Poor Correlation between Diaphragm Thickening Fraction and Transdiaphragmatic Pressure in Mechanically Ventilated Patients and Healthy Subjects

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Abstract

Background. The relationship between the diaphragm thickening fraction and the transdiaphragmatic pressure, the reference method to evaluate the diaphragm function, has not been clearly established. This study investigated the global and intra-individual relationship between the thickening fraction of the diaphragm and the transdiaphragmatic pressure. We hypothesized that the diaphragm thickening fraction would be positively and significantly correlated to the transdiaphragmatic pressure, in both healthy participants and ventilated patients.

Methods. Fourteen healthy individuals and 25 mechanically ventilated patients (enrolled in two previous physiological investigations) participated in the present study. The zone of apposition of the right hemidiaphragm was imaged simultaneously to transdiaphragmatic pressure recording within different breathing conditions i.e. external inspiratory threshold loading in healthy individuals and various pressure support settings in patients. A blinded offline breath-by-breath analysis synchronously computed the changes in transdiaphragmatic pressure, the diaphragm pressure-time product, and diaphragm thickening fraction. Global and intra-individual relationships between variables were assessed.

Results. In healthy subjects, both changes in transdiaphragmatic pressure and diaphragm pressure-time product were moderately correlated to diaphragm thickening fraction ($R=0.40$, $p<0.0001$ and $R=0.38$, $p<0.0001$, respectively). In mechanically ventilated patients, changes in transdiaphragmatic pressure and thickening fraction were weakly correlated ($R=0.11$, $p=0.008$), while diaphragm pressure-time product and thickening fraction were not ($R=0.04$, $p=0.396$). Individually, changes in transdiaphragmatic pressure and thickening fraction were significantly correlated in 8/14 healthy subjects ($\rho=0.30–0.85$, all $p<0.05$) and in 2/25 mechanically ventilated patients ($\rho=0.47–0.64$, all $p<0.05$). Diaphragm pressure-time product and thickening fraction correlated in 8/14 healthy subjects ($\rho=0.41–0.82$, all $p<0.02$) and in 2/25 mechanically ventilated patients ($\rho=0.63–0.66$, all $p<0.01$).

Conclusion. Overall, diaphragm function as assessed with transdiaphragmatic pressure was weakly related to diaphragm thickening fraction. The diaphragm thickening fraction should not be used in neither healthy subjects or ventilated patients when changes in diaphragm function are evaluated.

Introduction

The diaphragm acts as a piston within the chest, generating air flow as it descends and displaces the abdominal contents beneath and elevates the lower thorax. The pressure generated across the dome between the thoracic and abdominal cavities, the transdiaphragmatic pressure (Pdi), is proportional to the tension developed within the muscle fibers.[1] Pdi is commonly used as a surrogate of diaphragm function. Pdi is defined as the difference between esophageal (Pes) and gastric (Pga) pressures so that Pdi = Pga – Pes.[2] Measuring Pdi provides useful information in various clinical settings in which diaphragm dysfunction occurs as within the intensive care unit (ICU).[3-8] However, measuring Pes and Pga relies on the use of gastro-esophageal catheters (inserted through the nose or mouth of the patients), which explains that clinicians may be reluctant to use this technique. Alternatively, the diaphragm function can be non invasively explored by ultrasound. Through an intercostal approach, one can image the muscular layer of the diaphragm surrounded by two hyperechoic layers, i.e. the *pleura* and *peritoneum*, at the zone of apposition of the right hemidiaphragm.[9-11] One particular index derived from diaphragm ultrasound is known as the diaphragm thickening fraction (TFdi). The latter is calculated based on the change in diaphragm thickness during inspiration[11], and was first used in 1989 by Wait *et al.*.[12] Various studies reported that TFdi may guide clinicians in evaluating diaphragm function[13], and in predicting the outcome of weaning in mechanically ventilated patients[14-17], although the latter point is still debated.[11] Furthermore, several authors have suggested that TFdi may reflect the diaphragm function. Goligher *et al.*[18] reported the relationship between the changes in Pdi (ΔPdi) and TFdi in 5 healthy subjects. Although statistically significant, the authors noted large variability in TFdi for a given ΔPdi. Similarly, Umbrello *et al.*[19,20] and Vivier *et al.*[21] showed that esophageal and diaphragm pressure-time product (PTPdi) were significantly related to TFdi with also large differences in PTPdi values for a given TFdi value. This variability may arise from interindividual differences in

the ΔPdi – TFdi and PTPdi – TFdi relationships, that were not accounted for in the aforementioned studies. By contrast, Oppersma *et al.*[22] found no increase in TFdi during stepwise increase in inspiratory efforts from 0 to 50 % of maximal inspiratory pressure in healthy subjects. However, the aforementioned studies reported relationships between diaphragm function and TFdi based on averaged data for a given ventilation condition, thus ignoring ΔPdi and TFdi variability within the condition of ventilation tested and its impact on the ΔPdi – TFdi relationship. A breath-by-breath analysis may allow to better understand such relationships. Also, assessing the relationship between TFdi and diaphragm function at the patient level, instead of grouping patients altogether, could explain the high variability in TFdi observed in these previous works. In addition, TFdi has been reported to vary as much as 27 %.[18] This moderatel reliability may affect the strength of its relationship with diaphragm function as assessed using Pdi.[18] Taken together, these results emphasize that the relationship between diaphragm function and TFdi requires further investigation. Therefore, the objective of the study was to examine the within-individual relationship between ΔPdi and TFdi in healthy subjects and mechanically ventilated patients. By performing a breath-by-breath analysis, we hypothesized that TFdi would be positively and significantly correlated to ΔPdi and PTPdi.

Material and Methods

The current study includes participants from two previously published studies[23,24] registered on ClinicalTrial.gov (NCT03313141 and NCT03832231) and approved by local ethics committees (ID RCB: 2015-A00949-40 and 2018-A022311-54). We used data prospectively collected during these two physiological studies that were primary designed to investigate a new ultrasound technology (transient shear wave elastography) [23,24]. In the present work, a post-hoc analysis of unpublished data pertaining to diaphragm thickness and thickening fraction are reported. Written informed consent was obtained from all participants or their relatives. The studies followed the STROBE guidelines for observational studies.

All participants had to be older than 18 years old at the time of inclusion. Healthy subjects were free from any disease and non smoker. Mechanically ventilated patients had been intubated and ventilated for a minimum of 24 h, and failed a first spontaneous breathing trial. They could be included if they met the following readiness-to-wean criteria[25]: $SaO_2 > 90\%$ or $PaO_2/FiO_2 \geq 150$ mmHg with a fraction of inspired oxygen (FiO_2) $\leq 40\%$, no or minimal vasopressor, and positive end-expiratory pressure (PEEP) ≤ 8 cmH_2O. Patients under a legal protection measure, with known allergies to anesthetizing, pregnant, or with a contraindication to the insertion of a gastric-esophageal probe were not included.

Flow and pressure measurements

Different apparatus were used in healthy subjects and mechanically ventilated patients. In healthy subjects, two 8-cm balloon catheters (C76080U; Marquat Génie Biomédical, Paris, France), connected separately to differential pressure transducers (DP45-32; Validyne, Northridge, CA) were used to measure Pes and Pga. For flow measurement, healthy subjects wore a noseclip and were breathing through a mouthpiece, itself connected to a two-way valve and

pneumotachograph (3700 series, linearity range 0–160 L*min-1; Hans Rudolph, Kansas City, MO). Flow and pressure signals were digitized (Powerlab, ADInstruments, Sydney, Australia) and recorded at a sampling frequency of 2 kHz (Labchart, ADInstruments). In mechanically ventilated patients, the flow was measured using a flow sensor (Hamilton Medical, Bonaduz, Switzerland) connected to a spirometer (ADInstruments, Bella Vista, Australia). A double-balloon feeding catheter (NutriVentTM, Mirandola, Modena, Italy), connected to differential pressure transducers (DP45-32, Validyne, Northridge, CA), allowed the recording of Pes and Pga. Flow and pressure signals were digitized (Powerlab, ADInstruments, Sydney, Australia) and recorded at a sampling frequency of 1 kHz (Labchart, ADInstruments). A dynamic occlusion test was performed to validate esophageal balloon position allowing the visualization of a corresponding negative deflection in esophageal pressure and airway pressure during inspiratory effort.[26] To validate gastric balloon position, an increase in gastric pressure had to be observed when gently pressing the patient's abdomen. In both setting, Pdi was continuously obtained by the online subtraction of Pes from Pga.

Ultrasound imaging

In healthy subjects and patients, the zone of apposition of the right hemidiaphragm was imaged using the same linear transducer array (7-10 MHz, SL10-2, Supersonic Imagine, Aix-en-Provence, France) driven by an ultrasound scanner (Aixplorer, Supersonic Imagine). The diaphragm was imaged through the intercostal approach, with the probe placed on the 8^{th}-10^{th} intercostal space near the midaxillary line. A generous amount of gel was applied to the participant's skin to optimize acoustic coupling. The diaphragm was identified as a muscular layer in-between two hyperechoics lines (i.e. the *pleura* and *peritoneum*), superficial to the liver. Probe location was skin-marked as it is known to increase the reproducibility of TFdi measurement.[18] Ultrasound measurements were performed by a single trained-operator in healthy subjects (MD)

and mechanically ventilated patients (QF). Both operators had extensive experience in diaphragm ultrasound imaging and followed the aforementioned methodology to ensure the reliability of ultrasound recordings across participants and allow an accurate comparison of healthy subjects and mechanically ventilated patients.

Protocol

All participants (healthy and patients) were in a semi-recumbent position throughout the entire protocol.

Healthy subjects. Healthy subjects first performed a maximal isovolumetric inspiratory effort (Müller maneuver[27]) to determine their maximal transdiaphragmatic pressure and maximal inspiratory pressure at functional residual capacity. Briefly, participants were asked to perform maximal inspiratory efforts using a unidirectional valve allowing expiration only. At least 5 trials were performed and trials were repeated until three reproducible (<10 % variance) trials were recorded. They then went through a randomized series of stepwise inspiratory threshold loading from 10 to 50 % of maximal inspiratory pressure, with 10 % steps. As previously described[22,23], the inspiratory threshold loading was applied using an in-house developed apparatus modified from Chen *et al.*[28], generating a constant negative pressure that the subjects had to overcome. Participants were instructed to exert an outward motion of the abdomen during each inspiration, as such breathing technique optimizes diaphragm recruietment.[29] Each loading task was repeated twice with at least six respiratory cycles per recording. Participants were receiving visual feedback of their effort to ensure they reach the desired inspiratory pressure target.

Mechanically ventilated patients. In mechanically ventilated patients, recordings were performed under different conditions of ventilation. Patients were ventilated under pressure support ventilation mode. Four conditions of ventilation were applied in a randomized order: (i) initial

ventilator settings predefined by the attending physician, (ii) pressure support increased by 25% with baseline PEEP, (iii) pressure support decreased by 25 % with baseline PEEP and (iv) baseline pressure support and zero end-expiratory pressure. Each breathing condition was maintained for 10 min with 30-s acquisitions performed at 3 and 9 min within the condition. Eventually, recordings were performed during spontaneous breathing, where no assistance from the ventilator was provided. During this maneuver, patients were still connected to the ventilator but pressure support and PEEP were set to 0 cmH_2O. Maximal transdiaphragmatic pressure was measured during a Müller maneuver to assess maximal diaphragm function. Patients were briefly disconnected from the ventilator and attached to a one-way valve allowing expiration only. The occlusion was maintained for at least 20 seconds but not longer than 30 seconds, during which subsequent efforts of gradual intensity were recorded until a plateau in ΔPdi was observed. Patients were then immediately reconnected to the ventilator. Patients were conscious and did not receive sedatives while light dose of analgesics was allowed.

Data analysis

A controlling computer was used to trigger simultaneously the recording of the physiological signals (airway pressures, esophageal and gastric pressure) and ultrasound images. As a result during the offline analysis process, a given TFdi value could be directly compared to the ΔPdi of the same respiratory cycle. An overview of the setup, along with the acquired physiological and ultrasound parameters as well as the calculated variables are displayed in Figure 1. Data were analyzed offline using Matlab (Mathworks, Natick, MA, USA) scripts developed in-house. The offline analysis was performed by an operator blinded to the participant's identity and condition of ventilation. As previously reported[24], relying on the flow signal to demarcate respiratory cycles may mask the onset of inspiratory effort, especially in mechanically ventilated patients who need to overcome intrinsic PEEP.[30,31] For this reason, the operator delimited each

respiratory cycle by visually identifying the negative deflection in Pes associated with an increase in flow and Pga. ΔPdi was computed as the difference between the start of the increase in Pdi and the positive peak value of Pdi during inspiration. PTPdi per breath was computed as the area under the Pdi curve during the neural inspiratory time.[32] PTPdi per minute was calculated as the product between PTPdi and respiratory rate for a given breathing condition. Maximum transdiaphragmatic pressure was calculated as the difference between Pdi at functional residual capacity and maximal Pdi during the Müller maneuver. For each breathing cycles, the Gilbert Index (ΔPga / ΔPdi)[33] was calculated in order to quantifiy diaphragm contribution to inspiratory effort. The higher is this index, the higher is diaphragm contribution to inspiratory effort.[34]

For every recording, a time-motion (M-Mode) image was generated, on top of which the onset and end of inspiration for a given cycle were plotted (Figure 1). Subsequently, an experimented operator (TP), blinded to the condition of ventilation and participant identity, manually positioned a vertical electronic caliper at the internal border of the *pleura* and *peritoneum* membranes. Diaphragm thickness at end-expiration ($T_{di,ee}$) and peak inspiration ($T_{di,pi}$, i.e. maximal diaphragm thickness during inspiration) were defined as the distance between the internal border of the *pleura* and *peritoneum* membranes. TFdi was defined as the percentage change between $T_{di,ee}$ and $T_{di,pi}$, such that:

$$\text{TFdi (\%)} = \frac{T_{di,pi} - T_{di,ee}}{T_{di,ee}} \times 100 \qquad \text{(Eq. 1)}$$

Recordings of mechanically ventilated patients were potentially affected by cough, body movement, inferring with Pdi and ultrasound recordings. For this reason, the 3 cycles with the least variation in ΔPdi were considered as representative of a given ventilatory condition and selected for further analysis.[24] Asynchronous breaths, define as a mismatch between patient's effort and the ventilator, were excluded from the analysis. In healthy subjects, cycles that were affected by cough

or poor image quality were discarded. In both populations, breathing cycles for which any data was missing or not measurable were discarded so that a complete case analysis could be performed.

Statistical analysis

Descriptive statistics are expressed as median (25 to 75th percentile) unless stated otherwise. Since we used data pertaining to patients enrolled in two previous studies,[23,24] we estimated the sample size a posteriori. We calculated that at least 13 patients were needed to demonstrate a correlation of 0.70 between ΔPdi and TFdi. All statistical analysis were two-tails tested. Repeated measure correlations (R, 95 % CIs) were computed to determine overall ΔPdi – TFdi and PTPdi – TFdi relationships, using the 'rmcorr' R package.[35] This technique accounts for the inter-individual variability and the independence of repeated measures between individuals. Within-individual relationships were assessed using the non-parametric Spearman correlation coefficient (ρ), as variables failed the Shapiro-Wilk normality test. Spearman correlation coefficients were calculated using the base R 'cor.test' function. A mixed effect model was run to examine the Gilbert index × TFdi interaction effect on ΔPdi using the 'lme4' package in R.[36] If a significant interaction effect was found, repeated measure correlations were computed for breaths with a Gilbert index > 0.3 and for breaths with a Gilbert index < 0.3.[37] Reproducibility of TFdi, ΔPdi and PTPdi was assessed through each breathing condition by calculating standard errors of measurement and intraclass correlation coefficients to report absolute and relative reliability, respectively.[38] Analyses were performed separately in healthy participants and patients. Analyses were performed in the computing environment R.[39] Significance was set at $p<0.05$ for all tests.

Results

Population

Fourteen healthy subjects and 25 mechanically ventilated patients were studied. Table 1 presents the characteristics of participants at inclusion. ICU patients had been ventilated for 4 (3 – 7) days and were receiving a pressure support level of 10 (9 – 13) cmH_2O and a PEEP level of 5 (5 – 5) cmH_2O. In mechanically ventilated patients, 3878 respiratory cycles were recorded and 815 were considered for the analysis (i.e. corresponding to the 3 respiratory cycles with the least variation in ΔPdi). Of those, 383 were withdrawn from the analysis because of poor image quality, cough, expiratory muscles recruitment during prior expiration or patient movement. In healthy subjects, 813 cycles were recorded and 129 were discarded because of poor image quality, body movement or lung artefact. Eventually, a total of 684 and 587 respiratory cycles were analyzed for healthy subjects and mechanically ventilated patients, respectively. Maximal transdiaphragmatic pressure was 119 (108 – 142) cmH_2O in healthy subjects and 24 (15 – 35) cmH_2O in mechanically ventilated patients.

Group level relationship between TFdi and diaphragm function

TFdi, ΔPdi and PTPdi at all ventilatory condition in mechanically ventilated patients are presented in Figure 2. Likewise, Figure 3 displays TFdi, ΔPdi and PTPdi at all inspiratory load in healthy subjects. TFdi significantly correlated with ΔPdi in healthy subjects ($R = 0.40$, 95 % CIs [0.34 0.47], $p<0.0001$) and in mechanically ventilated patients ($R = 0.11$, 95 % CIs [0.03 0.19], $p=0.008$). Regarding TFdi – PTPdi relationships, a significant relationship was found at the group level in healthy subjects ($R = 0.38$, 95 % CIs [0.31 0.44], $p<0.0001$) but not in mechanically ventilated patients ($R = 0.04$, 95 % CIs [-0.05 0.12], $p=0.396$). Group level relationships between TFdi and ΔPdi, and between TFdi and PTPdi are shown in Figure 4 and 5, respectively.

Within individual relationships between TFdi and diaphragm function

Individual relationship, including all data points, between TFdi and ΔPdi, and between TFdi and PTPdi are presented in Figures SDC1-4. Individual correlations between TFdi and ΔPdi were significant in 8 (57 %) healthy subjects ($\rho = 0.30 – 0.85$, all $p<0.05$) and in 2 (8 %) mechanically ventilated patients ($\rho = 0.47 – 0.64$, all $p<0.05$). Individual TFdi – PTPdi relationship was significant in 8 (57 %) healthy subjects ($\rho = 0.28 – 0.84$, all $p<0.05$) and in two (8 %) mechanically ventilated ($\rho = 0.63 – 0.66$, all $p<0.01$). Table 2 displays the overall results of the relationships between TFdi and ΔPdi and between TFdi and PTPdi. Tables SDC1 and SDC2 present the standard error of measurement and intraclass correlation coefficients of TFdi, ΔPdi and PTPdi across breathing conditions in healthy subjects and mechanically ventilated patients, respectively.

Diaphragmatic contribution to inspiratory work

In healthy subjects, the mixed models did not reveal a significant interaction effect of the Gilbert index and TFdi on ΔPdi ($\beta = 0.02$, $p=0.648$). In patients, a significant and negative interaction effect of the Gilbert index and TFdi was found on ΔPdi ($\beta = -0.27$, $p<0.001$). Repeated measure correlation between ΔPdi and TFdi was not significant for breaths with a Gilbert index > 0.3 ($R = 0.08$, 95 % CIs [-0.06 0.22], $p=0.255$), but was significant for breaths with a Gilbert index < 0.3 ($R = 0.18$, 95 % CIs [0.08 0.28], $p<0.001$).

Discussion

This study investigated the relationship between simultaneously recorded diaphragm thickening fraction and diaphragm pressure production, both in healthy subjects and mechanically ventilated patients. Our results indicate that, at the group level, significant ΔPdi – TFdi relationships exist in both populations, although being only moderate in healthy subjects and weak in mechanically ventilated patients hampering the possibility to infer pressure output from ultrasound recordings. PTPdi – TFdi relationships exist in healthy subjects only. When considering the intraindividual relationship between diaphragm thickening fraction and diaphragm function, a significant relationship was found in approximately 50 % of healthy subjects and in less than 10 % of mechanically ventilated patients.

TFdi is the magnitude of the increase in diaphragm thickness during inspiration.[11] Several authors showed that TFdi increased with lung volumes[12,40,41], suggesting a relationship between TFdi and the intensity of diaphragm contraction. However, despite its extensive use in the ICU[3,10,11], little is known on the extent to which TFdi may reflect the transdiaphragmatic pressure, that is the physiological estimate of the diaphragm function.[42] Very few studies reported correlation values for the TFdi – ΔPdi relationship.[18,22] Goligher *et al.*[18] reported a significant TFdi – ΔPdi correlation from five pooled healthy subjects. On the other hand, Oppersma *et al.*[22] showed no effect of stepwise increase of inspiratory load on the change in TFdi. Our results show that, at the group level, TFdi was significantly correlated to ΔPdi in healthy participants. Nonetheless, one should note the moderate power of this relationship (i.e. R = 0.40). This result confirms previous findings, that either found a weak correlation between TFdi and ΔPdi[18], or no correlation at all.[22] Also, high inter-individual variability in TFdi was found for a given ΔPdi value, which may explain the moderate correlation found in healthy subjects (i.e. R = 0.40). Although significant, the

relationship between TFdi and ΔPdi was very weak in mechanically ventilated patients at the group-level (R = 0.11). Several factors may explain this finding. First, mechanically ventilated patients exhibited a much narrower range of ΔPdi values (0 – 40 cmH_2O) as compared to healthy subjects (0 – 120 cmH_2O). This may result in subtle changes in diaphragm function that TFdi may be not able to detect. Second, it cannot be ruled out that inspiratory work is redistributed across the various inspiratory muscles[29], which may partially explain the high inter-individual variability of TFdi.[43] This point highlights that Pdi does not solely depend on diaphragm activation[44], while TFdi does. Besides ΔPdi, PTPdi is another common index of diaphragm function. Other studies investigated the relationships between PTPdi and TFdi on pooled data at the group level. For instance, Vivier *et al.*[21] reported a significant PTPdi - TFdi correlation ($\rho = 0.74$, $p<0.001$) in non-invasively ventilated patients. Likewise, Umbrello *et al.*[19] reported similar results in mechanically ventilated patients ($r = 0.70$, $p<0.001$). Our findings partially support these studies as PTPdi - TFdi relationship at the group-level was significant in healthy subjects only. However, this relationship was not significant in mechanically ventilated patients. As illustrated in Figure 2, very little variation in diaphragm function were observed when varying ventilator settings in patients. This may partly explain why little to no changes were observed in TFdi from one condition of ventilation to another in this context whereas another study reported a significant change in TFdi in recently extubated patients, presumably characterized by high respiratory resistances.[21] Here again, the range of PTPdi being much wider in healthy subjects (3 – 279 $cmH_2O.s/breath$) than in mechanically ventilated patients (0 – 22 $cmH_2O.s/breath$), coupled with a possible redistribution of the inspiratory work across inspiratory muscles may partially explain why the TFdi – PTPdi relationship was not significant in patients. Also, it must be noted that, contrary to ΔPdi and TFdi, PTPdi is time-dependent. This means that the correlation between PTPdi and TFdi not only relies

on the level of Pdi but also on the inspiratory time. This may contribute to explain the weak correlation between the two parameters.

Group-level analyses provide a broad picture of the relationship between two variables. However, they do not account for the interindividual variability, which could partly explain the difference in ΔPdi or PTPdi for a given TFdi value. For this reason, we also performed a breath-by-breath analysis of the relationship between TFdi and physiological indices of diaphragm function. By doing so, we were able to investigate the direct link between the change in transdiaphragmatic pressure during a respiratory cycle and the TFdi for the very same respiratory cycle. To the best of our knowledge, this is seemingly the first study to conduct such an analysis. We found that intra-individual ΔPdi – TFdi relationships were significant in 8 (57 %) healthy subjects and 2 (8 %) mechanically ventilated patients. Accordingly, our findings suggest to be cautious when using TFdi as a surrogate of Pdi. Also, one must note that high intraindividual TFdi variability was observed for a given ΔPdi, even in participants exhibiting a significant ΔPdi - TFdi relationship. Beside, the slope of the relationship between ΔPdi and TFdi greatly differed from one participant to another, even when a significant correlation was found between the two parameters. Figure SDC5 illustrates the ΔPdi – TFdi relationship in two participants with a significant correlation, but distinct relationship slope. Likewise, seven healthy subjects and two mechanically ventilated patients presented with a significant PTPdi - TFdi relationship. Several reasons may be brought to explain these results. ΔPdi is not an actual force but rather the pressure change resulting from diaphragm contraction. Indeed, as the diaphragm contracts, its caudal displacement increases Pga, which acts as a reacting pressure to this caudal displacement.[45] In turn, different abdominal conformation would result in different gastric pressure reactions for a given diaphragm force production.[44] Also, and as mentioned above, intercostal and neck inspiratory muscles may be

responsible for a partial increase in the swing of Pes during inspiration, which would impact ΔPdi without affecting TFdi. One may also question whether the zone imaged is representative of the whole diaphragm. Previous research showed that the zone of apposition is the region of the diaphragm displaying the highest amount of active shortening.[46] Nonetheless, it cannot be ruled out that the force generated by the diaphragm may not be uniform across the muscle, particularly in patients.[47] In such case, imaging the zone of apposition may be inadequate to monitor diaphragm function. Finally, another source of uncertainty lies in the fact that TFdi depends on the manual measurement of diaphragm thickness. Goligher *et al.*[18] showed that TFdi was moderately repeatable, with an intra-operator variability of 16 %. Our results suggest that TFdi across respiratory cycles recorded in a given breathing condition are moderately to highly reliable, as demonstrated with intraclass correlation coefficients ranging between 0.50 and 0.93 (Table 3 and 4). Nonetheless, standard error of measurement for TFdi varied from 9.8 to 29.1 % in healthy subjects and from 6.4 to 11.6 % in mechanically ventilated patients, supporting previous studies reporting moderate repeatability of TFdi.[18] Both in healthy subjects and mechanically ventilated patients, we carefully skin-marked the position of the probe to ensure consistent imaging across trials. In addition, a single operator proceeded to the measurement of TFdi as between-operator variability is higher than within-operator variability.[18] Taken altogether, these factors may explain why ΔPdi - TFdi or PTPdi - TFdi relationships were not significant in a majority of participants.

The Gilbert index is commonly used to determine the diaphragmatic contribution to total inspiratory work.[34] Our mixed model analysis showed that there was a negative and significant interaction effect of the Gilbert index and TFdi on ΔPdi. This means that TFdi increases less and less as the Gilbert index increases. In other words, additional diaphragmatic contribution to inspiratory effort results in smaller and smaller increases in TFdi. When stratifying our correlation

analysis between breaths with a Gilbert index > 0.3 and breaths with a Gilbert index < 0.3[37], we showed that the repeated measure correlation was only significant for breaths with a Gilbert index < 0.3. Three main factors can explain such findings. First, the different correlations may be simply related to the low number of breathing cycles with a Gilbert index > 0.3 (n=221) as compared to breaths with a Gilbert index < 0.3 (n=363). Second, the range of ΔPdi and TFdi was substantially greater for cycles with a Gilbert index < 0.3 compared to cycles with a Gilbert index > 0.3 (0-40 cmH2O vs. 0-20 cmH2O for ΔPdi and 0-98 % vs. 0-73 % for TFdi, respectively). Third, the low albeit significant interaction effect of the Gilbert index and TFdi on ΔPdi is not powerful enough, restricting TFdi ability to detect an increase in diaphragmatic contribution to inspiratory work.

Strength and limitations

This work performed a breath-by-breath analysis of the diaphragm thickening fraction and transdiaphragmatic pressure prospectively collected during two previously published studies investigating the use of transient shear wave elastography to evaluate the diaphragm function. By synchronizing ultrasound images with the physiological signals, we were able to perform a straightforward comparison of TFdi and other indices of diaphragm function such as PTPdi. Data were analyzed offline, with the operator blinded to the condition of ventilation and participant identity, using standardized scripts allowing repeatable analysis across respiratory cycles. This study has several limitations. The first limitation is inherent to TFdi measurement, which is its moderate repeatability[18], although high care was taken to limit its impact on TFdi measurement. One should note that the correlation between two variables depends on the reliability of the correlated variables.[48] The poor relationship between TFdi and diaphragm function can therefore be related to the absence of relationship with diaphragm function, but also to the moderate reliability of its measurement. Therefore, the lower relationship between TFdi and diaphragm function in patients compared to healthy subjects may be partially attributed to the lower intraclass

correlation coefficients of TFdi in patients (Tables SDC1 and SDC2). In addition, potential bias may have influenced the measured correlations. One of them is the cycle selection in mechanically ventilated patients. Although we ensured that the selected cycles were representative of a given ventilatory condition, the observed relationships may slightly differ if different breathing cycles had been analyzed. For instance, cycle selection may have limited the range of ΔPdi and TFdi measured, which would ultimately affect the relationship between these variables. Various factor may also influence the magnitude of a relationship between two variables, such as the variability of the data.[49] As previously stated, the range of ΔPdi and PTPdi measured in patients was much narrower as compared to healthy subjects, which could partiatlly explain why the relationships between TFdi and diaphragm function were weaker in patients. Still, these data represent what ICU clinicians are faced with, and we reason this work could clear up the importance that should be given to TFdi when used for gauging diaphragm effort. Finally, we solely imaged the right hemidiaphragm, but previous studies reported that extra-diaphragmatic inspiratory muscles, such as the parasternal intercostal muscles, also thicken during inspiration in healthy subjects[50] and mechanically ventilated patients.[42] Because some individuals naturally excessively use their accessory inspiratory muscles[29], a sonographic evaluation of these accessory muscles may improve our understanding of the relationship between inspiratory muscles thickening and diaphragm function.

Generalization of findings

These findings have important implications in various research and clinical settings involving routine diaphragm monitoring. Because TFdi was related to ΔPdi in less than 10 % of mechanically ventilated patients, our results suggest that one should not use this ultrasound index as a surrogate of diaphragm function. One may argue that TFdi may be used for qualitative comparisons of diaphragm function within a given patient, but the large variability in TFdi for a

given ΔPdi hinders this approach. Also, it could be argued that more participants could have presented with significant relationships between TFdi and diaphragm function if an increased number of breathing cycles would have been analyzed. Although this is true, one must keep in mind that beside the presence or not of a significant relationship between two parameters, its magnitude may better depicts the actual relationship between them. In the present work, the magnitude of the relationships presented was only moderate, and especially in mechanically ventilated patients. Our results highlight the fact that TFdi poorly reflects transdiaphragmatic pressure. TFdi has been extensively studied as a potential criteria for predicting weaning outcome in mechanically ventilated patients[14,16,17], with cutoff TFdi values ranging from 25 to 36 % that have not been prospectively validated so far. Predicting weaning outcome based on TFdi is beyond the scope of this work, and our results do not reject any conclusion drawn from these latter works. However, the variability of TFdi for a given ΔPdi across patients may partially explain the different cutoff TFdi obtained in previous work. In addition, we showed that the TFdi was poorly related to the pressure generated by the diaphragm, although other measures are available to quantify diaphragm activity, such as diaphragm electrical activity. Recent work showed that the later was significantly related to TFdi (R^2 = 0.62).[51] TFdi may be more related to diaphragm electrical activity than the pressure it generates, although within-individual analysis are yet to be performed. We strongly encourage future studies to thoroughly describe respiratory cycles selection and analysis to provide readers with an exhaustive and reproducible method. We believe this may, at least partially, improve the comparison of TFdi-related results across studies. Combining TFdi with other ultrasound-based techniques such as shear wave elastography[23,24], speckle tracking[22,46], tissue doppler imaging[52,53], or ultrafast ultrasound imaging[54], may contribute to improving the non-invasive monitoring of the diaphragm function.

Conclusion

Overall, diaphragm function as assessed with transdiaphragmatic pressure was weakly related to diaphragm thickening fraction. The diaphragm thickening fraction should not be used in neither healthy subjects or ventilated patients when changes in diaphragm function are evaluated.

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Figure captions

Figure 1. Experimental setup used to synchronize the physiological parameters with the ultrasound recordings. The physiological and ultrasound recordings were simultaneously acquired using a controlling computer triggering both recordings. All data were then saved on the controlling computer in order to perform the offline blinded analysis. Changes in transdiaphragmatic pressure (ΔPdi) were computed as the difference between the start of the increase in Pdi and the positive peak value of Pdi during inspiration. Transdiaphragmatic pressure time product (PTPdi) per breath was calculated as the area under the Pdi curve during the neural inspiratory time. For each respiratory cycle, delimited by the vertical dotted lines, diaphragm thickness at end-expiration ($T_{di,ee}$) and at peak-inspiration ($T_{di,pi}$) were manually measured. TFdi was defined as the percentage change between $T_{di,ee}$ and $T_{di,pi}$.

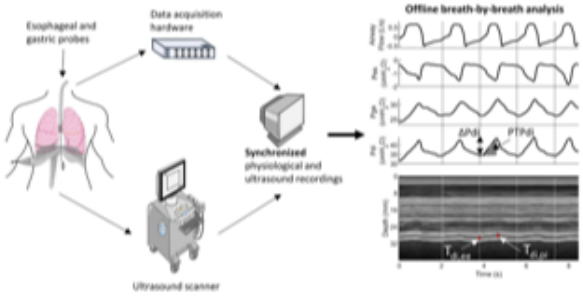

Figure 2. Diaphragm thickening fraction (TFdi, panel A.), changes in transdiaphragmatic pressure (ΔPdi, panel B.) and diaphragm pressure-time product per breath (PTPdi, panel C.) according to the condition of ventilation in mechanically ventilated patients. Box plots display the median and interquartile range. Whiskers represent the range. PS, Initial pressure support settings; +25PS, pressure support increased by 25 %; -25PS, pressure support decreased by 25 %; ZEEP, zero end-expiratory pressure with initial pressure support. SB, spontaneous breathing.

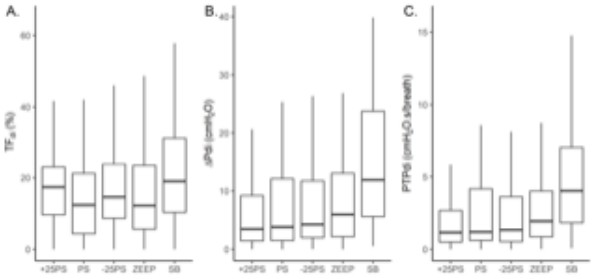

Figure 3. Diaphragm thickening fraction (TFdi, panel A.), changes in transdiaphragmatic pressure (ΔPdi, panel B.) and diaphragm pressure-time product per breath (PTPdi, panel C.) according to the inspiratory load in healthy subjects. Box plots display the median and interquartile range. Whiskers represent the range.

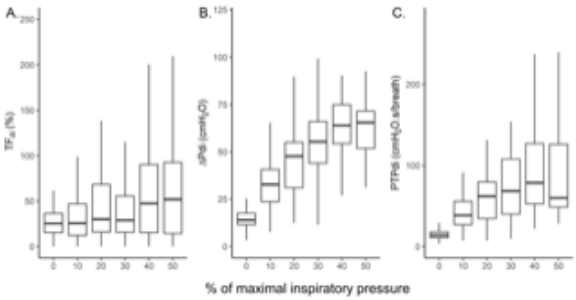

Figure 4. Group level relationships between diaphragm thickening fraction (TFdi) and changes in transdiaphragmatic pressure (ΔPdi) in healthy subjects (panel A.) and in mechanically ventilated patients (panel B.). Data are presented as mean ± SD. PS, Initial pressure support settings; +25PS, pressure support increased by 25 %; -25PS, pressure support decreased by 25 %; ZEEP, zero end-expiratory pressure with initial pressure support. SB, spontaneous breathing.

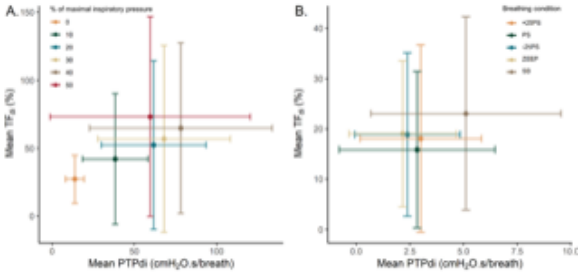

Figure 5. Group level relationships between diaphragm thickening fraction (TFdi) and diaphragm pressure-time product (PTPdi) in healthy subjects (panel A.) and in mechanically ventilated patients (panel B.). Data are presented as mean ± SD. PS, Initial pressure support settings; +25PS, pressure support increased by 25 %; -25PS, pressure support decreased by 25 %; ZEEP, zero end-expiratory pressure with initial pressure support. SB, spontaneous breathing.