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Guiding Intramuscular Diaphragm Injections Using Real-time Ultrasound & Electromyography

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Abstract

Introduction—We describe a unique method that combines ultrasound and electromyography to guide intramuscular diaphragm injections in anesthetized large animals.

Methods—Ultrasound was used to visualize the diaphragm on each side of spontaneously breathing, anesthetized beagle dogs and cynomolgus macaques. An electromyography needle was introduced and directed by ultrasound to confirm that the needle entered the muscular portion of the diaphragm, and methylene blue was injected. Injection accuracy was confirmed upon necropsy by tracking the spread of methylene blue.

Results—All methylene blue injections were confirmed to have been placed appropriately into the diaphragm.

Conclusions—This study demonstrates the feasibility and accuracy of using ultrasound and EMG to guide injections and to reduce complications associated with conventional blind techniques. Ultrasound guidance can be used for clinical electromyography of the diaphragm. Future applications may include targeted diaphragm injections with gene replacement therapy in neuromuscular diseases.

Keywords

diaphragm; electromyography; intramuscular injection; ultrasound; real-time

Introduction

Needle electromyography (EMG) of the diaphragm is usually done blindly using techniques guided only by anatomic landmarks.^[1] It can be challenging due to the thin size of the diaphragm muscle and its proximity to the lung and liver. The possible complications include pneumothorax caused by inadvertent lung puncture or liver hematoma.^[2] Ultrasound imaging provides excellent non-invasive direct and real-time visualization of soft tissue with good distinction of anatomic landmarks, fascial planes, and neurovascular structures adjacent to the intended target. Recent advances in technology have made high-resolution ultrasound machines affordable, portable, and widely available for a variety of clinical applications. Direct visualization of the diaphragm in real time using ultrasonography can be particularly helpful, given the dynamic nature of the diaphragm muscle. The utility of diaphragm ultrasound has been reported recently in animals.^[3-5] We describe a unique method that combines real-time ultrasound and EMG to guide intramuscular diaphragm injections in anesthetized large animals.

Material and Methods

The Animal Care and Use Committee at Wake Forest School of Medicine approved this study. Ultrasound (ACUSON Sequoia 512 Ultrasound System, Siemens, USTM) was used to visualize the diaphragm on each side of spontaneously breathing, anesthetized beagles (n=3) and cynomolgus macaques (n=3) using the B mode with a 15 MHz linear probe.^[6] The animals were anesthetized, and EMG-guided injections were performed as part of a separate independent study.^[7, 8] The ultrasound transducer was placed at the lowest intercostal space in the midaxillary line and directed towards the midline through the liver and spleen windows.^[6] The lateral intercostal approach was used to visualize the diaphragm in the zone of apposition (in the area where the diaphragm abuts the rib cage).^[6] The long axis of the transducer was aligned with the intercostal space to avoid any bone shadows from the ribs and to allow visualization of the needle at all times. The diaphragm muscle was identified by its deep location, curved geometry, and its appearance as 2 echogenic layers of peritoneum and pleura sandwiching a more hypoechoic line of the muscle itself.^[9] In addition, it was seen to move and thicken during inspiration as a result of muscular contraction.^[10] Liver on the right side and spleen on the left side were visualized deep to the diaphragm as homogeneous, low-intensity structures punctuated by fenestrating blood vessels (Figure 1). Lung was seen as a bright, high-intensity tissue downwardly displacing the diaphragm and the underlying liver or spleen during inspiration (Figure 1).^[11-13] We evaluated all lower intercostal spaces to identify the space that provided best visualization of the diaphragm muscle at end-inspiration, where the muscle is thickest, with minimal encroachment of the descending lung during inspiration.

The EMG needle was introduced and directed by ultrasound to confirm that the visualized image was the muscular portion of the diaphragm and that the tip of the EMG needle entered the muscle (Figure 1). A 5-cc syringe with a 20-gauge injectable needle was inserted parallel to the transducer using a long axis approach to allow direct visualization of the injection needle throughout the examination while simultaneously monitoring the pleural space descending into the field of view as the animal inspired.^[11, 14] Crisp motor unit potentials,

with rapid rise times on EMG signaled that the needle was in the diaphragm muscle. Methylene blue was then injected. Following injection, the animals were euthanized humanely as part of the original study that included necropsy as a part of its protocol, and diaphragm tissue was collected immediately to assess injection sites for methylene blue staining. [7, 8]

Results

All methylene blue injections were confirmed upon necropsy to have been placed appropriately into the diaphragm muscle (Figure 1). The site of injections was confirmed by tracking the spread of injected methylene blue.

Discussion

Ultrasound is a non-invasive, portable technology with no risk of radiation that is becoming increasingly popular for assessment of diaphragm muscle structure and function. The muscular portion of the diaphragm can be visualized easily in the zone of apposition through the lateral intercostal approach on B mode ultrasonography.^[4, 6] Many studies using animal models of neuromuscular diseases are using directed diaphragm injections or implantation of tracers to assess diaphragm function.^[15-17] Currently, targeted diaphragm injections in animals require laparotomy, which can be cumbersome and is fraught with inherent complications of anesthesia and surgery.^[18] There is emerging clinical interest in ultrasound-guided EMG to isolate the intercostal space and approximate the depth of EMG needle placement to optimize the safety of diaphragm EMG in human clinical studies and practice.^[10, 11, 14] We undertook this study to demonstrate the feasibility and practicality of using real-time ultrasound-guided needle EMG and injections in large animal models in an effort to reduce complications associated with conventional blind technique.^[2] The use of ultrasound facilitates direct visualization during needle placement and enhances the safety and accuracy of this examination. Augmenting EMG monitoring with ultrasound ensures that targeted injections enter the muscular portion of the diaphragm.

Conclusion

The safety and accuracy of needle EMG of the diaphragm can be enhanced by visual guidance using ultrasonography. This study demonstrates the feasibility of using real-time ultrasound guidance for targeted diaphragm therapy. We have evaluated the accuracy of ultrasound-guided injections in non-human primates or dogs. This novel method for performing diaphragmatic injections with ultrasound and EMG guidance may prove useful as a clinical tool in the delivery of targeted gene or stem cell therapy to the diaphragm.

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Abbreviations

EMG electromyography

References

1. Hutten GJ, van Thuijl HF, van Bellegem AC, van Eykern LA, van Aalderen WM. A literature review of the methodology of EMG recordings of the diaphragm. *J Electromyogr Kinesiol.* 2010; 20:185–190. [PubMed: 19423360]
2. Hodges PW, Gandevia SC. Pitfalls of intramuscular electromyographic recordings from the human costal diaphragm. *Clin Neurophysiol.* 2000; 111:1420–1424. [PubMed: 10904223]
3. Spattini G, Rossi F, Vignoli M, Lamb CR. Use of ultrasound to diagnose diaphragmatic rupture in dogs and cats. *Vet Radiol Ultrasound.* 2003; 44:226–230. [PubMed: 12718361]
4. Sarwal A, Cartwright MS, Walker FO, Mitchell E, Buj-Bello A, Beggs AH, Childers MK. Ultrasound assessment of the diaphragm: preliminary study of a canine model of x-linked myotubular myopathy. *Muscle Nerve.* 2014 In Press.
5. Tidwell AS. Ultrasonography of the thorax (excluding the heart). *Vet Clin North Am Small Anim Pract.* 1998; 28:993–1015. [PubMed: 9698623]
6. Sarwal A, Walker FO, Cartwright MS. Neuromuscular ultrasound for evaluation of the diaphragm. *Muscle Nerve.* 2013; 47:319–329. [PubMed: 23382111]
7. Childers MK, Joubert R, Poulard K, Moal C, Grange RW, Doering JA, et al. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. *Sci Transl Med.* 2014; 6:220ra10.
8. Grange RW, Doering J, Mitchell E, Holder MN, Guan X, Goddard M, et al. Muscle function in a canine model of X-linked myotubular myopathy. *Muscle Nerve.* 2012; 46:588–591. [PubMed: 22987702]
9. Gerscovich EO, Cronan M, McGahan JP, Jain K, Jones CK, McDonald C. Ultrasonographic evaluation of diaphragmatic motion. *J Ultrasound Med.* 2001; 20:597–604. [PubMed: 11400933]
10. Boon AJ, Harper CJ, Ghahfarokhi LS, Strommen JA, Watson JC, Sorenson EJ. Two-dimensional ultrasound imaging of the diaphragm: quantitative values in normal subjects. *Muscle Nerve.* 2013; 47:884–889. [PubMed: 23625789]
11. Amirjani N, Hudson AL, Butler JE, Gandevia SC. An algorithm for the safety of costal diaphragm electromyography derived from ultrasound. *Muscle Nerve.* 2012; 46:856–860. [PubMed: 23042084]
12. Toledo NS, Kodaira SK, Massarollo PC, Pereira OI, Dalmas JC, Cerri GG, et al. Left hemidiaphragmatic mobility: assessment with ultrasonographic measurement of the craniocaudal displacement of the splenic hilum and the inferior pole of the spleen. *J Ultrasound Med.* 2006; 25:41–49. [PubMed: 16371554]
13. Toledo NS, Kodaira SK, Massarollo PC, Pereira OI, Mies S. Right hemidiaphragmatic mobility: assessment with US measurement of craniocaudal displacement of left branches of portal vein. *Radiology.* 2003; 228:389–394. [PubMed: 12819344]
14. Boon AJ, Alsharif KI, Harper CM, Smith J. Ultrasound-guided needle EMG of the diaphragm: technique description and case report. *Muscle Nerve.* 2008; 38:1623–1626. [PubMed: 19016552]
15. Petrof BJ. Respiratory muscles as a target for adenovirus-mediated gene therapy. *Eur Respir J.* 1998; 11:492–497. [PubMed: 9551759]
16. Lessa TB, Carvalho RC, Francioli AL, de Oliveira LJ, Barreto RS, Reder D, et al. Muscle reorganisation through local injection of stem cells in the diaphragm of mdx mice. *Acta Vet Scand.* 2012; 54:73. [PubMed: 23231953]
17. Zhang YN, Zhang C, Yu MJ, Wang SH, Li MS, Huang H, et al. Dystrophin expression and pathology of diaphragm muscles of mdx mice after xenogenic bone marrow stem cell transplantation. *Nan Fang Yi Ke Da Xue Xue Bao.* 2006; 26:53–58. [Chinese]. [PubMed: 16495176]

18. Mantilla CB, Zhan WZ, Sieck GC. Retrograde labeling of phrenic motoneurons by intrapleural injection. *J Neurosci Methods*. 2009; 182:244–249. [PubMed: 19559048]

Figure 1.

Diaphragm images at necropsy showing spread of methylene blue in beagles and cynomolgus macaques (left images A-D). All methylene blue injections were confirmed upon necropsy to have been placed appropriately into the diaphragm. Representative image of ultrasound-guided insertion of EMG needle in a human cadaver using a linear 18 MHz probe on B mode ultrasound (right image E).