Direct Effect of Caffeine on Diaphragmatic Muscles in Preterm Babies Through Ultrasonographic Examination

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Abstract

Background: Caffeine is frequently used for the treatment of apnea in preterm babies. Its mechanism of action includes not only antagonism of adenosine receptors on central nervous system but also an increase in electrical activity of the diaphragm. Caffeine's direct effect on diaphragm was investigated via electromyography, but ultrasound has not been used to show visible changes in muscles after the treatment before.

Objective: We aimed to assess the effect of caffeine on diaphragmatic function through ultrasonographic examination.

Methods: Fifty-six participants receiving nasal continuous positive airway pressure who were assigned caffeine with less than or equal to 32 weeks' gestational age born were enrolled. Diaphragmatic thickness, amplitude of excursion and velocity of movement were measured by two observers before and within 5 minutes after caffeine loading dose and compared to each other. Interobserver variability was also investigated. The protocol was registered with ClinicalTrials.gov Identifier NCT04483492.

Results: Right diaphragmatic thickness and diaphragmatic velocity of movement did not differ after the treatment. However, amplitude of excursion of the diaphragm was found significantly higher after caffeine loading dose (8.7 mm, 10mm, respectively, $p<0.05$). Interobserver variability was not detected.

Conclusion: Diaphragm excursion increased after caffeine treatment in preterm babies, and this finding was supported the direct effect of the caffeine on diaphragm. The amplitude of excursion has also been suggested as a sensitive US parameter for determination of diaphragmatic function in preterm babies.

What In Known

- Transient increase in electrical activity of the diaphragm after caffeine assignment has been shown.
- Increase in electrical activity after caffeine was indicated the caffeine's direct effect on diaphragmatic muscle.

What is New

- There was a significant increase in the amplitude of diaphragm excursion after caffeine assignment.
- The findings have suggested that amplitude of excursion would be used as a sensitive marker for diaphragmatic function in preterm babies.

Introduction

Caffeine is frequently used for prevention and treatment of apnea of prematurity. It also facilitates extubation [1,2] and reduces the duration of mechanical ventilation [3] and the rate of bronchopulmonary

dysplasia (BPD) [3]. Caffeine therapy has also been demonstrated to have beneficial effects against acute kidney injury [4] and neurodevelopmental impairment [3,5,6].

Caffeine is a methylxanthine such as theophylline and aminophylline. Their primary mechanism of action in apnea of prematurity includes antagonism of adenosine receptors which inhibit inspiratory neurons [7]. Thus, they increase responsiveness of hypercarbia in central nervous system (CNS) [1,7]. Besides their impact on CNS, the direct effect on diaphragmatic contractility has also been demonstrated in a few experimental and clinical studies [2,8]. Caffeine activated diaphragmatic contraction and increased tension in hamsters and dogs [8,9]. In comparison with other methylxanthines, caffeine was shown to have a greater effect on diaphragm than theophylline in both experimental and clinical studies [10,11]. The treatment also increased diaphragmatic activity, tidal volume, lung function and muscle strength in preterm babies [2,12,13]. There were two studies included preterm infants and showed transient increase in electrical activity of the diaphragm after caffeine assignment [12,13]. Its beneficial effects on lung compliance and function were also demonstrated by measurement of lung volume, respiratory system compliance and resistance [2]. That improvement on muscle strength clarified the effect on facilitation of weaning, as well. However, besides electrical activity, changes in diaphragmatic muscle after caffeine have not reported before.

Diaphragm function can be evaluated by electrical phrenic nerve stimulation with electromyography (EMG) and fluoroscopy [14–16]. Recently, ultrasonography (US) has been considered as a promising technique [13–17]. It has many advantages over other methods. It is non-invasive in contrast to EMG and it is radiation-free in contrast to fluoroscopy. Increase in diaphragmatic thickness on US was considered to be a possible indirect marker of muscle fiber contraction, since contraction muscle fiber has shortened and caused muscle thickening [16]. Also, with M-mode, diaphragm dysfunction was assessed and found as a useful predictor for weaning [15]. Therefore, ultrasound provides information about not only muscle changes, but also its function. We aimed to evaluate the direct effect of caffeine on diaphragm by using US parameters including diaphragm thickness, excursion and its velocity. To the best of our knowledge, it is the first study that has investigated the diaphragmatic function after caffeine with a guidance of US in preterm babies.

Material And Methods

This study was designed as a prospective and observational study at an academic tertiary NICU between 2018–2021. The present study was approved by our institutional ethical committee (KA-20029) and written consent was obtained from parents. The protocol was registered with ClinicalTrials.gov Identifier NCT04483492.

Only babies receiving nasal continuous positive airway pressure (nCPAP) who were assigned caffeine with less than or equal to 32 weeks' gestation at birth were enrolled to the study. Infants with congenital anomalies and perinatal asphyxia were excluded. Fifty-six patients were enrolled to ensure that the power

of the test was 80% to detect a difference at a 0.05 significance level. Our caffeine protocol includes 20-minute loading dose of caffeine citrate (20 mg/kg) to the babies less than or equal to 32 weeks' gestation required mechanic ventilation or the babies without any respiratory support, but having more than one episode of apnea [18].

Demographic data were collected including gender, antenatal exposure to corticosteroids, maternal history of preeclampsia, gestational age, mode of delivery, multiple gestation, birth weight, APGAR, mode of ventilation prior to caffeine administration.

Ultrasonographic examinations to evaluate diaphragm function were done with measuring diaphragm thickness, amplitude of excursion and diaphragmatic velocity of movement. US was performed before and within 5 minutes after caffeine loading dose to be able to show the caffeine affect by two unblinded observers having experience in diaphragm ultrasound (observer 1-pediatric radiologist/fellow and observer 2-neonatologist/fellow). Right and left sides of diaphragm thickness were assessed using B-mode with a 11.4 MHz broadband linear transducer (VF13-5, Acuson X300, Siemens, Erlangen, Germany) placed on the intercostal space in the anterior axillary line while baby was in the supine position. The diaphragm was viewed between the two echogenic layers of the pleura and peritoneum in the transition zone from lung to liver or spleen. Diaphragm thickness was measured as the perpendicular distance between the pleural and peritoneal reflections (Fig. 1). The amplitude of diaphragmatic excursion was measured on the vertical axis of the M-mode US tracing from the baseline to the point of maximum inspiration with a 2.7 MHz broadband sector array transducer (P8-4, Acuson X300, Siemens, Erlangen, Germany) placed below the costal margin between the mid-clavicular and anterior axillary line for the only right diaphragm, with the liver serving as an acoustic window (Fig. 2) (Video 1). There was difficulty in imaging the left diaphragm due to the lung obscuring the view and smaller window of the spleen as compared with the liver window on M-mode US. Therefore, only right side excursions and velocities were measured. On the horizontal axis, the time of right diaphragmatic contraction was noted. It begins at the beginning of inspiration and ended when the peak was reached. The diaphragmatic velocity of movement (mm/s) was calculated by dividing the amplitude of excursion to the time of contraction. The two observers independently recorded their measurements and each observer was blinded to the measurements of the other.

Primary outcome was to investigate changes in the ultrasound measures of diaphragm muscle after caffeine administration. These measurements included both sides of diaphragmatic thicknesses, amplitudes of right diaphragm excursion and right diaphragm velocities obtained before and after the therapy were compared with each other. Also interobserver variability was assessed. Secondary outcome was to show the utility of ultrasound to evaluate diaphragm muscle functions in preterm babies.

Statistical analysis

The assumption of normality was assessed with Shapiro-Wilk test. If the data was normally distributed, data are presented with mean (standard deviation) and differences assessed for statistical significance

using paired sample t-test for changes after caffeine administration or interobserver variability. If the data was non-normally distributed, data are presented with median (interquartile range) and differences evaluated for statistical significance using Wilcoxon rank-sum test for changes after caffeine administration. The intraclass correlation coefficient (ICC) was used to assess inter-observer reproducibility. SPSS 26.0 program (SPSS Inc., Chicago, IL, USA) was used in the statistical analyzes and $p < 0.05$ was considered statistically significant.

Results

Maternal and infant characteristics of the study cohort are presented in Table 1. Total 56 newborns were enrolled. Median gestational age was 30 weeks (IQR 28–31) and median birth weight was 1160 gr (IQR 835–1065). All babies were in their first day of life.

Table 1
Characteristics of infants

Variable	Study group (n = 56)
Maternal characteristics	
Antenatal steroids, n (%)	44 (78.6%)
Multiple gestation, n (%)	22 (39.3%)
Cesarean delivery, n (%)	50 (89.3%)
Chorioamnionitis, n (%)	1 (1.8%)
Preeclampsia, n (%)	12 (21.4%)
Gestational diabetes mellitus, n (%)	7 (12.5%)
Neonatal characteristics	
Female/Male sex, n (%)	26/30 (46.4%/53.6%)
Gestational age, week, median (IQR)	30 (28–31)
Birth weight, g, median (IQR)	1160 (835–1065)
APGAR score at 5 min, median (IQR)	8 (7–9)
Small for GA, n (%)	10 (17.9%)
Cord blood analysis	
pH, mean (SD)	7.3 (± 0.4)
Lactate, median (IQR)	2.5 (1.8–3.4)
Base deficient, mean (SD)	-2.6 (± 2.8)
IQR, interquartile range. SD, standard deviation.	

Interobserver variability

There wasn't any statistically significant difference in both sides of diaphragm thicknesses, right diaphragm excursion and diaphragmatic velocity between interobservers' evaluations before and after caffeine administration (Table 2).

Table 2
Interobserver variability

	Observer 1	Observer 2	p
Before caffeine administration			
Right diaphragm thickness (mm)	0.75 ± 0.42	0.75 ± 0.43	0.7 [x]
Left diaphragm thickness (mm)	0.71 ± 0.39	0.73 ± 0.40	0.5 [x]
Right diaphragm excursion (mm)	8.7 ± 3.30	8.7 ± 3.84	0.5 [t]
Right diaphragmatic velocity (mm/second)	0.03 ± 0.01	0.04 ± 0.05	0.1 [x]
After caffeine administration			
Right diaphragm thickness (mm)	0.77 ± 0.43	0.78 ± 0.44	0.5 [x]
Left diaphragm thickness (mm)	0.71 ± 0.36	0.78 ± 0.43	0.1 [x]
Right diaphragm excursion (mm)	10.09 ± 3.56	8.94 ± 3.36	**< 0.05** [t]
Right diaphragmatic velocity (mm/second)	0.04 ± 0.02	0.03 ± 0.01	0.1 [x]
[t] paired sample t-test, [x] Wilcoxon test			

Before and after caffeine administration

There was not any statistically significant difference in right side of diaphragm thicknesses and right diaphragmatic velocities between before and after the treatment (Table 3). Left side of diaphragm thickness and right side of diaphragmatic excursions increased significantly after caffeine administration.

Table 3
Right and left diaphragm thicknesses, right diaphragm excursions and velocities before and after caffeine administration.

	Before caffeine	After caffeine	p
Right diaphragm thickness (mm)	0.75 ± 0.41	0.79 ± 0.46	0.2 [x]
Left diaphragm thickness (mm)	0.73 ± 0.40	0.77 ± 0.43	**< 0.05** [x]
Right diaphragm excursion (mm)	8.7 ± 3.30	10.09 ± 3.56	**< 0.05** [t]
Right diaphragmatic velocity (mm/second)	0.03 ± 0.01	0.04 ± 0.02	0.3 [x]
[t] paired sample t-test, [x] Wilcoxon test			

Discussion

Significant differences in diaphragmatic excursions and left diaphragmatic thicknesses were supported the direct effect of caffeine on diaphragm. However, there was not any statistically significant difference in right side of diaphragm thicknesses and right diaphragmatic velocities before and within 5 minutes after caffeine loading dose.

Kaaijenga et al [12] measured diaphragmatic activity by using transcutaneous EMG of the diaphragm (dEMG) and plethysmography before and after caffeine-loading dose. They found rapid and significant increases in dEMG amplitude within 5 minutes correlated with the change in tidal volume [12]. Therefore, in our study the immediate evaluation within 5 minutes was chosen for re-evaluation of diaphragm activity to identify the change after caffeine loading dose. On the other hand, Williams et al [13] evaluated 32 infants under 34 gestational week born by using dEMG before and after caffeine loading dose and identified a significant increase in dEMG amplitude at 25 minutes that peaked at 30 minutes [13]. Therefore, the evaluation at 5 minutes rather than at 25 minutes may be the reason of no change in the right diaphragm thicknesses and velocities observed in our study. Besides, another reason of this discrepancy may be the fact that an increase in electrical activity may not reflect the muscle activity directly and thus some changes in dEMG cannot be visualized by US. However, the visual proof of differences on the diaphragm muscle should be clinically more important than differences solely on electrical activity.

In a previous study, diaphragmatic thickness was measured by M-mode during both inspiratory and expiratory time in asymptomatic term and preterm babies [19]. Thicknesses were found less in preterm babies than term newborns with 1.2 and 1.6 mm, respectively. Rehan et al [20] figured out correlations between postmenstrual age, body weight and diaphragmatic thicknesses. They found 1.09 ± 0.08 mm at 26–28 gestational weeks, and 1.25 ± 0.07 mm at 29–31 gestational weeks. These were similar with our

findings. Rehan et al [21] also demonstrated that diaphragmatic thicknesses and amplitude of excursion can be affected by CPAP at different levels. Therefore, respiratory support could alter the measurements.

Heyman et al found that 43% higher diaphragmatic excursions in neonates treated with aminophylline which is another methylxanthine like caffeine [22]. Similarly, in our study, excursion of the diaphragm increased significantly after caffeine administration. This finding is suggested that evaluating excursion can be a sensitive method for determination of diaphragm function in preterm babies. Indeed, a previous study investigating successful extubation in preterm babies by diaphragm US reported higher thicknesses and excursions in the successfully extubated group [23]. The researchers also stated that the excursion was the most useful indicator for successful extubation. This also supported the superiority of excursion in the demonstration of diaphragm function.

To our knowledge, diaphragmatic velocity of preterm babies was evaluated by M-mode US for the first time in the current study. Diaphragmatic velocity of contraction was also considered to be a marker of diaphragmatic strength [16]. However, in this study there was not any significant difference between the measurements before and after the therapy.

There were some limitations in this study. For instance, sample size may be increased with further studies for a better understanding. Including re-evaluation within 25 minutes may also provide more information in other parameters. Moreover, both sides of diaphragmatic excursion could be included. However, only right side was evaluated in the current study due to the difficult visualization of the left diaphragm through the smaller window of the spleen and the lung obscuring the image on M-mode US [14].

Conclusion

This study supported the direct effect of caffeine on diaphragmatic contractility with a significant increase in the amplitude of diaphragm excursion. It could also be considered as a sensitive US parameter for reflecting the changes in diaphragmatic function in preterm babies.

Abbreviations

BPD: Bronchopulmonary dysplasia

CNS: Central nervous system

dEMG: Electromyography of the diaphragm

EMG: Electromyography

US: Ultrasonography

Declarations

Declarations: N/A

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Conflict of Interest: The authors have indicated they have no potential conflicts of interest to disclose.

Availability of data and material: The data that support the findings of this study are available on request from the corresponding author.

Code availability: N/A

Authors' contributions:

Dr Gozdem Kayki and Dr Hasan Tolga Celik conceptualized and designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. Dr Ercan Ayaz, Dr Ayse Tandircioglu collected data, carried out the initial analyses, and reviewed and revised the manuscript. Prof Berna Oguz designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. Prof Sule Yigit and Prof Murat Yurdakok coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Ethical approval: The present study was approved by local institutional ethical committee (KA-20029).

Consent to participate: Written consent was obtained from parents.

Consent for publication: Consent was obtained.

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Figures

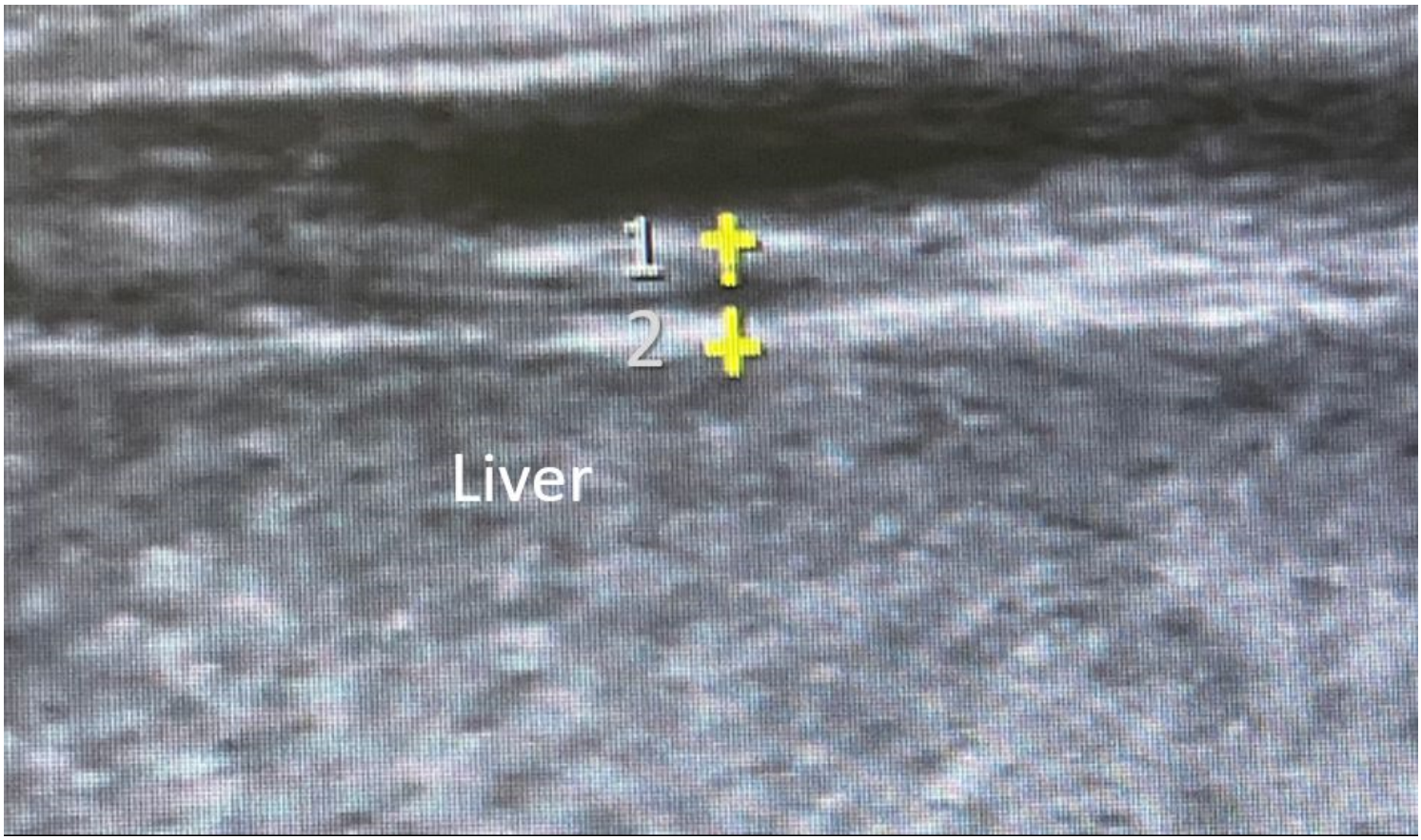

Figure 1

B-mode US image shows the measurement of the right diaphragm thickness as the perpendicular distance *(marks)* between the pleural *(1)* and peritoneal *(2) e*chogenic layers.

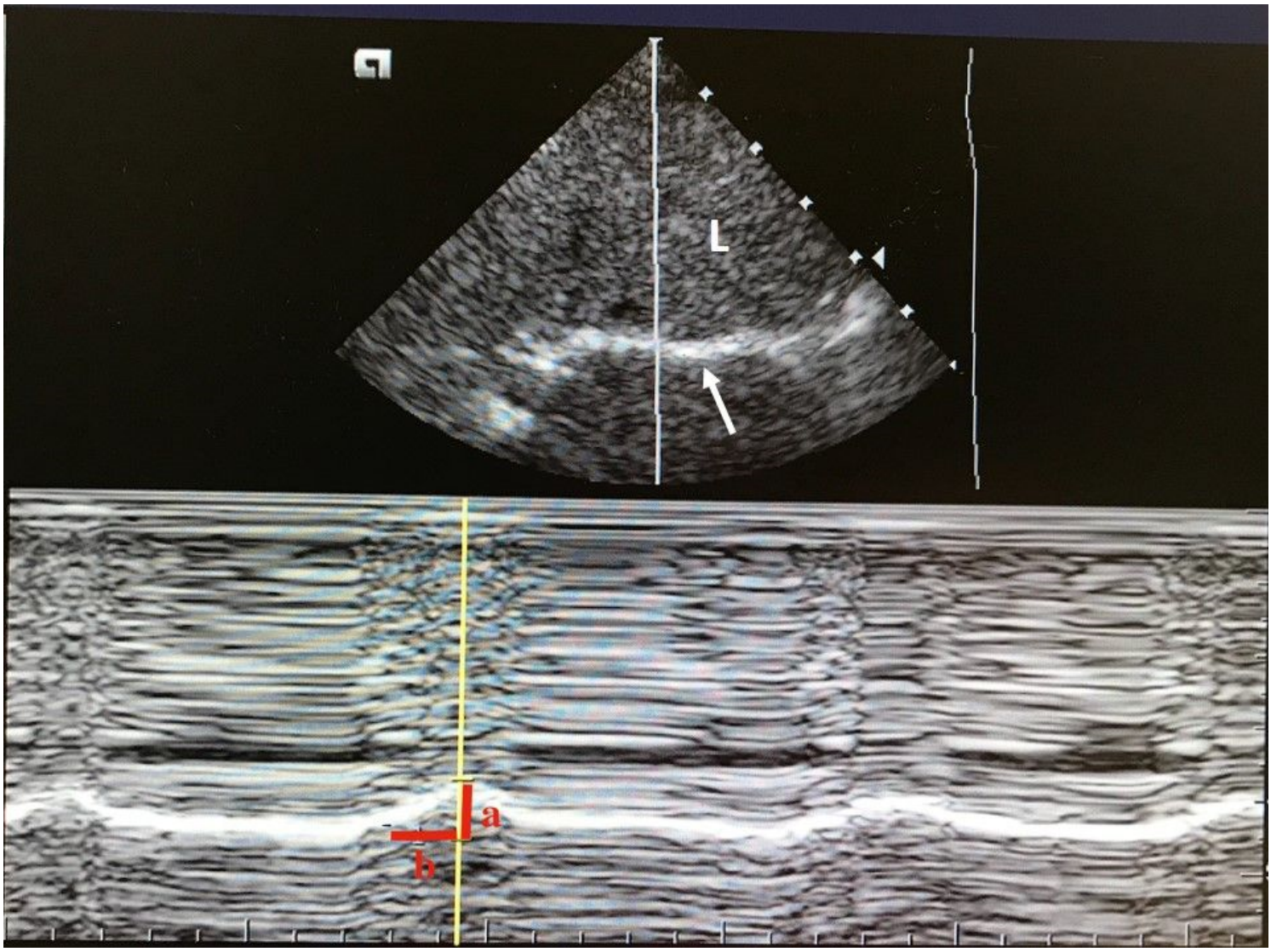

Figure 2

US image shows M-mode view of the right diaphragm *(arrow)* motion. **"a"** represents the amplitude of excursion of the diaphragm and **"b"** represents the time frame of diaphragm contraction, which is used to calculate velocity of diaphragm movement. Velocity (mm/s) = a/b. L: liver

Supplementary Files

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