Shear Wave Elastography, a New Tool for Diaphragmatic Qualitative Assessment A Translational Study

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A Translational Study

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Abstract

Rationale: Prolonged mechanical ventilation is often associated with either a decrease (known atrophy) or an increase (supposed injury) in diaphragmatic thickness. Shear wave elastography is a noninvasive technique that measures shear modulus, a surrogate of tissue stiffness and mechanical properties.

Objectives: To describe changes in shear modulus (SM) during the ICU stay and the relationship with alterations in muscle thickness. To perform a comprehensive ultrasound-based characterization of histological and force production changes occurring in the diaphragm.

Methods: Translational study using critically ill patients and mechanically ventilated piglets. Serial ultrasound examination of the diaphragm collecting thickness and SM was performed in both patients and piglets. Transdiaphragmatic pressure and diaphragmatic biopsies were collected in piglets.

Measurements and Main Results: We enrolled 102 patients, 88 of whom were invasively mechanically ventilated. At baseline, SM was 14.3 $\pm$ 4.3 kPa and diaphragm end-expiratory thickness was 2.0 $\pm$ 0.5 mm. Decrease or increase by more than 10% from baseline was reported in 86% of the patients for thickness and in 92% of the patients for SM. An increase in diaphragmatic thickness during the stay was associated with a decrease in SM ($\beta = -9.34 \pm 4.41$; $P = 0.03$) after multivariable analysis. In the piglet sample, a decrease in SM over 3 days of mechanical ventilation was associated with loss of force production, slow and fast fiber atrophy, and increased lipid droplets accumulation.

Conclusions: Increases in diaphragm thickness during critical illness is associated with decreased tissue stiffness as demonstrated by shear wave ultrasound elastography, consistent with the development of muscle injury and weakness.

Clinical trial registered with www.clinicaltrials.gov (NCT03550222).

Keywords: diaphragmatic dysfunction; diaphragmatic ultrasonography; mechanical ventilation; transdiaphragmatic pressure

*These authors contributed equally to this work.

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Author Contributions: A.F. and Y.A. performed all the ultrasound measurements, analyzed the results, and participated in the manuscript editing. F.G. and D.C. contributed to the study design and patient enrollments. M.C. performed all transdiaphragmatic pressure measurements and contributed to the piglet model. F.R. performed all histological analyses. K.K. contributed to the study design. A.L., P.E., and S.M. reviewed some of the measurements and participated in the analysis results. S.J. contributed to the study design, analysis results, and manuscript editing. N.M. and L.G. performed statistical analysis. B.J. designed the study, participated in manuscript editing, and approved its final version.

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Scientific Knowledge on the Subject: Diaphragm weakness is associated with poor outcomes in critically ill patients. Both decrease (atrophy) and increase (supposed injury) in diaphragmatic thickness are associated with prolonged ventilation. Shear wave ultrasound elastography provides additional information about muscle quality at rest by measuring shear modulus, a quantifiable measurement of tissue stiffness. Diaphragm mechanical properties, assessed by shear wave ultrasound elastography, have never been investigated in the critically ill population.

What This Study Adds to the Field: Our results show that the increase in diaphragmatic thickness in one-third of patients during their ICU stay is associated with a decrease in diaphragmatic shear modulus. Injurious changes in muscle, including muscle fiber atrophy and weakness, are associated with a decrease in shear modulus. These results suggest that shear modulus can detect an alteration in diaphragmatic mechanical properties and are in line with the diaphragmatic myotrauma concept.

Diaphragmatic weakness occurs in 30–60% of critically ill patients (1, 2). It can preexist ICU admission (3) or can appear during the ICU stay (4–6). Prolonged mechanical ventilation (MV) duration (6), increased incidence of weaning failure (3, 5, 6), prolonged ICU stay (3, 7), and long-term mortality (8) are associated with diaphragmatic weakness. MV and sepsis are the main risk factors (6, 9–11), and human biopsies have shown that diaphragmatic weakness is associated with muscle fiber lipid accumulation, inflammatory infiltrate, sarcomere injury, autophagy, and fiber atrophy (4, 12–14).

Ultrasonography is a user-friendly, noninvasive tool to explore the diaphragm at the bedside. Ultrasonography has been used to explore diaphragmatic contractile activity by measuring its excursion, thickening fraction, the velocity of diaphragmatic muscle motion (tissue Doppler imaging), and diaphragm mass using its thickness as a surrogate (15–19). In a landmark study, Goligher and colleagues reported that both diaphragmatic decrease of thickness and increase of thickness are associated with poor outcomes (20, 21). The authors then developed the "myotrauma" concept in which abnormal diaphragm function is associated with either overassistance (disuse atrophy) or underassistance myotrauma (muscle overuse leading to inflammation, edema, and injury) (22).

Shear wave ultrasound elastography (SWE) allows direct and real-time quantification of the mechanical properties of tissues and additional information about muscle quality at rest (23). Briefly, tissue deformation is created by an intrinsic focused push beam generated by the ultrasound probe. This results in the propagation of shear waves parallel to the surface of the probe. The velocity of these shear waves varies with mechanical properties of tissues (i.e., stiffness). Stiffness is then quantified by shear modulus (SM), which is calculated through shear wave propagation ($SM = \rho.v2$, in which ρ is the tissue density, equal to 1,000 kg/m^3 in the human body, and v is the shear wave velocity [m^2s^{-2}]) (24). By using inversion algorithms, this method maps the created wave velocity into elastograms and determines stiffness of the tissue by measuring the SM (25). A higher SM value corresponds to higher tissue stiffness. SWE provides a spatial representation and quantifiable measurement of the mechanical properties of the tissue (23). Although shear wave speed only depends on stiffness for large organs such as breast or liver, for thin structures such as the diaphragm, the shear wave travels approximately 50% faster when the structure's thickness doubles (26). Therefore, if the SWE decreases while thickness increases, it strongly suggests that the muscle's stiffness is decreased. Most of the available SWE studies have been published in the fields of limb muscles and tendons in chronic myopathy and in sports medicine (23, 27). Recently, two studies have evaluated SWE during diaphragmatic contraction and have suggested that SM can be a surrogate for diaphragm contractile activity in healthy volunteers (28, 29). Diaphragmatic SWE exploration is feasible in the critically ill population (30) with a previously reported reference value in this specific population at admission of 13.7 kPa (±4.4) (31), and healthy subjects' values at rest ranging from 6.2 kPa (±3.6) to 12 kPa (±2) (29, 30). Fossé and colleagues investigated changes in SM during diaphragm contraction in mechanically ventilated patients as a potential surrogate to transdiaphragmatic pressure (Pdi) (31). Changes in diaphragm SM at rest over time have never been investigated in the critically ill population. We hypothesized that change in SM, either higher or lower, may help to better describe the histological changes occurring in diaphragm muscle during critical illness (32). A drop in stiffness could reflect muscle fiber atrophy (33) or a combination of muscle edema, inflammatory or lipid accumulation, and fiber atrophy (34). An increase in SM could be due to exacerbated muscle fibrosis as in late-stage Duchenne's myopathy (35) or cerebral palsy (36).

The aim of this translational study was first to describe diaphragm thickness and SM changes during the ICU stay and then to explore the relationship between ultrasound measurements, diaphragmatic histology, and force production using diaphragm biopsies in a mechanically ventilated piglet model. Results were partially presented at the American Thoracic Society Annual Meeting (Dallas, Texas, May 17–22, 2019) (37).

Methods

Human Study Population and Setting

This prospective observational study was conducted in the medical ICU at the University Hospital of Montpellier, France, from December 2017 to June 2018 and included all consecutive patients with at least one organ failure (defined by the Sepsis-related Organ Failure Assessment score) (38) (*see* online supplement for details). Patients were included within 24 hours of admission regardless of the need for MV. Images in B mode for thickness and SWE mode for SM were obtained at baseline (Day 0), and repetitively every other day up to Day 28, discharge from the ICU, or death. Ultrasound diaphragm thickness may increase or decrease according to the combination of changes in all its components including the muscle fibers but also connective tissue, the vessels, inflammatory or lipid accumulation, and muscle edema. Institutional ethical approval was obtained (2017-CLER-MTP-09-16). We followed the STROBE guidelines for observational studies (39).

Shear Wave Elastography Imaging Procedure

In biomechanics, stiffness is defined by the proportional relationship between stress (the external force or compression) and strain (deformation) applied to it. Transmission of a longitudinal pulse leads to tissue displacement, which is detected by pulse echo ultrasound, and allows for the measurement of shear wave velocity (V in $m \cdot s^{-1}$) in the tissue. Shear wave velocity is proportional to SM (also named μ and expressed in kPa) using the formula: $\mu = \rho.v^2$ (in which ρ is the tissue density, equal to 1,000 kg/m^3 in the human body). Hard tissues have a higher SM and V than soft tissues (24).

An Aixplorer ultrasonic scanner (SuperSonic Imagine) was used with a 4- to 15-MHz linear transducer (SL15-4; SuperSonic Imagine) in both B and SWE modes with musculoskeletal preset. All images and measurements were performed by two operators (Y.A. and A.F.) according to a standardized and reproducible protocol (30) (*see* Methods, Figure E1, and Video E1 in the online supplement).

Patients were assessed in supine position, half sitting at 30°. Conscious subjects were instructed to remain relaxed and to breathe as calmly as possible throughout the procedure. We performed all measurements in triplicate on the right hemidiaphragm. Because contractile activity increases SM, we performed all the measurements at the end of expiration when the diaphragm is at rest. Conscious subjects were instructed to remain relaxed, to breathe as calmly as possible throughout the procedure, and to remain apneic at FRC for SWE acquisition as long as possible. For mechanically ventilated patients, an end-expiratory pause was applied during SWE acquisition. When a stable image with good quality criteria (homogeneous SWE color map) was not possible to collect, owing to high respiratory rate for example, the measured SM was not used for data analysis. Proofreading of the images was done offline and blinded by the two operators (A.F. and Y.A.). To enable replication of image location on repeated ultrasound assessments, a mark was carefully drawn on the subject's chest during the first measurement procedure and a standardized protocol was applied.

During image acquisition, transducers were placed with minimal compression on top of a generous amount of at least 5 mm of coupling gel to avoid distortion of the underlying tissue (40). The transducer was placed via an intercostal approach at the zone of apposition, in the sagittal plane perpendicular to the diaphragm muscle, and between the right anterior and midaxillary lines. The diaphragm was identified as a three-layered structure comprising two hyperechoic lines (pleural and peritoneal membranes) and a middle hypoechoic layer (diaphragm muscle fibers). Subsequently, the probe was oriented approximately 90° and finely adjusted to obtain a longitudinal view with the probe (and acoustic beam) parallel to the direction of the diaphragm muscle fascicles, and to obtain maximal echo intensity from diaphragm membranes. When activating the SWE mode, a color-coded box representing the region of interest was superimposed on the image. The widest area possible was drawn in the most homogeneous zone of the region of interest using built-in software of the ultrasound system (Q-Box trace) to avoid measurement bias, and SM was collected (Figure E1 and Video E1).

All acquired images and data were stored and secondarily analyzed as recommended by Nijholt and colleagues using Osirix DICOM Viewer software (Pixmeo) (41). The whole procedure, with image acquisition in B-mode frames and SM elastograms in triplicate, required 4–5 minutes at the patient's bedside

Mechanically Ventilated Piglet Studies

After approval by the University of Montpellier Institutional Ethics Committee for Animal Research and Care and according to the recommendations of the Helsinki Declaration, we conducted a piglet study to describe the association between diaphragm thickness and SM. Twelve piglets (25–35 kg) were sedated and mechanically ventilated in volume-controlled mode for 3 days as previously described (42). The detailed protocol is provided in the electronic supplement. Briefly, we used Pdi as the reference method for the assessment of diaphragm function (1). Pdi was calculated from esophageal and gastric pressure measurements. Pressure–frequency curves (Day 0–3) were measured daily by stimulating diaphragm contractions at end-expiration and using transvenous pacing catheters to achieve phrenic nerve stimulation with frequencies ranging from 20 to 100 Hz in a serial manner (43).

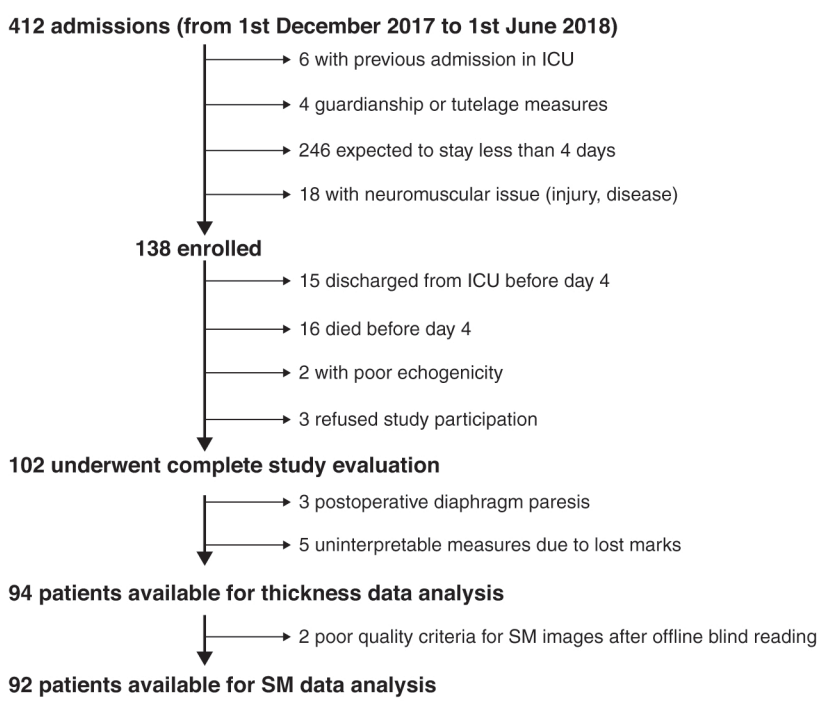

Figure 1. Study flow chart of critically ill patients. This figure shows the flow chart of patients admitted to our unit during the study. Consecutive patients with at least one organ failure and an expected duration of ICU stay ⩾3 days were enrolled. Of 412 adults, 138 were enrolled, 36 were excluded, and 102 completed the study evaluation. Ninety-four patients were available for analysis with at least three diaphragm evaluations. SM = shear modulus.

Diaphragm ultrasound-acquired images for thickness and SM measurement were recorded daily at the end of expiration when the diaphragm was at rest for all 12 piglets. Finally, diaphragm biopsies were obtained before euthanasia at Day 3 for 10 piglets (*see* Methods in the online supplement). Briefly, segments of dissected diaphragm strips were either immediately fixed for electronic microscopy examination and the quantification of intramuscular lipid droplets accumulation, or stained with hematoxylin/eosin for inflammatory cells infiltration and fiber types determination. Computer images captured from randomly selected microscopic fields were analyzed.

Endpoints

The two coprimary endpoints were to describe the evolution of diaphragm SM during the ICU stay and the association between diaphragm SM (as a surrogate for diaphragm stiffness) and thickness. Diaphragm thickness is a surrogate for the whole diaphragm muscle mass including the muscle fibers but also connective tissue, the vessels, inflammatory or lipid accumulation, and muscle edema.

The exploratory endpoints were to describe the association between diaphragm SM and the patients' clinical outcomes, and between diaphragm SM, fiber size, muscle inflammatory cells infiltration, muscular

Table 1. Demographics, Clinical Characteristics, and Outcomes of the Study Population

Characteristics	Study Population (*n* = 92)	Change in Diaphragm Shear Modulus during the First Week			*P* Value
		>10% Increase (*n* = 47)	<10% Change (*n* = 7)	>10% Decrease (*n* = 38)	
Age, yr	60 ± 17	62 ± 17	59 ± 16	55 ± 17	0.46
Sex, M	63 (70)	34 (72)	3 (43)	26 (72)	0.29
Body mass index, kg/m^2	26 ± 16	24 ± 5	24 ± 7	24 ± 6	0.64
SAPS II	58 ± 19	62 ± 20	50 ± 10	55 ± 18	0.11
SOFA score at admission	8 (5–11)	9 (5–11)	6 (5–6)	8 (6–11)	0.22
Comorbidities					
COPD	16 (17)	9 (19)	1 (14)	6 (16)	0.90
Ischemic heart disease	11 (12)	5 (11)	0 (0)	2 (5)	0.46
Chronic heart failure	17 (19)	5 (11)	0 (0)	12 (32)	0.02
Diabetes mellitus	20 (22)	7 (15)	1 (14)	12 (32)	0.16
Chronic kidney disease	13 (14)	9 (19)	1 (14)	3 (8)	0.33
Cancer	31 (34)	13 (28)	2 (29)	16 (42)	0.36
Cirrhosis	5 (5)	1 (2)	2 (29)	2 (5)	0.02
Inflammatory disease	7 (8)	5 (11)	0 (0)	2 (5)	0.47
Chronic corticosteroid treatment	21 (23)	13 (28)	1 (14)	7 (18)	0.20
Malnutrition	22 (24)	9 (19)	2 (29)	11 (29)	0.35
Sepsis-3 criteria within first week	72 (78)	35 (74)	6 (86)	31 (82)	0.87
Primary reason for admission					
Respiratory distress	52 (56)	27 (57)	4 (57)	21 (55)	0.98
Cardiac arrest	9 (10)	4 (8)	1 (14)	4 (11)	0.87
Acute heart failure	3 (3)	2 (4)	0	1 (3)	0.41
Coma – Encephalopathy	10 (11)	5 (11)	2 (29)	3 (8)	0.27
Sepsis	12 (13)	5 (11)	0	7 (18)	0.32
Other	6 (7)	4 (9)	0	2 (5)	0.64
Clinical management during ICU stay					
Use of NMBA	55 (60)	28 (60)	6 (86)	21 (55)	0.32
Duration of NMBA infusion, d*	3 (2–5.5)	3 (2–4.5)	8 (6–18)	3 (2–6)	0.30
Use of corticosteroids	51 (55)	32 (68)	3 (43)	16 (42)	0.35
Duration of corticosteroid infusion, d*	8 (4–12.3)	8 (3.8–11)	8 (4–12.5)	5 (4.3–8)	0.62
Use of aminoglycosides	29 (32)	14 (30)	0 (0)	15 (39)	0.11
Use of sedatives	79 (86)	40 (85)	6 (86)	33 (87)	0.50
Duration of deep sedation, d*	5 (3–9)	5.5 (2.3–8)	8 (4–8.5)	4 (3–9)	0.41
Duration of invasive MV, d*	7 (4–15)	7.2 (2.8–13.5)	8 (7.4–18)	7 (4–15.2)	0.41
Duration of CMV, d*	2.8 (1.1–5.7)	2.7 (0.9–5.4)	4.4 (3.3–6)	2.8 (1.6–5.6)	0.41
No need for ventilation	13 (14)	7 (15)	1 (14)	5 (13)	0.38
Need for vasopressors	78 (85)	41 (87)	4 (57)	33 (87)	0.11
Duration of vasopressor infusion, d*	4 (3–7)	4 (3–6)	4 (2.8–7.8)	4 (3–7)	0.41
Need for dialysis	24 (26)	14 (30)	1 (14)	9 (24)	0.62
Duration of dialysis, d*	6 (3–8.5)	7 (4–12.5)	1 (1–1)	4 (3–7)	0.28
Cumulative fluid balance at Day 3, L	4.7 ± 4.4	4.4 ± 4.7	3.7 ± 2.6	5.2 ± 4.3	0.83
Cumulative fluid balance at Day 7, L	5.5 ± 5.4	5.1 ± 5.7	4.4 ± 4.9	6.1 ± 5.3	0.81

Definition of abbreviations: CMV = controlled mechanical ventilation; COPD = chronic obstructive pulmonary disease; MV = mechanical ventilation; NMBA = neuromuscular blockade; SAPS II = Simplified Acute Physiology Score; SOFA = Sequential Organ Failure Assessment.
Data are presented as *n* (%), mean ± SD, or median (interquartile range). Two out of 94 patients could not have shear modulus measurements and were therefore removed from this table.
*Results are presented for patients who received the mentioned therapy.

lipid droplets accumulation, and force production (measured by Pdi) in mechanically ventilated piglets.

Statistical Analysis

The study population was divided into three groups (increase, decrease, or stable) based on the overall change in diaphragm thickness (thickness groups) or SM (SWE groups), from the baseline measurement to the last measurement obtained during the first week in the ICU using a 10% cutoff value (20). This cutoff was chosen because it was clinically relevant according to previous studies about SWE in limb muscles (23, 35) and was in agreement with the measurement of variance identified in our previous study (30).

Descriptive statistics are reported as numbers (%), means ± SD, or medians (interquartile range) and compared as appropriate. A mixed linear regression was used for the analysis. Repeated measures correlation coefficient (R) was used for determining overall relationships between variables. Covariates were tested in the model (if $P < 0.15$ in the univariate analysis), selected in a backward selection procedure, and then presented as a regression coefficient with 95% confidence intervals. A nonlinear effect for continuous variables was tested using a polynomial model. Akaike information and Bayesian information criteria were used to assess the nonlinearity. For all final comparisons, a P value $\leqslant 0.05$ was considered statistically significant. Statistical analysis was conducted using SAS version 9.3 (SAS Institute).

Results

Demographics and Ultrasound Measurements at Baseline

Between December 2017 and June 2018, 102 critically ill patients were enrolled and at least three ultrasound evaluations of thickness and SM were respectively available for 94 and 92 of them (Figure 1). Demographic data and clinical characteristics are reported in Table 1. At Day 0, 67 patients (71%) were mechanically ventilated. The duration of MV was 7 (4–15) days, 2.8 (1.1–5.7) days being spent in controlled mode. We collected 637 and 631 ultrasound measurements for thickness and SM, respectively, after excluding 42 and 38 images for poor quality criteria. On ICU admission, diaphragm end-expiratory thickness was 2.0 ± 0.5 mm and SM was 14.3 ± 4.3 kPa. In the 12 mechanically ventilated piglets, baseline end-expiratory thickness and SM were 2.3 (2.1–2.5) mm and 16.4 ± 4.3 kPa, respectively.

Change in Diaphragm Thickness and Diaphragm SM Over Time

In critically ill patients overall, diaphragm thickness did not significantly change during the first week of the ICU stay (2.0 ± 0.5 mm on admission vs. 1.8 ± 0.4 mm at Day 7; $P = 0.24$). Diaphragm thickness change showed heterogeneous patterns in the critically ill patients. It remained unchanged in 13 patients (14%), decreased by more than 10% in 46 patients (49%), and increased by more than 10% in 35 patients (37%) (Figure E2). In critically ill patients, diaphragm SM remained unchanged in 7 patients (8%), decreased by more than 10% in 38 patients (41%), and increased by more than 10% in 47 (51%) patients (Figures 2 and E3). In multivariable analysis, diaphragm SM dropped over time in older patients ($P < 0.05$), in patients treated for sepsis ($P = 0.03$) or with steroids ($P = 0.01$), and in patients showing increased diaphragmatic thickness during the ICU stay ($P = 0.03$) (Table 2). Time spent under controlled MV was associated with a decrease in SM in comparison with time spent under pressure support ventilation or without ventilatory assistance ($P < 0.05$) (Table 2).

In piglets, diaphragm SM decreased between Day 0 and Day 3 (16.4 ± 4.3 kPa vs. 14.1 ± 3.1 kPa [$P = 0.07$]), when diaphragm thickness did not (2.3 ± 0.4 mm vs. 2.3 ± 0.4 mm, $P = 0.18$) (Figure 3A). When using the same 10% cutoff change in SM as in critically ill patients, SM remained unchanged in six piglets and decreased by more than 10% in six piglets. No correlation was found between absolute diaphragm thickness and SM values (Figure 3B).

SM, Muscle Fiber Size, Inflammatory Cells Infiltration, Lipid Accumulation, and Pdi

To better understand the drop in SM over time, we evaluated the relation between SM and diaphragm ultrastructure in mechanically ventilated healthy piglets for 3 days. A decrease in SM during the 3-day experiment was associated with a smaller fast- and slow-twitch fiber cross-sectional

Table 2. Factors Associated with Changes in Diaphragm Shear Modulus and Thickness during ICU Stay: Adjusted Multivariable Analysis

		Diaphragm Shear Modulus as a Quantitative Value		
Risk Factors	**(/ Reference)**	**β**	**SD**	***P* Value**
Sepsis	Yes / No	−1.79	0.82	0.03
Ventilation mode				<0.01
	Assisted / Controlled	1.45	0.40	
	No MV / Controlled	2.85	0.47	
Age		−0.05	0.02	<0.05
Days under deep sedation		0.14	0.07	0.05
Days on corticosteroids		−0.11	0.04	0.01
Diaphragm thickness		−8.92	4.46	0.03
		Diaphragm Thickness as a Quantitative Value		
Sex	M / F	0.03	<0.01	<0.01
Diaphragm shear modulus		<−0.01	<0.01	<0.01
Days of invasive MV		<−0.01	<0.01	<0.01

Definition of abbreviation: MV = mechanical ventilation.
Adjusted on day of measurement, and global number of days under MV. Deep sedation was defined as a Richmond agitation sedation scale of −3 to −5. Variables included in the model are those with $P < 0.15$. *See* Table E1 for thickness and Table E2 for shear modulus. Shear modulus model variables: sepsis, difficult weaning, ventilation mode, age, ventilation-free days, days under vasopressors, days under deep sedation, days on corticosteroids, and diaphragm thickness. Thickness model variables: sex, ventilation mode, diaphragm shear modulus, days under deep sedation, days of invasive MV, and septic shock.

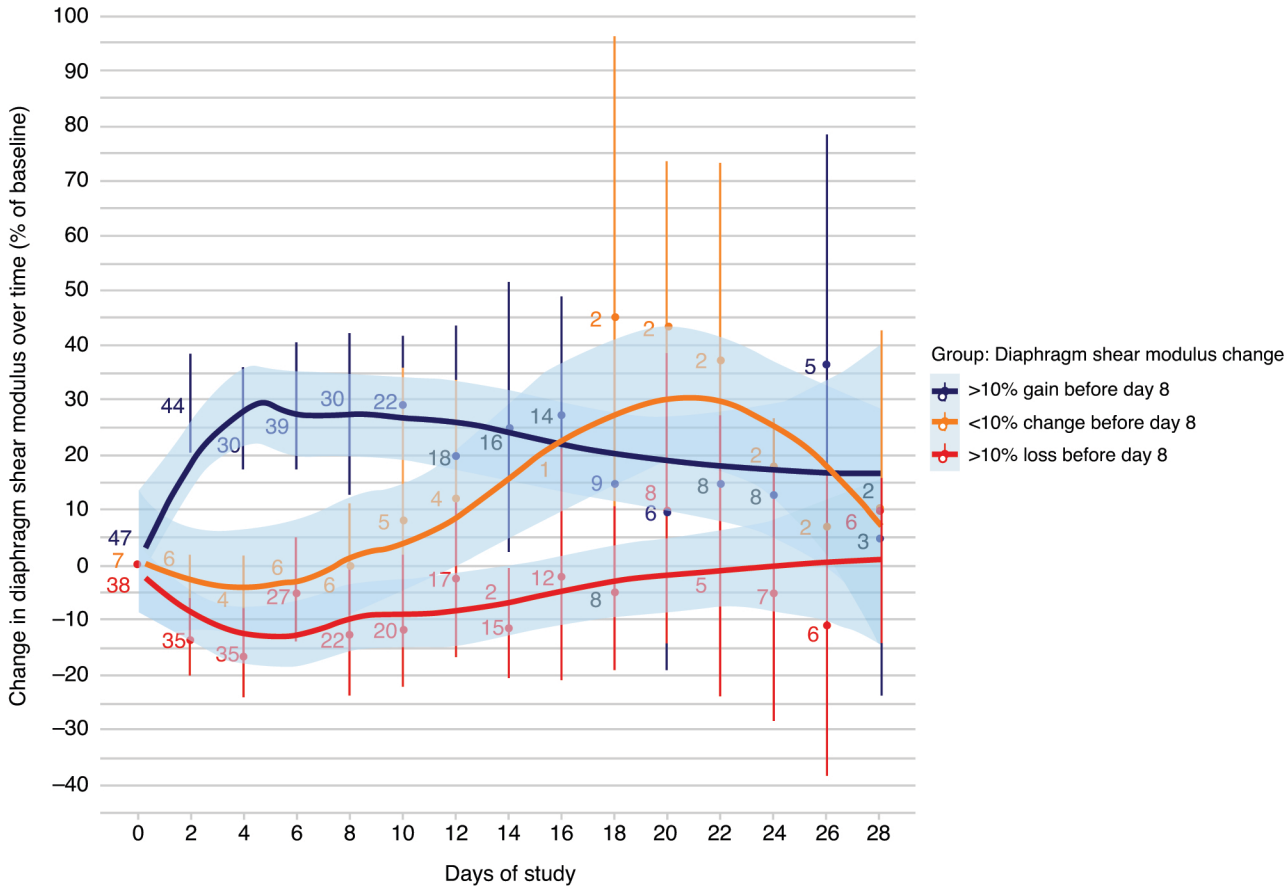

Figure 2. Variation in diaphragm shear modulus over time obtained in critically ill patients. Percentage of change from baseline (enrollment day) is represented on the *y*-axis and days of follow-up on the *x*-axis. Subjects are categorized into three groups according to the magnitude and direction of change in diaphragm shear modulus during the first week of the ICU stay using a 10% cutoff value. Mean and SD are plotted for each group on each study day. Numbers shown next to each data point indicate the number of measurements on each study day. Shared area indicates confidence intervals. Trend line and confidence intervals (shaded area) were fitted by Loess smoothing.

area demonstrating diaphragm fiber atrophy (Figures 3C and 3D) and with higher lipid vacuoles accumulation (Figures 3F and 3G). No difference was found with respect to inflammatory cells infiltration (Figures 3H and 3I).

Furthermore, the decrease in SM was associated with diaphragm loss of force measured by Pdi (Figures 3E and E5).

Patient Outcome

Ventilator-free days, ICU length of stay, weaning difficulty, and mortality at Day 28 were not significantly associated with change in diaphragm SM or thickness (Tables 3 and E5).

Discussion

The study demonstrates a heterogeneous pattern of changes in SM during the first week of ICU stay, in which SM does not change in 8%, increases in 51%, and decreases in 41% of patients. Changes in SM were negatively related to changes in diaphragm thickness in critically ill patients after multivariate analysis. Decrease in SM was associated with diaphragm fiber atrophy, diaphragm lipid accumulation, and loss of force production in a healthy mechanically ventilated piglet model. The apparent increase in diaphragm thickness during the ICU stay may therefore be linked with an alteration of diaphragmatic ultrastructural and mechanical properties, as suggested in the diaphragm myotrauma concept.

SWE provides a reliable quantifiable measurement of muscle mechanical properties and muscle stiffness (40, 44) and therefore has been suggested as a tool to evaluate muscle quality and changes of *in vivo* mechanical properties over time (23, 40). SWE is routinely used to evaluate tendon injuries in sports medicine and muscle mechanical properties in muscular diseases (45, 46). According to muscle stiffness change occurring during diseases, SM would be either increased or decreased. For instance, increased SM has been observed in late-stage Duchenne's myopathy, which is associated with muscle fibrosis (35), or in the affected and stiff limb consecutive to cerebral palsy (36). Conversely, conditions associated with inflammation, edema, and muscle swelling (e.g., ultramarathons, myositis) have been associated with loss of stiffness and decreased SM (34, 46). Decreased SM has also been associated with denervated muscle atrophy (33). Although recent studies reported that SM of the diaphragm was correlated with diaphragm force production during inspiratory efforts (28, 29, 31), no study has evaluated SWE of the diaphragm in the

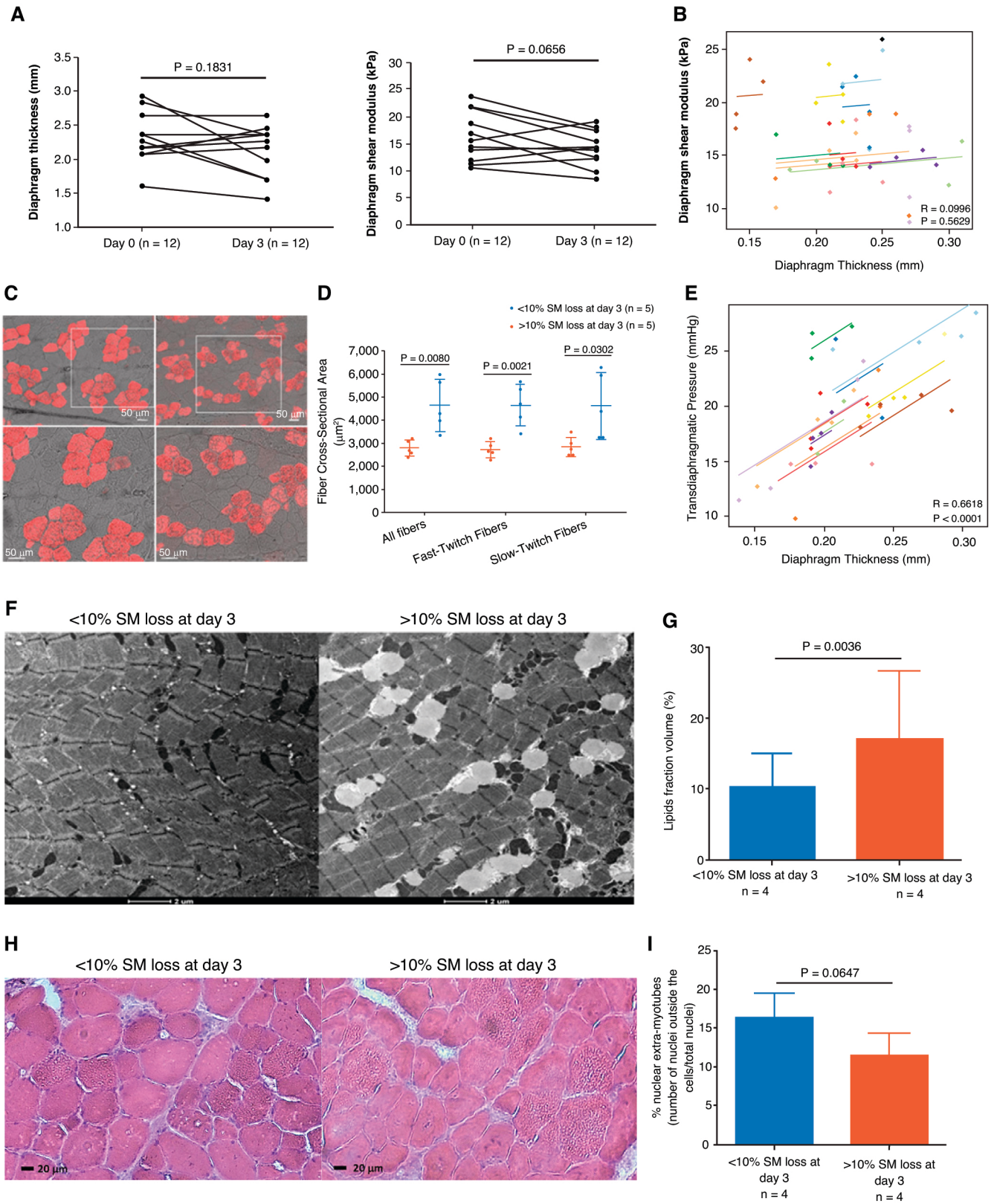

Figure 3. (*A–I*) Relationship between diaphragm shear modulus, (*A* and *B*), diaphragm thickness, (*C* and *D*) diaphragm muscle fiber size, (*E*) diaphragm force production, (*F* and *G*) diaphragm lipid accumulation, and (*H* and *I*) diaphragm inflammatory infiltration obtained in mechanically ventilated healthy piglets. (*A*) Quantitative analyses of the diaphragm thickness (left panel) and the diaphragm shear modulus (SM) (right panel) at Day 0 (circles) and after 3 days of mechanical ventilation (MV) (squares). (*B*) Relationship between thickness and SM for all

Table 3. Univariate Analysis of Outcomes According to Change in Diaphragm Shear Modulus over Time

Outcomes	Study Population ($n = 92$)	Change in Diaphragm Shear Modulus during the First Week			*P* Value
		>10% Increase ($n = 47$)	<10% Change ($n = 7$)	>10% Decrease ($n = 38$)	
Ventilator-free days to Day 28*	18.4 (3.8–24.1)	19.2 (4.1–25.1)	5.3 (0–20)	15 (3.8–24)	0.21
Ventilator-free days to Day 28† ($n = 79$)	15.5 (3.6–22.2)	16.9 (3.7–22.6)	5.2 (0.1–19.8)	13.7 (3.5–22.7)	0.48
Prolonged or difficult weaning† ($n = 70$)	35 (50)	16 (44)	4 (80)	15 (52)	0.30
Tracheostomy*	4 (4.4)	1 (2)	1 (14)	2 (5)	0.31
ICU mortality*	21 (23)	9 (19)	2 (29)	9 (24)	0.74
Day-28 mortality*	19 (20)	9 (19)	2 (29)	8 (22)	0.83
ICU length of stay, d*	12.4 (7.1–19)	12.2 (7.5–18.6)	12 (8.5–39)	13.1 (6.3–19.6)	0.82

Data are presented as *n* (%) or median (interquartile range).
*Results are presented for the total study population (92 patients).
†Results are presented for patients who received the mentioned therapy.

critically ill population as a surrogate for muscle quality or to follow up its architectural changes over time.

In the present study, diaphragm SM as well as thickness changes over time did present different patterns (Figures 2 and E2). After adjustment for day of measurement and number of days on MV, thickness and SM changes over time appeared inversely associated (Table 2). Assuming the theoretical increase in shear wave speed when thickness increases (26), a positive correlation was expected if mechanical properties and stiffness of the analyzed tissue remained similar. Thus, the present negative correlation between diaphragm thickness and SM changes over time is even more valuable. These results indeed demonstrate that the diaphragm's stiffness is reduced because the shear wave speeds decrease with increasing thickness. After multivariable analysis, aging, sepsis, steroids, duration of MV, and the absence of spontaneous breathing cycles were associated with a decrease in diaphragm SM (Table 2). Conversely, one-fourth of the patients did show an increased SM and decreased thickness, which may represent a combination of decreased muscle mass, myofibrillar destruction, fiber atrophy, and muscle fibrosis (Table E9 and Figure E7).

Aging is associated with sarcopenia, myosteatosis, and myofibrosis. In a clinical study involving different age groups of volunteers, aging was associated with a decline in skeletal SM correlated with muscle weakness, which is in line with the present study (47). The decrease of SM in patients with sepsis in our study may be the consequence of the combination of atrophy, inflammation, edema, and lipid accumulation that reduce diaphragm stiffness (48). The effects of short-term corticosteroids on the diaphragm in critically ill patients is complex because corticosteroids both downregulate protein synthesis and promote atrophy but may also limit disease-related myositis thus with an unpredictable effect on muscle stiffness (11). A recent study showed that SWE detected decreased muscle stiffness associated with weaker muscles over time in patients on long-term glucocorticoid (49). Interestingly, we observed that changes in diaphragm thickness and in SM occurred in 80% of the 14 nonventilated patients, confirming that both MV and critical illness target the respiratory muscles before (3) or during the ICU stay (50).

To better understand the link between diaphragm histology and SM, we performed a 3-day MV piglet study and biopsied the diaphragm at the end of the experiment. We report herein for the first time that the decrease in SM was associated with both slow and fast fiber atrophy (Figures 3C and 3D), higher intramuscular lipid vacuoles content (Figures 3F and 3G), and diaphragm loss of force production (Figures 3E and E4).

The lack of difference in inflammatory cells infiltration between SM groups in piglets may be related with the healthy state of this model, with less systemic inflammation like sepsis or circulatory shock (51). These findings are in line with the compelling diaphragm myotrauma concept in which underassistance may lead to muscle injury, edema, and inflammation (22). The model of healthy mechanically ventilated piglet is a model of diaphragm overassistance that may explain the increased diaphragm lipid content. Picard and colleagues showed that resting diaphragm during controlled MV may indeed be associated with a state of relative energetic oversupply versus demand that would trigger mitochondrial dysfunction

Figure 3. (*Continued*). values during 72 hours of MV. (*C*) Representative images of transverse frozen sections stained with antibodies directed against slow isoforms of myosin heavy chain (red), according to SM decrease at Day 3: specimens with a decrease in the SM of <10% (left panels) and a decrease in the SM of >10% (right panels). Scale bars, 50 μm. (*D*) Quantitative analyses of diaphragm fiber cross-sectional area (median and quartiles) according to SM decrease at Day 3. (*E*) Relationship between transdiaphragmatic pressure after supramaximal phrenic nerve stimulation at a frequency of 100 Hz and diaphragm SM for all values during 72 hours of MV. (*F*) Representative electron micrographs showing diaphragm lipid droplets accumulation according to SM decrease at Day 3: SM decrease <10% (left panel) and SM decrease >10% (right panel). Scale bars, 2 μm. (*G*) Quantitative analyses of diaphragm lipid droplets accumulation (mean ± SD) according to SM decrease at Day 3. (*H*) Representative images of hematoxylin and eosin–stained sections for inflammatory cells infiltrate according to SM decrease at Day 3: SM decrease <10% (left panel) and SM decrease >10% (right panel); extracellular nuclei of an inflammatory cell are colored in purple. (*I*) Quantitative analyses of diaphragm inflammatory cells infiltrate (mean ± SD) according to SM decrease at Day 3. All extracellular nuclei outside the muscle fibers were assumed as inflammatory cells, and their presence was expressed as a percentage of the extracellular nuclei out of total nuclei in the area.

and oxidative stress (13). Diaphragm inflammation and edema may be the main hypothesis for decreased SM in critically ill patients with overuse injury (34, 52). We summarized the different hypothesis for diaphragm SM and thickness changes in Table E9.

The perspective of the present results and the recent literature (29, 31) is that diaphragm SM evaluation in critically ill mechanically ventilated patients may be used as a tool in future interventional studies that would aim to promote diaphragm protective strategies. It may also help in phenotyping patients according to their diaphragm profile using both diaphragm mass surrogate (thickness) and diaphragm mechanical properties (SM). Finally, SM has been described as a surrogate of inspiratory muscles effort in spontaneously breathing patients.

Our study presents limits that should be discussed. First, we did not perform serial biopsies of the diaphragm in patients, a dangerous procedure outside the operating room. We therefore could only describe the association between ultrasound measurements and biopsy findings in the piglet model. Diaphragm fiber atrophy was associated with a decreased SM in piglets. Second, to ensure the highest reproducibility, we performed the ultrasound measurements at the end of expiration (and carefully checked the absence of diaphragm contraction) (20). However, because this study was designed as a "real life" study, we did not use Pdi monitoring to make sure none of the measurements were performed above the FRC. Third, we performed more than 600 ultrasound examinations from ICU admission to discharge, increasing the statistical power of the analysis, but we analyzed a combination of different ventilatory conditions (Figure E5). To date, longitudinal studies have been heterogeneous, with follow-up being cut off right after extubation (53) at Day 5 (50) or at Day 14 (20). Our study design is a limit but also better reflects the journey of the ICU stay. Finally, although the present combination of both critically ill patients mechanically ventilated for 7 days and healthy piglet model ventilated for 3 days illustrate the myotrauma concept, it may be difficult to extrapolate some data from one model to the other.

In summary, we show for the first time that both diaphragm thickness and stiffness change vary heterogeneously during the ICU stay. The increase in global diaphragm thickness during the ICU stay in one-third of the patients is associated with a drop in diaphragm stiffness. In a mechanically ventilated piglet model, we report that the decrease in diaphragm stiffness from Day 1 to Day 3 is associated with fast and slow fiber atrophy, diaphragm lipid accumulation, and loss of diaphragm force production. Our results could be another building block to corroborate the myotrauma concept in which increase in diaphragm thickness marks a different yet not independent type of diaphragm injury than the better-known decreased thickness. Further studies focusing on better understanding cellular and molecular changes associated with changes in diaphragm thickness and stiffness over time are warranted to investigate whether increased thickness associated with decreased stiffness is a noninvasive indicator of diaphragmatic inflammation, lipid accumulation, injury, and edema, associated with the fiber atrophy previously described in piglets. ■

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