

Diaphragmatic Evaluation

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INTRODUCTION

a) Anatomy and Physiology

The diaphragm is a dome-shaped membrane that delimits the thoracic from the abdominal cavity. In adults it covers an area of about 900 cm². It represents the principal inspiratory muscle and it can shorten itself to 40% from Residual Volume (**RV**) to Vital Capacity (**VC**). The diaphragm has extensive functional reserve; in fact, a unilateral paralysis of the phrenic nerve generates only a mild respiratory dysfunction [4].

It is made of a peripheral muscular component subdivided into sternal, costal and lumbar group and a central fibrous tendon made up of the right, left and middle leaflet [1]. The muscular component originates from surrounding structures (lumbar vertebrae, ribs and xiphoid process) and insert centrally on the central fibrous tendon. It is primarily composed of fatigue-resistant slow-twitch type I and fast-twitch type IIa myofibers [5]. It is characterized by several attachments to neighboring structures: posteriorly the two diaphragmatic crura insert as muscle bundles into the superior lumbar vertebrae (L1-L3 on the right and L1-L2 on the left side) [1]. The median and lateral arcuate ligaments blend to L1-L2 and to T12 and the 12th rib, respectively. Muscle fibers insert at the front into the xiphoid process and continue laterally attaching to the costal margin of the ribs from 6th to 12th.

The left and right phrenic arteries which arise directly from the aorta, together with branches of the internal mammary arteries and provide the arterial blood supply to the diaphragm. The venous system drains in the inferior vena cava via the phrenic veins.

In normal conditions the external Zone of Apposition (**ZOA**) between the inner part of the ribcage and the diaphragm allows free diaphragmatic excursions due to the parietal pleura and to the absence of interposed lung tissue. At Functional Residual Capacity (**FRC**) and in orthostatism the ZOA include 55% of the diaphragmatic surface [4].

The diaphragmatic contraction results in an increase in lung volumes. The underlying mechanism is most likely made up of a combination of a lowering of the central part of the diaphragm with a reduction of the ZOAs and a raising in tension of the diaphragmatic dome with reduction in curvature. The change in the shape, the lowering and the anterior-posterior motion of the entire diaphragm results in the caudal displacement of the abdominal content and increase in the abdominal pressure in the ZOA end expansion of the lower rib cage, with consequent physiologic action on lung volumes [4,5].

b) Innervation

The diaphragm is innervated exclusively by the left and right phrenic nerves, which originate mainly from the fourth (C4) and partially from the third (C3), and the fifth (C5) cervical nerves roots [6]. They carry both sensory and motor fibers to the diaphragm. The phrenic nerves emerge close to upper part of the lateral border of the Scalenus Anterior Muscle (**SAM**) and run obliquely in the lateral compartment of the neck, from lateral to anterior-medial direction, toward the thorax [2,6].

The right phrenic nerve crosses anteriorly the internal mammary artery in the upper thorax, then runs along the innominate vein and the superior vena cava and on the anterior surface of the pericardium until it passes through the diaphragm, laterally to the caval hiatus. The left phrenic nerve runs anteriorly to the left subclavian artery and posteriorly to the thoracic duct, crossing in its course the left mammary artery and the aortic arch, then it passes over the pericardium of the

left ventricle and it reaches the diaphragm laterally to the left heart. Left and right phrenic nerves separate then in four branches to innervate right and left diaphragm surfaces [6,8].

c) Openings

Three openings allow the thorax-abdominal passage of as many major vascular and gastrointestinal structures. The first is the caval opening; it is situated in the middle of the central fibrous tendon, at T8 level and the inferior vena cava and the branches of the right phrenic nerve pass through it. The esophagus passes through the esophageal opening, at T10 level, together with the vagus, fibers from sympathetic nerves and branches of the left gastric artery [9]. At T12 level the descending aorta, the azygos and emiazygos vein and the thoracic duct pass through the aortic hiatus, which represents the third opening [1,2].

DIAPHRAGMATIC DYSFUNCTION [2,5,10]

The diaphragm is the principal inspiratory muscle, as outlined in the previous paragraph. Moreover, it helps in regulating other physiologic functions, such as vomit, the expulsion of feces and urine and the prevention of gastroesophageal reflux [2].

Frequently the first suspect of a Diaphragmatic Dysfunction (**DD**) comes accidentally from radiological findings (elevation of hemidiaphragm); in fact, DD is often asymptomatic, especially when it is unilateral. In order to avoid false positives, it is mandatory to remember that the normal frontal chest X-ray appearance is characterized by a right hemidiaphragm one intercostal space higher than left hemidiaphragm [2,9].

Diaphragmatic dysfunction can be classified as weakness or paralysis and can affect one (unilateral) or both (bilateral) hemidiaphragms.

The unilateral DD is often asymptomatic; dyspnoea during exercise and in supine position are common findings. Symptoms are more frequent in patients with comorbidities that can affect respiratory or muscular function or abdominal pressures such as obesity, lung disease (especially with airflow obstruction), reduction in muscular strength of various etiology and heart failure.

Patients with bilateral DD present with symptoms of respiratory failure such as dyspnea at rest, in supine position or on exertion; they may suffer from hypoventilation in their sleep [2,5].

A special consideration merits eventration as differential diagnosis. Diaphragmatic eventration consists in a congenital or rarely acquired disease characterized by a thinning or absence of the muscular part of the eventrated portion. It can be bilateral, unilateral or localized and it can be mistaken for diaphragmatic paralysis [11].

The etiology of diaphragmatic dysfunction can involve any of the structures that interest the neuromuscular axis from the phrenic nerve to the diaphragm, which begins with the brain and ends to the muscle itself, in a cranial to caudal point of view. Central nervous system diseases such as multiple sclerosis, stroke, amyotrophic lateral sclerosis and spinal cord injury above C5, with

partial functional preservation in lesion from C3 to C5, can result in DD. Phrenic nerve lesions caused by Guillain-Barré syndrome, external compression from neoplastic masses, neuropathy of several etiology including critical-illness polyneuropathy and chronic inflammatory demyelinating polyneuropathy might lead to an abnormal or absent diaphragm unilateral or bilateral neuromuscular stimulation. Among respiratory system disorders Chronic Obstructive Pulmonary Disease (**COPD**) and asthma might impair the diaphragm by shortening the muscle and making contraction less favorable due to a hyperinflation mechanism. Similarly, any disease able to increase the intra-abdominal pressure might possibly create a mechanically unfavorable condition for diaphragmatic contraction. The neuromuscular junction might be impaired due to acquired diseases, such as miastenia gravis. Botulinum and organophosphates intoxication or drugs like neuromuscular blocking agents administered during anesthesia and/or mechanical ventilation affect diaphragm contraction. The muscle itself can be involved in congenital (like muscular dystrophies) or acquired diseases, such as myositis, nutritional defects, corticosteroid therapy and disuse atrophy [5,10,12]. In addition, hypothermic, mechanical and ischaemic injury are common and well-known causes of phrenic nerve damage during cardiac surgery [6]. A special mention should deserve diaphragmatic weakness caused by inflammation and oxidative stress that lead to an imbalance between proteolysis and protein synthesis the typical condition of the critically ill patient [13].

US EVALUATION

Ultrasonographic evaluation of diaphragmatic function (**D-US**) is a feasible, reproducible, relatively inexpensive and safe technique to assess respiratory muscle function at bedside. Comparing to the other diagnostic testing, US does not require to use ionizing radiations like thoracic CT or chest X-Ray, it is totally non-invasive and it can be performed anytime according to clinical changes or physician's needs.

Ultrasonographic evaluation of diaphragmatic function (**D-US**) can be done on spontaneous breathing patients or during mechanical ventilation, based on the patient's condition, the type of measurements and the specific etiology of diaphragmatic dysfunction. Furthermore, D-US has been used to support weaning and identify diaphragmatic impairment [14-20] or to optimize ventilator-patient's interaction in mechanically ventilated patients [21].

Diaphragmatic motion, abdominal organ evaluation and muscle thickness measurement during respiratory cycle represent the main consolidated methods to assess respiratory function with US. Several techniques have been proposed over years to achieve codified procedures and the most reliable and reproducible of them are described later in this chapter.

The type and frequency of the probe, the choice of US modality (2D B-mode or time-motion M-mode), the patient's position, the ecographic approach to the left and to the right hemidiaphragm, the modality of ventilation (spontaneous breathing, invasive or non-invasive mechanical ventilation) and the degree of patient's collaboration for volitional tests represent the most important technical aspects to deal with in the D-US assessment.

Diaphragmatic Thickness

Historical perspective

The first methodological descriptions in healthy subjects came in 1989 by Wait [25], followed by Houston in 1992 [26] and by Ueki in 1995 [27], who compared US measurements with maximum inspiratory pressure index (Pimax) values and proposed reference ranges. Subsequently Cohn [28] introduced again the notion of thickening fraction (TF_{di}) instead of absolute thickness (T_{di}), while McCool [29,30] showed the correlations between T_{di} findings, anthropometric data and Pimax values in normal subjects. In the same years De Bruin [31,32] described T_{di} in patients affected by Duchenne Muscular Dystrophy (**DMD**) and chronic asthma; Gottesman [33] extended the research on Paralyzed Diaphragm (**DP**) whereas Ayas [34] in a single-case report focused on the role of the US to evaluate the T_{di} in a case of muscular atrophy caused by spinal injury and the preventing role of exercise to preserve respiratory function. In two prospective trials Gorman [35] focused on the relationship between ribcage diameters and coronal diaphragm length in COPD patients and Goswami [36] employed US to define the role of diaphragmatic weakness in Graves's disease and its reversibility with carbamazepine therapy. Thickening fraction was also evaluated in patients affected by Cystic Fibrosis (**CF**) and exposed to inspiratory muscle training, by Enright [37]. There followed a case report on Amyotrophic Lateral Sclerosis (**ALS**) patients by Yoshioka [38] and two prospective studies in 2008 by Chiappa [40] in chronic heart failure patients. Summerhill in 2008 [41] and Crausman in 2009 [42] relayed then US in patients with DP, the first trying to find a valid diagnostic tool and the last to monitor the effects of a novel therapy. In the same years Dufresne tried to correlate the degree of T_{di} with inflammation in a CF cohort compared to controls [43]. Since T_{di} measurements have been studied only almost in the erect position, Baldwin established in 2011 a US method using semi-recumbent or supine postures, demonstrating its validity and accuracy in healthy participants [44]. The first study on Invasively Mechanically Ventilated (**IMV**) patients came from Grosu who demonstrated diaphragm muscle thinning at 48h after intubation but he did not find a correlation with muscle strength [45]. At the same time Vivier conducted a preliminary study on 12 ICU patients undergoing Non-Invasive Ventilation (**NIV**): the authors used M-mode for T_{di} and TF_{di} evaluation (21). The first large study, which provided an extensive database of normal T_{di} and TR values with respiration came from Boon in 2012 [46]. They measured T_{di} and TR in 150 healthy subjects in supine position, partitioned according to age, sex, Body Mass Index (**BMI**) and history of smoking. The same authors in 2014 provided a class II evidence that diaphragmatic US is a feasible and accurate diagnostic test to identify neuromuscular diaphragmatic respiratory failure, declaring a sensitivity 93% and a specificity 100%. Additionally, they analyzed the differences between US and other techniques, such as fluoroscopy and chest X-ray [47].

In the same year Baria concluded that normal values for T_{di} and TR found in healthy subjects are comparable with the same variables measured in COPD patients [22]; in a similar cohort of

patients Smargiassi suggested diaphragmatic US as a useful tool in determining the degree of hyperinflation [48].

In 2014 again US was used by Santana [49] to help with the diagnosis of the rare acute neuralgic amyotrophy in a single-case report, while Souza [49] and West [50] expanded the knowledge in the field of US employment in the evaluation of training programs efficacy in age-related weakness and in cervical spine cord injury, respectively. Moreover, the usefulness of US in the assessment of diaphragmatic dysfunction in patients affected by sepsis and undergoing mechanical ventilation, has been well described by Baldwin [51] in an ICU clinical context. They investigated differences in thickness and strength of respiratory and peripheral muscles and they concluded that the diaphragm is relatively spared from wasting compared to peripheral muscles. The B-Mode US was also used to find a new index able to predict weaning success or failure from IMV. Some evidence that TF_{di} may be of some use to predict extubation success or failure during spontaneous breathing trials was achieved by DiNino [43] in 63 intubated patients and by Ferrari on 46 tracheostomyzed subjects [20]. In 2015 Goligher first validated T_{di} and TF_{di} US measurement in ICU mechanically ventilated patients, defining normal reference values comparing to normal healthy volunteers [17]. Goligher again analyzed then the evolution of diaphragm thickness during IMV, assessed by M-mode US and compared with non-ventilated, control subjects. They concluded that changes in T_{di} and TF_{di} that may be associated with diaphragmatic weakness are common in ICU IMV patients and that the titration of the inspiratory effort may prevent these changes [23]. Schepens in an observational cohort study including 54 IMV patients described the Ventilator-Induced Diaphragmatic Dysfunction (**VIDD**) with US and found a correlation between the degree of VIDD and the length of IMV [18].

Description of technique

Diaphragm B-mode or M-mode thickness evaluation is a feasible, reproducible and non-invasive technique to assess diaphragm activity and to evaluate the impact of mechanical ventilation on patients in terms of anatomical changes and muscle strength.

Diaphragmatic thickness (T_{di}) at different lung volumes, the diaphragmatic thickening fraction during inspiration (TF_{di}) and the Thickening Ratio (**TR**) represent the main parameters that have been used for this purpose. T_{di} , TF_{di} and TR are measured in the Zone of Apposition of the diaphragm to the ribcage (**ZOA**).

T_{di} represents the distance in mm or cm from the pleural to the peritoneal line measured in B-mode or M-mode. This value can be obtained with the diaphragm relaxed at Functional Residual Capacity (**FRC**), at Total Lung Capacity (**TLC**), at Residual Volume (RV) or at Pi_{max} . Ueki and Ferrari measured T_{di} from the middle of the pleural line to the middle of the peritoneal line, [20,27] while Cohn defined T_{di} as the distance between the outer edge of the limiting membranes [28]. On the contrary Boon and Baria measured thickness by placing calipers inside the hyperechoic layers [22,47].

The TF_{di} can be obtained as the ratio between the T_{di} at end inspiration (or at Pi_{max}) minus the T_{di} at end expiration (at FRC) and the T_{di} at end expiration. This value can be expressed as absolute number or as percentage [21,28,33,40].

Another parameter that has been proposed is the Thickening Ratio (**TR**), obtained as a ratio between T_{di} at Pi_{max} or at the end of maximal inspiration (TLC) and T_{di} at FRC [27,31,37,46].

The correlation between US measurement and real anatomical structure was showed first in 1989, by Wait, and then in 1997, by Cohn, who measured T_{di} by US and by ruler in dead patients during necropsy [25,28]. The relationship between changes in T_{di} and diaphragm shortening during inspiration has also been described. (28)

In order to evaluate T_{di} , TR and TF_{di} , all the authors used a high-resolution 7.5-15 MHz US linear probe, because of its high superficial spatial resolution, in B-mode or M-mode. The transducer should be placed on an intercostal space perpendicular to the chest wall and among the antero- and midaxillary line. The marker has to be directed caudally with the US beam exploring a vertical anatomic section. The costophrenic sinus can be seen as a transition zone between the lung cranially, identified by specific artifacts (A lines if well aerated), and the liver or the spleen caudally. The Zone of Apposition (**ZOA**) is located 0.5-2 cm below the costophrenic sinus. The diaphragm in the ZOA presents itself as a hypoechoic layer between two hyperechoic bright, vertical and parallel lines, which represent the pleural and peritoneal membranes. In some people it is possible to visualize a third hyperechoic line in the core portion of the muscle.

The patients can be on spontaneous breathing in room air or on O_2 -therapy, or mechanically ventilated with different level of ventilation assistance, from spontaneous breathing to total support.

Reference ranges for T_{di} , TR and TF_{di} are summarized on table 1. It is known that the diaphragm is estimated to shorten by 25-35% from RV to TLC [28]. Boon et al. in a large prospective trial defined a $T_{di} > 0.14$ cm and a TR > 1.2 (obtained as Tdi at maximal inspiration ratio Tdi at FRC) as "normal"; Gottesman suggested a value for $T_{di} > 0.2$ cm in healthy subjects combined with a TF_{di} of at least 20% at maximal inspiration [33,47]. Basing on Cohn and Ueki findings, in a recent review Matamis et al. suggested to consider normal T_{di} range, values between 0.18 and 0.3 cm at FRC, T_{di} increase from RV to TLC of 54 % (range 42-78 %) and a TR of 2.6 (obtained as Tdi at Pimax ratio Tdi at FRC) [52].

Table 1: Limit values for diaphragmatic thickness and thickening.

Author (year)	Subjects (n)	Variables	Values
Ueki (1995)	Healthy (13)	T _{di} (mm) TLC FRC RV	4.5 ± 0.9 1.7 ± 0.2 1.6 ± 0.2
Cohn (1997)	Healthy (9)	T _{di} (mm) 25% _{VC} TF (%)	2.8 ± 0.4 54% (range 42-78%)
De Bruin (1997)	DMD (10)	T _{di} (mm) Pi _{max}	DMD Healthy 2.62 ± 0.70 3.50 ± 0.85
	Healthy (9)	TR FRC	1.74 ± 0.21 1.48 ± 0.20 1.6 ± 0.5 2.3 ± 0.4
De Bruin (1997)	Asthma (9)	T _{di} (mm) FRC	Asthma Healthy 2.2 ± 0.4 1.7 ± 0.3
	Healthy (9)	TR Pi _{max}	4.3 ± 0.9 4.0 ± 1.2 2.0 ± 0.3 2.2 ± 0.3
Gottesman (1997)	DP (15)	T _{di} (mm) FRC	Bilateral DP Healthy 1.8 ± 0.2 2.8 ± 0.4
	Healthy (15)	TF (%)	-1 ± 15% 37 ± 9
Boon (2012)	Healthy (150)	T _{di} (cm) FRC TR	0.33 ± 0.1 (R) 0.34 ± 0.18 (L) 1.8 ± 0.5 (R) 1.9 ± 0.6 (L)
Vivier (2012)	Healthy NIV (14)	T _{di} (cm) EI	SB PS10 3.08 (2.77–4.77) 2.73 (2.39–3.25)
		TF (%) EE	2.19 (1.94–2.76) 2.29 (1.84–2.52) 47.5 (35.9–63.2) 22.0 (9.6–28.2)
Baldwin (2014)	Sepsis (16)	T _{di} (mm) EE	Healthy Sepsis 1.73 ± 0.40 1.61 ± 0.43
	Healthy (16)		
Baria (2014)	COPD (50)	T _{di} (mm) FRC	COPD Healthy 0.28 ± 0.16 (R) 0.32 ± 0.17 (L) 0.32 ± 0.13 (R) 0.34 ± 0.18 (L)
	Healthy (150)	TR	1.9 ± 0.5 (R) 1.9 ± 0.7 (L) 1.8 ± 0.5 (R) 1.9 ± 0.5 (L)
Ferrari (2104)	Weaning (46)	T _{di} (mm) TLC	Success Failure 0.38 (0.29 to 0.45) 0.30 (0.20 to 0.40)
		TF RV	0.25 (0.19 to 0.28) 0.24 (0.17 to 0.30) 0.56 (0.38 to 0.64) 0.26 (0.22 to 0.30)
Smargiassi (2014)	COPD (32)	T _{di} (mm) FRC	3.3 ± 0.66
		TLC	3.6 ± 0.71 6.0 ± 0.96
Schepens (2015)	IMV (54)	T _{di} (mm) EE	Baseline 72h IMV 1.9 ± 0.4 1.4 ± 0.4

Data are presented as mean ± SD or median with IQR (25-75). Values for right hemi diaphragm unless otherwise specified; DT=diaphragmatic thickness; TR=thickening ratio (T_{di}-Pi_{max} or TLC/T_{di}-FRC); TF=(T_{di}-end exp-T_{di}-endinsp)/T_{di}-endexp*100); TLC=total lung capacity; FRC=functional residual capacity; RV=residual volume; VC=vital capacity; Pi_{max}=Pi_{max} manoeuvre; DP= diaphragmatic paralysis; CF=cystic fibrosis; Chronic heart failure; IC=inspiratory capacity; SB=spontaneous breathing; PS15=pressure support 15cm H₂O; (R)=right; (L)=left; EI=end inspiration; EE=end expiration; IMV=invasive mechanical ventilation.

Clinical applications

T_{di}, TR and TF_{di} have been used for research and clinical purpose in several pathological conditions responsible of diaphragmatic dysfunction. TR at FRC represents a good indicator of diaphragm strength [27], while TF_{di} is linearly related to inspired volumes, with a variation

among subjects and approximates the degree of diaphragm shortening [25,28]. It has also been demonstrated that T_{di} increases with height and body weight and that it is also enhanced in trained weight-lifters [30]. T_{di} at TLC and TR also improved with exercise in patients with CF [37]. Although in many studies US evaluation was performed in the erect position, supine posture does not affect measurement, as showed by Baldwin [44].

In the context of neuro-muscular disease, patients with DMD showed an increased T_{di} at rest, probably analogous to the pseudo-hypertrophy typical of limb muscles, with an impaired TR [31]. Opposite findings were reported in ALS patients, who were affected by diaphragmatic atrophy and absence of variation in T_{di} from FRC to TLC [38]. In athletes with cervical spinal cord injury, the inspiratory muscle training increased T_{di} in comparison to placebo [50].

Asthma and COPD can lead to diaphragmatic dysfunction by shortening the muscle at rest. Asthmatic patients have slightly increased T_{di} values compared to normal control subjects [32]. In COPD T_{di} was found to be similar to healthy subjects [22,35]. In addition, T_{di} at TLC was found to be a useful tool to estimate lung hyperinflation [48]. In CF patients T_{di} was greater than in control subjects, in particular when T_{di} at FRC was normalized by fat free mass. [43].

In chronic heart failure T_{di} at end-inspiration and TF_{di} are reduced and both improve with inspiratory muscle training, simultaneously with an improvement in P_{imax} [40].

In patients with unilateral or bilateral DP regardless of the etiology, T_{di} and TF_{di} were significantly reduced compared to the normal hemi diaphragm for unilateral DP or to control subjects for bilateral DP. Consequently, Gottesman concluded that “paralyzed diaphragm is atrophic and does not thicken during inspiration” [33]. Furthermore Summerhill proposed to use only the TF_{di} criteria (cutoff <20%) to diagnose DP, because T_{di} at FRC is influenced by height and body weight. (53) Ultrasound was also used to monitor the efficacy of antiviral therapy in idiopathic DP [42]. T_{di} and TR measurements performed by well-trained clinicians demonstrate a high rate of sensitivity (93%) and specificity (100%) to identify patients with neuromuscular diaphragmatic respiratory failure [47]. In septic patients, T_{di} did not differ from control subjects [51].

Changes in T_{di} are common in Mechanically Ventilated (**MV**) patients, and may be associated with diaphragmatic dysfunction [23]. In fact MV caused a mean decrease of 6% per diem in T_{di} with the most meaningful reduction in the first 72h [18,45]. In these patients, right hemidiaphragm measurements were obtained on 95 % of cases whereas left hemidiaphragm showed more variability [17]. TF_{di} can help to quantify diaphragmatic contribution to respiratory effort during NIV and during pressure support IMV; in particular, TF_{di} is inversely proportional to pressure support [21,54]. Moreover US were also used during weaning from MV as a support to predict extubation failure; a $TF_{di} \geq 30\%$ predicted extubation success with a sensitivity of 88% and a specificity of 71% [19]. In tracheostomized patients, a $TF_{di} > 36\%$ was associated with successful weaning with a sensitivity of 82% and a specificity of 88% [20].

Diaphragmatic Motion

Historical perspective

In a single case report of diaphragmatic dysfunction due to motorcycle accident with neck injury and subsequent phrenic nerve lesion Park in 1981 performed a qualitative US evaluation in the acute phase and in the follow-up to monitor recovery [55]. One of the first attempts to find a reference range for DM values came in 1983 by Harris, who described the normal right hemidiaphragm movement in 50 healthy subjects, compared to Pulmonary Function Tests (**PFT**) [56].

The evaluation of DM was also applied in the newborn population, first in a single case report by Ambler in 1985 [57], then in a prospective trial in 46 subjects, in 1988 by Laing [58]. Diament in 1985 described the US evaluation of diaphragm as a useful tool in the evaluation of 14 patients with suspected diaphragmatic abnormalities, [59]. The year after, Drummond introduced the method in a surgical context to assess the effect of induction of anesthesia on DM in spontaneously breathing subjects [60]. In 1989 Loring [61], who outlined in 1985 the relationship between diaphragmatic length and lung volumes [62], analyzed with US the consequences of pleural sclerosis on DM. In 1992 Jousela defined the DM US differences between spontaneous and mechanical ventilation [63] while Houston tried to establish the quantitative assessment of DM with US in healthy subjects [64]. The latter in 1994 compared the US DM evaluation with fluoroscopy reaffirming the reliability of US over fluoroscopy saying that “it should be the method of choice in the investigation of suspected hemi diaphragmatic movement abnormality” [65]. Coronary Artery Bypass Graft (**CABG**) or Cardiopulmonary Bypass (**CPB**) became soon a matter of interest because of the well-known postoperative respiratory complications, involving the diaphragm. Fedullo [66] and DeVita [67], in two separate trials in 1992 and 1993 prospectively evaluated with DM US 48 and 96 patients respectively who underwent CABG operation. Fedullo concluded that DM US evaluation might help in diagnosing unilateral diaphragmatic paresis, while DiVita found that sonography was the best predictor of phrenic nerve injury, which accounted however only for 57% of diaphragm motion abnormalities. The firsts to prove the relationship between DM and lung volumes were Cohen and Houston in 1994 on 10 and 14 healthy subjects, respectively [65,68]; the first one used a special device to fix the probe to eliminate operator movement bias and analyzed the right DM with a sector probe in M-mode; the second one relied on the B-mode analysis on both hemi diaphragm. In the same year Cohen again evaluated the DM with US in a cohort of hemiplegic patients [69] and in 1995 Houston did the same in 50 patients with stroke and respiratory failure with 40 controls [70]. US DM evaluation was also used by Mills to assess the efficacy of unilateral phrenic nerve stimulation on diaphragmatic contraction together with other methods [71]. The pediatric population was matter of study again in 1998: in fact Riccabona described the DM with US in M-mode in pediatric patients with diaphragmatic palsy [72]. In the same year Blaney carried out a reliability study in

12 healthy subjects [73]. In 1999 Darnley measured the velocity of DM in addition to standard measurement in a little prospective trial on patients affected by ischemic heart disease [74]. Diaphragmatic motion during mechanical ventilation was evaluated again by Akiyama in a little case report of 4 patients with damage to the central nervous system; the novelty was that right hemi diaphragm was insonated in three different position with the patient in four different positions (supine, left or right decubitus and sitting) [75]. In 2001 Ayoub studied US DM before and after cholecystectomy compared to lung function tests to assess the involvement of diaphragm on post-operative respiratory disorders [76] and in 2002 he made use of US DM evaluation to monitor the effects of an abdominal respiratory device in DMD patients [76]. The same author had already provided a US DM evaluation about the influence of spirometric technique on the lung function test itself in 1997 [77]. In 2001 Caruana demonstrated with US that the position and function of the diaphragm is altered in chronic heart failure patients [78]. In that year Gerscovich carried out a prospective trial in which first evaluated the DM by M-mode US in 23 healthy volunteers, then he drew up the same analysis on 22 patients with diaphragmatic abnormalities. They concluded that US is a feasible and useful technique in evaluating DM enough to replace fluoroscopy in clinical practice [79]. A novel method in the US DM evaluation was purposed by Toledo in 2003; he measured the craniocaudal displacement of left branches of portal vein and associated it to the right hemi diaphragm movement [80]. This technique was used to evaluate the origin of diaphragmatic impairment in 54 COPD patients, compared to controls by Yamaguti in 2008 [39]. The first large study, which aim was to quantify the reference values of US DM in healthy subjects, has been performed by Kantarci, in 2004 [81]. They also provided a correlation between some anthropometric variable and DM values. Furthermore, a large retrospective analysis was performed on 278 children with suspected diaphragmatic paralysis, by Epelman in 2005 [82]. Scott in 2006 provided a comparison of DM US findings with pulmonary function tests in 36 patients; they showed that DM US might be useful to predict static lung volumes, but it cannot replace plethysmography to perform dynamic measurements [83]. Moreover, Lloyd employed DM US in patients with suspected diaphragmatic paralysis and defined it a relatively simple and accurate test [84] and Merino-Ramirez used DM US to help in characterize the causal mechanism of phrenic neuropathy after CABG [85]. In the surgical field, Ferrandière reported a single case of intraoperative DM US to assess the efficacy of Non-Invasive Ventilation (**NIV**), used as rescue therapy in a morbid obese who underwent spinal anesthesia for urological surgery [86]. DM US was also successfully used in 2007 by Patel in a single case of lifelong dyspnoea of uncertain origin [87]. In 2008 Boussuges posed a milestone in determining the reference values for DM for both hemi diaphragm in 210 healthy subjects, assessing also the in inter- and intra- observer reproducibility. Their findings have been used and reported until nowadays by many authors to describe the US technique [88]. A case report of 3 tetraplegic patients evaluated with DM US followed in 2009 [89], together with another important research in the field of cardiac surgery by Lerolle, who stated the role of DM US as a support in the diagnosis of severe diaphragmatic

dysfunction in patients requiring prolonged mechanical ventilation after surgery [14]. In addition, DM US evaluation allowed Renes to assess the reduction in incidence of diaphragmatic dysfunction in interscalene plexus block performed with US compared to nerve stimulation guidance [90]. In upper abdominal surgery, DM US evaluation has been defined a practical method to investigate postoperative diaphragmatic abnormalities by Kim [91]; similar findings were showed in a prospective trial on pediatric cardiac surgery by Sanchez de Toledo in 2010 [16]. Then Unlu applied the DM US evaluation as a strategy to examine patients with ankylosing spondylitis [92]. Testa in 2011 proposed an easy, fast, reliable and reproducible method to evaluate diaphragmatic kinetics by US during spontaneous breathing. He provided a defined and reproducible placement point for the transducer and easy to find B-mode images and M-mode traces for quantitative evaluation [93]. In the following years, this technique was applied to hemiplegic patients by De Almeida [94] and Voyvoda [95] and to COPD by Kang [96], who related DM to hypercapnia. While Yamaguti [97] used this technique to assess the efficacy of breathing training programs on diaphragm activity in COPD patients. An appealing approach was proposed by Kim, who found a relationship between DM US and the risk of difficult weaning in a cohort of 88 mechanically ventilated patients in ICU [15]. In 2013 Subotic analyzed the DM in 27 patients who underwent a lung resection for cancer. He found that DM might influence the accuracy of the postoperative lung function prediction [98]. In the same year Soilemezi evaluated diaphragmatic kinetics under the influence of variable respiratory loads [99]. Concurrently many papers were published in 2014 analyzing the DM US evaluation in different clinical context and with various technical approaches. Tenorio used the US monitoring of right hemi diaphragm to evaluate the influence of inspiratory muscle training on diaphragmatic mobility in morbidly obese individuals [100]. Hatam introduced and compared to M-mode a new application in DM US called Speckle tracking echocardiography [101]. Patients affected by combined pulmonary fibrosis and emphysema were studied by He, who concluded that emphysema but not fibrosis might limit diaphragmatic motion [102]. Johnson reported a novel combined approach of phrenic nerve conduction studies with DM US, establishing that this approach enhances diagnostic accuracy in phrenic neuropathy [103]. Then Jung showed a correlation between DM and pulmonary function in hemiplegic patients and proposed to perform such evaluation prior to the rehabilitation therapy [104]. In a large observational trial on 124 patients, Zanforlin introduced the concept of M-mode index of obstruction to evaluate, compared with spirometry, obstructive diseases [105]. DM together with thickness was evaluated by Souza to assess the effects of inspiratory muscle training in elderly women [49]. In 2015 Park analyzed the DM with US in stroke patients with dysphagia compared to healthy subjects; in the study group he found a decreased diaphragm excursion during voluntary coughing [106]. In the same year Pasero introduced a new technical concept with the comparison between anatomical M-mode versus traditional M-mode, in the DM evaluation after cardiac surgery. They pointed out that the so far used M-mode technique might lead to a lack of recognition of diaphragmatic dysfunction in this cohort of patients [107].

Description of technique

Several techniques have been proposed to evaluate DM with US with many attempts of standardization. The major concerns are to determine the probe, the frequency, the posture of the patient, the site of insonation, the direction of the US beam, the US landmarks, the technique of measurement and the reference range for the evaluated parameters.

Another distinction shall be made between patients on spontaneous breathing and those on several degrees of mechanical ventilation, from c-PAP to invasive totally controlled mechanical ventilation.

The most cited Boussuges' description [88] will be used as a comprehensive example of this method. DM US evaluation was carried out in standing position with a sector 2.5-3.5 MHz probe; 2-D B-mode was used to identify the best image and quantitative analysis was performed in M-mode, using the liver and the spleen as acoustic windows for the right and the left hemidiaphragm, respectively. Other author used convex low-frequency probes with similar results [81,93,104] and with supine [79,104] or semirecumbent [93] patients. The B-mode appearance of the diaphragm is a curved and concave upward hyperechoic line which lies on the corresponding hypochondriac organ used as acoustic window. On the right, the probe was placed in the subcostal area between the midclavicular and anterior axillary lines; the US landmark was directed medially, cranially and dorsally, to insonate the posterior third of the hemidiaphragm. Many studies have shown in fact that diaphragmatic excursion increases from anterior to posterior diaphragmatic region [56,108]. On the left the probe was placed in the subcostal or low intercostal area and more laterally, between the anterior and mid axillary lines. Toledo et al. evaluated the correlation between US measurement of craniocaudal displacement of the left intrahepatic branches of the portal vein and right DE, stating that this method could be a useful surrogate to assess right hemidiaphragmatic mobility [80].

The diaphragmatic excursion (DE- expressed in cm or mm) is the main parameters that have been investigated; it can be obtained in B- or M-mode modality. Physiological diaphragm movement is caudal (directed to the probe) during inspiration and cranial (away from the probe) during expiration. Inspiratory, expiratory and total cycle times (in seconds) and the velocity of displacement (slope, in cm/s) are additional values that can be derived from M-mode trace. Slope can be measured during a maximal sniff, intended as a short and fast maximal inspiratory effort through the nostrils with closed mouth. In table 2 reference ranges from the main scientific papers are given. Boussuges indicated as normal an excursion of 1.8 ± 0.3 cm for men and of 1.6 ± 0.3 cm for women on quiet breathing. To cover the different physiological changes in lung volumes in relation to diaphragmatic activity, patients are evaluated during quiet or deep breathing or during a sniffing manoeuvre.

Table 2: Limit values for Diaphragmatic Excursion (DE).

Author (y)	Subjects (n)	DE	Values	
Cohen (1994)	Healthy (10)	DB (cm)	6.0 ± 0.7	
Ayoub (1997)	Healthy (8)	QB (cm)	1.34 ± 0.18	
Kantarci (2004)	Healthy (164)		<i>Men</i>	<i>Women</i>
		DB (mm)	52.73 ± 11.03 (R) 54.12 ± 12.65 (L)	46.93 ± 10.37 (R) 47.57 ± 10.36 (L)
Scott (2006)	Dyspnoea (36)	QB (mm)	14.7 ± 4.1	
		VS (mm)	14.8 ± 3.9	
Boussuges (2009)	Healthy (210)		<i>Men</i>	<i>Women</i>
		QB (cm)	1.8 ± 0.3 (R) 1.8 ± 0.4 (L)	1.6 ± 0.3 (R) 1.6 ± 0.4 (L)
		VS (cm)	2.9 ± 0.6 (R) 3.1 ± 0.6 (L)	2.6 ± 0.5 (R) 2.7 ± 0.5 (L)
		DB (cm)	7.0 ± 1.1 (R) 7.5 ± 0.9 (L)	5.7 ± 1 (R) 6.4 ± 1 (L)
Testa (2009)	Healthy (49)		<i>Men</i>	<i>Women</i>
		QB (mm)	18.7 ± 7.7	18.1 ± 7.7
		DB (mm)	77.9 ± 12.8	79.5 ± 14.0
Soilemezi (2013)	Healthy (40)	QB (cm)	1.7 ± 0.5	
		PT (cm)	2.3 ± 0.9	
		IL (cm)	2.1 ± 0.9	
Jung (2014)	Healthy (16)		<i>Men</i>	<i>Women</i>
		QB (cm)	2.30 ± 0.43 (R) 2.43 ± 0.51 (L)	2.67 ± 0.59 (R) 2.37 ± 0.66 (L)
		VS (cm)	3.64 ± 0.98 (R) 3.89 ± 0.14 (L)	4.11 ± 0.84 (R) 3.71 ± 0.65 (L)
		DB (cm)	7.16 ± 0.95 (R) 7.16 ± 0.56 (L)	6.50 ± 0.91 (R) 5.78 ± 0.76 (L)
Johnson (2014)	Healthy (10)		<i>Right hemiplegic</i>	<i>Left hemiplegic</i>
		QB (cm)	2.21 ± 0.90 (R) 2.36 ± 0.35 (L)	2.86 ± 0.43 (R) 1.59 ± 0.37 (L)
		VS (cm)	3.39 ± 1.41 (R) 3.57 ± 0.64 (L)	5.12 ± 1.08 (R) 1.83 ± 1.97 (L)
		DB (cm)	3.90 ± 1.64 (R) 4.90 ± 1.46 (L)	7.38 ± 1.59 (R) 3.23 ± 1.08 (L)
		QB (mm)	29.2 ± 8.5 (R) 29.5 ± 7.7 (L)	

Data are presented as mean ± SD. Values for right hemi diaphragm unless otherwise specified. DB= deep breathing; QB= quiet breathing; VS=voluntary sniffing; PT= patients connected to a pneumotachograph; IL=50 cm H₂O/L/s inspiratory load; (R) = right hemi diaphragm; (L) = left hemi diaphragm.

Clinical applications

US measurement in both Quiet (QB) and Deep (DB) breathing has been defined as a reliable quantitative and qualitative method to assess diaphragmatic motion, even when compared to fluoroscopy, which has been demonstrated to be less sensitive. Left to right DE ratio (normal values 0.5-1.6 on DB) has been purposed as an indicator of unilateral paresis. [26,79,93,109] A linear relation between tidal volume or inspired volumes and DE has been demonstrated [65,68]. DE measurement can be affected by several variables such as sex, body mass index, waist circumference, and age [81]. Despite DM-US may help to predict static lung volume, it cannot provide a specific or sensitive measure of pulmonary function in relation to whole body plethysmography [83]. DM-US has been successfully used in association to Phrenic Nerve Conduction Studies (PNCS) to improve accuracy and sensitivity as a diagnostic tool for phrenic neuropathy[103].

In hemiplegic patients, a unilateral reduction in hemi diaphragmatic motion was registered in the affected side during volitional breathing, in particular on DB or VS test [69,104]. However, other author did not find such strong correspondence [94,95]. In one study DE on DB was reduced bilaterally after acute stroke, but isolated hemiparesis did not occur [70]. In addition, stroke patients with dysphagia showed a more pronounced reduction in diaphragmatic motion compared with non-dysphagic stroke subjects [106]. In patients with ankylosing spondylitis it was not possible to demonstrate a compensatory increment of DE compared to control subjects [92]. In patients with DMD DE evaluation has been used to assess the efficacy of an abdominal respiratory device [76].

In the context of respiratory system disorders, COPD patients suffered from an impairment of diaphragmatic mobility mainly due to air-trapping. This was measured with the craniocaudal displacement of the left branch of the portal vein method [39]. DE reduction in COPD has also been associated with hypercapnia, airway obstruction and pulmonary hyperinflation [96]. Patients affected by combined pulmonary fibrosis and emphysema showed even lower values for DE [102]. The effects of a diaphragmatic breathing training program improved diaphragmatic movement and functional capacity in COPD patients [97].

To assess the degree of airway obstruction with US, an index called “M-Mode Index of Obstruction (**MIO**)” and based on the expiratory portion of the M-mode trace has been purposed [105].

US proved also to be a valid complement to spirometry; in fact, wearing of a nose clip and breathing through a mouthpiece and a pneumotachograph induced measurable changes in diaphragmatic motion, with an increment in DE and a reduction in the velocity of contraction [77,99].

Phrenic nerve injury following cardiac surgery has been extensively studied with D-US, in particular in patients requiring prolonged postoperative mechanical ventilation [14,85,110]. DE from FRC to TLC is a useful tool to identify diminished left unilateral diaphragmatic motion in asymptomatic patients after CABG, better than the X-Ray film [66]. In a pediatric population, US were perfectly accurate in predicting fluoroscopy result for abnormal diaphragmatic motion after cardiac surgery [16]. Although it can help with diagnosis it is not possible to distinguish between phrenic nerve injury and other etiologies of diaphragmatic dysfunction with US alone [67].

Anatomical M-mode has been evaluated recently for DM-US measurements in cardiac surgical patients; this novel technique reduces the overestimation of DE that occurs with M-mode alone in this clinical context. In fact, proper alignment of the M-mode US beam is difficult to obtain in patients after cardiac surgery, because of surgical scars and dressings [107].

In a cohort of patients with ischemic heart disease without heart failure, inspiratory muscle training resulted in increased velocity of diaphragm during QB and Voluntary Sniffing (**VS**) [74].

In patients with CHF DE on QB or VS was increased compared to controls as well as velocity of contraction, probably for an adaptive response to cardio-thoracic ratio variations due to cardiomegaly [78].

In patients referred for DP, DM-US technique was found to be a useful tool for diagnosis, even at bedside [84]. In unilateral DP the affected hemi diaphragm exhibits paradox (cephalad) motion on inspiration, in particular in the upright position, because of the transdiaphragmatic pressure generated by the normal hemi diaphragm. (87) Instead, a reduced caudal DE is pathognomonic of diaphragmatic weakness.

In the surgical context, US were used to demonstrate the impairment in DE on QB and DB after laparoscopic or open cholecystectomy despite normal Vt at spirometry [111]. After liver lobectomy, a linear correlation between DE values and vital capacity measured by spirometry has been demonstrated [91]. DM-US was helpful to diagnose diaphragmatic dysfunction in a case of acute respiratory failure in a liver transplant patient [112]. In a COPD, obese patients undergoing spinal anesthesia DE measurement allowed to quantify the efficacy of intraoperative NIV application [86]. When performing an interscalene brachial plexus block, the US guidance and the administration of lower concentration of local anesthetic bupivacaine resulted in a reduction of the incidence of DP [90,113].

DM-US technique proved also to be useful in ICU patients in predicting difficult weaning from MV. In fact, in mechanically ventilated subjects with diaphragmatic dysfunction diagnosed during spontaneous breathing trials, frequent early and delayed weaning failures were reported [15]. Recently in a cohort of postoperative patients undergoing spontaneous breathing trial with different levels of pressure support it was concluded that DE should not be used to quantitatively assess diaphragm contractile activity [54].

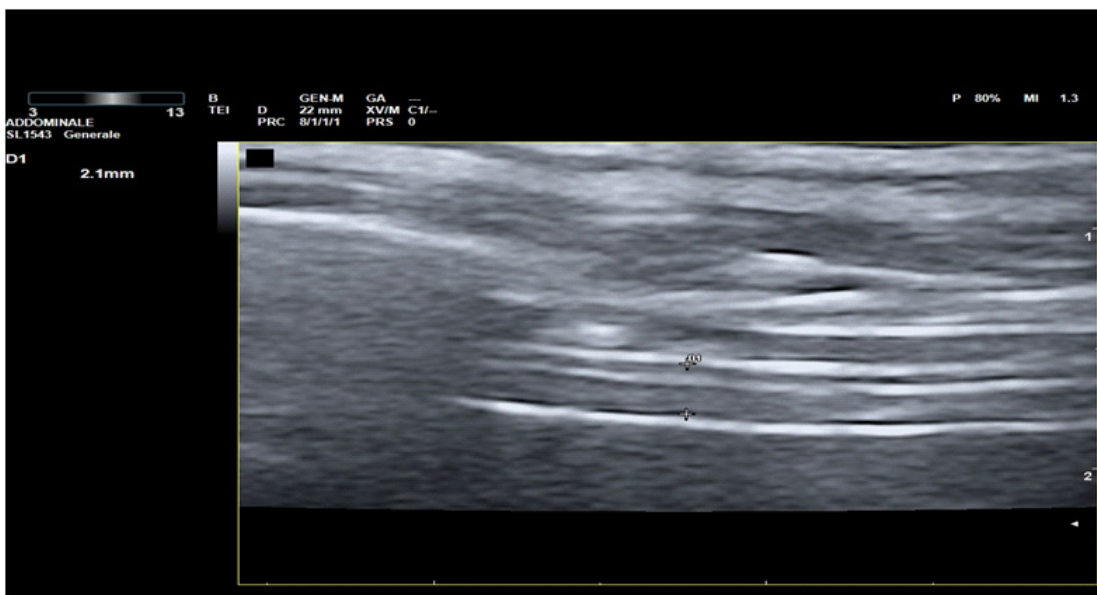


Figure 1: Right inner-layer T_{di} at FRC.

Right hemi diaphragmatic T_{di} measurement in the Zone of Apposition (**ZOA**). US landmark was directed cephalad. T_{di} was obtained with the caliper as the distance between the inner edge of the pleural (on the top) and the peritoneal (on the bottom) diaphragmatic layers. The measurement was obtained at the end of a relaxed, passive expiration (Functional Residual Capacity - **FRC**).

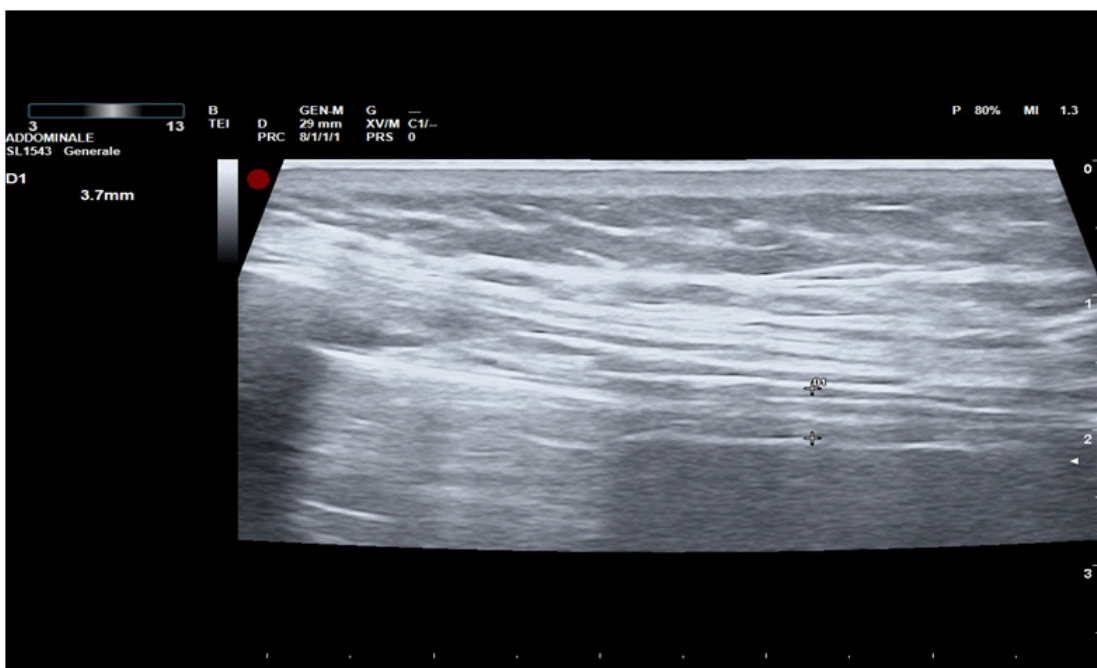


Figure 2: Right inner-layer T_{di} at TLC.

Right hemidiaphragmatic T_{di} measurement in the Zone of Apposition (**ZOA**). US landmark was directed cephalad. On the left side lung artifacts with A line are shown. T_{di} was obtained with the caliper as the distance between the inner edge of the pleural (on the top) and the peritoneal (on the bottom) diaphragmatic layers. The measurement was obtained at the end of a deep, maximal inspiration (Total Lung Capacity - **TLC**).

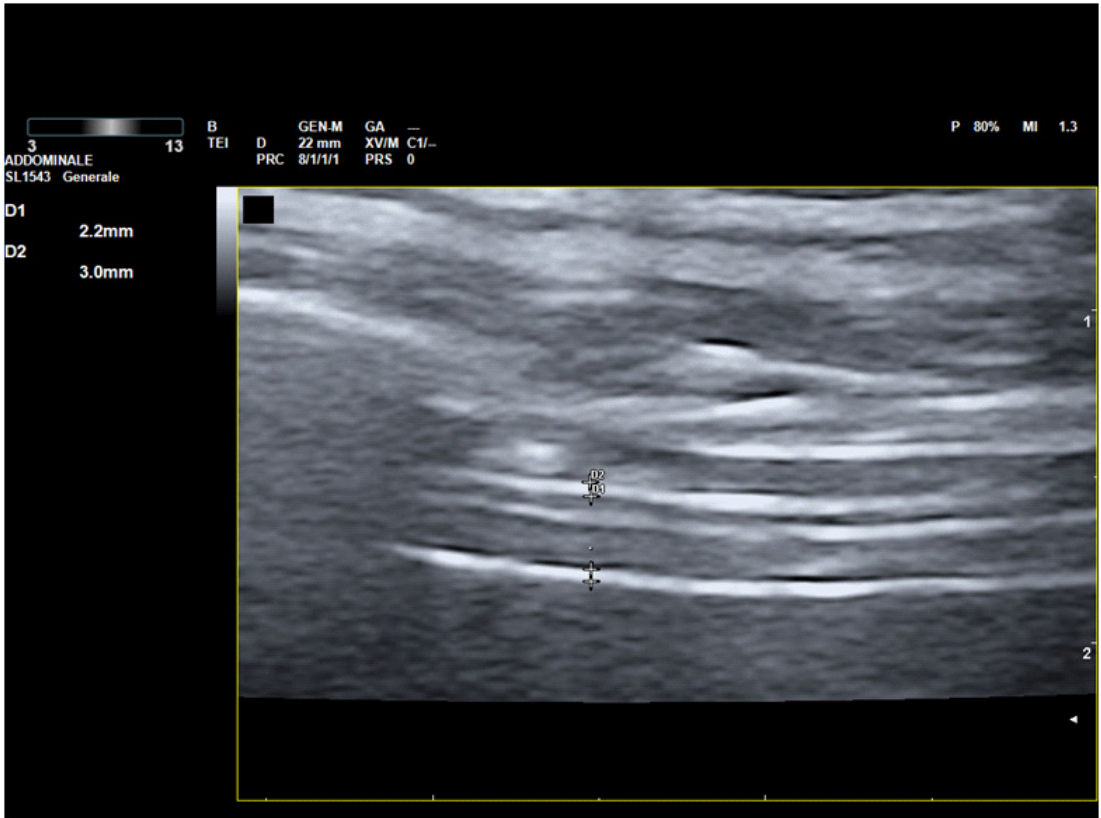


Figure 3: Right inner- and outer-layer T_{di} at FRC.

Right hemidiaphragmatic T_{di} measurement in the Zone of Apposition (**ZOA**). US landmark was directed cephalad. T_{di} was obtained with the caliper as the distance between the inner and the outer edge of the limiting membranes, respectively. The measurement was obtained at the end of a relaxed, passive expiration (Functional Residual Capacity - **FRC**).

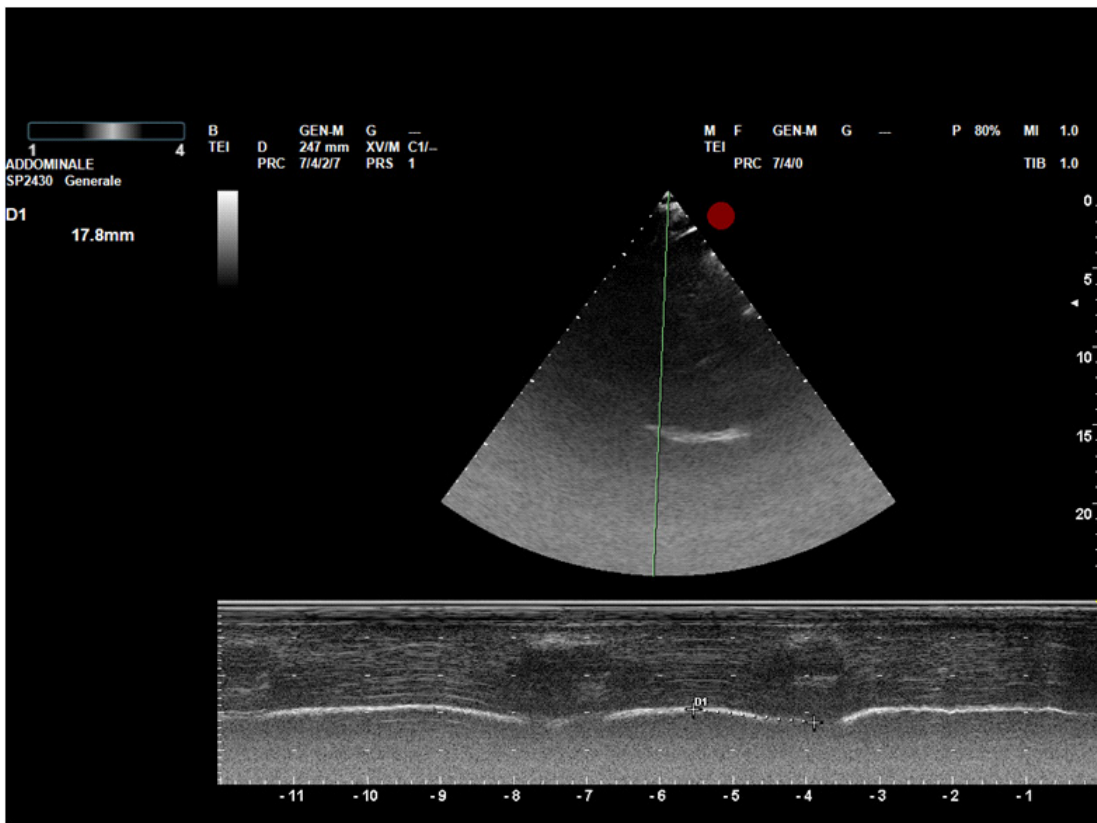


Figure 4: Right hemidiaphragmatic excursion during a quiet breath.

Right hemidiaphragmatic excursion (**DE**) measurement. US landmark was directed cephalad. Liver was used as acoustic windows. In order, from the top to the bottom a B-mode frame and an M-mode trace with DE measurement are shown, respectively. DE was obtained with the caliper as the distance between the higher and the lower point of the M-mode hyperechoic diaphragmatic line. The measurement was obtained during a quiet breath, from FRC to the end of a quiet inspiration.

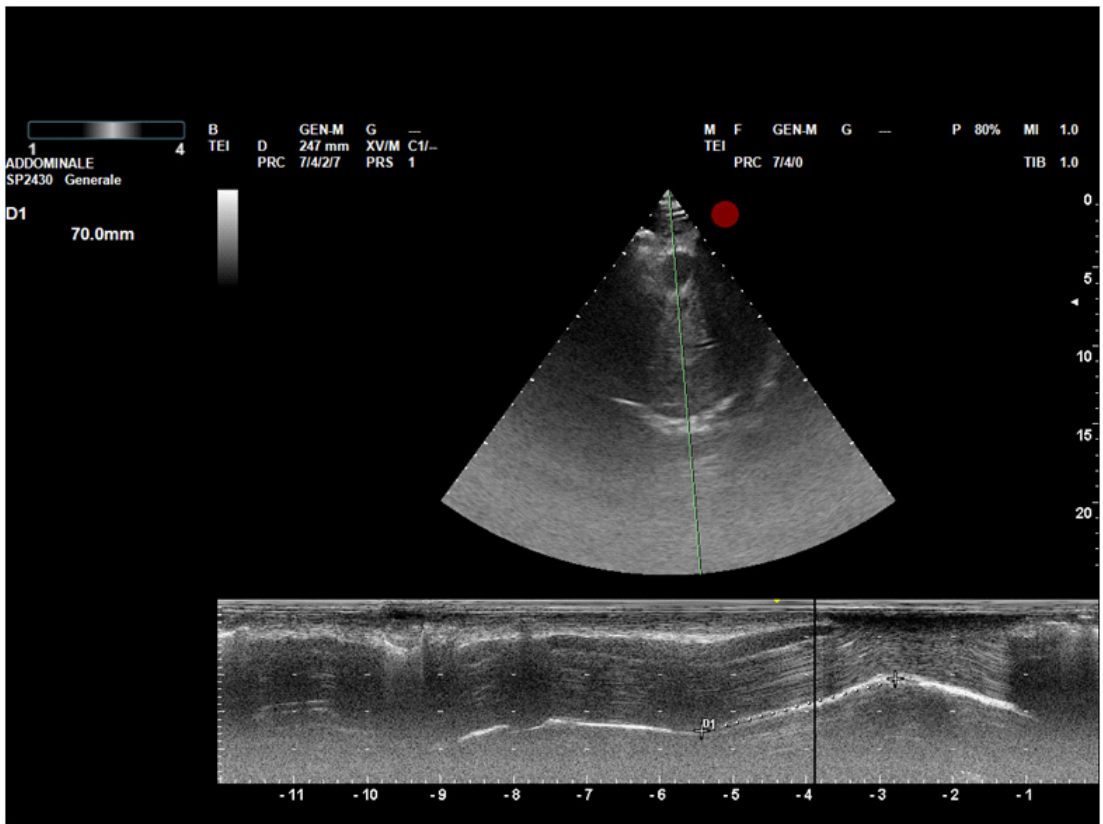


Figure 5: Right hemidiaphragmatic excursion during a quiet breath.

Right hemi diaphragmatic excursion (**DE**) measurement. US landmark was directed cephalad. Liver was used as acoustic windows. In order, from the top to the bottom a B-mode frame and an M-mode trace with DE measurement are shown, respectively. DE was obtained with the caliper as the distance between the higher and the lower point of the M-mode hyperechoic diaphragmatic line. The measurement was obtained during a deep breath, from FRC to TLC.

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