



Ultrasound assessment of the diaphragm during the first days of mechanical ventilation compared to spontaneous respiration: a comparative study.

Évaluation échographique du diaphragme pendant les premiers jours de ventilation mécanique par rapport à la respiration spontanée: étude comparative.

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ABSTRACT:

Introduction: In critically ill patients, the diaphragm is subject to several aggressions mainly those induced by mechanical ventilation (MV). Currently, diaphragmatic ultrasound has become the most useful bedside for the clinician to evaluate diaphragm contractility.

Aim: To examine the effects of MV on the diaphragm contractility during the first days of ventilation.

Methods: Two groups of subjects were studied: a study group (n=30) of adults receiving MV versus a control group (n=30) of volunteers on spontaneous ventilation (SV). Using an ultrasound device, we compared the diaphragmatic thickening fraction (DTF). Secondly, we analysed the relationship between DTF and weaning.

Results: comparatively to SV group, patients of MV group have a higher end expiratory diameter (EED) (2.09 ± 0.6 vs. 1.76 ± 0.32 mm, $p=0.01$) and a lower DTF ($39.9 \pm 12.5\%$ vs. $49.0 \pm 20.5\%$, $p=0.043$). Fourteen among the 30 ventilated patients successfully weaned. No significant correlation was shown between DTF and weaning duration ($Rho = -0.464$, $p=0.09$). A DTF value $> 33\%$ was near to be significantly associated with weaning success ($OR=2$; $95\% CI=[1.07-3.7]$, $p=0.05$) with a sensitivity at 85.7% . Conclusions: diaphragmatic contractility was altered from the first days of MV. ADTF value $>32,7\%$ was associated to the weaning success and that may be useful to predict successful weaning with sensitivity at 85.7% .

Keywords: Respiratory mechanics, ultrasound, inspiratory muscle, ICU, weaning

RÉSUMÉ:

Introduction: Chez les patients de réanimation, le diaphragme est sujet à plusieurs agressions principalement celles induites par la ventilation mécanique (VM). Actuellement, l'échographie diaphragmatique est devenue le chevet le plus utile pour le clinicien pour évaluer la contractilité du diaphragme.

Objectif: Examiner les effets de la VM sur la contractilité du diaphragme pendant les premiers jours de ventilation.

Méthodes: Deux groupes de sujets ont été étudiés: un groupe d'étude (n=30) de malades adultes recevant la VM contre un groupe témoin (n=30) de volontaires en respiration spontanée (RS). À l'aide d'un appareil échographique, nous avons comparé la fraction d'épaississement diaphragmatique (FED). Secondairement, nous avons analysé la relation entre la FED et le sevrage.

Résultats: comparativement au groupe RS, les patients du groupe VM avaient un diamètre télé-expiratoire (DTE) plus élevé ($2,09 \pm 0,6$ versus $1,76 \pm 0,32$ mm, $p=0,01$) et une FED plus faible ($39,9 \pm 12,5\%$ contre $49,0 \pm 20,5\%$, $p=0,043$). Quatorze des 30 malades ventilés ont réussi leur sevrage. On n'a pas démontré de corrélation significative entre la FED et la durée du sevrage ($Rho = -0,464$ et $p=0,08$). Une valeur FED $> 33\%$ était proche d'être significativement associée à un succès de sevrage ($OR = 2$; IC à $95\% = [1,07-3,7]$, $p=0,05$) avec une sensibilité à $85,7\%$.

Conclusions: la contractilité diaphragmatique était altérée dès les premiers jours de VM. Une valeur de FED $> 32,7\%$ était associée au succès de sevrage et cela peut être utile pour prédire un sevrage réussi avec une sensibilité de $85,7\%$.

Mots clés: Mécanique respiratoire, échographie, muscle inspiratoire, soins intensifs, sevrage

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INTRODUCTION

In critically ill patients, the diaphragm is subject to several aggressions [1,2]. For a longtime, diaphragmatic dysfunction was considered as a part of critical illness polyneuropathy[1]. Furthermore, it may be a negative consequence of frequent events in intensive care units, such as hyper catabolism, magnesium and phosphorus deficiency, the use of neuromuscular blockers and corticosteroids [1,2]. Nevertheless, mechanical ventilation (MV) is mainly the major cause of diaphragmatic dysfunction due to the diaphragm “withdrawal”. Recent findings have individualized the entity of ventilator-induced diaphragm dysfunction that it is associated with poor prognosis [3-8]. Experimental data from histological and physiological studies report that MV induces structural lesions such as myofibrillar disruption, increased lipid vacuoles in sarcoplasm and small abnormal mitochondria, oxidative stress, and remodelling of diaphragmatic muscle fibres [8-12]. These microscopic structural abnormalities were detected from the earlier days of MV and induce a decrease in the diaphragm resistance. Until the mid-90s, diaphragm function and contractile fatigue are best assessed with either electrical or magnetic phrenic nerve stimulation [13]. On the other hand, diaphragmatic motion is readily studied by fluoroscopy, and this technique can be of value in the diagnosis of diaphragmatic paralysis. Nevertheless, fluoroscopy requires patient transportation and uses ionizing radiation. An alternate and interesting method is sonography [14,15]. The diaphragmatic ultrasound is a functional and morphological tool that provides non-invasive indices describing the structure / function of this muscle.

There is still limited data on the topic of the timing of the morphological changes leading to the diaphragmatic dysfunction in intensive care patients. We hypothesized that ventilator-induced diaphragm dysfunction could appear since the first days of MV.

Our aim was to compare two groups: volunteer subjects on spontaneous ventilation (SV) vs. subjects who received MV for a short duration (between 48 hours and five days). Diaphragmatic ultrasound was used to evaluate diaphragmatic contractile activity for both groups. In a second step, we examined the influence of diaphragm thickening on the weaning from the ventilator.

METHODS

Study design and ethical status: it was a comparative study with prospective longitudinal follow-up. It was conducted in the medical intensive care unit of la Rabta hospital, over a six month- period (March - August 2019). The group of SV volunteers gave their oral consent for participation. Patient's legal representatives gave oral consent to include them in the MV group. The approval was obtained from the local ethics committee of la Rabta hospital and the study was in accordance to the Declaration of Helsinki for experiments involving humans. Study population: We selected two groups: a group of patients on MV (study group) versus a group of volunteer subjects on SV (control group). For the study group, any patient aged 18 years or over whose management required MV was included. Diaphragmatic ultrasound was performed within a minimum of 48 hours and a maximum of five days since the beginning of MV. Assisted volume controlled and/or pressure support ventilation were the modalities of interest. For the control group, they were independent from study group and met these criteria: healthy volunteers, age > 18 years, in spontaneous breathing, who approved their participation. In fact, they corresponded to members of the medical and paramedical staff which not meeting the non-inclusion criteria. Non-inclusion criteria were neuromuscular disease, diseases involving the abdomino-pelvic compartment (such as intra-abdominal masses, subocclusive syndromes, Ogilvie), severe obesity (body mass index over than 35 kg/m²), pregnancy and the use of neuromuscular blockers. Indeed, these factors may interfere with the effect of MV in the genesis of diaphragmatic dysfunction [1,2,16].

Exclusion criteria were targeted duration of MV less than 48 hours, a history of MV for more than 48 hours in the previous six months and poor quality of ultrasound images.

Study protocol (figure 1):

- Device description and diaphragm ultrasound circumstances: The same operator trained in diaphragmatic ultrasonography performed the diaphragm exploration. The ultrasonic device correspond to the Model of “Aloka-ARIETTA V60” [manufacturing Year: 2014, Company: Hitachi, Ltd, manufacturing country Chiyoda, Tokyo, Japan], equipped with a piezoelectric linear probe of high frequency (10 MHz) and providing incidences in B (two-dimensional) mode, in time/movement mode and Tissue Doppler Imaging mode.

According to the techniques described elsewhere [17-22], diaphragmatic thickness assessment can be obtained at the zone of apposition; level of seventh–ninth intercostal spaces. We chose to explore the right hemi-diaphragm

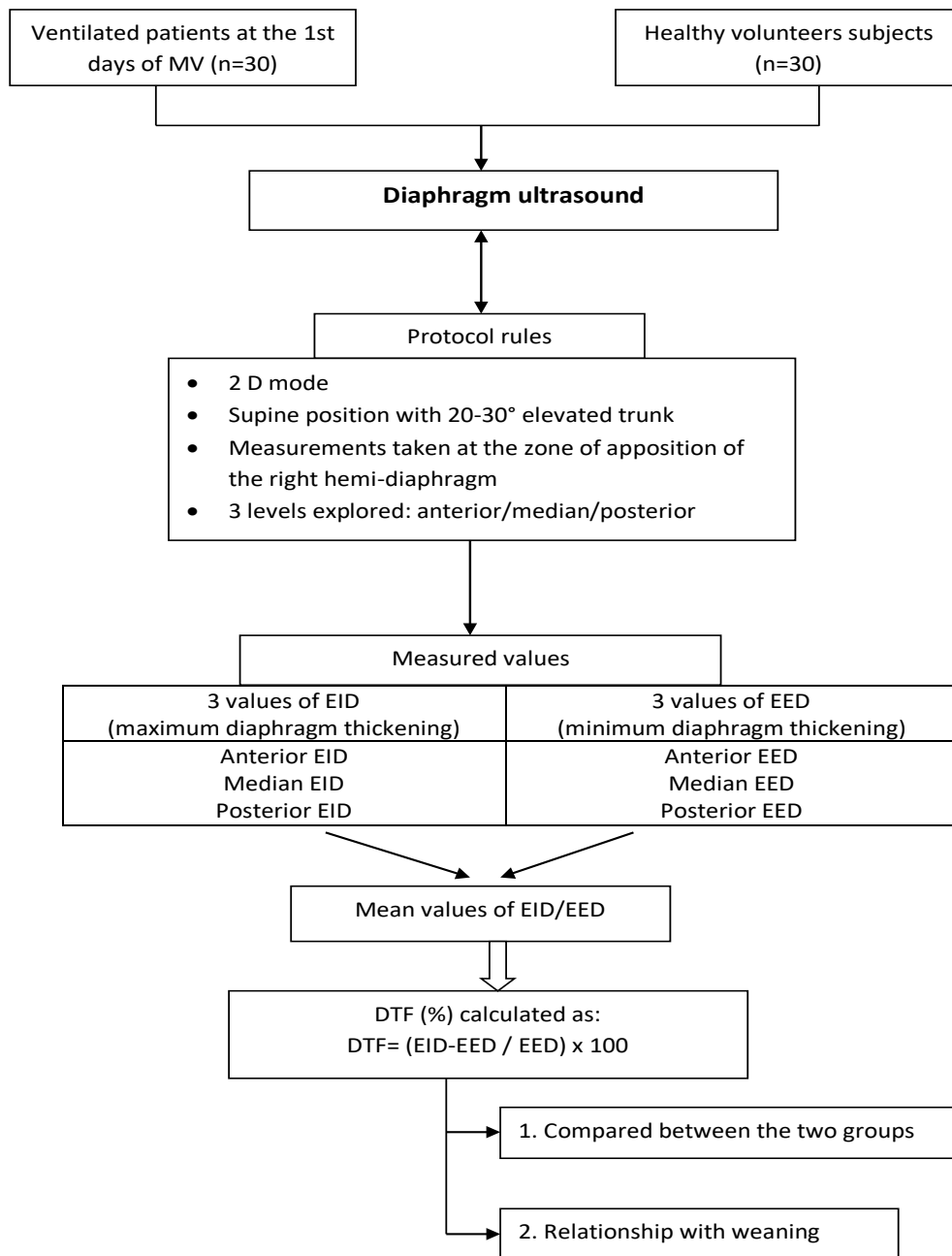


Figure 1. Study flowchart

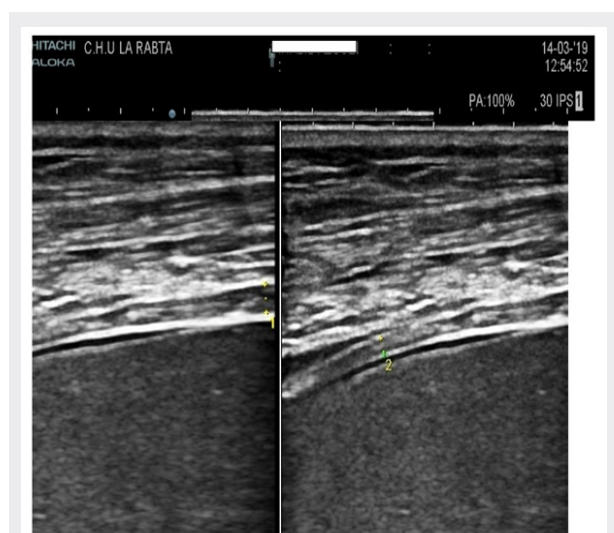
DTF: diaphragmatic thickening fraction, EED: end expiratory diameter, EID: end inspiratory diameter, MV: mechanical ventilation,

Figure 1. Study flowchart

DTT: diaphragmatic thickening fraction, CCD : end expiratory diameter, CID : end inspiratory diameter, MV : mechanical ventilation.

side since it is the most studied in literature [17,19-21]. The probe was inclined such that the ultrasound beam reaches perpendicularly the posterior part of the diaphragm.

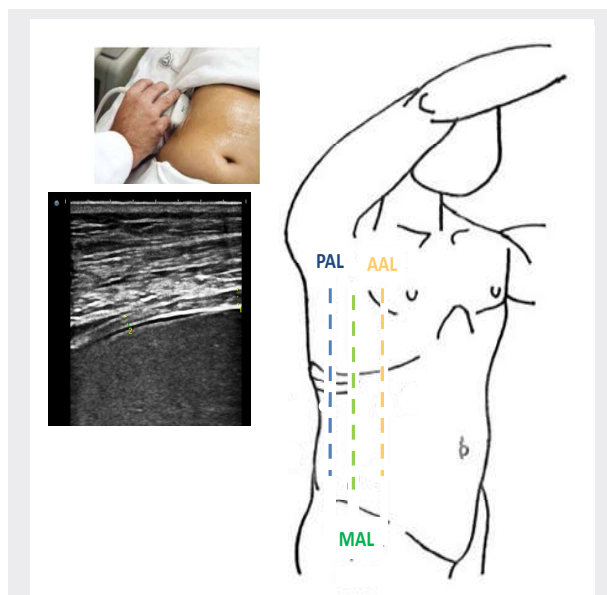
• Obtained images and measurements: when approached by the method described above, the diaphragm appeared as a three-layered structure composed of two parallel hyper-echogenic layers: diaphragmatic pleura at the top and peritoneal pleura at the bottom, sandwiching a hypo-echogenic muscle layer, which is the diaphragmatic muscle. Variation of the diaphragmatic thickness reflecting the two time-respiratory movements was recognized. By the two dimensional mode, the freeze image allows to measure the end inspiratory diameter (EID) and the end expiratory diameter (EED) of the diaphragm (annex 1).



Annex 1: Measures of EID (end inspiratory diameter) and EED (end expiratory diameter)

Measures 1 and 2 correspond to EID (maximum) and EED (minimum). The measurements were taken in B mode (video moved and frozen) by positioning the calipers between the lower edge of the pleural line and the upper edge of the peritoneal line delimiting the hypoechoic zone which is the diaphragmatic muscle.

For both groups, we took all measurements on three levels in relation to the axillary line (anterior, median and posterior) according to the probe pointer direction (annex 2). Thus, we obtained EID and EED as the averages values from the three levels and the diaphragmatic thickening fraction (DTF, %) was determined according to the following formula: $((EID - EED) / EED) \times 100$ [17,20].



Annex 2: Directions of linear probe pointer for measures of post, median and anterior EID/EED

The 3 measurements: anterior, median and posterior of the EID and EED were taken by deflecting the direction of the linear probe pointer (positioned at the apposition zone of the right hemi-thorax): posterior directed towards PAL, median towards MAL and anterior to AAL. The same directions were used in PP.

(EID: end inspiratory diameter, EED: end expiratory diameter, PAL: posterior axillary line, MAL: median axillary line, AAL: anterior axillary line, PP: prone position).

Data collection: For both groups, an electronic database recorded all clinical parameters and ultrasound measurements and DTFs. For the ventilated patients, we noted the details of hospitalization and MV; the severity scores (Simplified Acute Physiology Score II [23], Acute Physiology and Chronic Health Evaluation II [24] and Sequential Organ Failure Assessment [25], the lung/pleura conditions at the time of ultrasound practice, the weaning data and outcome.

Statistical analyses:

Sample size: to the best of our knowledge, no previous study compared DTF between voluntary subjects in SV versus that of MV patients. Hence with DTF as the primary outcome and considering:

- a mean DTF value in SV group at 35%

- an estimated DTF dropout rate of 10%, corresponding to difference Δ at 3.5% (i.e.; minimal interesting difference to detect)
- and a standard deviation σ at 5,

At a statistical power of 80% ($Z_{1-\beta}$ equal to 0.842) and at a risk of alpha error (in two-tailed test) equal to 0.05 ($Z_{\alpha/2}$ equal to 1.96), the sample size of each group was calculated to be 32 (based on the following formula):

$$\begin{aligned} n \text{ per group} &= 2 \times \sigma^2 (Z_{\alpha/2} + Z_{1-\beta})^2 / \Delta^2 \\ &= 2 \times 5^2 (1.96 + 0.842)^2 / 3.5^2 \\ &= 32/\text{group} \end{aligned}$$

Quantitative variables were expressed as means (standard deviation) for Gaussian distribution or median (interquartile 25-75) for non-Gaussian distribution and compared using Student's t-test or the Mann-Whitney U test as appropriate. The normality test was verified by the Shapiro-Wilk test. Comparisons between multiple measures were made using analysis of variance. Categorical variables were expressed as percentages and compared using the Chi 2 test or Fisher's exact test where appropriate.

- After defining the cut-off of the discriminative value of the DTF in the weaning success, the multivariate analysis was applied for assessing the association between DTF and weaning success. The entered factors were DTF (below or above 32,7%) age (below or above 65 years), co morbidities, pleural-lung disease, hypoxemia and sepsis. The correlation between weaning time and DTF was analyzed by the Spearman correlation coefficient. The receiving operating characteristic curve was used to determine the predictive value of DTF in the weaning. In order to assess the effect of the MV on DTF on the one hand and the effect of DTF on the ability of weaning from MV on the other hand, we calculated the effect size using the Cohen's D – Effect Size for T-Test. Means and standard deviations of each independent group were introduced and Cohen's d was calculated. Cohen's d is interpreted as $d = 0.20$ indicates a small effect, $d = 0.50$ indicates a medium effect and $d > 0.80$ indicates a large effect.

Data entry and processing were realized with IBM SPSS STATISTICS software (version 20). The level of significance was set at $p=0.05$.

RESULTS

The initial sample included 68 subjects (35 in the MV group, 33 in the SV group). After applying inclusion and non-inclusion criteria, data of 60 subjects (30 in the MV group;

30 in the SV group) were retained for statistical analysis.

Baseline characteristics: Compared to the MV group ($n=30$), the SV group was younger with a major female sex. All clinical data and circumstances of diaphragm ultrasound are displayed in table 1. Comparison of ultrasound measurements :

- EID: The observed EIDs at the three levels were similar between the two groups. For the average of the three EID measurements, no significant difference was shown ($p=0.09$). Likewise, it was not found a difference between the three values of EID nor in MV group nor SV nor between the six values (Figure 2).

- EED: EEDswere higher in MV group with a significant difference for the median level. The mean of the three EED measurements was higher in ventilated patients ($p=0.01$). It was found a significant difference between the six values (Figure 3)

- DTF: All individual values of DTF are shown in Figure 4. Overall, DTF was significantly lower in early-stage MV patients compared to SV subjects with an average reduction of ten percent. The effect size calculation of the early-stage MV on the DTF values reinforced this result (Cohen's $d=2.06$ which was a huge effect).

- For MV patients, the ventilation mode had no effect on DTF ($40.2 \pm 13\%$ for assisted volume control mode vs. $38.6 \pm 9\%$ for pressure support ventilation mode, $p=0.8$).

Relationship between DTF and weaning:

- Correlation between weaning duration and DTF: In MV patients who have successfully weaned ($n=14$), no significant correlation between the duration of weaning (in days, from the cessation of sedation to extubation) and DTF values (%) was found ($Rho = -0.464$ and $p=0.09$). No correlation was found between the ventilator days and DTF ($Rho = -0.262$ and $p=0.2$).

- The multivariate analysis showed that a DTF value $> 32.7\%$ was close to be a significant factor associated with successful weaning (OR=2; 95% CI= [1.07-3.7] and $p=0.05$).

- Reliability of DTF in predicting weaning: as shown in Figure 5, the performance that a DTF $> 32.7\%$ predicted successful weaning was not significant (AUC-ROC=0,679, $p=0,09$) with a sensitivity at 85.7%.

- The effect size calculation of DTF on the ability to wean from MV was moderate (Cohen's $d=0.52$).

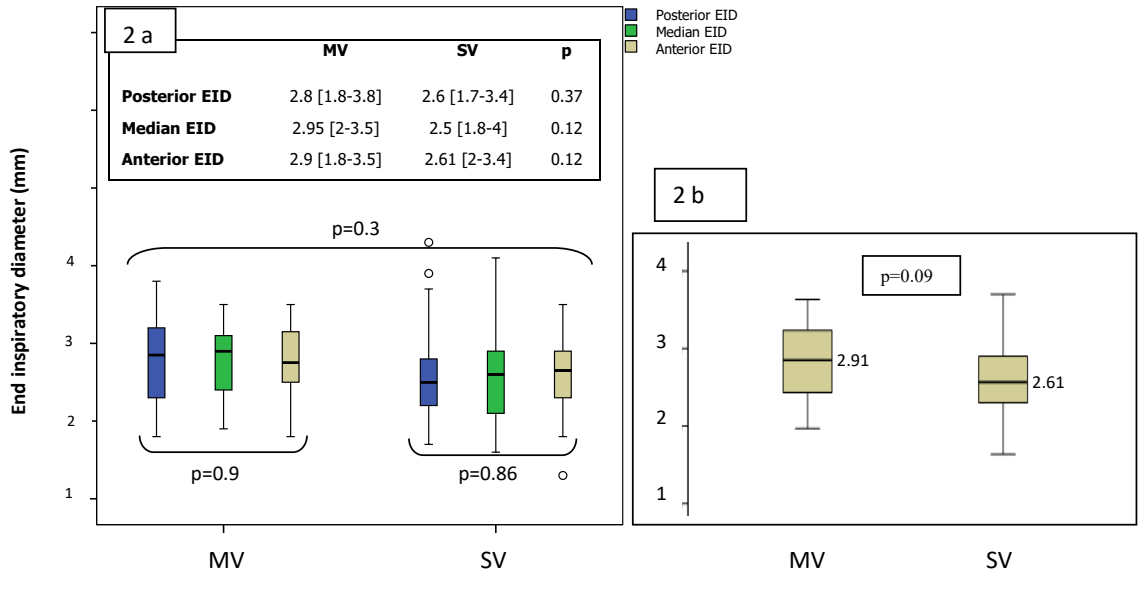


Figure 2. Comparison between end inspiratory diameters at the three levels of mid-axillary lines (2 a) and between their averages (2 b)

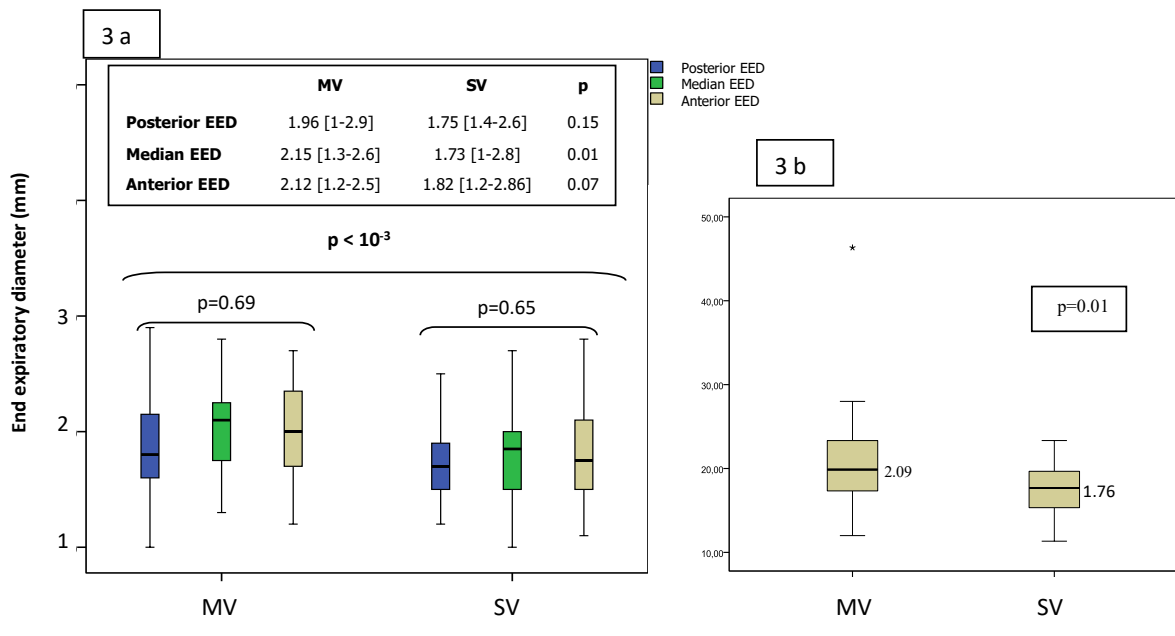


Figure 3. Comparison between end expiratory diameters at the three levels of mid-axillary lines (3 a) and between their averages (3 b)

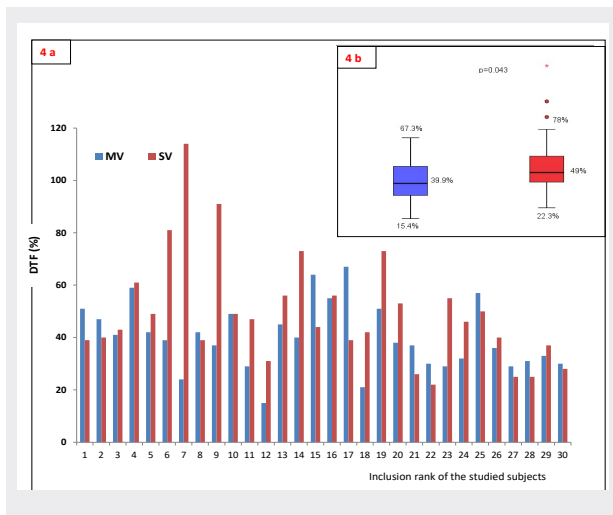


Figure 4. Individual values (4 a) and box plot (4 b) of DTF in the study groups

DTF : diagnostic thickening fraction, MV : mechanical ventilation, SV: spontaneous ventilation

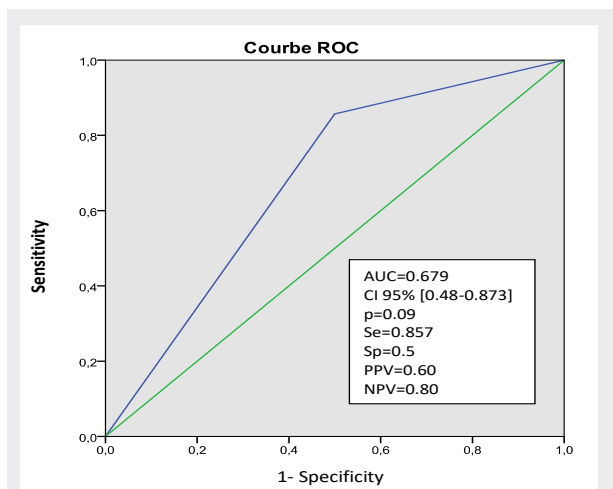


Figure 5. Reliability of DTF > 32.7% in predicting successful weaning

AUC : area under the curve, CI : confidence interval, DTF : diaphragmatic thickening fraction, Se : sensitivity, Sp : specificity, PPV : positive predictive value, NPV : negative predictive value.

DISCUSSION

We demonstrate that diaphragmatic contractile activity was altered from the first days of MV with a DTF that dropped by ten percent in average. A DTF value exceeding 33%, may be useful for predict a successful weaning with a sensitivity equal to 85.7%.

Diaphragmatic dysfunction in critically ill patients: The diaphragm, a pivotal organ in breathing, is subject to

several attacks in intensive care units, which cause a morphological deterioration of the striated muscle resulting in diaphragmatic atrophy [3-5,7,11]. These abnormalities are commonly observed during septic phenomena, hypoxia, respiratory acidosis, hyperglycaemia, malnutrition and ionic disorders (hypophosphatemia, hypomagnesaemia, hypokalaemia, hypocalcaemia) [26-28]. That is why diaphragmatic dysfunction has been integrated for a long time in the critical illness polyneuropathy/myopathy [1,3]. However, these abnormalities were also described in abdominal/cardiac surgeries and that was imputed to direct lesion of the phrenic nerve [29]. The ventilator's own aggression was confirmed by experimental histological data, which report that ventilation induces structural lesions such as myofibrillar disruption, increased lipid vacuoles in the sarcoplasm and abnormal small mitochondria, and remodelling of muscle fibres [8-12]. Regarding clinical studies, the literature remains poor for histological data. With the use of ultrasound, the ventilator-induced diaphragmatic dysfunction phenomenon (VIDD) became more individualized.

Epidemiology and impact of VIDD: A decrease in diaphragmatic muscle strength was shown in 64% of the patients during the first 24 hours of intubation [30], between 23 and 80% during the weaning [4,5,31-35], and in 84% among patients with prolonged ventilation [36]. Referring to these studies, a DTF value below 30% was considered to evoke DD. As a result, our frequency was 23% (seven among 30 ventilated patients had DTF < 30%), a value comparable to that reported by DiNino et al. at the time of weaning (15/66) [33]. Animal studies suggest that the onset of VIDD depends on the animal model: from the 6th hour of MV in the mice [37], and the 24th hour of MV in rabbits [38], to the 72nd hour in piglets [39]. In humans, the literature has not yet defined the precise time of occurrence of a VIDD. Although our study was not designed for this purpose, since we did not have daily measurements of DTF, it suggests that VIDD occurred precociously (at the first five days of MV). Daily ultrasound measurements have shown that the diaphragmatic thickness decreases within 48 hours of MV, and continue to decrease by six percent every day [40].

Regarding the impact of VIDD, it prolonged the duration of MV and the length of stay in resuscitation unit with a high risk of re-intubation and tracheotomy [31,41,42]. In addition, it was correlated to mortality [30]. In our series, these seven patients who met the definition of VIDD (DTF < 30%), didn't have a

Table 1. Baseline characteristics

	MV group (n=30)	SV group (n=30)	p
M/F (sex-ratio)	20/10	9/21	0.009
Age, years (mean±SD)	47±17	35±13	0.002
BMI, kg/m ² (mean±SD)	24.9±3.9	24.6±3.4	0.79
Tobacco, n (%)	13 (44%)	7 (24%)	0.17
Co-morbidities, n (%):			
• Diabetes mellitus	4 (13.5%)	1 (3.5%)	0.38
• Chronic respiratory disease	9 (30%)	5 (17%)	0.49
• Cardiac disease	7 (23.5 %)	4 (13.5%)	0.5
• Renal failure	3 (10%)	0	-
Origin:			
• ED	24 (80%)	-	
• Medical service	6 (20%)		
ICU admission reason:			
• ARF	14 (47%)		
• Coma	14 (47%)	-	
• Septic shock	1 (3%)		
• Cardiogenic shock	1 (3%)		
Severity scores:			
• SAPS II, med [IQR]	41 [25-47]		
• APACHE II, med [IQR]	14 [11-21]	-	
• SOFA, med [IQR]	4 [3-6.5]		
Diaphragm ultrasound circumstances:			
• Delay from MV:			
○ Day 2	10 (34%)		
○ Day 3	8 (27%)		
○ Day 4	7 (24%)		
○ Day 5	5 (15%)		
• Sedation	25 (84%)		
• Ventilator mode and settings:			
○ Assisted Control volume:	25 (84%)	-	
▪ inhaled O ₂ fraction, med [IQR]	0.4 [0.3-0.6]		
▪ Tidal Volume (ml, med [IQR])	400 [375-437]		
▪ RR (c/mn), med [IQR]	18 [16-22]		
▪ PEEP (cm H ₂ O), med [IQR]	6 [5-8]		
○ PSV:	5 (16%)		
▪ inhaled O ₂ fraction, med [IQR]	0.3 [0.3-0.4]		
▪ pressure support (cm H ₂ O), med [IQR]	10 [9-12]		
▪ PEEP (cm H ₂ O), med [IQR]	6 [3-6]		
Lung and pleura status:			
• Pneumonia	16 (54%)		
○ Right lung pneumonia	10/16	-	
• Right pleurisy	7 (24%)		
Haemodynamic status:			
• HR (b/mn), med [IQR]	96 [80-110]		
• SBP (mm Hg), med [IQR]	125 [118-143]	-	
• DBS (mm Hg), med [IQR]	64 [61-85]		
• Vasopressors, n (%)	9 (30%)		
Corticosteroids	12 (40%)	-	
Successful weaning	14 (47%)	-	
Ventilator days? med [IQR]	7 [4-14.5]	-	
ICU length of stay, med [IQR]	11 [7.5-24]	-	
Survivors	16 (54%)	-	

Abbreviations du table 1: APACHE: Acute Physiology and Chronic Health Evaluation, ARF: acute respiratory failure, BMI: body mass index, DBP: diastolic blood pressure, ED: emergency department, F: female, HR: heart rate, ICU: intensive care unit, M: male, MV: mechanical ventilation, PEEP: positive end expiratory pressure, PSV: pressure support ventilation, RR: respiratory rate, SAPS: Simplified Acute Physiology Score, SBP: systolic blood pressure, SD: standard deviation, SOFA: Sequential Organ Failure Assessment, SV: spontaneous ventilation

longer ventilator days (12.5 d [9-25] in the VIDD subgroup vs. six days [4-11] in those without VIDD, $p=0.46$) nor a longer length of stay (13 days [8-28] vs. 10 days [7-20], $p=0.1$). For mortality, it was higher without statistical significance in the VIDD subgroup: 5/7 (71%) vs. 9/23 (39%), $p=0.2$. The lack of significant poor impact may be explained by the precocity of the measurements, since our objective was to assess the early effect of MV on diaphragmatic contractility.

Interpretation of the study results: An increase in the diaphragmatic thickness of 20% at forced inspiration reflects a good trophicity of the muscle [43]. The thickening fraction oscillated between 28 and 96% in a normal subject [43]. A diaphragmatic thickness of less than two mm and a thickening ratio of less than 20% were found during diaphragmatic paralysis [44,45]. Brown et al. [46] compared EID, EED and DTF in healthy subjects between supine, sitting and standing positions. They found that EID and DTF increased significantly between the three positions (for EID: from 35 to 46 to 65, $p=0.01$ and for DTF: from 60 to 97 to 174%, $p<0.001$); however, no change in EED was found (22 to 24 to 25 mm) [46]. Overall, these values, although taken in healthy subjects, are higher than ours and than those reported in literature [33-36]. Goligher et al. [47], by a daily assessment of DTFs over eight days of ventilation, showed that in the subgroup with decreased DTF, it ranged from 47% and 21% from day one to day eight. Our MV group's DTF measured in a median time of three days was at 39.9%. What is "weird" for our measurements is that with the alteration of the diaphragmatic contractility in ventilated patients, the expiratory diameters were higher in ventilated patients. Histological aggressions of the ventilator on the diaphragm (increased proteolysis, oxidative stress, structural damage and remodeling of muscle fibres) eventually lead to atrophy. However, at an early stage (such as the delay of our measurements), it is rather the oedematous-inflammatory phenomena preceding the evolution to atrophy which predominates. This explains the thicker diameter in these ventilated patients.

Usefulness of diaphragmatic ultrasound to predict weaning success/failure: The influence of VIDD on the weaning remains a controversial topic. For some authors, it is considered to be one of the main underestimated causes of weaning failure associated with prolonged stay in intensive care unit [5,42]. For others, diaphragmatic dysfunction is not necessarily associated with weaning failure [4]. In general, the diaphragmatic thickening fraction is markedly lower in patients with weaning failure [48,49]. Several studies have attempted to define the threshold

value of DTF that predicts a weaning success. It varied from 20% to 36% according to Dres et al. [5], DiNino et al. [33], Farghaly and Hasan [48], Blumhof et al. [49] and Ferrari et al. [50]. In our study, a minimum DTF value of 32.7% was nearly to be discriminatory in predicting weaning success ($p=0.05$).

Strength and weakness: The original conception of our study is that we focused mainly on the early assessment of the ventilator effect on the diaphragm contractility, by comparing a group of patients from their first days of MV to that of volunteers in spontaneous breathing. Our results consolidate further the relationship (cause/effect) between the ventilation at its beginning and diaphragmatic dysfunction.

Nevertheless, certain limitations reduce the generalization of our results: first, the control group was younger (12 years average difference), in good health (zero severity scores) and predominantly female. Subgroup analysis by sex was not performed here. In previous results (at post-mortem) [51], the diaphragm was thicker in male taking into account body weight. Yet, it is not clear that this necessarily supports the idea that the diaphragm contractility is higher in males in vivo. More studies [43,52] did not find a difference in thickening between sexes, which reinforces our decision not to split the analysis of participants by sex. Second, the MV group was not homogenous since 10/30 patients have a right lung pneumonia, which could alter the diaphragm mechanic and therefore explain a large part of the results observed. Other factors may be considered as a source of heterogeneity: the patients had their diaphragm ultrasound on four different days from the onset of MV and received two types of ventilator modalities (assisted volume control mode and pressure support ventilation mode). It was designed to explore the diaphragmatic function during the early days of MV that it was set between a minimum of 48 hours and a maximum of five days. For the ventilation mode, it could not be an influencing factor since the DTF did not differ according to the mode ($p=0.8$).

Third, we did not obtain a series of histological diaphragm tissue samples in order to correlate structural changes with diaphragm thickness variations observed with ultrasound exploration.

We conclude that the diaphragm contractile activity was altered since the first days of ventilation with a poor impact on the weaning progress. That corresponds to a current clinical challenge. Advances in ultrasonic technology allow to clinicians to screen precociously, patients at risk of diaphragmatic weaknesses. Future studies should

consider integrating ultrasound into algorithms for the decision-making procedure in the weaning process.

Conflict of Interest: The authors declare they have no conflict of interests.

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