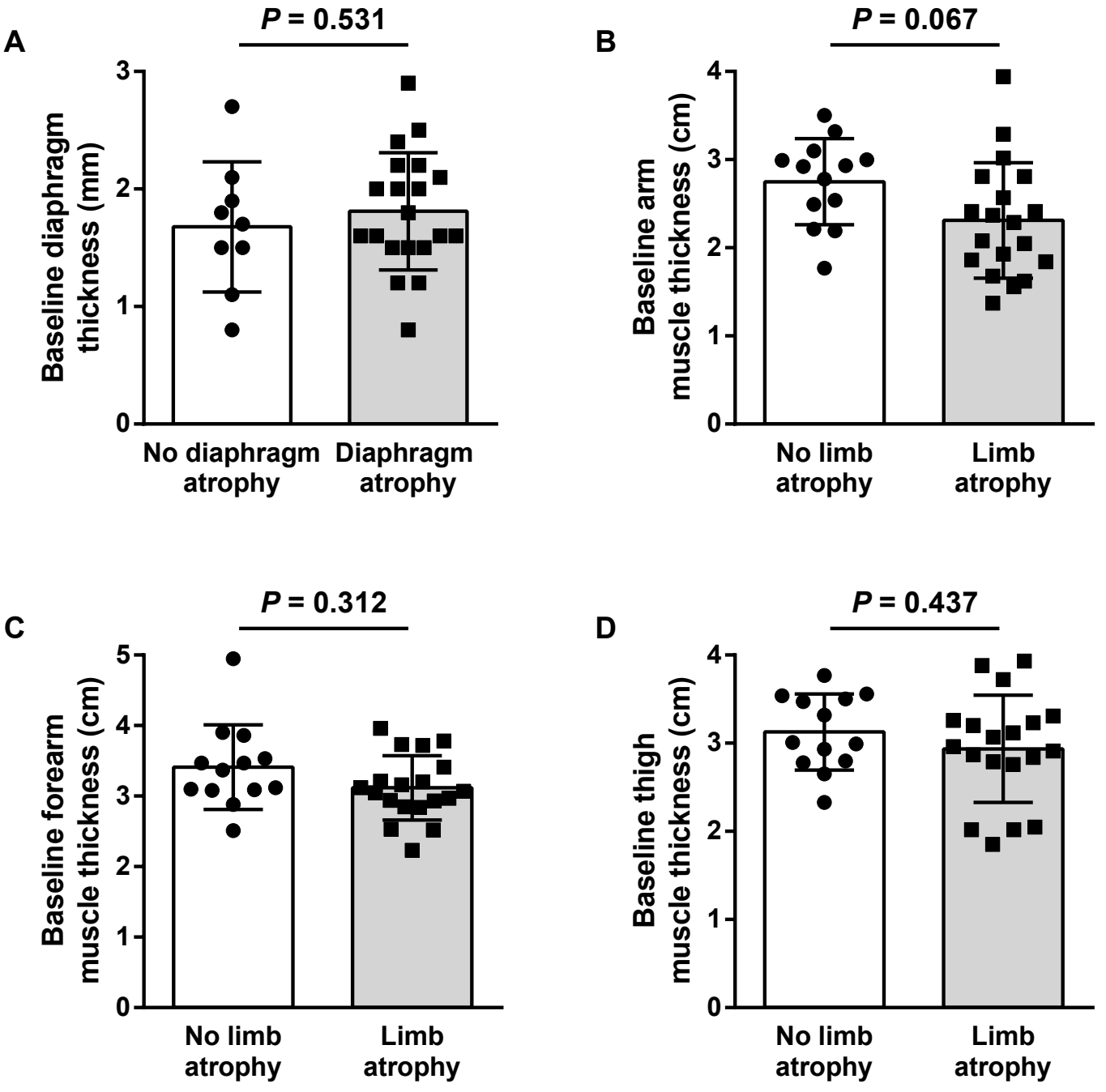
Data are presented as n (%) for categorical variables and median (25^{th}, 75^{th} percentile) for continuous variables, unless otherwise indicated.

The comparison between patients with or without combined muscle atrophy was performed including the 29 patients in which the evolution of both diaphragm and limb muscle thickness could be determined.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3