Optimising muscle biopsy: ultrasound-guided technique for enhanced accuracy and safety

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Abstract

Objective

Muscle ultrasound (US) has emerged as a valuable tool for assessing neuromuscular disorders, offering real-time imaging that enhances diagnostic accuracy and procedural safety. Ultrasound-guided needle muscle biopsy (UGNMB) optimises site selection, improves needle placement precision, and minimises complications compared to conventional biopsy techniques. This manuscript describes the UGNMB technique using a 14G guillotine-type semiautomatic biopsy needle, highlighting the continuous role of ultrasound in selecting the biopsy site and guiding the entire sample collection process.

Methods

A structured approach is followed, including patient preparation, pre-procedural assessment, and systematic ultrasound evaluation to ensure optimal needle trajectory, while avoiding tendons and neurovascular structures. This approach leverages ultrasound not only to assist in selecting the target muscle but also to guide the entire procedure in real-time.

Results

A previous study from our group demonstrated that UGNMB achieves a high diagnostic success rate with minimal discomfort. Continuous real-time visualisation allows dynamic needle tracking and more accurate targeting of viable muscle tissue while avoiding fascia, vessels, and areas of advanced damage.

Conclusion

Continuous real-time ultrasound guidance thereby addresses a key limitation of conventional mark-and-biopsy approaches and potentially reduces false-negative sampling reported with traditional methods.

Key words

ultrasound-guided muscle biopsy, idiopathic inflammatory myopathies, myositis, diagnostic procedure, safety and feasibility

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Introduction

Imaging plays a crucial role in evaluating muscle disorders and myopathies, aiding in the detection of structural changes and providing insight into muscle composition. Initially developed between the late 1960s and early 1970s (1), muscle ultrasound (US) was one of the earliest imaging techniques applied to muscle evaluation. This imaging technique is particularly advantageous due to its non-invasive nature, absence of contraindications, and ability to offer real-time, dynamic muscle assessment directly at the bedside. Its ease of use and patient-friendly approach make it a valuable tool for clinicians in diagnosing and monitoring neuromuscular conditions (2). Precisely because of its dynamic nature, muscle ultrasound also serves as a useful guide for various diagnostic procedures, such as muscle biopsies. Muscle biopsy has historically been a fundamental tool in diagnosing myopathies (3). Although advancements in serological and genetic testing have expanded diagnostic capabilities, microscopic examination of skeletal muscle remains essential for evaluating patients with unexplained muscle weakness (4). Examining muscle tissue obtained through biopsy can aid in establishing a precise diagnosis, confirming or ruling out a clinically suspected condition, and supplying crucial samples for research in this field. When selecting a muscle for biopsy, the choice is usually directed toward areas likely to be affected by the disease. This decision is generally based on observed muscle weakness, findings from electromyography (EMG) or structural abnormalities identified through imaging methods such as magnetic resonance imaging (MRI) or US (4). There are multiple methods for obtaining a muscle biopsy. The chosen technique depends on institutional protocols and expertise, with common approaches including open surgical biopsy, conchotome biopsy and needle muscle biopsy. The technique of percutaneous muscle biopsy is widely recognised and has served as a foundation for advancing various lines of investigation across both preclinical and clinical research contexts (5). Two of the most used techniques are performed with the Bergström needle or the Well-Blakesley conchotome (5). The original Bergström needle consists of two cylinders (Fig. 1a). Upon insertion of the outer needle into the muscle tissue, the surrounding muscle fibres are displaced and protrude into the exposed sampling chamber, positioning them for collection. The Well-Blakesley conchotome consists of an alligator forceps that opens with a scissor grip (Fig. 1b). Opening of the conchotome forceps after muscle penetration tends to cause greater disruption to the surrounding tissue compared to the Bergström needle (5). Additionally, the use of the conchotome is frequently associated with increased intramuscular bleeding compared to the Bergström technique (5).

In a systematic literature review (SLR), the diagnostic utility of needle, conchotome, and open muscle biopsies was compared in the assessment of neuromuscular disorders (6). The authors concluded that, despite the smaller tissue yield, moderate- to large-gauge needle and conchotome muscle biopsies had equivalent diagnostic utility to that of open surgical muscle biopsies (6). Only a limited number of studies in this SLR systematically examined the use of imaging for selecting muscle biopsy sites. The potential of imaging to assist in choosing a representative muscle for histological analysis, thereby enhancing the diagnostic value of muscle biopsies, is an area of interest.

A recent paper from our group (7) assessed the safety and efficacy of UGNMB and showed that histological analysis was possible in most samples (90%). UGNMB appeared to be a well-tolerated procedure, with an average Visual Analogue Scale (VAS) for pain of 3.8±2.2, and a safe method for collecting muscle samples, with only mild adverse events rarely reported. In this paper, we aim to describe in detail the technique for performing a muscle biopsy using a 14-G guillotine-type semiautomatic biopsy needle (Fig. 1c). This technique utilises US not only for muscle selection and entry point determination but also for guiding the entire sample collection process. We also aim

to emphasise the procedural novelty of continuous real-time ultrasound guidance, as opposed to ultrasound-assisted skin marking alone, and to provide a reproducible protocol that may be adopted in appropriately trained centres. This manuscript is intended as a practical methods/protocol paper describing a standardised approach rather than as a report of a new prospective cohort.

Pre-biopsy checklist for muscle sampling

To ensure the proper execution of an UGNMB, a structured approach must be followed. In the authors' department, the process begins with a comprehensive checklist. This includes patient reception, screening for contraindications, obtaining written informed consent, and collecting clinical, demographic, and procedure-related data through patient interviews and electronic medical record retrieval. Patients taking oral anticoagulants are advised to switch to low-molecular-weight heparin or discontinue anticoagulation 24 hours before the procedure. Several conditions are considered a contraindication for the procedure, such as fever or other signs of infection, particularly, but not exclusively, on the potential biopsy site, such as cellulitis or other skin infections; severe thrombocytopenia (platelets <50,000/μL); coagulation disorders (such as haemophilia); pregnancy; or any other condition which in the investigator's opinion could increase the risk of complications. Additional diagnostic tests, particularly muscle MRI and EMG, are also reviewed, as they may assist in selecting the most appropriate muscle for biopsy.

The biopsy procedure should be conducted in an operating theatre or a clean procedure room with adequate space to accommodate a sterile trolley, a patient bed, an ultrasound machine, the physician, and a dedicated procedure nurse. Although the procedure can be performed in an operating theatre, one of the practical strengths of this technique is that, in experienced hands, it may also be safely undertaken in a dedicated clean procedure room or specialised rheumatology outpatient setting, potentially improving logistical efficiency and resource use compared with surgical biopsy pathways.

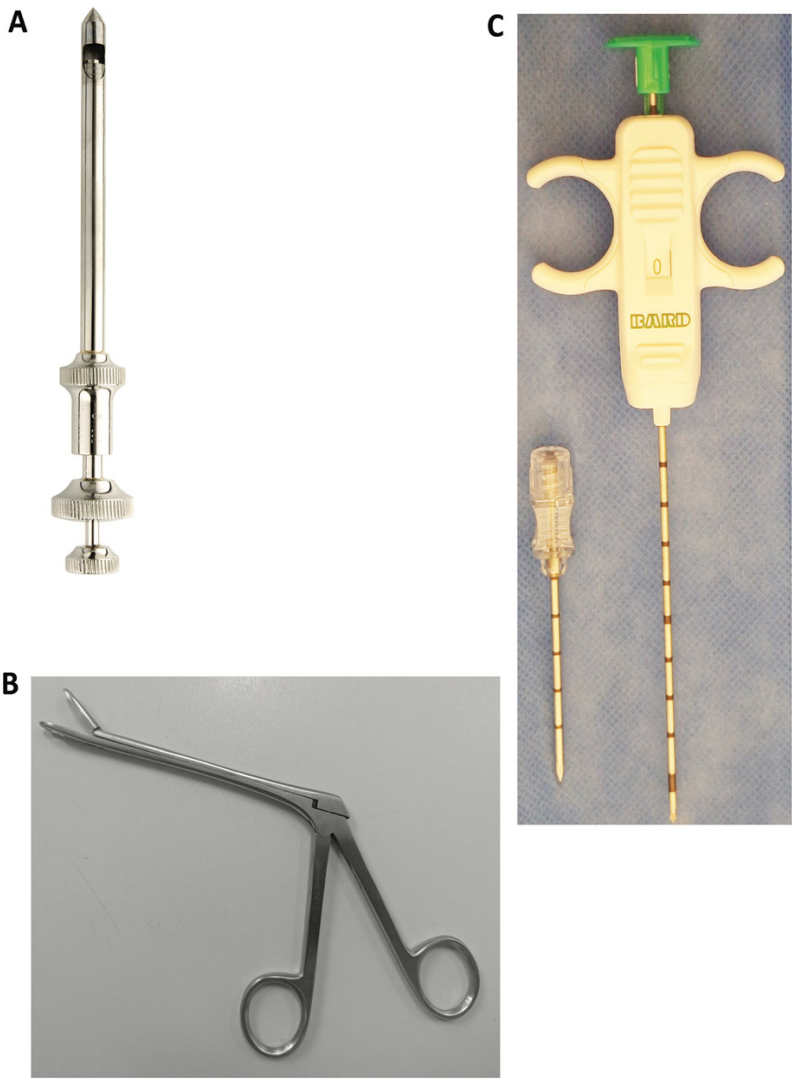


Fig. 1. Examples of instruments used for muscle biopsy:
A. The Bergström needle*; **B.** The Well-Blakesley conchotome; **C.** 14-G guillotine-type semiautomatic biopsy needle. *Image courtesy of Stille AB. © Stille AB. All rights reserved.

Criteria for selecting the muscle to biopsy

A multifactorial approach guides the selection of muscles for biopsy. This includes clinical evaluation based on patient symptoms and muscle involvement identified through MMT-8, as well as complementary data from EMG and muscle MRI when these exams are available. This thorough evaluation is crucial in selecting the most appropriate region for biopsy. However, the final decision relies on identifying the most altered muscle section through a brief ultrasound examination, which also determines the optimal needle trajectory to avoid tendinous and neurovascular structures.

On grayscale US, normal muscle typically appears hypoechoic compared to the surrounding subcutaneous tissue (8). When viewed in the longitudinal plane, hyperechoic bands corresponding to the perimysium or aponeurosis can be observed within the muscle. In the transverse plane, these bands create a more dotted pattern, often referred to as the "starry night" appearance (8). The echotexture of muscle on ultrasound can vary depending on muscle depth, fibre orientation, and fibre size, influencing how different muscle groups appear on imaging (9). Multiple studies have described higher muscle echogenicity in myositis cases (10).

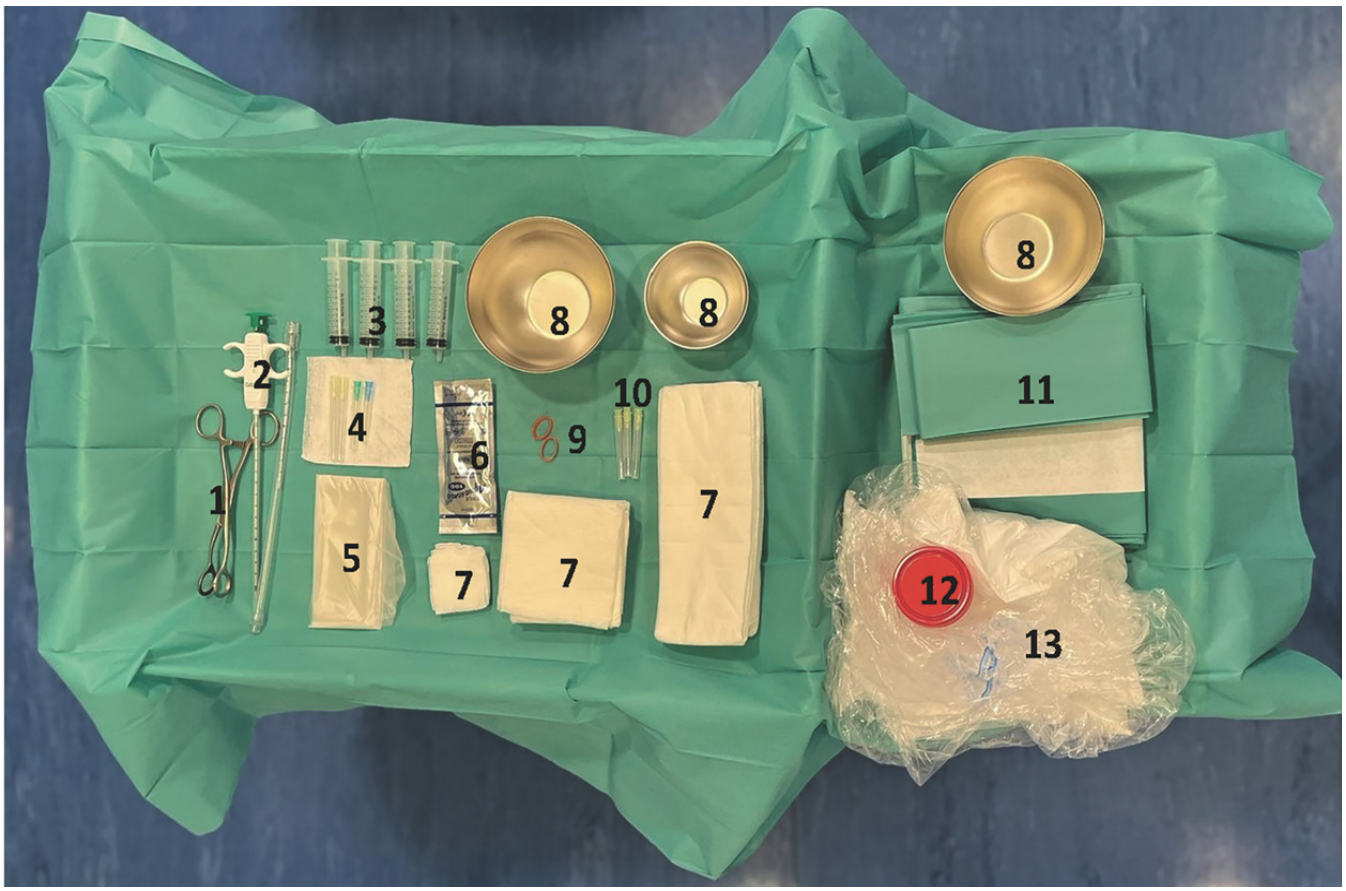


Fig. 2. Biopsy material.
1: Sponge forceps; 2: 14G guillotine-type semiautomatic biopsy needle; 3: 10cc syringes for anaesthesia administration; 4: 21G needle for anaesthesia administration; 5: Sterile ultrasound probe coat; 6: Sterile ultrasound gel; 7: Gauze pads in different sizes; 8: Stainless steel bowl used for antiseptic solution and sharp instruments; 9: Sterile elastic bands to secure the ultrasound probe inside the sterile cover; 10: Dilution needles; 11: Sterile drapes to isolate the entry area and cover the patient; 12: Recipient for tissue storage; 13: Ice bag to preserve muscle fragments.

However, certain factors must be considered when interpreting these findings. In the acute phase, muscle oedema can often result in a paradoxical decrease in echogenicity, making the muscle appear hypoechoic (11). Furthermore, this oedema may also lead to a loss of clear muscle definition and an increase in muscle thickness (8). The Siena Myositis Ultrasound Grading Scale highlights that oedema and power Doppler activity are strongly associated with the early and active phases of myositis, indicating that inflamed muscle is likely to appear swollen, thickened, and exhibit increased vascularity on Doppler imaging. In contrast, muscle atrophy and increased echogenicity indicate disease chronicity, correlating with both patient age and overall disease severity (12). Based on the above score, acute myositis presents as markedly hypoechoic and swollen muscle on ultrasound, aligning with MRI findings of oedema. This suggests that hypoechoic regions represent the most suitable biopsy sites (12). Ultrasound machines with lower-frequency transducers may be necessary for deep-seated muscles, but in most cases, a linear multifrequency probe is adequate.

Detailed technique description of UGNMB

A support table with all required equipment and materials is prepared, and a sterile technique is implemented (Fig. 2). Sterile gel or chlorhexidine solution must be used as a contact medium. The in-plane approach, which ensures that the needle remains as parallel as possible to the probe's long axis, is the optimal technique, as it provides better visualisation of the entire needle trajectory, thereby enhancing precision and safety during the procedure. After selecting the anatomical site, local anaesthesia is administered under US guidance up to the muscle fascia. To preserve the structural integrity of the muscle, anaesthetic infiltration within the muscle itself is avoided, as studies on other tissue types have suggested that this may alter its architecture and compromise histological analysis (13). Up to 10 ml of a local anaesthetic like lidocaine using 21G or 23G needles is injected into the subcutaneous tissue and fascial plane (Fig. 3A). A 21-G needle is then used to perforate the fascia under real-time ultrasound guidance (Fig. 3B). The needle's position is continuously monitored as it advances toward the fascia, ensuring precise placement while avoiding major vascular structures. This approach facilitates a smoother passage of the 14-G-guillotine-type semiautomatic biopsy needle into the muscle compartment under

minimal pressure and minimises the risk of complications (14). For deeper muscles, a suitable coaxial outer needle may be used in conjunction with the 14-G biopsy needle to facilitate repeated needle entry through a longer path. Once the needle is positioned at the targeted muscle site for biopsy, the stylet is advanced 2 cm, and the biopsy device is triggered to obtain a muscle sample (Fig. 3C-D). This process is repeated until a minimum of six fragments are collected for submission to the neuropathology laboratory. Within the same muscle, we aim to target different areas by adjusting the needle position and rotating it 180º to obtain multiple muscle samples. This is always performed under real-time ultrasound guidance to ensure accuracy and optimal tissue collection. Each collected sample is visually examined to confirm the presence of muscle tissue before being placed in a dry-cooled universal container and delivered to the neuropathology laboratory of our institution within one hour, where the samples are frozen and prepared for histological analysis (Fig. 4). Samples for biobank storage are also collected and preserved for RNA sequencing (RNAlater®), in formaldehyde, and in a dried tube (at least one sample for each) and stored at -80ºC.

After the procedure, compression of the entry site is followed by the application of a small adhesive dressing (Fig. 5). As shown in Figure 5, the entry site is very small, making skin sutures unnecessary. Instead, only an adhesive dressing is required. When the procedure is completed, any immediate complications are recorded, and the patient is asked to grade their tolerance to the intervention. Following the procedure, patients are instructed to rest the affected area for 48 hours. Cryotherapy is recommended for 10-minute intervals every two hours during the first two days, except while sleeping. For pain management, paracetamol or non-steroidal anti-inflammatory drugs are prescribed as needed, provided there are no contraindications.

The entire procedure has an average duration of less than 50 minutes, with approximately 30 minutes allocated to pre-procedure preparations. These preparatory steps include completing a checklist that covers patient reception, assessment of contraindications, obtaining written informed consent, and collecting clinical data, such as current treatments and a muscle strength evaluation. Additionally, a brief US examination is performed, along with necessary preparations for the operator, equipment, and patient. The biopsy itself is completed within 20 minutes.

Between one and two weeks post-biopsy, patients are requested to assess their pain levels using a VAS for pain and to report any complications experienced during this period. In exceptionally rare cases where a serious complication is suspected, patients are promptly referred for an in-person clinical evaluation. We highlight, as previously reported (7), that we have experienced only mild adverse events. Additionally, each patient who undergoes the procedure is asked to provide feedback on the existence of any scarring and indicate whether they would be willing to undergo the procedure again in the future.

Muscle sample collection and processing

A minimum of 6 samples is collected in a dry-cooled universal container and delivered to the neuropathology laboratory of our institution within one hour. Each tube is labelled with the following information: patient's full name, date of birth, date of biopsy, biopsied muscle and detailed clinical information, as it is essential for the accurate interpretation of the biopsy results. The biopsy sample is approximately 5–10 mm in length. Each sample is mounted on cork discs, which contain a small amount of tragacanth gum to hold the sample in place. When mounting, each sample is properly aligned, with muscle fibres oriented perpendicularly. The sample is then readily immersed in an isopentane solution for 10-20 seconds. The isopentane solution was previously frozen using liquid nitrogen at -160°C, then allowed to warm until it reached a semi-solid state, with both solid and liquid phases present. Once frozen, the sample can be sectioned using a cryostat; each section is 8–12 μm, cut at a temperature of -23 to -25°C. The histological diagnosis is established by an experienced neuropathologist, who considers the clinical data and histological analysis. Hematoxylin/Eosin, Gomori Trichrome, PAS (periodic acid-Schiff), NADH-TR (Nicotinamide adenine dinucleotide, Tetrazolium Reductase), SDH (Succinic acid dehydrogenase), COX (cyclooxygenase)/SDH, Acid Phosphatase, ATPase, Myosin Heavy Chain, MHC (major histocompatibility complex) class I and Class II, MxA (myxovirus protein A), and complement (C5b-9) staining are routinely performed. In the presence of inflammatory infiltrates, anti-CD3, CD4, CD8, CD20, and CD68 are added as needed. For selected cases, based on morphological features, oil red O, Congo red, and/or myophosphorylase are also performed.

Adverse events related to UGNMB

Adverse events are uncommon in UGNMB and are usually mild and transient. This technique offers a minimally invasive alternative to open muscle biopsy, allowing for the sampling of deep muscles while requiring only a minimal skin incision and minimising the need for operating room resources. In our experience, the procedure is generally well-tolerated, with an average VAS for pain of 3.8±2.2 out of 10 during the procedure (7). The most frequent short-term adverse event is pain. Long-term adverse events are rare (in our experience, only one patient reported persistent pain after the biopsy, and another one reported persistent numbness, but both symptoms disappeared after a few months). It is important to highlight that we have not observed any cases of infection or scarring at the biopsy site. The procedure is efficient, well-tolerated, and can be conducted under local anaesthesia in an outpatient setting (7). An additional strength is the possibility of performing the biopsy under local anaesthesia without intramuscular anaesthetic infiltration, thereby preserving muscle architecture while maintaining patient comfort.

Discussion

This method enables the biopsy of virtually any skeletal muscle in the body,

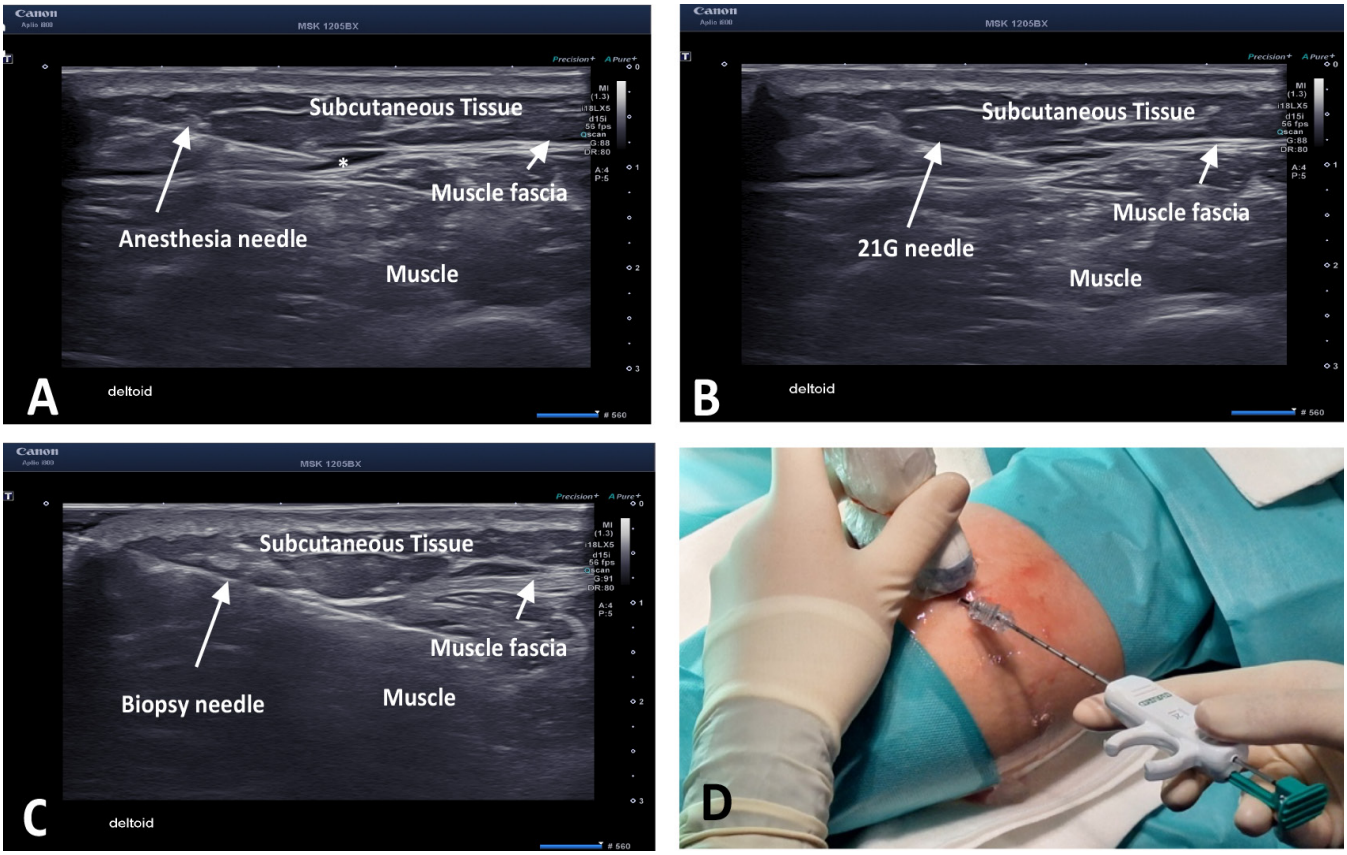


Fig. 3. Ultrasound-guided muscle biopsy.
A. The needle is inserted up to the muscle fascia and used to administer anaesthesia to the biopsy path. The asterisk shows the hypoechogenic areas resulting from the fluid accumulated after anaesthesia administration. **B.** A 21G needle is then used to perforate the fascia. **C.** Depicts the biopsy needle inserted in the muscle for sample retrieval. **D.** Image showing the position of the probe and the entry point of the 14G guillotine-type semiautomatic biopsy needle.

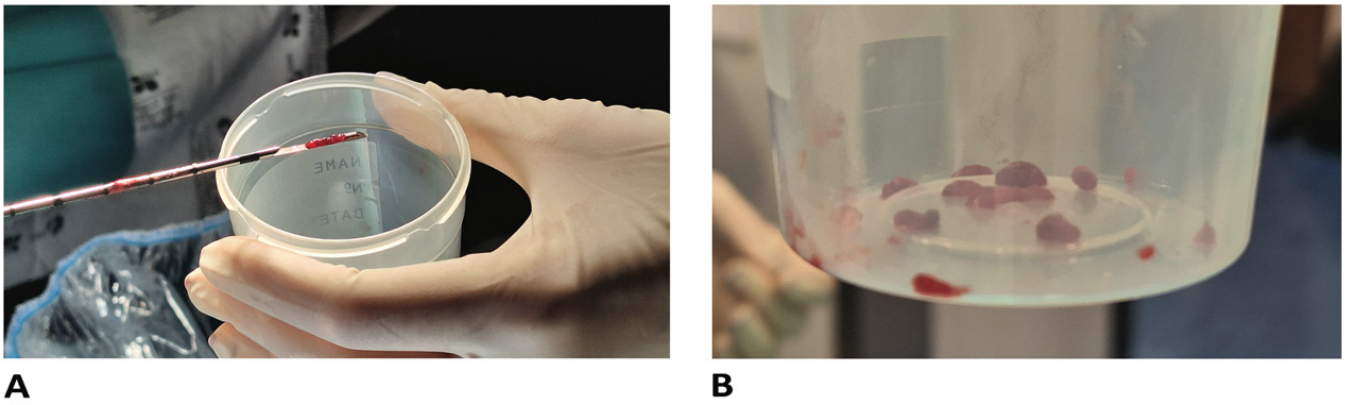


Fig. 4. Muscle sample collection.
A. Muscle sample being collected from the biopsy needle. **B.** Muscle fragments ready to be delivered to the Neuropathology Laboratory.

providing a versatile, safe, and effective approach for retrieving muscle tissue. Real-time US imaging enhances precision by identifying optimal biopsy sites, avoiding critical structures, and reducing the likelihood of complications, such as bleeding (15, 16). Some previously described techniques utilise different biopsy needles, such as the Bergström® needle or vacuum-assisted devices, which require larger incisions, thereby increasing the risk of tissue trauma, hematomas and sample distortion (17, 18). When comparing needle, conchotome and open muscle biopsies, Ross *et al*. (6) concluded that all techniques were generally well tolerated with a low complication rate for all adverse events except haematoma or ecchymosis. Haematoma and ecchymoses were reported in up to one-third of patients undergoing large-gauge needle muscle biopsy (14). Infections at the biopsy site were predominantly observed in studies on open surgical muscle biopsies, with an incidence ranging from 1% to 5% (19). Long-

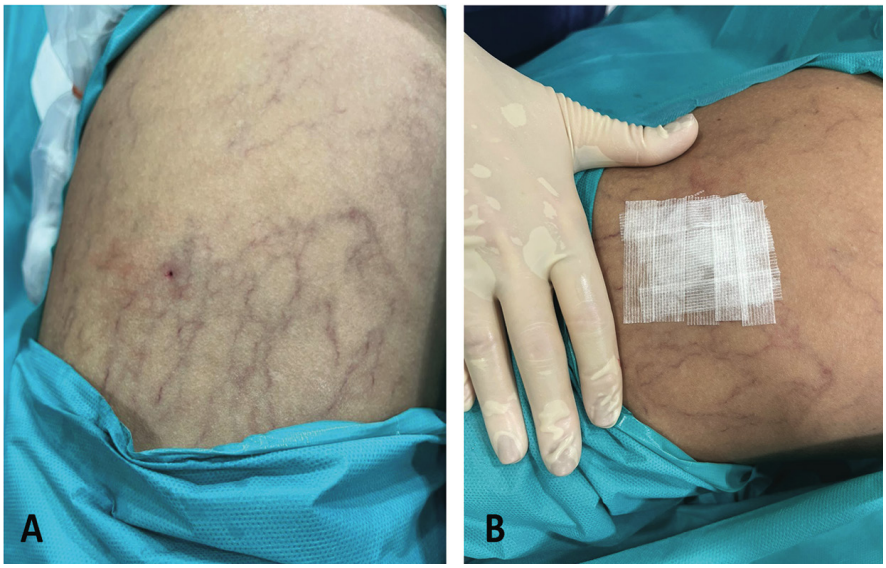


Fig. 5. Biopsy site without active bleeding after the biopsy (vascular changes were present prior to the biopsy). **A.** Biopsy site small entry. **AB** Biopsy site with adhesive dressing.

term complications after muscle biopsy were rarely documented, with one open surgical biopsy study reporting a 3.1% incidence of keloid scarring (20).

The needle choice may influence sample size, handling characteristics, and potentially tissue architecture preservation. We also note that while the Bergström needle has traditionally been widely used and may provide larger specimens, the 14G guillotine needle offers practical advantages in the real-time ultrasound-guided setting, particularly in terms of manoeuvrability and precision, although direct comparative data regarding preservation of tissue architecture between these approaches remain limited. Although needle biopsy yields smaller individual muscle samples compared to open muscle biopsies, it presents several advantages. This technique enables the collection of tissue from multiple regions within the same muscle through a single, minimal incision, thereby enhancing sampling diversity while maintaining a less invasive approach. In open biopsies, sutures require a more prolonged recovery and limited activity for 10-14 days (21, 22). In studies where incision lengths were documented, 75% of open biopsy procedures involved cuts of at least 2 cm (23-26), while 89% of percutaneous biopsies required incisions of 1 cm or less (27-30). We acknowledge, however, that surgical biopsy may still be preferable in selected scenarios, particularly when larger tissue volume is required, such as in suspected vasculitis, very focal disease, or when ultrasound visualisation and target accessibility are suboptimal.

The 14G guillotine-type semiautomatic biopsy needle was selected as a practical compromise between tissue adequacy for histopathological and ancillary studies, patient tolerability, and manoeuvrability under real-time ultrasound guidance. Available muscle-biopsy literature supports the use of moderate- to large-gauge percutaneous needles for diagnostic sampling, but direct comparative data across different gauges in ultrasound-guided muscle biopsy remain limited (7, 16, 31). Table I summarises the main muscle biopsy techniques and their reported characteristics, advantages, and limitations based on the available literature. However, a direct comparison among the different techniques, as well as among the needles used, has not yet been undertaken.

In a recent study by Sutu *et al*. (30), 102 patients reported their experiences and perspectives after undergoing muscle biopsy. Most participants reported having an open biopsy (68.6%) performed by a surgeon (73.5%). Most respondents (80.4%) mentioned that they were "asleep" as the procedure was performed using anaesthesia or sedation. A few respondents experienced post-procedural pain lasting >72 h (27/102, 26.5%), increasing weakness post-biopsy (13.7%), numbness at the biopsy site (18/102, 17.6%) and a recovery time beyond 3 days (36/102, 35.3%). Two-thirds (67%) of all respondents reported a scar >2 cm. The study authors suggested that the burden of morbidity associated with these open procedures may be more significant than is generally recognised. Participants reported higher-than-expected rates of pain and perceived complications when compared to those documented in previous studies. These results underscore the importance of developing strategies to better identify individuals who may be at increased risk for post-procedural adverse outcomes and delayed recovery. Some participants expressed concerns regarding a perceived disconnect between the clinician responsible for their diagnosis and the operator performing the biopsy, which contributed to uncertainty and anxiety about the appropriateness of the muscle selected for sampling. In this context, the ability to perform a focused pre-procedural US screening of the most clinically affected muscles, combined with real-time ultrasound guidance throughout the procedure, may help alleviate these concerns. This integrated approach has the potential to enhance patient confidence and improve overall compliance with the biopsy process. This may be maximised if the patient can see the abnormal muscle or follow the whole procedure on the ultrasound machine screen.

Muscle MRI or prior US imaging helps determine the biopsy site. Yet, even when imaging is used, in most centres, the procedure is generally guided by external skin markings rather than real-time imaging (19, 28, 32, 33). This technique can result in false-negative findings in up to 15% of cases (34). Percutaneous muscle biopsy performed under US guidance has been shown to yield diagnostic accuracy comparable to that of traditional open biopsy (19). The methods previously described in the literature differ from the approach presented in this manuscript, primarily in US utilisation and needle design. In most prior studies, US was used only to locate and mark the muscle before

Table I. Summary of the main muscle biopsy techniques and their reported characteristics, advantages, and limitations based on the available literature.

Technique	Specimen characteristics	Procedural setting	Main advantages	Main limitations
Open biopsy	Typically provides the largest specimen and the broadest architectural assessment	Usually performed in a surgical or operating room setting	May be advantageous when a larger tissue sample is required	More invasive; larger incision; greater logistical burden; wound-related complications and scarring may occur
Fine-needle biopsy (*e.g.*, 14–16G)	Smaller specimen volume	Usually performed under local anaesthesia in an outpatient, bedside, or procedure-room setting	Minimally invasive; may be image-guided	May be associated with a higher rate of inadequate sampling and may provide less tissue for full architectural assessment
Moderate- to large-gauge needle biopsy (*e.g.*, 9–10G or Bergström-type techniques)	Moderate-to-large specimen volume, generally sufficient for histological assessment	Usually performed under local anaesthesia, often in an outpatient or bedside setting	Less invasive than open biopsy; in the available literature, diagnostic utility appears broadly comparable to open biopsy	Provides less tissue than open biopsy; pain, haematoma, or sensory symptoms have been reported
Conchotome biopsy	Moderate specimen volume	Usually performed under local anaesthesia in an outpatient, bedside, or procedure-room setting	Useful percutaneous alternative; available literature suggests diagnostic utility broadly comparable to open biopsy	Tissue disruption, bleeding, persistent pain, or, rarely, persistent weakness have been reported

This summary is intended as a practical comparison only; direct head-to-head evidence remains heterogeneous across studies.
G: gauge. Adjusted from Ross *et al*. (31).

the procedure rather than to provide continuous guidance during the biopsy. One study assessed US-assisted muscle biopsies in patients with acute neuromuscular conditions and reported diagnostic accuracy comparable to that of open biopsies (93-95%) (19). Another study evaluated UGNMB but with a modified Bergström needle in a group of 11 patients, all of whom had sufficient muscle samples for histological evaluation (16). In our view, the distinction between ultrasound-assisted and genuinely ultrasound-guided biopsy is clinically relevant: continuous visualisation throughout the procedure allows ongoing adjustment of the needle trajectory and may help mitigate the false-negative sampling associated with static pre-marking strategies.

A separate study from Australia compared open muscle biopsies with percutaneous biopsies, where US was used only to mark the site rather than to guide the entire procedure. The results showed that open biopsies were more effective (89% *vs*. 74%) (35). However, unlike our proposed technique, real-time US guidance was not employed throughout the biopsy, which may have impacted the overall success rate.

MRI is traditionally considered the reference imaging modality for IIM. However, it is limited by its cost, long imaging times and accessibility (8). Compared to CT and MRI, US presents several advantages, including broader availability, no radiation exposure (unlike CT), and no interference from strong electromagnetic fields (as seen in MRI). It is also better tolerated by patients and allows for real-time, dynamic muscle assessment. However, US is not without its drawbacks, such as limited penetration depth, particularly in medium-range machines, which can make deep muscles imaging more challenging, and the absence of standardised assessment protocols, which may affect consistency in muscle evaluation (8).

Strengths of the present technique include real-time targeting of abnormal but viable muscle, the possibility of performing the procedure in a specialised outpatient setting, minimal scarring, reduced procedural invasiveness, and avoidance of intramuscular anaesthetic infiltration. Limitations include dependency on operator expertise, the currently unquantified learning curve, and the lack of formal comparative inter-operator reproducibility data. Diagnostic success is likely to remain operator-dependent, and this manuscript does not include a formal analysis of inter-observer reliability or outcomes across clinicians with different levels of experience; this should be addressed in future multicentre studies. This procedure requires familiarity with musculoskeletal ultrasound anatomy, dynamic needle visualization, and recognition of biopsy-relevant structural changes within muscle. Rather than being regarded as an extension of basic ultrasound training alone, it is best performed by clinicians with prior competency in musculoskeletal ultrasound and experience in ultrasound-guided procedures (16, 36, 37). Nevertheless, it is likely to be learnable within a structured training environment, and adequate training and supervised practice are therefore important for safe and effective implementation, although formal studies specifically addressing the learning curve remain lacking. Obesity may reduce ultrasound image quality and increase technical difficulty because of greater tissue depth (38), while severe muscle atrophy and/or advanced fatty replacement may make selection of a suitable biopsy target more challenging (39). More broadly, patient-related characteristics, including body habitus, degree and distribution of muscle involvement, and stage of disease, may influence both technical

feasibility and the likelihood of obtaining an informative sample; however, these factors were not formally analysed in the present study, as this was not one of its primary objectives. We also note that, in selected cases, open biopsy may still retain a complementary role, particularly when ultrasound visualisation is suboptimal or when sampling requirements favour a surgical approach (31).

Conclusion

UGNMB is a precise and minimally invasive technique that may enhance the accuracy and safety of muscle sampling. By providing real-time visualisation, it ensures optimal site selection, reduces the risk of complications, and allows for dynamic monitoring throughout the procedure. Unlike conventional approaches, which rely on pre-marked anatomical landmarks, our technique enables continuous ultrasound guidance, supporting accurate needle placement and improved diagnostic yield. The use of this technique facilitates access to deep muscle groups while requiring only local anaesthesia, making it a valuable alternative to open surgical biopsy in the assessment of neuromuscular diseases. Its main added value lies in the use of continuous ultrasound guidance rather than pre-procedural muscle localisation alone, supporting more accurate and safer sampling in routine clinical practice when performed by appropriately trained operators.

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