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The effect of breathing on the *in vivo* mechanical characterization of *linea alba* by ultrasound shearwave elastography

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ABSTRACT

The most common surgical repair of abdominal wall hernia consists in implanting a mesh to reinforce hernia defects during the healing phase. Ultrasound shearwave elastography (SWE) is a promising non-invasive method to estimate soft tissue mechanical properties at bedside through shear wave speed (SWS) measurement. Combined with conventional ultrasonography, it could help the clinician plan surgery. In this work, a novel protocol is proposed to reliably assess the stiffness of the *linea alba*, and to evaluate the effect of breathing and of inflating the abdomen on SWS.

Fifteen healthy adults were included. SWS was measured in the *linea alba,* in the longitudinal and transverse direction, during several breathing cycle and during active abdominal inflation.

SWS during normal breathing was 2.3 [2.0; 2.5] m/s in longitudinal direction and 2.2 [1.9; 2.7] m/s in the transversal. Inflating the abdomen increased SWS both in longitudinal and transversal direction (3.5 [2.8; 5.8] m/s and 5.2 [3.0; 6.0] m/s, respectively). The novel protocol significantly improved the reproducibility relative to the literature (8% in the longitudinal direction and 14% in the transverse one). Breathing had a mild effect on SWS, and accounting for it only marginally improved the reproducibility.

This study proved the feasibility of the method, and its potential clinical interest. Further studies on larger cohort should focus on improving our understanding of the relationship between abdominal wall properties and clinical outcomes, but also provide a cartography of the abdominal wall, beyond the *linea alba.*

1. Introduction

Abdominal wall hernias are a debilitating condition that can only be treated surgically. Although this treatment is one of the most commonly performed surgical procedures [1], recurrence rates can be as high as 24%, and postoperative complication rates and chronic morbidity following an incisional hernia can affect up to 2/3 of patients [2–4]. The most common type of surgical repair uses a mesh, which has lower complication and recurrence rates than meshless approaches [5,6]. However, incisional hernia repair still has a high failure rate with long-term recurrence rates of over 30 %, even with mesh repair [7].

Research and development of meshes has mostly focused on maximizing tensile strength, while the mismatch between mesh and soft tissue compliance has often been neglected [8]. However, the mechanical properties of meshes can affect postoperative pain and foreign body reactions [9], and addressing this mismatch could inform the selection of an appropriate biomaterial for hernia repair. While tuning the mechanical properties of meshes is feasible, to some extent, characterizing the complex composite structure of the abdominal wall, in order to assist the surgeon in selecting the most appropriate mesh and planning surgery, remains a challenge. Several *in vitro* studies have been performed on animal samples, in different species [10–12] as well as in humans [13–15]. *In vitro* studies can provide the range of compliance of the different regions of the abdominal wall, but they cannot replace patient-specific assessment, especially because current studies have not found strong correlations between abdominal wall mechanical properties and patient characteristics [8,16].

Magnetic resonance imaging has proven to be a promising tool to assess abdominal wall deformation, which can represent a first step towards the determination of its compliance [17,18]. Optical methods, coupled with intrabdominal pressure measurement, were also applied to estimate abdominal wall mechanical properties [19–21].

Ultrasound shearwave elastography (SWE) is a non-invasive ultrasound-based method to estimate soft tissue mechanical properties [22]. It is easier to access in clinical routine and less time-consuming than conventional MRI and more easily available than MRI-elastography.

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SWE is based on the measurement of shear wave speed in the tissue, which in turn is related to the tissue's shear modulus. The method was applied to estimate the mechanical properties of the abdominal wall, and studies reported that shear wave speed (SWS) in patients with incisional hernia was higher than in controls [23]. However, poor reproducibility was reported, between 7.7% and 41 % uncertainty (in terms of coefficient of variation) [24], which limited SWE applicability in clinics.

Previous studies on abdominal muscles have shown that breathing can have a significant effect on ultrasound measurements [25,26]. In previous studies on the intercostal soft tissues, we have also shown that accounting for the breathing cycle can significantly reduce the uncertainty [27]. In this study, we developed a novel protocol to assess the abdominal wall stiffness with ultrasound-based SWE and applied it to measure the *linea alba* mechanical properties, in order to evaluate the effect of breathing and abdominal inflation on SWS. The protocol is designed to be compatible with clinical routine, in order to facilitate its translation to medical applications.

2. Methods

2.1. Subjects and imaging

Fifteen healthy adults (6 females, 9 males, 33 [26; 40] years old, 24 [20,27] kg/m^2 body mass index, BMI) were included prospectively. Medical conditions or previous treatment concerning the abdominal wall were exclusion criteria. Subjects weight and height were noted. Subjects signed an informed consent, and the data collection was authorized by the ethical committee (CPP Ile-de-France VI 6001).

SWE acquisitions were performed with a Mach30 device (Supersonic Imagine, Aix-en-Provence, France) and a SL 18-5 probe. SWS was acquired using the "General" mode and "resolution" frequency optimization (which uses the higher end of the 5–18 MHz spectrum of the probe). Subjects lied supine, and measurement were performed 1 cm below the navel (Fig. 1); first the location of the *linea alba* was determined with a transversal scan, then transversal and longitudinal measurements were repeated three times. Each measurement corresponded to a video of sufficient duration to span at least 4 breathing cycles (about 40 s), so about 12 breathing cycles were recorded for each subject.

Subjects were then asked to take a deep breath and inflate their abdomen as much as possible, and to keep the position for 5 s. During this time, a 5-s clip was acquired. The maneuver was repeated 3 times to acquire SWE in the transverse direction and 3 times in the longitudinal direction with inflated abdomen.

Fig. 1. Elastographic measurement of the *linea alba* in the longitudinal direction.

2.2. Image processing

Elastographic frames were recorded with a frequency between 1 and 3 Hz (depending on depth and size of the elastographic frame). These frames were extracted from the videos and processed to compute the average SWS in the *linea alba* (Fig. 2) as previously described [28].

Furthermore, an arbitrary region of the abdominal wall, at the interface between fat and muscle, was tracked during the whole video using custom software. The vertical displacement of this region provided a sinusoidal signal corresponding to the breathing movements (Fig. 3). The peaks and valleys of this signal were detected, and each full oscillation (from valley to valley) was extracted and resampled to have a length between 0 and 100%, representing the breathing cycle. The elastographic frames were then placed in this time-frame to assign them to a specific phase of the breathing cycle.

Finally, one random frame (at minimal lung volume) was selected in each measurement to measure the thickness of the *linea alba* in the longitudinal and transverse direction, which were then averaged.

2.3. Statistics

Data from the literature [24] was used to preliminary estimate that a cohort of 9 subjects could suffice to detect a difference between subject position (rest vs inflated abdomen, in the present work), so a cohort of 15 subjects was considered ($\alpha = 0.05$, $\beta = 0.95$, [29]).

In order to assess the protocol's reliability, measurements were repeated by a second operator in a subset of 6 subjects. Intra-operator repeatability and inter-operator reproducibility were calculated according to the ISO 5725 standard, and they were expressed in terms of standard deviation of uncertainty. Differences were analyzed with Wilcoxon signed rank paired test or Mann-Whitney test (for non-paired comparisons), while correlations with Spearman's test. Significance was set at $\alpha = 0.05$. Results were reported as median [1st;3rd quartile]. All processing was performed in Matlab 2022a (The Mathworks, Natick, MA, USA).

3. Results

3.1. Study participants

Subjects were 6 female and 9 males, with median age of 33 [26; 40] years and median body mass index (BMI) of 24 [20,27] kg/m^2. One subject was underweight (BMI <18.5 kg/m^2), six were overweight ($25 <$ BMI <29.9 kg/m^2) and one was obese (BMI >30 kg/m^2).

3.2. Shearwave elastography

The measurement was feasible in all subjects, irrespective of their BMI. Median SWS during normal breathing was 2.3 [2.0; 2.5] m/s in longitudinal direction and 2.2 [1.9; 2.7] m/s in the transversal one. The difference was not significant ($p > 0.05$). When the abdomen was inflated, SWS was 3.5 [2.8; 5.8] m/s in the longitudinal direction and 5.2 [3.0; 6.0] m/s in the transversal (longitudinal vs transversal, $p = 0.035$, Table 1). SWS increased in all subjects when inflating the abdomen, both in the longitudinal and transversal directions (at rest vs inflated, $p < 0.001$).

SWS in the longitudinal direction was weakly correlated with subject's height and weight, but not BMI (Fig. 4), while the transversal direction was only weakly correlated with weight.

Males had slightly higher SWS both in the longitudinal (2.4 [2.2; 2.9] m/s vs 2.1 [1.8; 2.4] m/s) and transversal direction (3.0 [2.4, 5.2] vs 2.3 [2.1; 2.8] m/s) than females, but the difference was not significant ($p > 0.05$). No correlation was observed with age ($p > 0.05$).

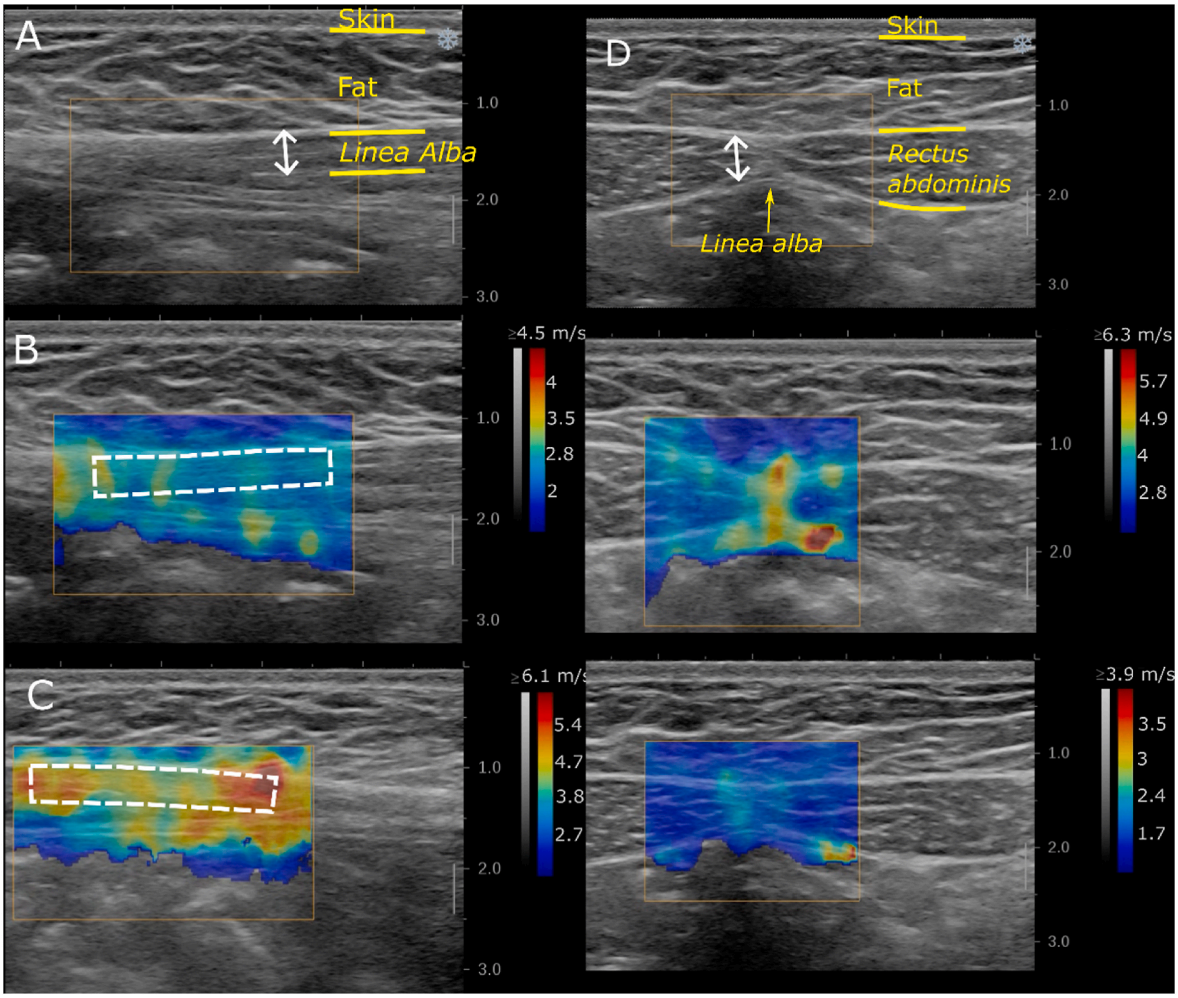

Fig. 2. Typical examples of elastographic measurements of the *linea alba* in the longitudinal direction (A, B, C) and transverse direction (D, E F). Panels A and D show reference conventional ultrasonography, where different layers of the abdominal wall were labeled. Elastography measurements were performed at rest (B, E) and with an inflated abdomen (C, F). Average shear wave speed was calculated in the regions of interest (marked by white rectangles). The white double arrow represents the thickness of the *linea alba*.

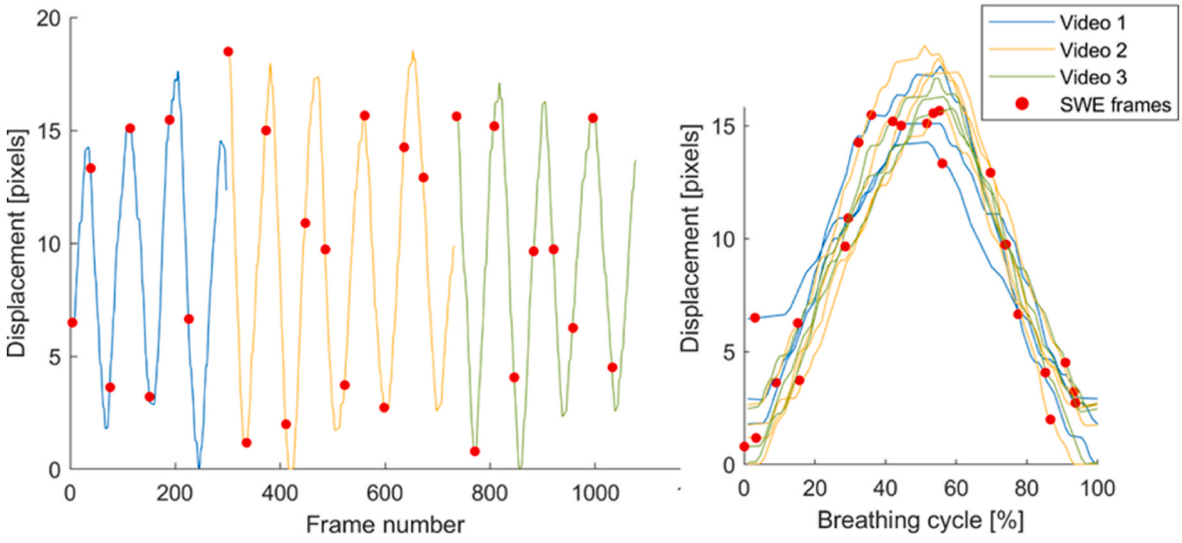

Fig. 3. Breathing cycle measured in three videos for one typical patients. Red dots represent the frames where the elastography signal changed, and the average SWS of the *linea alba* was extracted. Peaks and valleys of the breathing cycles were extracted to make each elastographic frame correspond to a specific instant of the breathing cycles (between 0 and 100%).

Table 1
Shear wave speed (SWS) in the longitudinal and transversal direction in different phases of breathing cycle or with inflated abdomen. SWS was significantly different when the abdomen was inflated ($p < 0.001$).

Direction	Phase	Median SWS [quartiles]
Longitudinal	Overall median	2.3 [2.1; 2.5] m/s
	Inspiration (40–60%)	2.3 [2.0; 2.6] m/s
	Exhalation ([10–40%] and [60−100]%	2.3 [2.1; 2.5] m/s
	Inflated abdomen	3.5 [2.8; 5.8] m/s
Transversal	Overall median	2.2 [1.9; 2.7] m/s
	Inspiration (40–60%)	2.2 [1.9; 2.6] m/s
	Exhalation ([10–40%] and [60−100]%	2.2 [1.9; 2.6] m/s
	Inflated abdomen	5.2 [3.0; 6.0] m/s

3.3. *Effect of breathing*

Variations of SWS according to the phase of breathing cycle were visible in some subject, but not all of them (Fig. 5). When comparing the average SWS over the whole breathing cycle with the peak values (the average SWS between 40% and 60% of the cycle), results were not significantly different: 2.3 [2.0; 2.6] m/s in the longitudinal and 2.2 [1.9; 2.6] m/s in the transversal direction (peak value vs overall average, $p > 0.05$).

Measurement reproducibility was mildly affected by breathing: when calculated on the overall average values, inter-operator reproducibility was 0.19 m/s (8%) in the longitudinal direction and 0.33 m/s (14%) in the transversal one. However, when reproducibility was calculated during maximal inspiration (i.e., between 40 and 60% of the breathing cycle), it slightly improved to 0.15 m/s (6%) and 0.30 (13%) in the longitudinal and transversal direction, respectively. However, median SWS was not affected by breathing cycle phase (Table 1).

3.4. *Linea alba thickness*

Median thickness of the *linea alba* was 2.5 [2.3; 2.8] mm. Thickness was not correlated with SWS, nor with patient characteristics (age, sex, weight, height, BMI).

4. Discussion

A novel protocol to assess the abdominal wall stiffness using ultrasound-based SWE has been proposed and applied it to evaluate the effect of breathing and abdominal inflation on SWS of the *linea* alba. SWS is a biomarker of intrinsic soft tissue stiffness, because it is directly related the shear modulus of the tissue and, under certain assumptions, to its elastic modulus [22]. SWE has also been shown to be effective in

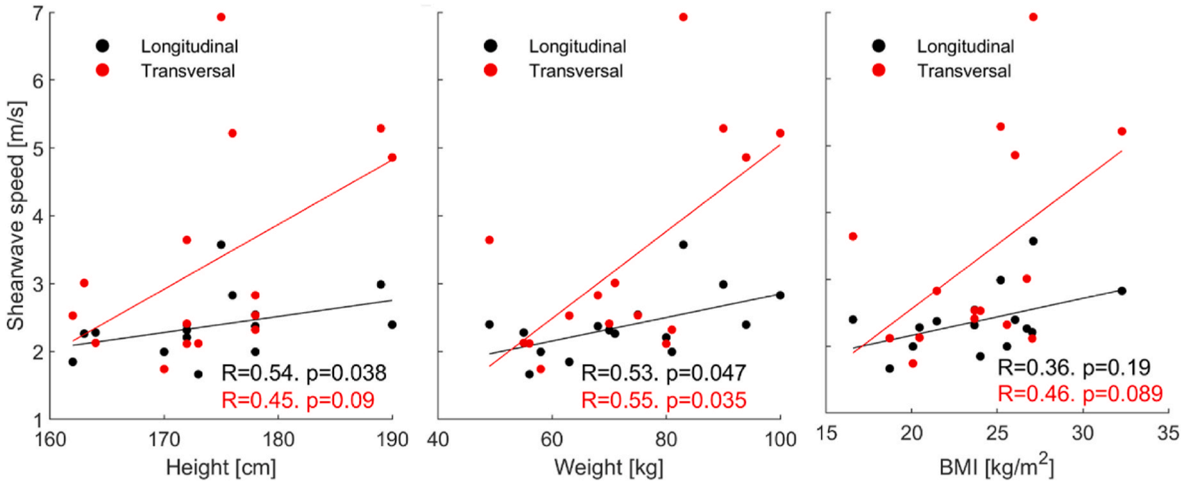

Fig. 4. Correlation between shearwave speed in the longitudinal and transversal direction with subject height, weight and body mass index (BMI).

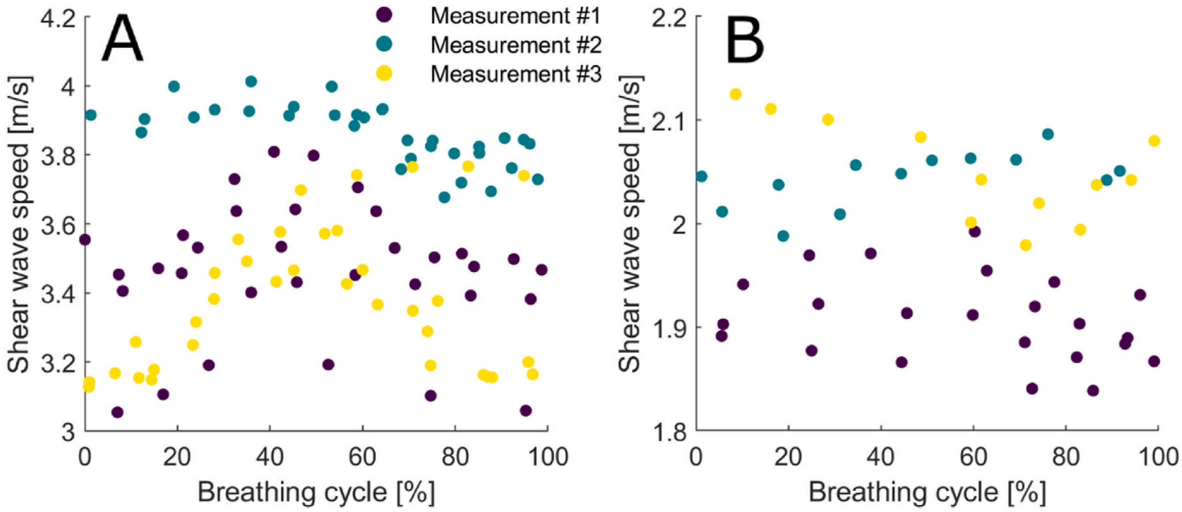

Fig. 5. Elastography for two subjects during several breathing cycles. Three repeated measurements are shown. Variations of shearwave speed are visible for subject A but not for subject B.

measuring anisotropic mechanical properties of soft tissue [30,31]. The results of the present study show that actively inflating the abdomen significantly increased SWS (i.e., increased tissue stiffness) in both the longitudinal and transverse directions of the *linea alba*, and that normal breathing had little effect on SWS. SWS increased during inspiration in some patients (Fig. 5), but these variations were generally smaller than the uncertainty between measurements, and they were not detected in all subjects. Nevertheless, the main finding of the present work is that the averaging long acquisitions over several breathing cycles can significantly improve the reproducibility compared to the literature [24]. Furthermore, taking the breathing cycle into account improved the reproducibility by 1–2%, which may be relevant depending on the clinical application considered.

This result represents an important step forward as it paves the way for further studies and, more importantly, clinical applications of SWE on healthy and pathological abdominal wall. SWE could be a good candidate for the clinician to assess the quality of native abdominal wall tissue preoperatively, and thus providing a decision aid to adapt the surgical strategy and material to subject-specific mechanical properties. Such an approach could help to reduce complications due to mechanical mismatch.

The longitudinal and transverse direction showed a similar SWS at rest, which is consistent with previous results obtained with *in vitro* mechanical testing [32]. However, their difference became significant when the abdomen was inflated, which is also consistent with other *in vitro* studies [13,33,34], which reported that *linea alba* is stiffer in the transverse direction than longitudinal, and that the difference decreases at lower loading. Nevertheless, these latter studies also suggested that the apparent elastic modulus of the tissue can remain significantly different at lower loading. The non-significant difference of SWS at rest could be explained by the fact that this parameter is not directly related to the elastic modulus, rather it depends on the shear modulus μ, through the well-known relationship $SWS = \sqrt{\mu/\rho}$, where ρ is the mass density. Furthermore, the relationship between shear and elastic modulus can be complex, especially in an anisotropic fibrous tissue. On the other hand, the increase of SWS in the inflated abdomen was expected; like many biological tissues, the *linea alba* stiffens as it is loaded (like during abdomen inflation), hence its shear modulus likely increases as well as the SWS.

The equation to transform SWS in shear modulus requires certain assumptions on the propagating medium (non-dispersive, infinite, etc) which are usually false in biological tissues. Therefore, in this work it was decided to report values in terms of SWS, and not as elastic or shear modulus. Nevertheless, it is still possible to apply it to have a rough estimation of the tissue's shear modulus, which would yield values between 5.8 and 13.5 kPa in the longitudinal direction and 5.3 and 29.7 kPa in the transverse direction.

Only mild correlations with height, weight and sex were observed, which is consistent with the existing literature [8]. However, these mild correlations in a relatively small cohort are promising. Since this study proved the feasibility of the method, and its potential clinical interest, further studies on larger cohort should focus on improving our understanding of the relationship between abdominal wall properties and subject characteristics, but also provide a cartography of the abdominal wall, beyond the *linea alba*.

Mikołajowski et al. [25] used a face mask to measure the air pressure during the breathing cycle and acquire SWE images at specific phases of the breathing cycle. However, this method requires an external device to synchronize the acquisitions with the breathing cycle, and it does not allow to measure full breathing cycles. The main limitation of this work is that subjects were not instructed to use abdominal breathing rather than thoracic breathing. In our previous experience in assessing the effect of breathing on SWE [27], we noted that not all subjects are able to easily choose one type of breathing over the other, and therefore in the present study it was decided to allow the subject to breath naturally. It is possible that instructing them to breath with their diaphragm and reach maximum abdominal amplitude [17] may have increased the effect of breathing on SWE. Maximal abdominal contractions, or controlled compressions, could also have been tested to further highlight the nonlinear mechanical nature of the abdominal wall. Another limitation is the relatively small cohort, although it was sized using a statistical approach.

Finally, the device used in the present work provides a direct measurement of shearwave speed, which is obtained on the assumption that shear waves propagate in the tissue. However, this hypothesis is only valid in relatively large tissues, whereas the *linea alba* is thin. Assuming a frequency (f) between 300 and 800 Hz [35] and a SWS of 2.3 m/s (c), a wavelength of $\lambda = c/f = 2.8$–7.7 mm could be expected, which is similar or larger than the thickness of the *linea alba* (2.5 [2.3; 2.8] mm). Therefore, the waves propagating in the *linea alba* could be guided by the tissue, and their speed could be overestimated in the present results [36]. However, a guided wave tends to be slower in a thinner guide, and since no correlation was found between SWS and *linea alba*'s thickness, one can assume that this phenomenon may be negligible.

5. Conclusion

This study demonstrated that SWE provides reproducible results to assess the stiffness of the *linea alba* at the bedside and that in vivo loading of this tissue by active inflation of the abdomen can highlight its non-linear mechanical properties. The breathing cycle had a relatively small effect on the SWS but taking it into account improved the reproducibility compared to the literature. SWE also allowed to detect the anisotropy of the loaded tissue. Further studies should provide a cartography of the SWS in the abdominal wall, with the further aim of comparing healthy and pathological tissue, with the aim of providing the surgeon with new tools to plan surgery and improve patient outcome.

Role of the funding source

The funding source had no role in this work.

Declaration of competing interest

No conflict to disclose.

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